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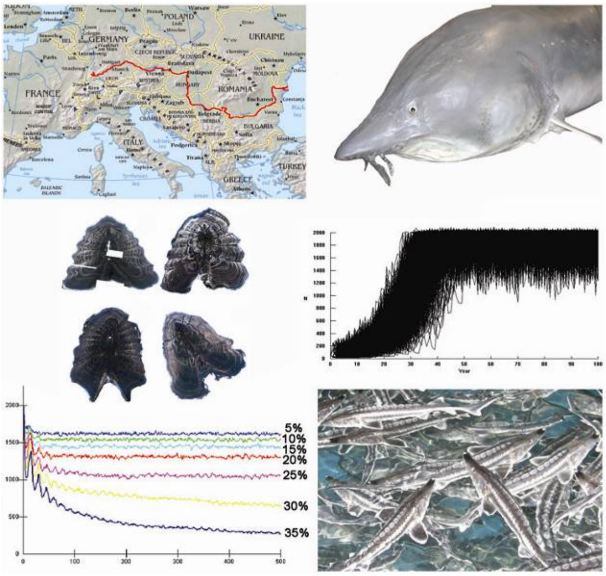
Population viability analysis of the Danube sturgeon populations



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Abstract

Populations of six sturgeon species in the Danube River (Beluga, Russian sturgeon, Stellate sturgeon, Sterlet, Ship sturgeon and Atlantic sturgeon) have experienced severe decline during the last several decades, mostly due to the unsustainable fishery, river fragmentation and water pollution. In the present study, the question of viability of Danube sturgeon populations and the poor knowledge of their ecology have been addressed through three different research activities: 1) statistical methods that infer extinction based on sighting records have been used to assess probability of extinction of the Ship and Atlantic sturgeon, whose presence n the Danube basin is uncertain; 2) assessment of microelement accumulation in sturgeon pectoral fin rays, especially of strontium and calcium, has been conducted as a method that can reveal migration patterns of anadromous sturgeons; 3) population viability analysis in a Vortex simulation model has been conducted in order to assess the state of the six Danube sturgeon species, their future risk of extinction and to determine the most suitable conservation and management measures. Methods for inferring extinction based on sighting records provided a significant probability that the Atlantic sturgeon is extinct, with extinction occurring somewhere between 1966 and 1970, and that the Ship sturgeon is probably still present in the Danube basin, but that extinction may occur within a few decades. A new model has been also developed within the present study, able to infer probability of extinction based on the trends in sighting intervals, since this issue was not adequately considered in the existing models. Although the analysis of the strontium to calcium ratio in sturgeon pectoral fin rays has revealed changes that might indicate probable migration of juvenile fish from the river to the sea, further studies are needed for improvement of this method. Population viability analysis has revealed a large sensitivity of the Danube sturgeon populations to changes in the natural mortality, fecundity, age at maturity and spawning frequency. It was also confirmed that the sturgeons are highly susceptible to even moderate levels of commercial fishery, and that their recovery is a multi-decadal affair. Stocking with adult individuals was shown to produce considerably greater effect on population persistence than stocking with juveniles, but the latter approach is probably still preferable since it avoids many inherent problems of aquaculture cultivation. This study represents the first population viability analysis of the Danube sturgeons.

Key words: *Acipenser*, *Huso*, sturgeon, PVA, extinction risk, life history, modelling, pectoral fin microchemistry, Sr : Ca ratio, sighting records

Contents

Introduction

When a person mentions sturgeons, two thoughts usually come to mind to those that are even slightly familiar with this group of fish. One is the huge size of some sturgeon species, that can reach several tons in weight and up to eight or even nine meters in length, and the other is of sturgeons as the source of caviar, their eggs that are one of the most prized alimentary products worldwide. Unfortunately, another association that is nowadays becoming more common is the endangered state of these species, some of them even being on the brink of extinction. These large fish, which have been object of fishery for thousands of years and that have once represented the basis of large-scale industry in many parts of the world, have reached a point when they can be no longer considered as a resource of economic significance in most of their former area of distribution (Birstein *et al.* 1997; McDowall 1999). It is clear and well known that human activities, such as unsustainable exploitation, pollution and river regulation and fragmentation through construction of hydropower plants (Figs. 1 & 2), have caused their worldwide decline (Birstein *et al.* 1997; Pikitch *et al.* 2005). However, the actual extent of each of those negative factors is not well studied, nor is the response of different sturgeon populations to them.

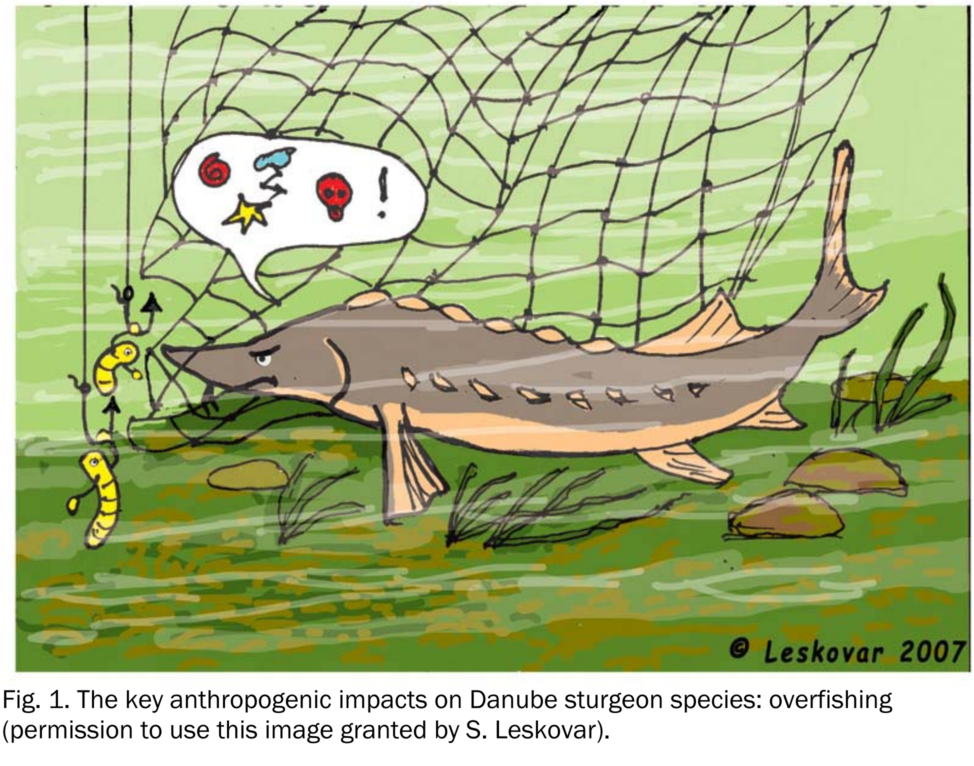


Fig. 1. The key anthropogenic impacts on Danube sturgeon species: overfishing (permission to use this image granted by S. Leskovar).

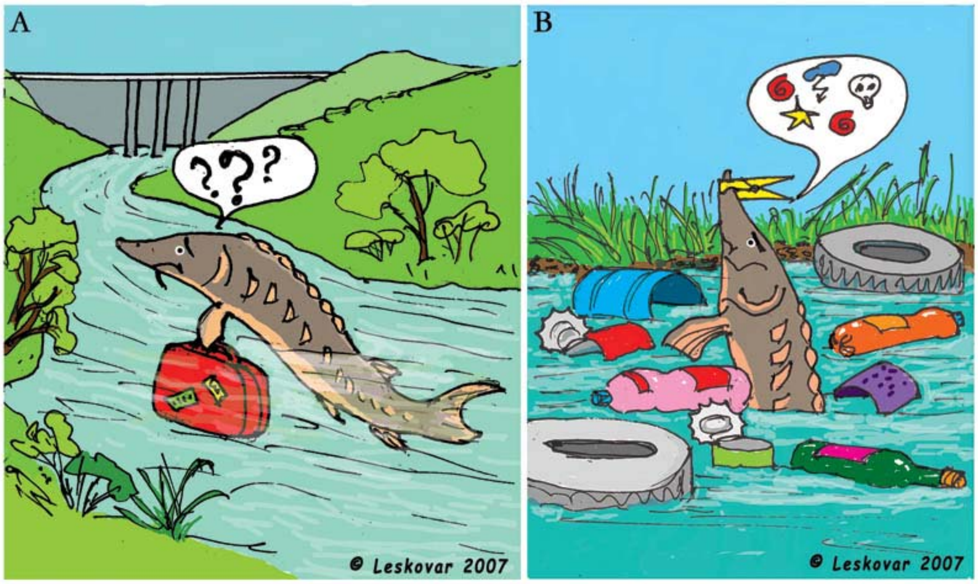


Fig. 2. The key anthropogenic impacts on Danube sturgeon species: A) habitat fragmentation by construction of dams and B) water pollution (permission to use this image granted by S. Leskovar).

Sturgeons (family Acipenseridae) are a group of 27 extant species that inhabit the northern hemisphere. They represent an old group of fish that has evolved for more than 250 million years, and that has successfully survived several mass extinction events throughout the history (Nikcevic *et al.* 2004). However, sturgeons have experienced the same fate as the majority of other economically important fish worldwide that are being either fully exploited, overharvested or completely depleted (Birstein *et al.* 1997; Botsford *et al.* 1997). It is generally considered that the same life history strategy, involving long lifespan, delayed maturation, large size and anadromy, which has made them resilient to global environmental changes in the past (Bemis *et al.* 1997), is at the same time making them highly susceptible to extinction under anthropogenic impacts (Jager 2001).

The Danube River basin and the Black Sea (Fig. 3), originally inhabited by six sturgeon species, are considered as the key habitat of European sturgeons (Williot *et al.* 2002; Lenhardt *et al.* 2006:1). However, due to a whole spectre of anthropogenic impacts, Atlantic sturgeon (*Acipenser sturio*) and Ship sturgeon (*Acipenser nudiventris*) have nowadays almost disappeared from the region, while populations of Beluga (*Huso huso*), Russian sturgeon (*Acipenser gueldenstaedtii*), Stellate sturgeon (*Acipenser stellatus*) and Sterlet (*Acipenser ruthenus*) are experiencing severe decline (Fig. 4, Reinartz 2002; Williot *et al.* 2002; Lenhardt *et al.* 2006:2).

Fig. 3. The Danube River. Black points numbered 1 and 2 represent locations of hydro-power plants Djerdap I and II (source: www.wikipedia.org).

