

Insight into Danube sturgeon life history: trace element assessment in pectoral fin rays

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Abstract Sturgeon populations in the Danube River have experienced severe decline during the last several decades, mostly due to the poorly regulated fishery, river fragmentation and water pollution. This study focuses on gaining better understanding of sturgeon life history primarily by addressing the assessment of microelement accumulation in sturgeon pectoral fin rays, especially of strontium and calcium, as a method that can reveal migration patterns of

anadromous sturgeons. Analysis was performed on pectoral fin samples of three anadromous Danube sturgeon species (beluga, Russian sturgeon and stellate sturgeon) by the use of a Nuclear Microprobe technique. The most frequent pattern in analyzed samples was represented by a low Sr:Ca ratio in the innermost annuli, followed by an increased ratio in the middle annuli segment, and often with a decreased ratio in the outermost annuli. Probability density estimate has revealed three distinguished maxima of the Sr:Ca ratio, 7.08×10^{-3} , 8.98×10^{-3} and 9.90×10^{-3} , which might correspond, respectively, to fresh, brackish and saltwater. Although the analysis of the Sr:Ca ratio in sturgeon pectoral fin rays has revealed changes that might indicate probable migration between habitats with different water salinity, further studies are needed for improvement of this method. This study represents the first analysis of this kind that was conducted on sturgeon species from the Black Sea basin.

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Introduction

Sturgeons are one of the most endangered groups of fish and, due to a specter of different anthropogenic impacts, such as poorly regulated fisheries, habitat

fragmentation and pollution, their populations have experienced serious decline (Lenhardt et al. 2008). The majority of 27 extant sturgeon species are nowadays considered to be endangered or critically endangered (IUCN 2008). The Danube River basin represents one of the centers of sturgeon diversity (Williot et al. 2002). It used to be inhabited by six sturgeon species: Atlantic sturgeon (*Acipenser sturio*), ship sturgeon (*Acipenser nudiventris*), beluga (*Huso huso*), Russian sturgeon (*Acipenser gueldenstaedtii*), stellate sturgeon (*Acipenser stellatus*) and sterlet (*Acipenser ruthenus*). Unfortunately, the Atlantic and ship sturgeon are, respectively, considered to be extinct and possibly extinct within the Danube basin, while the latter four species are facing different levels of extinction risk (Reinartz 2002; Williot et al. 2002; Lenhardt et al. 2006; Jarić et al. 2009).

Most sturgeon species are anadromous, which means they spend a larger part of their lives in the marine environment, and once every several years they migrate to rivers to reproduce (Bemis et al. 1997). Beluga, Russian sturgeon, stellate sturgeon and Atlantic sturgeon are anadromous species, while the sterlet spends its whole life cycle in a freshwater environment. Although different populations of the ship sturgeon can be both anadromous and potamodromous, the Danube population is believed to stay exclusively in the river (Reinartz 2002). These reproduction intervals are considered to be species specific, and range from 2 to 3 year intervals for the Atlantic sturgeon to 5–6 year intervals for beluga (Reinartz 2002; Vassilev 2006; Jarić et al. 2010).

However, patterns of sturgeon movements between the sea and the river environment have been thus far poorly studied, and the lack of better understanding of their migrations presents a significant obstruction for development of adequate conservation and fishery management measures (Veinott et al. 1999; Arai et al. 2002). Field studies of migration patterns are often difficult to conduct, since they are expensive and time consuming (Veinott et al. 1999). Fortunately, recent development of microelement analysis of fish bone structures has offered new opportunities for getting better insights in the ecology of diadromous fish species. Since fish can grow throughout their life, their bone structures annually experience creation of visible growth zones. Each growth zone represents 1 year of life of an individual, so the technique of counting growth zones on fish scales or bones has

gained wide application in fish age assessment. The pectoral fin ray is commonly used for age assessment studies on sturgeons (Stevenson and Secor 2000).

Certain elements, such as strontium, accumulate in fish bones proportionally to their concentration in the environment (Limburg et al. 2001). Since the level of strontium in an aquatic environment is directly correlated with water salinity, growth zones in fish bone structures will contain different strontium concentrations depending on the salinity of the water where they have resided in a certain year of their life (Veinott et al. 1999; Arai et al. 2002). In this way, Sr:Ca ratios in each growth zone of certain bone structures can reveal whether the individual has spent a specific year of its life in the marine or freshwater environment (Limburg et al. 2001). A significant increase in the Sr:Ca ratio should signify the migration of the studied individual to a more saline environment, and vice versa, a substantial drop in the ratio would imply movement to freshwater (Limburg et al. 2001; Arai et al. 2002). Furthermore, transect scans of the Zn:Ca ratio can be potentially used as an additional method to distinguish growth zones in an otolith sample since they show regular variation that follows the annual cycle of seasons (Limburg et al. 2001). The most advanced method developed for microelement assessment in fish bone structures is the Nuclear Microprobe technique (Elfman et al. 1999; Elfman et al. 2000; Limburg et al. 2001).

In the present study, we have attempted to apply this approach for the assessment of migration patterns of the Danube sturgeon species, which should enable better understanding of their ecology and offer better estimation of the appropriate conservation measures. This study represents the first analysis of this kind that was conducted on sturgeon species from the Black Sea basin.

Methods

To assess migration patterns of Danube sturgeon species through the microchemical concentration assessment in pectoral fin rays, a number of fin samples were collected. Three sturgeon species were the subject of this research: beluga, Russian sturgeon and stellate sturgeon. All samples were acquired from individuals that had been archived from the commercial sturgeon fishery during the period 2001–2007. No individuals were harmed or sacrificed within this research.

Seven samples were caught by fishermen in 2004 near Tulcea in Romania (71 km of the river flow), and these samples were acquired from the collection of the Danube Delta National Institute in Tulcea, Romania. Two samples were caught by fishermen near the Prahovo in Serbia, below the Iron Gate II dam (861 km of the river flow), and these samples were acquired from the collection of the Institute for Multidisciplinary Research in Belgrade, Serbia. Basic data for all individuals that were used in this research is presented in Table 1.

All fin rays were removed from the surrounding tissue and air dried. A smaller, 2–3 cm long section was cut with a saw from each fin ray sample, close to the place of its articulation with the body. Final sample sectioning was performed at the Laboratory for Anthropology, Department of Anatomy, of the University of Belgrade. Samples were placed in moulds and mounted in epoxy resin, and then cut with a Leica SP 1 600 low-speed electric saw to 500 µm thick sections. Each section was mounted on a glass slide.

The age of each fish was assessed according to the method of Stevenson and Secor (2000). The fin ray sections were analyzed under the microscope. One growth zone (annulus) was defined as the combination of two consecutive zones, an opaque and a translucent band. The innermost area represents the first annulus and corresponds to the first year of life, and the outermost annulus to the last year of life of the studied individual (Stevenson and Secor 2000). As suggested by a number of authors (e.g., Jackson et al. 2007; Killgore et al. 2007), age assessment was performed by four independent readers, in order to reduce errors in the process of age reading. A final decision on the age and the border between growth zones was reached by consensus. If there was still a discordance and opinions about the counts differed by

1 year, the fish was assigned a higher age, as the age of older individuals is usually underestimated (Rien and Beamesderfer 1994; Paragamian and Beamesderfer 2003; Killgore et al. 2007).

Assessment of microelement concentrations in each fin ray section was performed by the use of a Nuclear Microprobe (NMP) at the Lund Nuclear Microprobe facility, Division of Nuclear Physics, Physics Department, Lund Institute of Technology at the Lund University. An NMP consists of a small accelerator, probe forming magnetic lenses and a irradiation chamber, where the sample is placed. While the NMP is similar in its methods to an electron microprobe, it generally has 50 to 100 times more sensitivity. A scanning beam produces maps of concentrations of selected elements in the sample, over an active area of 50 mm², by the use of the Particle Induced X-ray Emission (PIXE) method. Detailed information about the Lund NMP facility and methods applied was presented by Shariff et al. (2005a, 2005b) and Elfman et al. (2005).

NMP scanning conditions were described by Elfman et al. (1999) and Limburg et al. (2001). The standard 2.55 MeV proton beam and the X-ray detector (Kevex Si(Li)) of the 50 mm² active area were used in the analysis, and a thick mylar and aluminum adsorber was used to suppress X-ray peaks of calcium, thus enabling an increase of the current which enhances the signal of strontium and other trace elements (Elfman et al. 1999, 2000). While concentrations of different elements were checked (e.g. iron, manganese and cobalt), special emphasis was placed on concentrations of strontium, calcium and zinc, since the accumulation of these elements has been shown to provide the best information on environmental salinity and seasons (Limburg et al. 2001). The Sr:Ca

Table 1 Basic data for the nine sturgeon individuals whose pectoral fin rays were assessed for microchemical concentrations