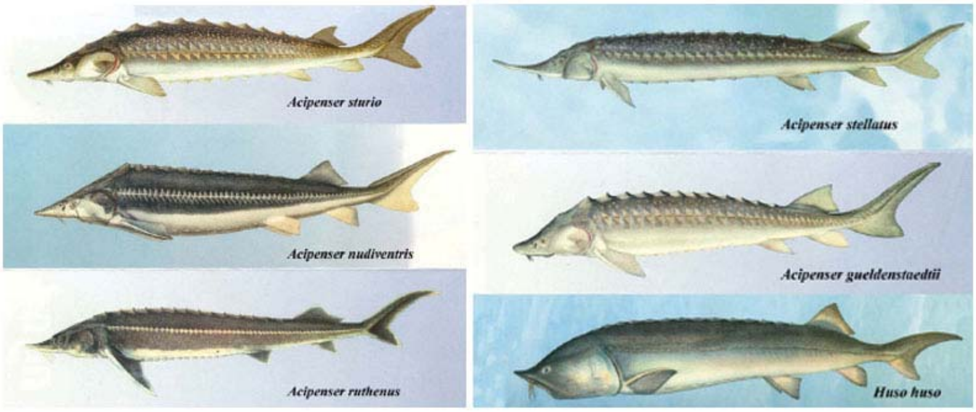


Fig. 4. The six Danube sturgeon species (Vassilev 2006).

Upstream spawning migrations of the Danube sturgeons were obstructed by the river regulation in the Djerdap gorge already at the end of 19th century (Petrović 1998), and after the construction of two hydro-power plants, Djerdap I in 1970 and Djerdap II in 1984, the migratory route of anadromous species in the Danube River was shortened to only 863 km (Lenhardt *et al.* 2006:2). The extent of water and sediment pollution in the Danube and their impact on sturgeons has been poorly studied so far (Bacalbaşa-Dobrovici 1997), but certain chronic and sublethal effects of pollution have been recently confirmed (Poleksic *et al.* 2009). The sturgeon fishery in the Danube reached its peak by the middle of the 20th century, followed by a rapid decrease, in Romania from

1 144 tonnes/year in 1940 to less than 8 tonnes/year in 1995, in Ukraine from 114.2 tonnes/year in 1952 to the lack of any catch (at least the legal one) since 1994 (Fig. 5, Navodaru *et al.* 1999). Unsustainable fishery levels in the Danube have been further compounded by a significant illegal fishery (Bacalbasa-Dobrovici & Patriche 1999) and, due to a lack of efficient regulation, fishing effort has increased during the last two decades (Navodaru *et al.* 1999). In 2006, Romania introduced a ten year moratorium on commercial sturgeon fishery (Suciu 2008), and Bulgaria started introducing moratoria by decrees issued every year (Vassilev pers. comm.). It is believed that the amount of illegal fishery has decreased in recent years, but there are no studies made that could confirm this. Another potential threat to Danube sturgeon populations, which only recently has been recognized, is the hybridization of natural populations with individuals of exotic species that have escaped from aquaculture (Ludwig *et al.* 2009). All sturgeon species are known to be able to hybridize (Reinartz 2002), and their autochtonous genetic diversity can become rapidly eroded if exotic genotypes are introduced (Ludwig *et al.* 2009).

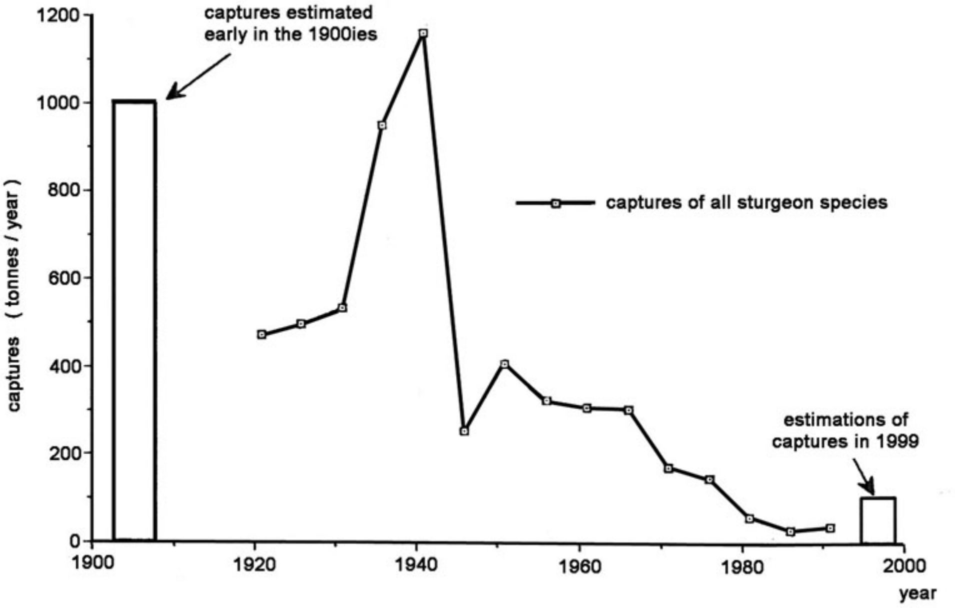


Fig. 5. Sturgeon captures in Romania from 1920 to 1990 (Bacalbaşa-Dobrovici 1991 citt. in Patriche *et al.* 1999).

Sturgeon aquaculture and stocking have been considerably used as conservation measures in the Danube and Black Sea basins during the last two decades (Vassilev 2006). Sterlet stocks in the Middle Danube have become dependant on stocking measures (Reinartz 2002), and there are ongoing stocking activities with the majority of fish released by Hungary (on average

100 000 fingerlings/year), and to a small extent by Slovakia, Bulgaria and Austria (Williot *et al.* 2002; Guti 2006; Holčik *et al.* 2006; Vassilev pers. comm.). In the Lower Danube, more than a million larvae, fingerlings and juveniles have been released so far, mostly Russian and Stellate sturgeon, and to a smaller extent Sterlet and Beluga (Fig. 6, Vassilev 2006). However, stocking efforts are considered to be insufficient to counter the present decline of Danube sturgeon populations (Vassilev 2006).

Fig. 6. Sturgeon aquaculture facility in Galati, Romania, used for supportive stocking of Danube sturgeon populations (Photos: I. Jarić, November 2008).

To make things worse, there is a present lack of knowledge on basic sturgeon demography, life history and relative effects of different negative factors, as well as a lack of efficient policy and management measures (Williot *et al.* 2002; Pikitch *et al.* 2005). Managers do not have time to wait for better knowledge, and management decisions must be made based on available information, even when it is limited, which is often the case for endangered species (Grogan & Boreman 1998). Effects of some important factors will never be completely predictable even when sufficient data are available, and many managers are uncomfortable to make choices with such uncertainties (Ludwig 1999).

Most sturgeon species are anadromous, which means that they spend larger part of their lives in the marine environment, and with intervals of certain number of years that are species specific they migrate to rivers to reproduce (Bemis *et al.* 1997). Beluga, Russian sturgeon, Stellate sturgeon and Atlantic sturgeon are considered as anadromous, while the Sterlet is considered to be potamodromous, which means that it spends whole life cycle in rivers, and makes only local upstream or downstream migrations within a river. While 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). However, patterns of sturgeon movements between the sea and the river environment have been so 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 one 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 spine is commonly used for age assessment studies on sturgeons (Stevenson & Secor 1999). Certain elements, such as strontium, accumulate in bones proportionally to their concentration in the environment, so growth zones in fish bone structures will contain different strontium concentrations depending on the water salinity, revealing whether the individual in question has spent that year of its life in marine or freshwater environments (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, I have tried to apply this approach for the assessment of migration patterns of the Danube sturgeons.

The lack of knowledge is even more pronounced regarding the question whether some of the species are still present in the Danube or already extinct. The presence of the Ship sturgeon and Atlantic sturgeon in the Danube during the 20th century has been indicated only through occasional chance sightings and, therefore, the lack of empirical data have prevented any quantitative approach for the assessment of the state of their populations. The Ship sturgeon used to be recorded in the middle Danube upstream to Bratislava, in the lower Danube, and occasionally in the Danube delta (Hensel & Holčik 1997). In the middle of the 20th century it became rare, with only a few specimens found over the last few decades (Hensel & Holčik 1997; Guti 2006). At the beginning of the 20th century, only a few specimens of Atlantic sturgeon were recorded in the Danube River (Reinartz 2002). The last record of this species in Danube was made in 1965 (Manea 1980).

So far, only a “rule of thumb” approach has been used to determine the status of these two species, which led to different conclusions regarding their presence in the Danube River. Different authors have considered the Ship sturgeon to be endangered (Simonović *et al.* 2005), critically endangered (Hensel & Holčik 1997; Guti 2006), possibly extinct (Kynard *et al.* 2002; Reinartz 2002) or likely extirpated (Pintér 1991). There is a stronger agreement regarding the Atlantic sturgeon in the Danube basin, since most authors designate this species as extinct or probably extinct in the Danube River (Bacalbaşa-Dobrovici & Holčik 2000; Kynard *et al.* 2002; Reinartz 2002; Pikitch *et al.* 2005).

Developments in the statistical approach in circumstances when data are scarce have resulted in new methods for the calculation of the probability that a species is extinct (Burgman *et al.* 1995). Indirect statistical methods for estimating the extinction probability from observation records seem to provide practical means of addressing that concern (Grogan & Boreman 1998), and can be useful when sightings are the only available data (Akçakaya & Sjögren-Gulve 2000). The strength of these methods is that they can be applied in situations where management decisions must be based on limited information, which is often the case for endangered species (Grogan & Boreman 1998). In the present study, indirect statistical methods for the assessment of the extinction probability of Ship sturgeon and Atlantic sturgeon in Danube were applied. However, appropriate caution is necessary in using these methods, because acquired probabilities for species presence or extinction should represent more an indicator of the real state of studied population, than to provide the final conclusion. McCarthy (1998) and Robbirt *et al.* (2006) suggested that it is prudent to use a number of complementary tests, since each can be sensitive to different characteristics of the collection/sighting records and their combination enhances the overall ability to detect extinction. Here, four different tests for estimation of the extinction probability (i.e. that the population is already extinct) were employed and one test for the estimation of the remaining time to extinction.

Assessments of the state of Danube sturgeon populations that have been the object of the commercial fishery (Beluga, Russian sturgeon, Stellate sturgeon and Sterlet) have so far mostly relied on the assessment of catch statistics (Lenhardt *et al.* 2006:2), which can be significantly biased by social and economic dynamics, and which does not consider illegal fishery that at certain points represented up to a 90 percent of the actual total catch in the Danube in the past (Bacalbasa-Dobrovici & Patriche 1999; Navodaru *et al.* 1999). The development of population models that are able to assess its viability and the extinction risks could be that tool that would bridge existing gaps in knowledge and understanding of sturgeon ecology and life history. Population viability analysis (PVA) is the method that has become the mainstream and indispensable tool in conservation biology during the last two decades (Boyce 1992), especially since it explicitly deals with uncertainties in ecological data and processes (Akçakaya & Sjögren-Gulve 2000). This approach has however not been applied to sturgeons in the Danube River basin.

There is a lack of consensus on the definition of PVA, and it has been used to designate methods that range from qualitative and verbal processes to mathematically sophisticated simulation models (Beissinger 2002; Reed *et al.* 2002). Shaffer *et al.* (2002) have defined PVA as a risk assessment methodology that is applied to the problem of species extinction. All methods have in common that they represent some form of examination of the interacting factors that place a population or species at risk (Shaffer 1990). The outcome of a PVA model can be an estimation of the risk of extinction or decline, quasi-extinction probability, expected time to extinction, chance of recovery, future population size and an assessment of different impacts of human activities or efficacy of different management scenarios (Boyce 1992; Ludwig 1999; Akçakaya & Sjögren-Gulve 2000; Reed *et al.* 2002; Reed *et al.* 2003).