No.	Species	Gender	Date of catch	Locality	SL (cm)	Weight (kg)
1	<i>A. gueldenstaedtii</i>	F	May 2004	Tulcea, RO	150	22.5
2	<i>A. gueldenstaedtii</i>	F	May 2004	Tulcea, RO	149	19
3	<i>A. gueldenstaedtii</i>	F	April 2004	Tulcea, RO	149	23
4	<i>A. stellatus</i>	F	April 2004	Tulcea, RO	106	5.9
5	<i>A. stellatus</i>	F	April 2004	Tulcea, RO	120	9.3
6	<i>A. stellatus</i>	M	May 2004	Tulcea, RO	106	5.9
7	<i>H. huso</i>	M	May 2004	Tulcea, RO	194	70
8	<i>H. huso</i>	M	Nov–Dec 2001	Prahovo, SR	/	110
9	<i>A. gueldenstaedtii</i>	M	Dec 2007	Prahovo, SR	50.3	1.3

SL standard length, F female, M male.

ratio was used as a potential proxy for salinity, and the Zn:Ca ratio as an indicator of seasons (Limburg et al. 2001). Elemental maps of 128×128 pixel size, that covered as much of the sample as possible, were obtained as the first step of the analysis. After checking the elemental maps for preliminary information, a transect traversing the fin ray section from the core to the outer edge was made on each sample. Since transects were made by performing a scan every 6–7 μm along the transect line, they were able to provide greater resolution and thus better information than the elemental maps. After the data collection, the data sets were normalized to counts per charge (Limburg et al. 2001).

Data analysis

Transect scans from all three sturgeon species were subjected to moving average analysis, in order to eliminate point to point fluctuations and display a clearer profile. Since sample 9 was a juvenile specimen, it was excluded from the moving average analysis. Different moving average window lengths (MAW) were tested, and optimal lengths chosen for further analysis. MAW length used for the Russian sturgeon and beluga was 22 points, for stellate sturgeon was 24 points. Data from these filtered profiles were treated as equal members of ensemble, and subjected to probability density estimate (PDE), supplied by MATLAB 6.5, in order to determine most probable concentration ratios for each of the three studied species. Local maxima from the three acquired PDE profiles were pooled and treated as a new statistical ensemble, which was again subjected to the PDE analysis, in order to determine most probable concentration ratios across all studied species. Different zones along the concentration transect scans were statistically compared using the Mann Whitney U test (SPSS software, version 15.0).

Fig. 1 Microscopic pictures of the analyzed pectoral fin ray sections of three different sturgeon species (Russian sturgeon, stellate sturgeon and beluga), samples 3, 4 and 7. Sample numbers are presented in the upper right corner of each image



Results

Several samples were placed in a less appropriate position during the scan, which made analysis of such samples difficult. Microchemical analyses were mostly performed in the upper part of the fin ray section, where the annuli are very densely positioned or even merged together, so in such cases it was difficult to distinguish between different annuli. Furthermore, some samples had areas close to the central part of the section where the calcified material was missing, which additionally complicated their analysis. This was especially true for sample 6, where significant parts of the transect line were located in such areas. On sample 4 and, partially, sample 7, the outermost annuli were missing from the scan. Unfortunately, it was impossible to repeat the scans on these samples, due to financial constraints.

The samples that were included in analysis were four fin rays of the Russian sturgeon, three of the stellate sturgeon, and two of the beluga. Since the annuli of some samples were difficult to distinguish (Fig. 1), initial conclusions of the independent readers on the age of the studied individuals significantly differed. Nevertheless, consensus on the age of each individual was reached after a repeated assessment. Age of all individuals is presented in Table 2.

Ratios of Sr:Ca along the transect scans showed only moderate variation, without clearly distinguished negative peaks that would show unambiguous migrations from saltwater into the river. However, the Sr:Ca ratio in most of the samples showed a clear and statistically significant distinction (Mann Whitney U Test, $p < 0.001$, see Table 2) between the transect section within the first annulus or first several annuli and the remaining annuli that were located within the transect. This change in Sr concentration can also be noticed on the Sr elemental maps of some samples,

Table 2 Results of statistical comparison of the average Sr:Ca ratios within different zones in the line transects, using the Mann Whitney *U* Test

Sample No.	Age	Zones compared ^a	Average Sr:Ca ratios		Mann Whitney <i>U</i> Test (<i>U</i> value) ^b	Sample size ^c
			First zone	Second zone		
1	28	Age 1–7/8–21	6.77×10^{-3}	7.81×10^{-3}	4 228	177/156
2	22	Age 1–4/5–14	7.08×10^{-3}	8.72×10^{-3}	2 467	125/172
3	24	Age 1–5/6–17	6.52×10^{-3}	8.04×10^{-3}	3 756	235/162
4	21	Age 1/2–21	7.14×10^{-3}	9.97×10^{-3}	756	211/148
5	19	Age 1/2–19	6.59×10^{-3}	7.82×10^{-3}	792	42/161
6	17	Age 1/2–17	7.43×10^{-3}	7.82×10^{-3}	2 890	59/146
7	19	Age 2/3–11	6.34×10^{-3}	7.53×10^{-3}	1 649	79/140
8	20	Age 1–5/6–20	8.80×10^{-3}	8.58×10^{-3}	NS	219/137
9	4	Age 1–2 /3	1.72×10^{-3}	2.00×10^{-3}	17 362	469/148

^a presents the age of the individual to which series of annuli in each compared zone correspond (first zone/second zone).