There are many possible classifications of PVAs but, according to their general structure, they may belong to the group of deterministic models, age and stage-structured models, spatially explicit models, individual based models, or represent some combination of these general types of PVA. The simplest PVAs are deterministic models (Boyce 1992). They can be, for instance, models that use deterministic population growth rates to make population projections (Dulvy *et al.* 2004). However, these models do not consider the stochasticity of the system and thus can create significant bias, proportional to the degree of unpredictable variance in the system (Boyce 1992; Burgman *et al.* 1988), so they can be useful only for heuristic purposes, or as a preliminary tool, for example in rapid assessments of a large number of populations (Boyce 1992; Dulvy *et al.* 2004). Spatial models, such as different occupancy models, are used for PVA of metapopulations and beside the spatial dimension they can include parameters like dispersal between subpopulations and habitat suitability (Sjögren-Gulve & Hanski 2000). Demographically structured models group individuals into distinct classes, either according to their age (age-structured models) or certain stages (stage-structured models), e.g. according to size, weight or developmental state (Akçakaya 2000).

Individual-based models (IBMs) represent simulations where each individual, its behaviour and fate, are separately modelled (Akçakaya & Sjögren-Gulve 2000). They are often performed through computer programming (Lacy 1993), and as simulations became more feasible, use of IBMs became much more frequent (Chambers 1993). IBMs have the flexibility to represent individual differences in age, size, spatial location, and other relevant attributes (Uchmański 1999; Jager *et al.* 2000), and provide a basis for merging genetics with demography (Chambers 1993). IBMs are the only PVA methods that can successfully model relevant genetic changes in a population, or effects of inbreeding depression (Jager 2001, 2005). Stochastic processes that are relevant to population viability can be categorized into demographic, environmental and genetic stochasticity, and catastrophic events (Lacy 1993). IBMs, demographically structured and spatial models often do have stochasticity included within the model, but not necessarily. Development of models that consider stochasticity of the system is considered crucial in order to increase reliability of the PVA (Boyce 1992).

PVA program packages have become popular in the field of conservation biology, and some programs like the RAMAS series and Vortex are being widely used (Lacy 1993; Akçakaya & Raphael 1998; Brook *et al.* 2000); for example, Vortex is now routinely used by the World Conservation Union (IUCN) to establish quantitative classification of endangered species (Brook *et al.* 1997).

However, even though the PVA as a method has experienced significant development and proliferation of models that have been applied to a large number of species and populations, there is an ongoing debate in the scientific community about the reliability of this approach and its ability to become an integral part of the conservation policy development. One of the key and intrinsic problems of PVA is that it normally deals with complex issues, for which our theoretical and practical knowledge is never complete (Shaffer 1990). The major limitation for successful development of PVA is probably the common lack of suitable and necessary data (Shaffer *et al.* 2002), especially when they are to be used in such detailed models as the Vortex (Boyce 2002). Field studies supply data on life history, habitat related parameters and management implementation, but this is a time-consuming activity and only a smaller number of species, usually those charismatic ones or those of significant economic interest, are studied well enough. Furthermore, stochasticity and genetics are representing two factors whose simulation is probably posing the biggest problems for PVA practitioners (Boyce 1992; Allendorf & Ryman 2002). Genetic factors are usually considered too poorly known to be included in the model, and as a result few PVAs incorporate genetic components. Lack of general consensus on criteria that should be used to measure viability, such as the thresholds for acceptable extinction risks and the time-frames applied, is another obstacle for successful implementation of PVA results (Shaffer *et al.* 2002). This is probably partly caused by the fact that the acceptable level of extinction risk is not so much a scientific decision, as it is a social and practical one (Lande 2002).

On the other hand, PVA has shown its value in many successful cases, and benefits coming from the use of this powerful tool have placed it beyond the need to prove its worth anymore. If PVA models are used with necessary caution, with the awareness about all the drawbacks and uncertainties related to its use, they can provide meaningful results that far surpass any kind of "rule of thumb" approach. It is generally accepted that, while it owns certain weaknesses, PVA is still probably the most rigorous conservation-planning tool available (Boyce 2002).

Moreover, PVA computer software packages have proven to be able to offer reasonable reliability in predictions (Boyce 2002). Brook *et al.* (2000) found a surprising accuracy of most commonly used program packages with a high concordance in their results. The main potential of PVA program packages is their ability to compare the efficacy of different management scenarios (Ebenhard 2000; Reed *et al.* 2002). As proposed by Ebenhard (2000), PVA should be generally oriented to three main fields of study – assessment of extinction risks of small and isolated populations, evaluation of conservation goals and the evaluation and design of management methods.

Study objectives

The main objective of this study was to assess the state of the sturgeon species populations in the Danube River, their future risk of extinction and to assess existing measures for protection and sustainable use of these populations.

Estimation of migration patterns of the Danube sturgeon species was another important objective, which should enable better understanding of their ecology and offer better estimation of the best conservation measures.

The probability of continued presence of the two sturgeon species that are considered to be on the brink of extinction in the Danube was assessed through the use of a number of statistical assessment methods that can infer population status using sighting records. One additional method that is sensitive to the trends in sighting records was also developed, in order to enhance the reliability of the predictions.

Material and methods

Extinction probability estimation for the Atlantic sturgeon and Ship sturgeon in the Danube River based on sighting records

In order to assess the probability that the Ship sturgeon and Atlantic sturgeon are already extinct in the Danube, four different tests based on sighting records were employed and one test for the estimation of the remaining time to extinction. McCarthy (1998) and Robbirt *et al.* (2006) suggested that it is prudent to use a number of complementary tests, since each can be sensitive to different characteristics of the sighting records and their combination enhances the overall ability to detect extinction. This represents the first quantitative approach for the assessment of the presence of Ship and Atlantic sturgeon populations in the Danube. Furthermore, a new model that infers extinction based on the average length and trend of sighting intervals is also presented.

Sighting records

Published sighting records in the Danube River were compiled for the two species under study. Only sightings starting from the end of the first half of the 20th century were used (1948 and 1949 for the Atlantic and the Ship sturgeon, respectively), because in the previous years data quality had been characterized by less reliable records and sightings, which had also been inconsistently noted and published. Documented sightings of the Ship sturgeon and Atlantic sturgeon are presented in Table 1. Only those sightings where the species was identified by an expert were used in this study, indirect or ambiguous sighting reports were omitted.

Statistical methods

Five complementary methods for the assessment of extinction probability based on sighting data were applied. An explicit assumption in each of the methods is that all observations of a species are incidental and made independently of each other. It is hence important to note that systematic or deliberate collections of the species cannot be used, because they violate the underlying statistical assumptions of the methods (Solow 1993:1; Burgman *et al.* 1995). Observations that can be considered as random may be landings bycatch, incidental observations during surveys designed to capture other species, stranding, and records on entrapment and mortality in power plants (Grogan & Boreman 1998). Data used in this study fulfil these criteria, as all records represented bycatch or accidental observations.

Another important assumption is that the average chance of incidentally collecting or observing a species does not change over time. According to

Solow & Roberts (2003), variation in sighting effort is an important potential source of variation in the sighting rate, but underlying assumptions of these tests may be reasonably met if sightings arise from accidental encounters. The Danube River basin has been an object of intensive fisheries during the whole period of observations (Reinartz 2002; Williot *et al.* 2002; Lenhardt *et al.* 2006:2), which justifies the assumption that the chance of catching specimens of either the Atlantic sturgeon or the Ship sturgeon has always been high.

Table 1. Documented sightings of the Ship sturgeon and Atlantic sturgeon in the Danube River, with reference for each record.

Atlantic sturgeon	References	Ship sturgeon	References
1948	Ristić 1963; Manea 1980	1949	Vásárhely 1957; Manea 1980
1949	Manea 1980	1950	Manea 1980
1950	Ristić 1963; Manea 1980	1951	Manea 1980
1951	Manea 1980	1952	Vásárhely 1957; Manea 1980
1952	Ristić 1963; Manea 1980	1953	Ristić 1963
1954	Ristić 1963	1954	Ristić 1963; Manea 1980
1960	Manea 1980	1956	Vásárhely 1957
1961	Manea 1980	1957	Vásárhely 1957
1962	Manea 1980	1961	Kálmán 1961
1963	Manea 1980	1975	Hensel & Holčik 1997
1964	Manea 1980	1989	Hensel & Holčik 1997
1965	Manea 1980	1992	Guti 2006
		1998	Harka & Sallai 2004
		2003	Simonović *et al.* 2005; Sallai pers. comm.
		2005	Guti 2006

Sighting records were arranged as a series of time units (t) with positive sightings within the observation period (with the first time unit labelled 1 and the last T), ordered from the earliest to the latest, $t_1 < t_2 < \ldots < t_n$. Multiple sightings within one time unit are recorded as a single sighting. The last time unit (T) corresponds to the year 2008. Separate analyses were run with the year 2009 as the last time unit, in order to test the potential scenario of lack of new observations in the current year. All equations express the probability of presence (p) of the investigated species, and most model authors employed a 0.05 probability as the threshold value, below which the species can be considered as extinct (Solow & Roberts 2003; McInerny *et al.* 2006; Roberts & Kitchener 2006; Carpaneto *et al.* 2007). A number of authors (Burgman *et al.* 1995; Solow & Roberts 2003) recommended omitting the first record when the time series of incidental observations has no definitive starting point, other than the first recorded observation, and this was applied in this study.

The series of time units with sighting records for the Atlantic sturgeon and the Ship sturgeon, respectively, are:

1, 2, 3, 4, 6, 12, 13, 14, 15, 16, 17

1, 2, 3, 4, 5, 7, 8, 12, 26, 40, 43, 49, 54, 56

The first method was introduced by Solow (1993:1), for inferring extinction of a species based on sightings over a continuous series of time units. Using the time of the last sighting (t_n), this equation expresses the probability of presence in relation to the number of time units in which the species was collected (n) within the period, given that sightings are equally likely to occur during the whole observation period (T):

$$p = \left(\frac{t_n}{T} \right)^n \qquad (1)$$

Burgman *et al.* (1995) developed a similar equation which, instead of using number of time units with sightings (n), introduces the total number of individuals (k) observed among the t_n time intervals. This method was not used in this study, because some of the published records did not include number of individuals.

The second method employed was a sighting rate probabilistic model, developed by McInerny *et al.* (2006), which gives the probability that another sighting will occur using the previous sighting rate (n/t_n) and the time since the last observation ($T - t_n$) with the following equation:

$$p = \left(1 - \left(\frac{n}{t_n} \right) \right)^{(T - t_n)} \qquad (2)$$

A low likelihood value ($p < 0.05$), means a low probability of discovering another record for the species based on the previous sighting rate.

The third method, Solow/Roberts non-parametric equation (Solow & Roberts 2003), can be advantageous in some cases, because it does not require a complete series of sighting records, as the number of sightings (n) is not required for its calculation. Using t_n, T and the time of the second most recent sighting (t_n-1), the equation generates the probability that another sighting will occur:

$$p = \left(\frac{t_n - t_{n-1}}{T - t_{n-1}} \right) \tag{3}$$

Equation 1 can result in a high *p* value if a single observation of the species was made relatively recently (as in the case of Ship sturgeon), regardless of the length of time intervals between previous sightings or of any evidence of population decline during the observation period (Burgman *et al.* 1995). Since population decline is often characterized by longer and longer periods during which the species is not observed, Burgman *et al.* (1995) applied a method that considers such a development. The fourth method, a so-called "runs test", calculates the probability that the species will be recorded again during the period that is as long as, or longer than the longest observed run of absence:

$$p = \binom{T}{n_0}^{-1} \sum_{x=1}^{\lfloor n_0 / r \rfloor} (-1)^{x+1} \binom{n_1 + 1}{x} \binom{T - rx}{n_1} \tag{4}$$

Here, *r* is the length of the longest run of consecutive time units without records, n_0 the number of time units without records, n_1 the number of time units with records, and $\lfloor\ \rfloor$ represents the integer part of the given value. Low acquired probability ($p<0.05$) implies extinction.

Finally a model that estimates the most probable extinction time (T_E) was employed, following the method developed by Solow (2005). If it is assumed that the species is extinct, this method estimates the time of extinction as:

$$T_E = \frac{n+1}{n} t_n \tag{5}$$

Solow (2005) also gives the estimation of the upper bound ($T^U{}_E$) of a 1-α confidence interval for T_E:

$$T^u{}_E = t_n / \alpha^{1/n} \tag{6}$$

All equations were generally analyzed for sensitivity to different future scenarios and possible biases.

Until recently, IUCN and CITES have arbitrarily decided on 50 years without sightings as the threshold value to declare a species as extinct (Reed 1996). However, the period that we are prepared to wait before we conclude that a species has become extinct should be based on the frequency with which it was seen before the last observation (Burgman *et al.* 1995). As stated by Solow (2005), a threshold value that implies extinction should be related to previous sighting rates, and for species with a high sighting rate, a relatively short period without sightings would indicate extinction, and vice versa. Since previous authors did not consider the effects of a trend in the time intervals between sightings, for example a general increase in the time between sightings, there is a need to introduce a model that would be sensitive to this aspect of the sighting record. Therefore, I have developed a new model that infers probability of extinction based on the average length of, and trends in, sighting intervals. In the Results section, the sighting trend model is described, with a sensitivity analysis, and then applied to the sighting records of the Atlantic sturgeon and Ship sturgeon in the Danube.

Assessment of microchemical concentrations in sturgeon pectoral fin spines

In order to assess migration patterns of Danube sturgeon species through the microchemical concentration assessment in pectoral fin spines, a number of fin samples were collected. Three species were object of this research: Beluga, Russian sturgeon and Stellate sturgeon. All samples were acquired from individuals that were already caught through the commercial sturgeon fishery during the period 2001-2007. No individuals were harmed or sacrificed within this research.

Table 2. Basic data for the thirteen sturgeon individuals whose pectoral fin spines were assessed for microchemical concentrations. SL – standard length, F – female, M – male.

No	Species	Gender	Date of catch	Locality	SL (cm)	Weight (kg)
1	*A. gueldenstaedtii*	F	May 2004	Tulcea, RO	150	22.5
2	*A. gueldenstaedtii*	F	May 2004	Tulcea, RO	149	19
3	*A. gueldenstaedtii*	F	April 2004	Tulcea, RO	149	23
4	*A. stellatus*	F	April 2004	Tulcea, RO	106	5.9
5	*A. stellatus*	F	April 2004	Tulcea, RO	120	9.3
6	*A. stellatus*	M	May 2004	Tulcea, RO	106	5.9
7	*H. huso*	F	Feb 2004	Tulcea, RO	245	166
8	*H. huso*	F	March 2004	Tulcea, RO	290	265
9	*H. huso*	M	May 2004	Tulcea, RO	194	70
10	*H. huso*	M	Nov-Dec 2001	Prahovo, SR	-	110
11	*H. huso*	M	Nov-Dec 2001	Prahovo, SR	-	110
12	*H. huso*	F	April 2004	Prahovo, SR	291*	221.6
13	*A. gueldenstaedtii*	M	Dec 2007	Prahovo, SR	50.3	1.3

* Total length

Nine samples were caught by fishermen in 2004 near Tulcea in Romania, and these samples were acquired from the collection of the Danube Delta National Institute in Tulcea, Romania. Four samples were caught by fishermen near the Prahovo in Serbia, below the Iron Gate II dam, 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 2.

All fin spines were removed from the surrounding tissue and air dried. A smaller, 2-3 cm long section was cut with a saw from each fin spine 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 (Fig. 7). Each section was mounted on a microscopic glass slide.

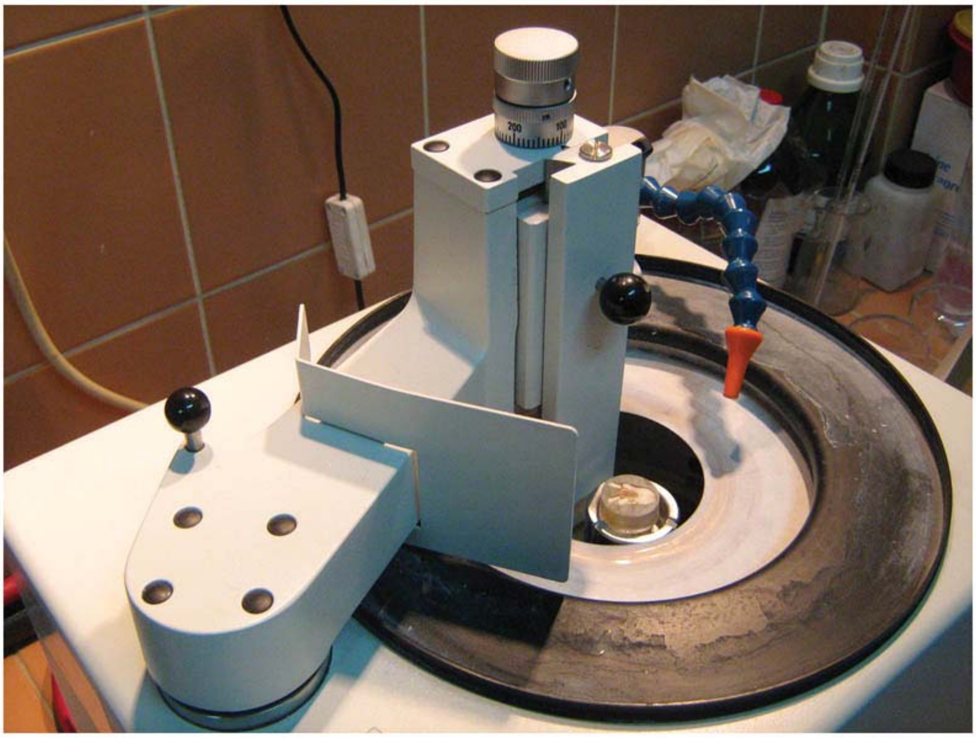


Fig. 7. Sturgeon pectoral fin ray sample, mounted in epoxy resin and placed in a Leica SP 1 600 low-speed electric saw for sectioning (Photo: I. Jarić, December 2008).

The age of each fish was assessed according to the method of Stevenson & Secor (1999). The fin ray sections were analyzed under the microscope. One growth zone (annulus) was defined as the combination of the 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 & Secor 1999). As suggested by a number of authors (e.g. Jackson *et al.* 2007; Killgore *et al.* 2007), age assessment was performed by five independent observers, 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 one year, the fish was assigned a higher age, as the age of older individuals usually is being underestimated (Rien & Beamesderfer 1994; Paragamian & Beamesderfer 2003; Killgore *et al.* 2007)

Assessment of microelement concentrations in each fin spine 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 (Fig. 8). While the NMP is similar in its methods to an electron microprobe, it has in general 50 to 100 times larger 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.* (2005:1, 2005:2) 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 aluminium 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 a number 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 strontium to calcium (Sr:Ca) ratio was assessed as a potential proxy for salinity, and the zinc to calcium (Zn:Ca) ratio as an indicator of seasons (Limburg *et al.* 2001). Elemental maps of 128 x 128 pixel size, that have 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 spine 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).

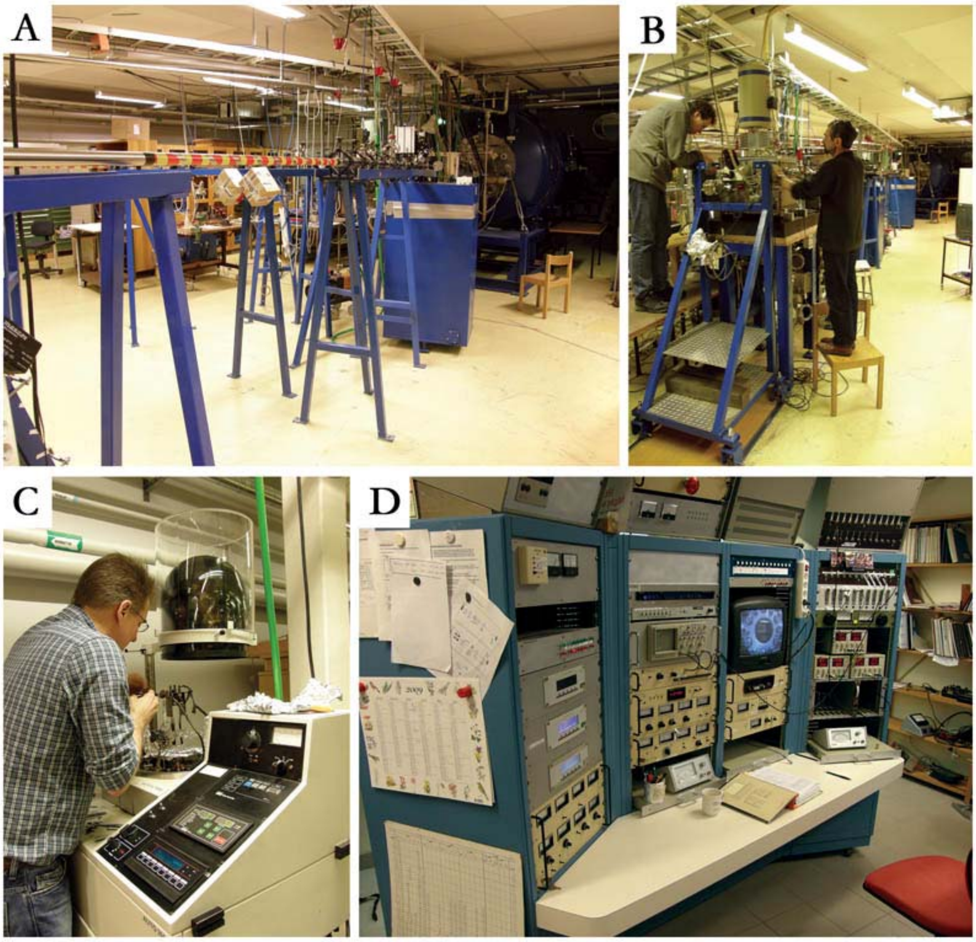


Fig. 8. Lund Nuclear Microprobe facility at the University of Lund: A) Accelerator and the beam line; B) Dr. Jan Pallon placing one of the samples in the experimental chamber; C) Dr. Mikael Elfman preparing sample for carbon coating; D) NMP command centre (Photos: I. Jarić, March 2009).