^b $p < 0.001$ in all comparisons (except for sample 8).

^c (number of scanned points) in the first zone/in the second zone.

The Table also includes the results of the age assessment for each individual. NS difference not statistically significant ($p > 0.05$).

with the weaker Sr concentrations located in the central parts of the sample (Figs. 2 and 3). The magnitude of change in the Sr:Ca ratio ranged from 1.04×10^{-3} in sample 1 to 2.83×10^{-3} in sample 4 (Table 2).

Two frequent types of general patterns could be distinguished among the changes of the Sr:Ca ratio along the transect line. The first type, which was present in samples 4–6 (Fig. 4b), was represented by a low Sr:Ca ratio in the inner annuli and then an increased ratio in the outer annuli (Table 2). Although sample 6 was difficult to assess, due to the lack of calcified material in some parts that resulted in gaps in the transect scan (Fig. 3), statistical comparison has also confirmed significant change in the Sr:Ca ratio ($p < 0.001$, Mann Whitney *U* test). All three samples with this type of pattern in Sr:Ca ratio change belonged to stellate sturgeon individuals.

The second type of pattern, present in samples 1–3 and 7 (Fig. 4a, c), was represented by a lower Sr:Ca ratio in the inner part of annuli, followed by increased ratio in a section at the middle of the transect, and then a decreased ratio in the outermost annuli (Table 2). While the inner and the outermost zone of transects in these samples had similar ratios and, except in sample 2, were not significantly different among themselves ($p < 0.001$, Mann Whitney *U* test), they significantly differed from the middle zone of

transect. Higher concentration of the central zone of transect, when compared to the inner and outer annuli, was also observed on elemental maps of strontium of these four samples (Figs. 2 and 3).

Changes in Sr:Ca ratio in sample 8 lacked any clear pattern. Due to less appropriate position of the transect, it was not possible to distinguish between annuli corresponding to ages 1–5. Differences between the inner and outer annuli were not significantly different ($p > 0.05$, Mann Whitney *U* test). Sample 9, which represents a juvenile Russian sturgeon, was caught in the Danube River at the life stage when it should reside in the sea. This sample was therefore included in the analysis to determine whether it remained in the river throughout its life, or whether it had migrated from the sea before its capture. While the difference in Sr:Ca ratios between the zones corresponding to age 1–2 and 3 was significant ($p < 0.001$, Mann Whitney *U* test), both were very low (Table 2), which should indicate that this individual has never left the freshwater environment. Results of comparisons of Sr:Ca ratios in different zones of each sample are presented in Table 2.

Each of the PDE profiles for the three studied species was characterized by distinct local maxima (Fig. 5). On the other hand, it was not possible to

Fig. 2 Elemental maps of the sturgeon pectoral fin ray sections, samples 1–5. Sample numbers and the element that was scanned are presented in the lower left corner of each image