Different zones along the concentration transect scans were statistically compared with the Mann Whitney U test (SPSS software, version 15.0).

Population viability analysis in a Vortex simulation model

Brief description of the Vortex simulation model

Vortex is a PVA computer model that makes an individual-based Monte Carlo simulation of a population, through the action of deterministic factors and environmental, demographic, and genetic stochasticity (Lacy 1993). It makes the simulation by moving through a series of discrete and sequential annual events that represent population processes, such as mate selection, reproduction, mortality and movement of individuals through different age classes, throughout the pre-determined number of years (or other time units applied) (Miller & Lacy 2005). There are some additional elements of population dynamics that can be optionally included, depending on the characteristics of the population in question, such as metapopulation dynamics, migration, catastrophes, inbreeding depression, and density dependence. As an individual-based model, Vortex keeps track of each individual in a population and simulates individual life events, such as sex determination, breeding, dispersal and mortality, as well as individual characteristics – sex, age, belonging to a certain sub-population, inbreeding coefficient and the number of alleles on specific loci (Lacy 2000). The outcome for each individual is determined by random processes, which gives the model its inherent demographic stochasticity.

Basic demographic and life history parameters that are required as scenario input are the initial population size and age distribution, carrying capacity, number of sub-populations, first and maximum age of reproduction, age specific natural mortality, sex ratio, mean and maximum number of offspring, and number of individuals that participate annually in reproduction. A number of parameters, such as the first age of reproduction and natural mortality, are defined separately for each gender. For some parameters, the program also provides inclusion of environmental variation, thus including another source of stochasticity in the simulation. Parameters related to the general model structure are the simulation duration (the number of time units simulated), definition of extinction criteria (whether it will be the actual extinction or pseudo-extinction) and the number of iterations simulated.

One important advantage of Vortex, when compared to some other PVA software packages, is that it has developed a platform for genetic considerations, and for the correlation of genetic factors with the demography. At the onset of a simulation, each individual is assigned two unique alleles at six different hypothetical loci, and during the simulation, the program tracks the alleles that individuals carry on each locus, as well as the ones transmitted to each of the offspring (Lacy 1993, 2000). Inbreeding coefficients for each offspring are established through maintenance of kinship coefficients between all living individuals during the simulation.

Simulation of different management scenarios in the Vortex functions mainly through two platforms, harvest and supplementation. The former represents removal of individuals during a simulation, that can mimic hunting, culling, and the research or translocation-related removals, while the latter represents adding of certain number of individuals to a population, thus simulating translocation or releases from a captive breeding program (Miller & Lacy 2005). Both types of scenarios can be defined to be gender or age specific, and to have different temporal dynamics.

Vortex can produce a number of different output values. Since it is a Monte Carlo simulation, the simulation is normally repeated for a certain number of iterations, and the resulting output variables represent the average values of all simulated iterations. The basic output variables are the probability of extinction within the specified time interval (or pseudo-extinction, if it was defined so in the model setup), the mean time to the extinction, resulting population size, resulting age distribution and deterministic (calculated) and stochastic (simulated) population growth rates. Main output variables that are related to the genetic state of the population are the average heterozygosity and the remaining number of alleles. Apart from the average values, the output also provides standard deviations of most output variables. If harvesting was applied the results also present the number of individuals caught during the simulation.

The version of the Vortex software package that was applied in this study was 9.72. For a more detailed description of Vortex, see Lacy (1993, 2000) and Miller & Lacy (2005).

Model parameterization

In order to define population parameter values for each of the six Danube sturgeon species, a literature survey was conducted. All publications that were found to contain important life history parameters, or information about the management of sturgeon populations in the Danube River and Black Sea basins, were included in the final determination of the parameter values that will be included in the model. On the basis of the data that was present in the available literature, data sheets were made for each Danube sturgeon species with the values for each of the basic parameters that are necessary for scenario building in Vortex (Appendix I). Available publications that describe PVA studies performed on other sturgeon species were also assessed, in order to get experience on the estimation of different life history parameters.

At the end of the initial phase of the literature survey, it became apparent that there are significant gaps in the knowledge about the sturgeon life history, and that for many parameters there were different values and ranges provided (see values presented in Appendix I). As a result, a number of experts on sturgeons from the Danube River basin were contacted and meetings with them were arranged. This was done because it was expected that the present lack and inconsistency of data on sturgeon life history parameters could be diminished through meetings and discussions about these issues with different experts on sturgeons, to try to obtain as much information, data and experience from them as possible. Due to time and budget constraints, meetings were organized only with sturgeon biologists in Romania and Bulgaria, while the communication with the other sturgeon experts in the region was established through the Internet.

Trips to Romania and Bulgaria were organized at the end of November and the beginning of December 2008. In Romania, meetings were made with Dr Neculai Patriche and Dr Jorga Valentin from the Institute for Research and Development of Aquatic Ecology, Fishing and Aquaculture in Galati, and with Dr Radu Suciu and Dr Ion Navodaru from the Danube Delta National Institute in Tulcea. In Bulgaria, meetings were made with Dr Milen Vassilev from the Institute of Zoology of the Bulgarian Academy of Sciences in Sofia, Dr Tania Hubenova from the Institute of Fisheries and Aquaculture in Plovdiv, Dr Angel Tsekov from the Department of Biology and Ecology at the Plovdiv University, as well as with Dr Ivan Dobrovolov from the Institute of Fisheries and Aquaculture in Varna (Fig. 9). The meetings proved to be useful, and provided new information and insight in sturgeon ecology, life history and management measures. On the other hand, all participants agreed that it would be hard to find more and better data than those already collected in data sheets from the available literature.

Fig. 9. Visiting sturgeon experts in Romania and Bulgaria: A) Fishery Institute in Galati, Romania; B) Dr. Ion Navodaru and Dr. Radu Suciu, Tulcea, Romania; C) Black Sea, Varna, Bulgaria; D) Dr. Angel Tsekov and Dr. Ivan Dobrovolov, Varna, Bulgaria; E) Danube River at Galati, Romania; F) Dr. Tania Hubenova and Dr. Milen Vassilev, Sofia, Bulgaria (Photos: I. Jarić, November-December 2009).

A number of other experts on sturgeons from the Danube River basin were contacted through the E-mail. Data sheets with sturgeon life history parameters were sent to them, and they were asked to mark the most probable values of each life history parameter, or to add new values and references if they were missing. However, this proved to be an inefficient approach since almost no replies were obtained, and Dr Patrick Williot, who provided some crucial parameters that were missing for the Atlantic sturgeon, was the only successfully established contact.

In general, for parameters that were provided by different authors as ranges or that were inconsistent in provided values, a most probable mean value was determined, and ranges were included in the model as environmental variation through establishing a standard deviation for the mean value. If ranges provided by different authors were inconsistent, values that were given in the majority of the publications were applied, and the other values were included in the sensitivity analysis (for details see subchapter Scenario development in Material and methods).

There is a significant uncertainty regarding the maximum age of reproduction. In most of the publications that are dealing with the ecology and life history of sturgeons, this issue has not even been raised or touched upon. Nearly all sturgeon experts that I have discussed with have claimed that sturgeons can reproduce throughout their whole life. On the other hand, according to some sources they stop reproducing significantly before reaching their maximum age (Williot *et al.* 2005; Lagutov & Lagutov 2008; Williot pers. comm.). I have applied the opinion of the majority of sources and set the maximum age of reproduction to be the same as the maximum longevity of the species. The effect of the existence of senescence on the results of simulation was assessed through the sensitivity analysis.

Although there are no real data on the type of mating system (how many males and females participate in a single spawning event), most interviewed experts claimed that it is polygynous (there are also some unpublished studies confirming this, for example Kynard in press citt. in Suciu pers. comm.), so this system was applied in simulations as well. As it most often has been defined in sturgeon PVAs (Pine *et al.* 2001), sex ratio was set to be equal, and effects of an unequal sex ratio were tested in the sensitivity analysis.

Sturgeons do not spawn every year (Reinartz 2002), because the egg development process (vitellogenesis) typically requires more than one year, so only a small fraction of the total population takes part in the annual spawning (Beamesderfer *et al.* 2007). The percentage of individuals of each sex that participate annually in spawning was established as $S=100/I$, where I represents the time between two spawning migrations.

When a studied species has too large mean and maximum number of offspring for an individual-based model like Vortex, it is recommended to redefine reproduction by condensing a series of mortality events at early life stages, and thus to include in the offspring only those that survive to a certain age (Kjos *et al.* 1998 citt. in Miller & Lacy 2005). Miller & Lacy (2005) have suggested that this approach could be useful for modelling fish populations, so it was applied in this study as well. For estimating the mean and the maximum number of offspring, mean and maximum fecundity for each of the studied sturgeon species was multiplied by the survival rate from age 0 to 1, and the resulting value was applied. Since the natural mortality for age 0 to 1 was thus already included in the model through the number of offspring, natural mortality for this age group was set to be 0.

It is a common opinion that the natural mortality is one of the most difficult vital rates to be determined (Boyce 1992; Beissinger & Westphal 1998; Beissinger 2002). This is unfortunately also true for the sturgeon species, and there are no data on the natural mortality for populations in the Danube River. As proposed by Boyce (1992), in such situations it is feasible to either use data from similar species or areas, or to explore behaviour of the model across the range of possible values. I have tried to apply both approaches here. For determination of the age 0 to 1 mortality, values from other sturgeon species and PVA studies were used. The mortality for egg to age 1 class was similar among all models and studied sturgeon species, it ranged from 0.9996 to 1.0 (Pine *et al.* 2001; Jager 2005, 2006:1; Bajer & Wildhaber 2007; Kennedy & Sutton 2007). These values were applied in this study as well; the middle value of the given range (0.9998) was used as the mean and 0.9996 as the minimum age 0 to 1 mortality rate. As was already described above, these values were incorporated in the model through the number of offspring.

It is believed that sturgeon longevity is characterized by a low mortality rate after the first few years of age, but there is no empirical data that would confirm this (Beamesderfer *et al.* 1995). Most of the authors of other sturgeon PVAs have used simple natural mortality distribution across age classes, i.e. the same values were used for both juveniles after the first year and for adults (Jager 2001, 2005, 2006:1; Jager *et al.* 2001; Heppell 2007; Beamesderfer *et al.* 2007; Kennedy & Sutton 2007). Such an approach was shown to be ecologically unrealistic when I tried to apply it in Vortex, since a uniform distribution of age specific mortality allowed the existence of only a small number of adults in a population, and prevented them from reaching the old ages that have been reported for these species. When I have tried to test behaviour of the model across different sets of mortality rates, as proposed by Boyce (1992), it became apparent that it would be necessary to place a larger mortality on the younger age classes and significantly lower mortality on adults, so that a certain number of older individuals can still be present in the population with the normal age distribution.