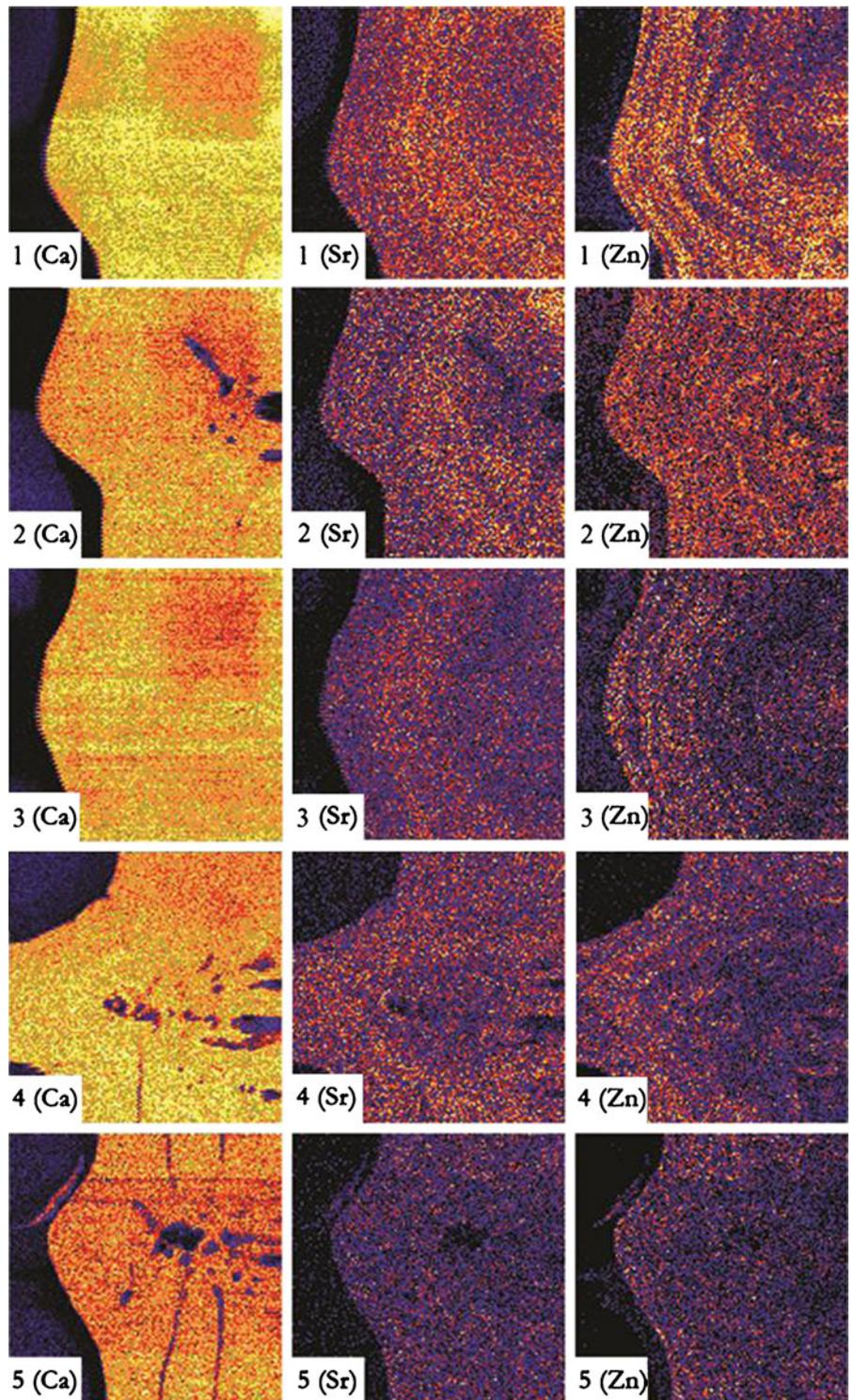
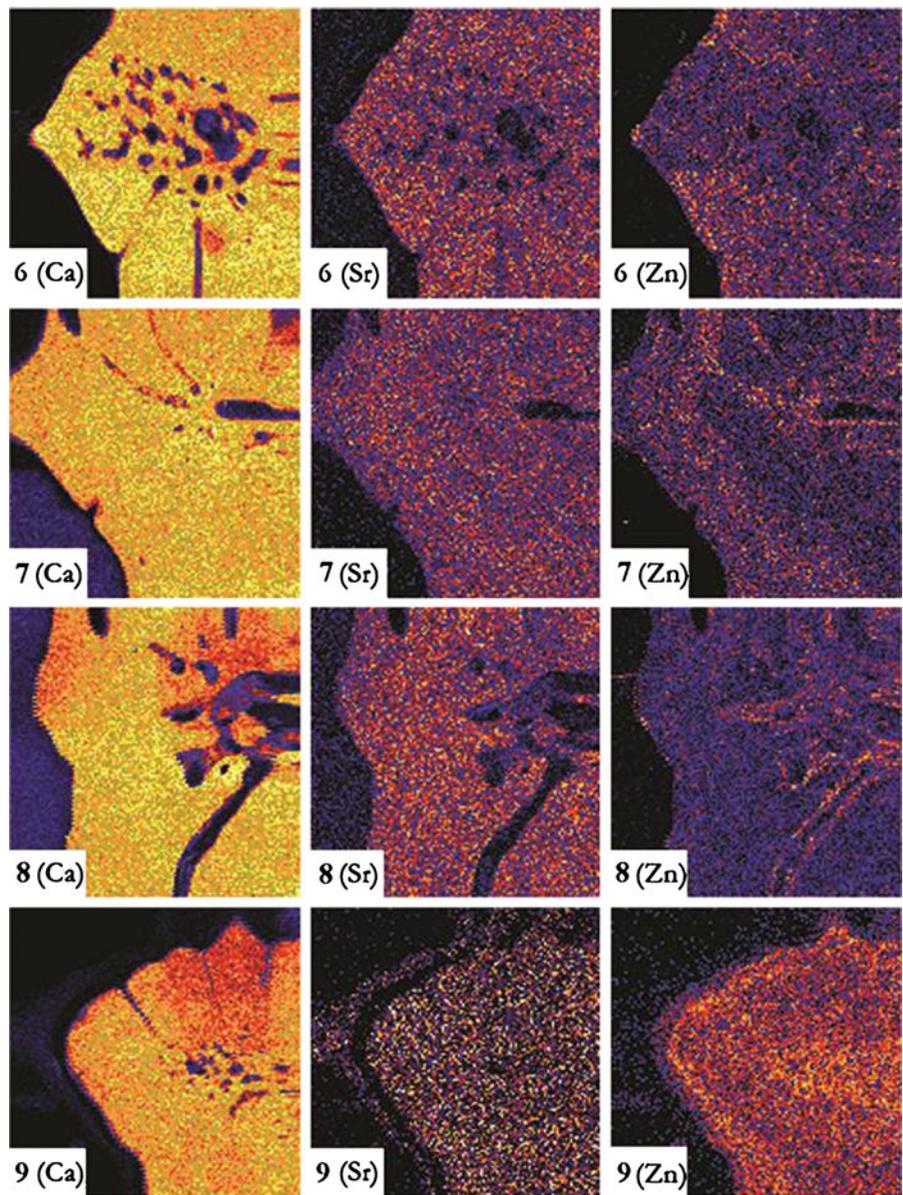