In order to define a starting point for the determination of age specific natural mortality, I have used Rikhter and Efanov's method for the estimation of natural mortality for fish species in temperate regions. This equation requires only the age at maturity of females as the input variable, and provides the total natural mortality rate, not the age-specific one. Calculations with Rikhter and Efanov's equation were made in the FISAT II (2000) computer program for fish stock assessments. Values obtained by this approach are presented in Table 3.

Table 3. Values of annual natural mortality obtained by the Rikhter and Efanov's method for each of the six studied species.

	Beluga	Russian sturgeon	Stellate sturgeon	Sterlet	Ship sturgeon	Atlantic sturgeon
Average age at maturity of females*	15	14	10	5	13	14
Natural mortality	0.056	0.067	0.130	0.317	0.080	0.067

* See the Appendix I for references regarding the age at maturity estimation

Upon obtaining these values, I have made a fitting of the natural mortality for each species, in order to get the most realistic distribution of mortality rates for different age classes. The fitting was performed with the aim to meet two basic criteria at the same time: 1) At the stable age distribution, populations had to contain enough adult specimens, of which a certain number had to be of higher age, close to the maximum longevity reported for these species. In other words, mortality had to be set in such a way to allow individuals to reach their maximum reported ages. 2) The deterministic population growth rate (*r*) should reach pre-determined levels.

Deterministic growth rate is one of the key demographic parameters, but the available data on the *r* value for sturgeon populations is poor, especially for the populations in the Danube River. According to some sources, the most common population growth rates for sturgeon populations are low, being close to 0.05 (Bruch 2008), 0.10 (Secor & Niklitschek 2002), or varying between 0.05 and 0.15 (Balnath *et al.* 2008). Based on this information, I have applied three different population growth rates for each species: 0.05, 0.10 and 0.15. In order to acquire such growth rates, age specific natural mortality was fitted so that each of these *r* values was met in different scenarios (see Appendix I for the natural mortality rates). All scenarios and simulations were tested against each of the three defined population growth rates.

According to Jager (2001), sturgeons are known to experience fluctuations in year class strength, and are unlikely to ever reach a true stable age distribution (Heppell 2007). Pine *et al.* (2001) stated that sturgeon probably demonstrate variable recruitment, where a few large year-classes make up a high percentage of the adult standing stock. In this study, age distribution at the beginning of each simulation was set to be stable, since the stochastic fluctuations in the age distribution and year-class strength already represent an integral part of simulations in the Vortex. This approach also makes the least number of assumptions since the stable age distribution is a direct function of the demographic rates chosen, whereas any other distribution must be supported by additional information. Furthermore, an unstable age distribution in itself creates population fluctuations that may mask the effects of other factors that were assessed. I have included the option of environmental concordance between reproduction and survival, in order to represent the actual situation in the environment, where bad years for reproduction (e.g. unsuitable hydrological regime in the river) are usually also bad years regarding survival. This approach was chosen since it as well meant the least number of assumptions, and makes the results more conservative, in the sense that it does not lead to underestimation of actual threats.

In order to simulate the environmental variation in natural mortality, I have assumed that younger age groups have much higher annual variability in natural mortality, and that it drops towards the adult age groups. I have applied the general rule that the standard deviation of the natural mortality of each of the age 1 to 3 age groups was equal to 40 percent of the natural mortality of those age groups. For the age groups from the age 4 to adulthood it was equal to 25 percent and for the adults it was 10 percent of the age specific natural mortality. However, if the mean mortality of a certain age plus two standard deviations resulted in a higher value than 100 (Mean + 2 x S.D. > 100), the standard deviation was reduced to the level where the expression was equal to 100. Variation in natural mortality of the age 0 to 1 was incorporated through the standard deviation of the mean number of offspring.

According to Allendorf & Ryman (2002), a population persisting over a certain number of years does not have to imply that it is viable, since it might have already experienced such significant changes that it is not likely to persist for additional period of time. Therefore, it is often prudent to employ a pseudo-extinction criterion, which represents a certain threshold population size below which the population is likely to experience dire demographic and genetic changes, leading to a significant extinction probability (Beissinger & Westphal 1998; Akçakaya 2000). According to Akçakaya (2000), the introduction of a pseudo-extinction threshold is also considered to be conservative, and can diminish the problem of having to incorporate factors that are dominant at low population sizes, such as inbreeding depression and Allee effects, for which there is usually insufficient knowledge. Based on the above described reasons, I have decided to use a pseudo-extinction criterion in this study, instead of scoring the risk of reaching absolute extinction. After running a number of test scenarios with varying population size for each species, in order to determine the relationship between the population size and the extinction probability, I have set the pseudo-extinction threshold to be at the population size of ten individuals, which was applied for all scenarios and all six species.

For the sake of greater simplicity and in order to reduce the number of assumptions already made in the model parameterization, some parameters like density dependence in reproduction and the existence of catastrophic events were not included in the model. While the demographic stochasticity is incorporated in the Vortex model, the problem of other Allee effects was partly resolved by introducing pseudo-extinction (Akçakaya 2000).

The number of iterations was set at 1 000 for all simulations, in order to increase the precision of the results. Since sturgeons are long lived species (some reaching even 100 years of age), a longer duration of simulations was necessary, so most scenarios were set to last for 500 years. Although the long-term projections can lead to serious statistical difficulties and the uncertainties are accumulating proportionally to the length of the simulation, they will do so much more slowly for species with a long generation time (Lande 2002). Exceptions were the stocking scenarios, where population recovery was monitored, which required much shorter time spans, usually less than 100 years.

The age 0 to 1 natural mortality was incorporated in the model through the proportional reduction of number of offspring produced, so young-of-the-year that have not survived up to the age 1 were not considered in the simulation. Therefore, it is important to note that in the future text, population size (*N*) does not include embryos and young-of-the-year that will not survive up to the age 1. For example, the population size of 2 000 individuals applied some scenarios represents a much larger actual population, since the number of offspring that will succumb to natural mortality during the first year of life can be up to few thousand times larger.

Except in the sensitivity analysis and the stocking scenarios, the carrying capacity was usually set to be equal to the initial population size, as was done by Reed *et al.* (2003).

Scenario development

The starting point of the Danube sturgeon PVA in the Vortex simulation model was the construction of basic scenarios. They were made for each of the six studied species, by applying the most probable values that describe their life history that are currently available (Appendix I). Each basic scenario was simulated with the three different fittings of age specific natural mortality, which were producing three different deterministic population growth rates (*r*), i.e. 0.05, 0.10 and 0.15 (for an explanation on the natural mortality fitting process see subchapter Model parameterization in Material and methods). Each of them was simulated at six different population sizes (50, 100, 200, 500, 1 000 and 2 000). Therefore, in order to cover different potential population growth rates and the behaviour of the model at different population sizes, there were 108 basic scenarios developed for each species. Carrying capacity was set to be equal to the initial population size, and the duration of the simulation was 500 years, so the model could be sensitive to the effects of those processes that are likely to emerge slowly. Main output values that were tracked in basic scenarios were the probability of pseudo-extinction, mean population size of extant populations, expected heterozygosity, the number of extant alleles and the mean time to extinction.

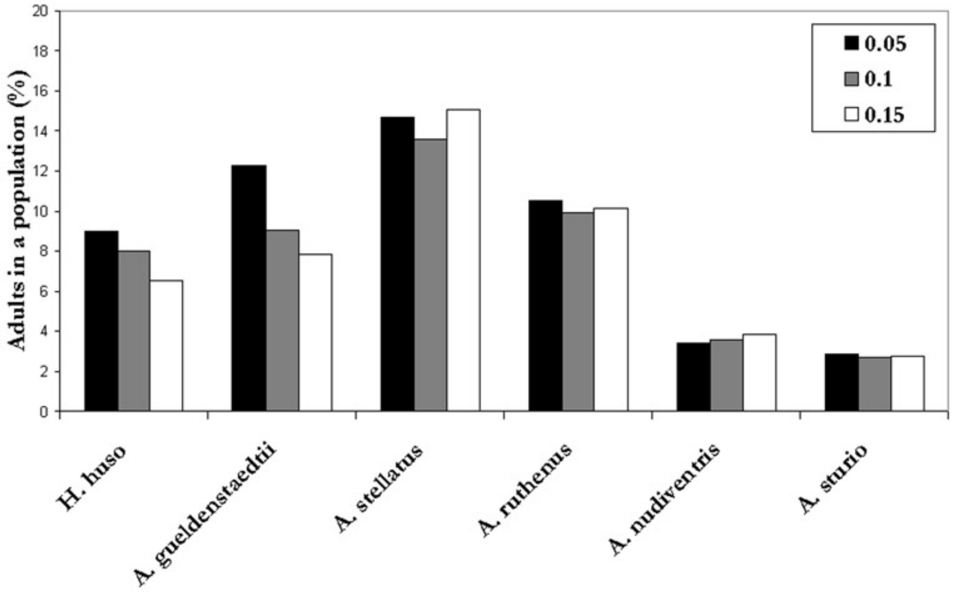


Fig. 10. Proportion of adults in the six studied sturgeon species, according to calculated stable age distributions based on demographic input data for simulation scenarios. Results for three rates of population increase (*r*) are shown.

There are two general approaches that can provide comparability among different sturgeon species. One is to set the carrying capacity equal for all studied species, with all age classes included in the population total. The other one would be to vary the carrying capacity based on the calculated stable age structure, with the aim to apply the ceiling to the number of female adults only disregarding the number of males, juveniles and subadults, and to achieve the same value for all species. Both approaches cannot be applied at the same time, since different life histories would produce different stable age distributions of the six Danube sturgeon species (Fig. 10). Therefore, another set of basic scenarios was developed that followed the latter approach. Since the ratio between adult males and females differs among species (due to the differing mortality between sexes, caused by different age at maturity), comparability can not be achieved for both genders in the same scenario, so females were chosen as the more critical gender in this case. As the initial step of the scenario development, a species was randomly chosen, and the initial population size was set to a level which gave large variation in output variables among scenarios. The stable age distribution was next calculated, to produce the corresponding number of adult females. For the remaining species, the initial population size was then increased or decreased to the level where the same number of adult females was reached. Simulations were run for 500 years, and the carrying capacity was set to be the same as the initial population size.