Fig. 3 Elemental maps of the sturgeon pectoral fin ray sections, samples 6–9. Sample numbers and the element that was scanned are presented in the lower left corner of each image



observe where the new pooled PDE maxima were positioned. This information was obtained by constructing pooled PDE profile for which three Sr:Ca ratio PDE maxima were detected: 7.08×10^{-3} , 8.98×10^{-3} and 9.90×10^{-3} (Fig. 6).

In the zinc elemental maps of some samples, especially in those of samples 1–3, it is possible to distinguish clear concentric rings, which resemble the pattern of annuli (Fig. 2). The Zn:Ca ratios in the transects scans generally had more frequent oscillations than Sr:Ca ratios. However, they were highly irregular, without a clear annual pattern, which made

it difficult to associate their pattern to the transitions between different annuli.

Discussion

Ratios of concentrations of Sr:Ca in annuli of nine analyzed sturgeon pectoral fin rays, which were assessed by the use of NMP technique, did not show as clear a pattern as those that were registered by Limburg et al. (2001) in fish otoliths. There was however a statistically significant increase in concen-

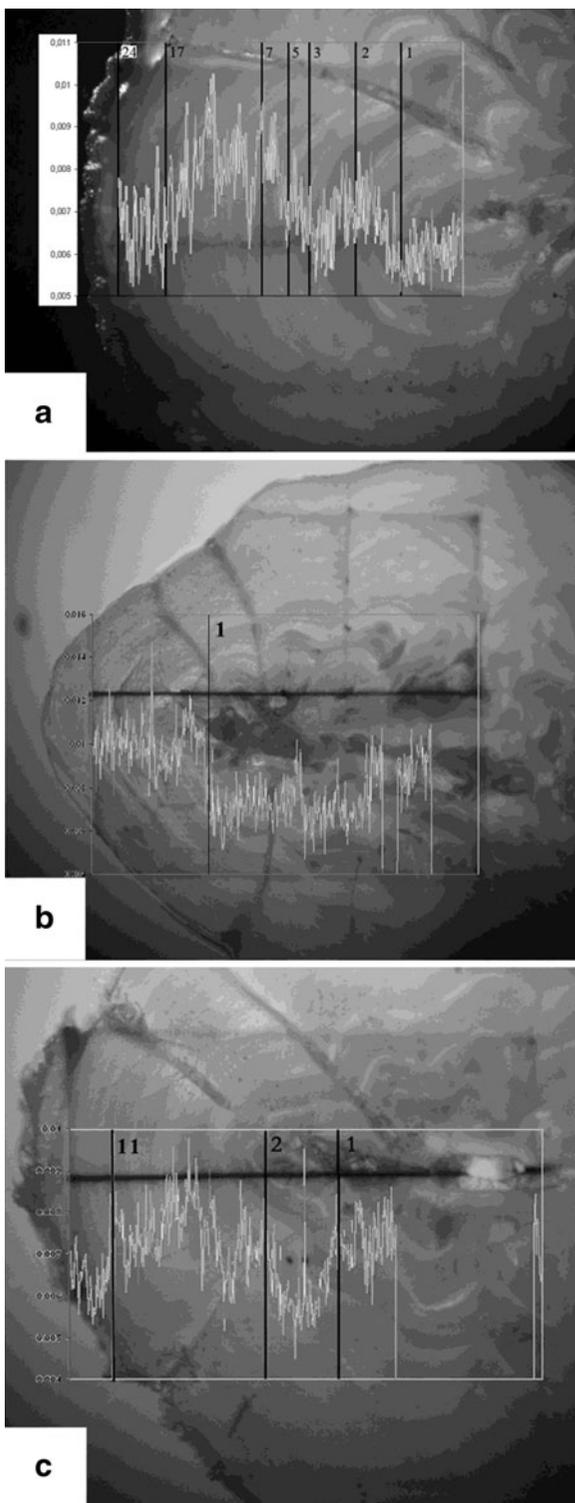


Fig. 4 Analyzed area of three species with the values of the Sr:Ca ratio transect scan (*gray broken line*) located above the scanned line (*black horizontal line*). **a** Sample 3–24 year old Russian sturgeon female; **b** Sample 4–21 year old stellate sturgeon female; **c** Sample 7–19 year old beluga male. Y axis—Sr:Ca ratio; vertical *black lines* mark the end of the annuli corresponding to the age presented beside each *line*