As stated by Miller & Lacy (2005), an important part of any PVA is the sensitivity analysis. It can reveal effects of uncertainty in applied parameters, as well as the relative influence of different parameters to the population dynamics and model projections (Miller & Lacy 2005). Therefore, in order to test the dependence of the model behaviour on changes in each of the life history parameters, as well as to span the ranges of life history parameter values provided by different authors, a sensitivity analysis was conducted. All simulations that were part of the sensitivity analysis were run at the population size N=50 and carrying capacity set at 2 000. Basic scenarios with this setup were also run for each of the studied species, in order to enable the comparison of the results. Since the initial testing had shown that most of the simulations have resulted in population recovery during the first 100 years of simulation, the duration of all sensitivity analysis scenarios was set to 100 years. In order to test the effect of change in any single parameter, only one parameter was being changed at a time. The age distribution of the initial population was kept constant in all scenarios that were compared, to avoid the effect of change in age distribution when life history parameters are being modified. The tested change in each parameter was either reflecting the ranges provided for that parameter by different authors, or some general amount of change was applied (e.g. ± 50 percent). As was done in the basic scenarios, sensitivity analysis of each parameter was also tested against three different population growth rates. Ranges of values applied for each life history parameter are presented in Appendix II. Output variables that were tracked during the sensitivity analysis simulations were the pseudo-extinction probability, deterministic and stochastic population growth rates, expected heterozygosity, and the mean time needed for population to reach half of the carrying capacity.

Relationships between the deterministic population growth rate and the probability of pseudo-extinction, as well as between the deterministic population growth rate and the expected heterozygosity, were assessed separately for each studied species, in order to determine the potential of the deterministic growth rate as a general predictor of the overall population viability. Since the variables lacked normality of distribution, they were compared with Spearman's non-parametric correlation test.

Results of the sensitivity analysis were assessed through the use of two distinct approaches, a ranking technique and regression analysis. For each studied species, absolute changes in output variables (pseudo-extinction probability, deterministic and stochastic population growth rates, expected heterozygosity, and the mean time to recovery), that were caused by changes in each of the life history parameters, were ranked from the highest to the lowest. This ranking was performed separately for each of the three population growth rates applied, and the ranks were then summed for each life history parameter (i.e. six rank values were summed for each life history parameter – results of the increase and decrease in parameter value, respectively, for each of the three growth rates). Life history parameters were then ranked separately for each output variable, thus revealing the relative influence of each parameter on the output variables. Ranks were also summed across output variables for each species, as well as across species for each output variable, in order to determine which life history parameters have the overall highest influence on sturgeons and their viability.

Regression was performed separately for each studied species and for each of the three population growth rates. Output variables (pseudo-extinction probability, deterministic and stochastic population growth rates, expected heterozygosity, and the mean time to recovery) acquired from the each sensitivity analysis scenario were used as the dependant variables and values of life history parameters applied in those scenarios were used as the independent variables. Regression analysis was performed in the SPSS software (version 15.0).

As the unsustainable fishery is considered to represent the most detrimental impact on the viability of Danube sturgeon populations (Reinartz 2002; Lenhardt *et al.* 2006:2), harvest scenarios made a significant portion of the simulations made within this study. The basic questions that were addressed in harvest scenarios were the determination of the threshold level of the harvest pressure below which population viability would still be preserved, as well as to compare the vulnerability of different sturgeon species to harvest. General model setup was similar as in the basic scenarios – both the initial population size and the carrying capacity were set at 2 000, and simulations were run for 500 years. The harvest was introduced in the model as the percentage of adults taken every year. The same pressures were always applied to both sexes, since it is believed that they are exposed to similar fishing efforts, due to lack of sexual dimorphism (Vassilev pers. comm.). The harvest was applied first at a 5 percent level (meaning that 5 percent of the adult part of the population was taken), and then increasing it in each new scenario by 5 percentage units, until population extinction was the most likely outcome. The harvest was performed each year, during the whole duration of the simulation. Since the fishery in the Black Sea, performed by Ukraine in the past, was also directed at subadults (Vassilev pers. comm.), as is the illegal Sterlet fishery in Serbia, special scenarios were developed where the two oldest age groups of subadults were also harvested, with the same harvest pressure applied as for the adults. Subadult harvest was introduced in the model through the increase of natural mortality of those age groups, because there was no way to introduce it as the percentage of subadults that were taken through the regular harvest platform. The age distribution of the initial population was kept constant. Main output variables that were tracked for the harvest scenarios were the pseudo-extinction probability, mean population size of extant populations, expected heterozygosity, the mean time to extinction, and the number of individuals that were harvested.

Supportive stocking is one of the main conservation measures applied to sturgeons worldwide, and this approach has also been widely applied in the Danube River basin (Reinartz 2002; Williot *et al.* 2002; Vassilev 2006). In order to test the effectiveness of this measure, a series of stocking scenarios was developed. The main goal was to test different approaches in supportive stocking, and their influence on the population viability. Since it was important to track the time that population needed to recover after the release of stocked individuals, the general setup of the model was similar to the one used in the sensitivity analysis – initial population size was 50, carrying capacity was set at 2 000, and the duration of simulations was 100 years. In general, stocking dynamics (e.g. time of the release, number of releases) varied between different scenarios along three main dimensions: total number of individuals that was released, age of released individuals (whether they were juveniles or adults), and the temporal scale of stocking (whether the same number of individuals was released in a single year, or during several years). The sex ratio of released individuals was always set to be equal. Initially scenarios were run with a smaller number of individuals released in the first year of simulation, and then the total number was increased in each new scenario until recovery was the most likely outcome. Each step was afterwards tested with two additional dynamics of release, but with the same total number of released individuals kept to be the same - release was conducted every second year or every year during the initial 10 year period of the simulation. Since the juveniles below age 1 are most often used in stocking activities, separate tests were run with juvenile and adult fish being released. Main output variables that were tracked for the stocking scenarios were the pseudo-extinction probability, mean population size of extant populations, expected heterozygosity, number of extant alleles, the mean time needed for population to reach half and 75 percent of the carrying capacity, and the stochastic population growth rate, both during and after the stocking period.

As proposed by a number of different authors (Ebenhard 2000; Beissinger 2002; Hanski 2002; Reed *et al.* 2002), results of all scenarios were used more to compare their relative effects, for instance to compare different management scenarios, then to use them as the absolute predictions. An extinction risk of $P<0.05$ is commonly applied as the threshold to evaluate viability (Beissinger & Westphal 1998), so it was used in this study as well.

Results

Models for inferring extinction probability based on sighting data

Values that were used in equations are given in the Table 4. Results of equations 1 - 4, applied on the documented sightings of the Atlantic sturgeon and the Ship sturgeon, provided the indices of probability that these species are still present in the Danube River basin (Table 5).

Table 4. Values used in equations for estimating probability of the Atlantic sturgeon and the Ship sturgeon presence in the Danube.

Value	Atlantic sturgeon	Ship sturgeon
T	60	59
n	11	14
t_n	17	56
t_{n-1}	16	54
n_1	11	14
n_0	49	45
r	43	13
α	0.05	0.05

Table 5. Probabilities (p) that the Atlantic sturgeon and the Ship sturgeon are still present in the Danube, according to the four methods employed.

Method	Atlantic sturgeon	Ship sturgeon
1	0.000	0.482
2	0.000	0.422
3	0.023	0.400
4	0.000	0.264

All equations provided a significant extinction probability for the Atlantic sturgeon ($p<0.05$), while for the Ship sturgeon they produced values that were well above the level of significance. In the case of absence of new records of the Ship sturgeon by the end of 2009, equations 1 - 4 would give the following probabilities, respectively: 0.381, 0.316, 0.333 and 0.287. Regarding the Atlantic sturgeon, the point estimate of the extinction time given by equation 5 is 1966-1967, and equation 6 gave the upper bound of the 0.95 confidence interval for extinction time as the year 1970. When applied to the Ship sturgeon, the point estimate of extinction time was 2009, with 2018 as the upper bound of the confidence interval.

Sensitivity analysis and influence of different future scenarios

Sensitivity analysis of equations 1 - 4 has been discussed in detail by different authors (Burgman *et al.* 1995; Grogan & Boreman 1998; Solow and Roberts

2003; McInerny *et al.* 2006). Specifically, an increase in time since the last observation is expected to increase the extinction probability. In the case of absence of Ship sturgeon observations in future years, equations 1 - 3 require different number of additional years to reach a significant level of probability of extinction (p<0.05). For equations 1 - 3, an absence of observations lasting until the year 2019, 2016 and 2044, respectively, would produce a likely extinction. In case of new sightings of Ship sturgeon in the near future, time to extinction would not be extended by more than a few years.

One of the potential biases in sighting records would be observations that were wrongly identified, or not recorded and published. Increased number of Ship sturgeon sightings during the observation period, before the last recorded sighting, would lead to increased probability of extinction in equations 1 - 3. According to Pintér (1991), fishermen did not always distinguish larger Ship sturgeon from Russian sturgeon, and small Ship sturgeon from Sterlet. However, this had limited effect regarding the adult Ship sturgeons upstream from Iron Gate dams, because the power plant construction prevented all big anadromous sturgeons from migrating upstream (Reinartz 2002), and the Sterlet, the only sturgeon found upstream nowadays, can never reach the dimensions of an adult Ship sturgeon.

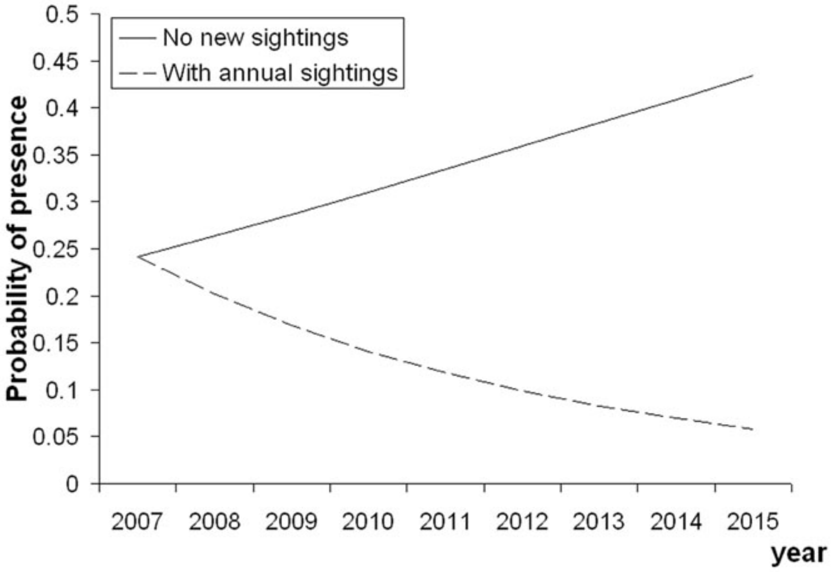


Fig. 11. Probabilities for the continued presence of Ship sturgeon given by Equation 4 in two potential future scenarios, in which the Ship sturgeon is recorded annually for the next seven years, or in which there are no new sightings.

Equation 4, which is sensitive to long periods without observations but less so to a short period since the last observation, gave the highest extinction probability for the Ship sturgeon. As Fig. 11 shows, in the case of a new observations in the following years this equation would give a greater probability of extinction, and a smaller probability without new sightings, given that the longest new run of years without observations is shorter than the longest run recorded so far ($r = 13$, between the time units 12 and 26, or 26 and 40). Although this might seem counterintuitive, it is caused by the fact that equation 4 is sensitive to the ratio of the longest run of years without sightings to the total period of observations, and not to the time since the last sighting.