trations at the end of the first or several first years of age, which should imply transition to an environment with higher water salinity. After the initial increase in the Sr:Ca ratio, further changes in most samples were more irregular. The drop of the Sr:Ca ratio in samples 1–3 & 7 near the end of the transect might be the result of a spawning migration. The decrease in ratio in samples 2 and 3 corresponds to age 12–16, which is in accordance with the age of first reproduction of the Russian sturgeon females, given by most authors (Bacalbaşa-Dobrovici 1991; Reinartz 2002; Ciolac and Patriche 2005; Bloesch et al. 2006). In sample 1, the decrease in Sr:Ca ratio however occurred somewhat later, approximately after age 18. The beginning of the decline in ratio in sample 7 is in the annulus that corresponds to age 11, and the minimum value is at the annulus corresponding to age 13. These values are in accordance with the values provided by most authors for the age at maturity of beluga males (Bacalbaşa-Dobrovici 1991; Reinartz 2002; Ciolac and Patriche 2005; Bloesch et al. 2006). However, these are all only gradual declines in ratio, covering several annuli, which might imply slow movement of these individuals from the more open sea towards the

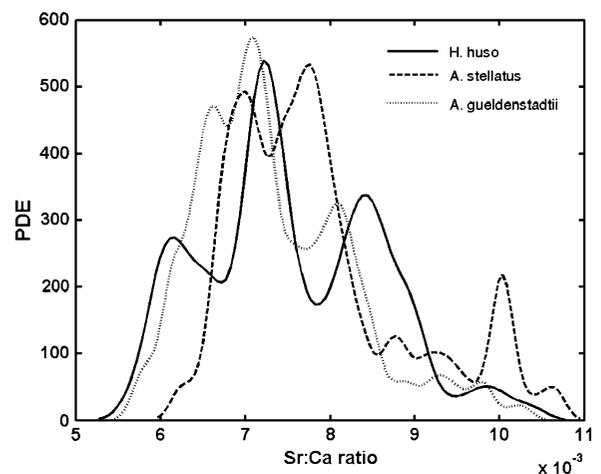


Fig. 5 Probability density estimate (PDE) profiles of the Sr:Ca ratio transect scans for the three studied species

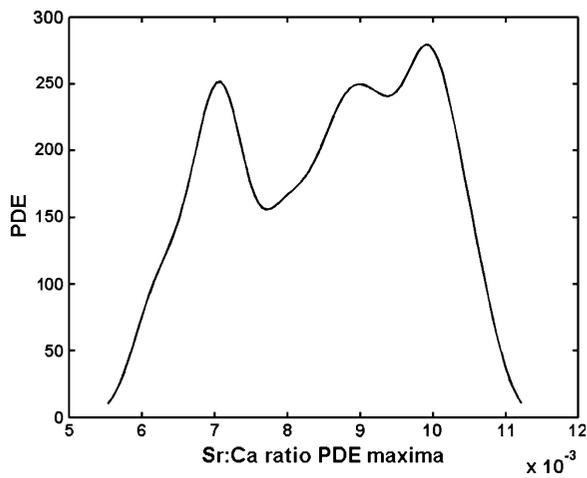


Fig. 6 Probability density estimate (PDE) profile of the pooled local maxima of the curves presented in Fig. 4

river mouth, where the water is more brackish, prior to the actual spawning migration. As was already described, the inner annuli in these samples and the ones within a section at the end of the transect had very similar average Sr:Ca ratios, which might indicate that these periods of life were spent in environments with the same salinity. An increased Sr:Ca ratio in the first annulus in comparison to the second one, as can be seen in sample 7, is often associated with maternally transmitted Sr (Limburg et al. 2001).

Arai et al. (2002) found changes of similar magnitude in pectoral fins of the Russian sturgeon from the Caspian Sea. However, observed ratios in the innermost annuli in their study were lower than those found in the present study. Most of the studied

sturgeons (samples 1–7) were caught near Tulcea in Romania, close to the river mouth. Therefore, larger ratios in the innermost annuli, when compared to those reported by Arai et al. (2002), could be explained either by a different chemical composition of the Danube River or by the possibility that the analyzed individuals had migrated to the river mouth soon after hatching, and stayed there during the initial year. In this way, the first annuli would only have the signature of brackish water in Sr:Ca ratio. PDE profile of pooled maxima (Fig. 6) shows three clearly distinguished maxima, which could correspond to three different salinities, fresh and saltwater with maxima for brackish water positioned between them.

Due to its isolation from the global ocean and a large influx of freshwater from numerous rivers, the Black Sea has such a low salinity that it can be even considered as a 1 : 1 mixture of marine and river water (Major et al. 2006). Since the Sr:Ca ratio in bones is directly related to the water salinity in the environment (Limburg et al. 2001), it might be presumed that the low level of oscillations in Sr:Ca ratio was the result of a small difference in salinity between the sea and freshwater. Furthermore, the lowest water salinity is located in the north–western part of the Black sea, where the Danube River mouth is situated (Fig. 7). These areas of low water salinity are strongly overlapping with the sturgeon feeding areas in the sea (Fig. 7), so this might imply that the sturgeons in the Black Sea actually never leave the low salinity areas during their life.

Analysis of Sr:Ca ratio in the fin ray of the juvenile Russian sturgeon caught below the Djerdap II dam

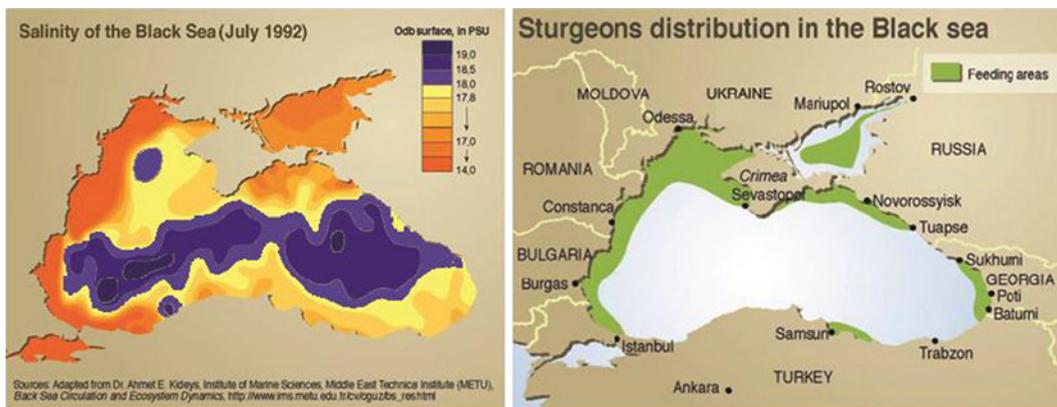


Fig. 7 Maps of the Black Sea, showing water salinity (left image) and sturgeon feeding areas (right image, green color) (source: Rekeawicz 2001a; 2001b)

indicates that this individual has never left the freshwater. This finding could be explained either by the theory of existence of a special resident, potamodromous form of the Russian sturgeon in the Danube (Hensel and Holčík 1997), or as a specimen escaped or released from some aquaculture facility. During the period 1998–2005, Bulgaria alone has released more than 670 000 Russian sturgeons into the Danube River, as a part of its supportive stocking activities (Hubenova et al. 2009). The latter theory might be an explanation for the significantly lower Sr:Ca ratio in the fin ray of this individual, when compared with the other studied individuals.

There is a present need for extensive studies on sturgeon ecology and life history that would provide better estimation of their life history parameters. The use of nuclear microprobe and PIXE methods on Sr:Ca ratios in sturgeon pectoral fin rays, that were assessed in the present study, may have a good potential to become a key approach used in future research efforts to determine some life history parameters of anadromous sturgeon species, such as the age at maturity and spawning frequency. Further studies should involve analysis of a larger number of samples, and thus enable determination of reference values for different salinities of the Danube River and Black Sea basin, and the relationship between the Sr:Ca concentrations in the environment and in sturgeon pectoral fins. Arai et al. (2002) proposed that this could be most effectively performed through studies that would involve a tagging and recapture approach.

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