Extinction time, based on equations 5 and 6, proved to be only moderately sensitive to new sightings, which would extend the extinction time by a few years for each new observation.

The new probability model

Solow/Roberts non-parametric equation (Equation 3, Solow & Roberts 2003) was used as a basis for the development of the new model. The Solow/Roberts model gives the probability that the species is still present using the time of the last sighting (t_n), the time of the second most recent sighting (t_{n-1}) and at the end of the observation period (T). In order to incorporate the average frequency of sightings within the model, the time between the last two sightings (t_n - t_{n-1}) was replaced with the average time elapsed between each two sightings.

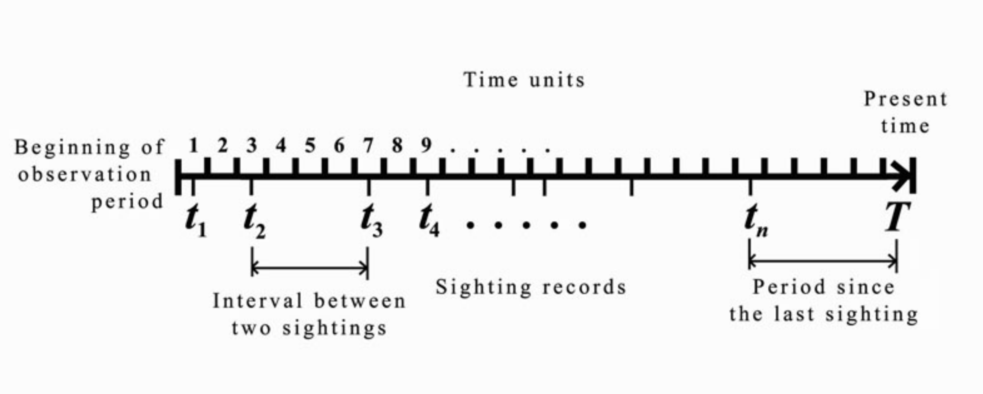


Fig. 12. A schematic representation of the observation period. The upper side of the timeline represents time units (e.g. years), the lower side represents sighting records (t_1, t_2, t_3, t_4,... t_n).

If time is considered as discrete, and sighting records are arranged as a series of time units (t) with positive sightings within the observation period (with the first time unit labelled 1 and the last T), ordered from the earliest to the latest, $t_1 < t_2 < . . . < t_n$ (Fig. 12), then the average time elapsed between each two sightings can be calculated as:

$$\Delta I = \frac{\sum_{x=2}^{n}\left(t_x - t_{x-1}\right)}{n-1} = \frac{t_n - 1}{n-1} \tag{7}$$

The probability that the species is still present would then be:

$$p = \frac{\frac{t_n - 1}{n-1}}{\frac{t_n - 1}{n-1} + \left(T - t_n\right)} \tag{8}$$

Here, n is the number of time units with sightings, and (T - t_n) represents the period since the last sighting; (t_n - 1)/(n - 1) represents the average length of the intervals between sightings. Multiple sightings within one time unit are recorded as a single sighting. The last time unit (T) corresponds to the final year of the time series.

In case of a species whose rate of sighting is changing over time, either increasing or decreasing, the equation can be modified in order to reflect this change. If the period between two sightings is expected to be longer than between the previous two, a longer time after the last sighting should be needed in order to infer extinction, and vice versa. Therefore, a coefficient of trend in sighting intervals (c) can be included in the equation, which represents the average change in length of intervals between each two consecutive sightings:

$$c = \frac{\sum_{x=2}^{n-1}\left[\left(t_{x+1} - t_x\right) - \left(t_x - t_{x-1}\right)\right]}{n-2} \tag{9}$$

Note that if the frequency (inverse time interval) increases, the coefficient is positive, otherwise it is negative.

The probability that the species is still present would then be:

$$p = \frac{\frac{t_n - 1}{n-1} + c}{\frac{t_n - 1}{n-1} + c + \left(T - t_n\right)} \tag{10}$$

The relative weight of c in the equation is arbitrary, depending on how much we want to stress different sections of the time series.

Similarly as for the models described in the subchapter Statistical methods in Material and methods, a 0.05 probability could be employed as the threshold value that infers extinction. In case the last sighting occurred in the present year, $(T - t_n)$ would reach the value of 0 and consequently p would assume the value 1. The two previously described assumptions, that observations are made independently of each other and that sighting effort should be constant through time, have also to be fulfilled here, if the model is expected to provide reliable results.

Sensitivity analysis of the new model and the illustration of its application

The sighting interval model (Equation 8) is sensitive to two variables, the number of time units in the time series since the last observation $(T - t_n)$, and the average length of sighting intervals $(t_n - 1)/(n - 1)$. The Equation 10 is also sensitive to the trend of sighting intervals (c). Sensitivity of probability of extinction to these factors was examined, and results are presented in Table 6.

Table 6. Probability (p) that the species is still present (based on Equations 8 and 10), given the average sighting interval and the total number of time intervals since the species was last observed $(T - t_n)$. Significant probabilities ($p<0.05$) are shown in italics. Average sighting interval is calculated as $[(t_n - 1)/(n - 1)]$ for Equation 8 and $[(t_n - 1)/(n - 1) + c]$ for Equation 10.

$T - t_n$	Average sighting interval				
	1	2	3	4	5
1	0.50	0.67	0.75	0.80	0.83
2	0.33	0.50	0.60	0.67	0.71
3	0.25	0.40	0.50	0.57	0.63
4	0.20	0.33	0.43	0.50	0.56
5	0.17	0.29	0.38	0.44	0.50
6	0.14	0.25	0.33	0.40	0.45
7	0.13	0.22	0.30	0.36	0.42
8	0.11	0.20	0.27	0.33	0.38
9	0.10	0.18	0.25	0.31	0.36
10	0.09	0.17	0.23	0.29	0.33
20	0.05	0.09	0.13	0.17	0.20
30	*0.03*	0.06	0.09	0.12	0.14
40	*0.02*	0.05	0.07	0.09	0.11
50	*0.02*	*0.04*	0.06	0.07	0.09
60	*0.02*	*0.03*	0.05	0.06	0.08
70	*0.01*	*0.03*	*0.04*	0.05	0.07
80	*0.01*	*0.02*	*0.04*	0.05	0.06
90	*0.01*	*0.02*	*0.03*	*0.04*	0.05
100	*0.01*	*0.02*	*0.03*	*0.04*	*0.05*

As shown in Table 6, the model produces significant probabilities that the population does not exist anymore ($p<0.05$) when the period since the last sighting exceeds 20 times the average sighting interval (as modified by the trend of sighting intervals in Equation 10). The previous IUCN threshold of 50 years without sightings (Reed 1996) would then correspond to species whose average sighting interval was less than 2.5 years or, according to Equation 10, for species whose average sighting interval was initially larger than this value but steadily decreasing in time.

It is important to note that although the extinction probability is sensitive to the average length and trend in sighting intervals, it is much less sensitive to the number of observations (n) or the length of the observation period (i.e. the value provided by this model can remain unchanged with different lengths of the observation period or with different number of observations, as long as the average sighting rate remains the same). This ability of the model to be unaffected by the total length of the sighting period, is similar to the sighting rate model developed by McInerny *et al.* (2006). This characteristic of the model can be advantageous, because inequalities that arise between records of different species in the number of sightings and in the length of the entire observation period can obstruct comparability of sighting records among species, and thus the applicability of methods that are sensitive to these features (McInerny *et al.* 2006).

The parameter capturing trends in sighting intervals (c) in Equation 10 provides sensitivity of the model to different patterns of the sighting record. For species whose sighting frequency is decreasing (longer and longer periods between sightings), c would reach a positive value. Equation 10 would, therefore, require a longer period since the last sighting to reach a level of significance ($p<0.05$), and vice versa. This is in accordance with the real situation, since a decreasing, but still extant, population implies that the next sighting should appear after the longer period than between the last two sightings, and that probability of extinction should be lower even if the species is not recorded for several years. In other words, the model would return a higher probability of presence for a species with decreasing sighting frequency, than for a species with stable sighting frequency, if the time since the most recent sighting is the same for both species.

However, it should be taken in consideration that this model is not sensitive to the distribution of the change in the trend, since it can not distinguish between changes in length of sighting interval that appeared at the beginning or at the end of the observation period. The p value would be influenced to a lesser or higher degree by the amount of change in sighting frequency, if c is given a different weight than the one used in this particular formulation.

As an illustration of model application, sighting records for the Atlantic sturgeon and Ship sturgeon were analyzed using Equations 8 and 10. The year 2008 was considered as the endpoint of the observation period. Values that were used in equations are given in the Table 7.

Table 7. Values used in the two sighting trend models for estimating the probability of presence of the Atlantic sturgeon and the Ship sturgeon in the Danube.

Value	Atlantic sturgeon	Ship sturgeon
T	61	60
n	12	15
t_n	18	57
ΔI	1.545	4
c	0	0.077
α	0.05	0.05

It is important to note that the models described in the subchapter Statistical methods in Material and methods use other information within the same datasets, and the first sighting is often omitted in them (Solow 2005), therefore causing T, n, and t_n to assume lower values for both studied species. Since the sighting trend model described here is mostly sensitive to the sighting frequency and less so to the length of the sighting period, I have not considered omitting the first record as necessary.

Equations 8 and 10 provided the same probability of Atlantic sturgeon presence, p=0.035. This was a result of the lack of a trend in sighting frequency (c=0). For the Ship sturgeon, Equation 8 provided the probability of presence p=0.571. Since the c value had positive value for the Ship sturgeon sighting record (0.077), indicating an average decrease in sighting frequency, the probability of presence provided by the Equation 10 was slightly larger, p=0.576.

In the case of absence of new records of both species by the end of 2009, probabilities provided by Equations 8 and 10 for the Atlantic sturgeon would be 0.034, and for the Ship sturgeon 0.500 and 0.505, respectively. Both equations of the sighting trend model presented in this paper have provided higher probabilities of presence for the two studied species than the other methods that were employed (Table 5). This is probably due to the fact that this model is mostly dependent on overall sighting frequency, which was especially low for the Ship sturgeon throughout the observation period (on average, one sighting every 4 years). On the other hand, probabilities provided for the Atlantic sturgeon have confirmed extinction of this species (p<0.05), and the values provided for the Ship sturgeon, although not near the threshold level, are certainly low enough to be alarming.

Assessment of the microchemical concentrations in sturgeon pectoral fin spines

Several samples have been 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 the sample 6, 11 and 12, where significant parts of the transect line were located in such areas. On sample number 4 and, partially, sample number 9, the outer edge and annuli located there were missing from the scan. On the sample 7, annuli corresponding to ages above the 10th year of life were damaged and missing in that area of the fin. Unfortunately, it was impossible to repeat the scans on these samples, due to the financial constraints, so the samples 8, 11 and 12, which were most problematic for assessment, were omitted from further analysis.

Fig. 13. Microscopic pictures of the analyzed sturgeon pectoral fin ray sections, samples 1-4. Sample numbers are presented in the upper right corner of each image.

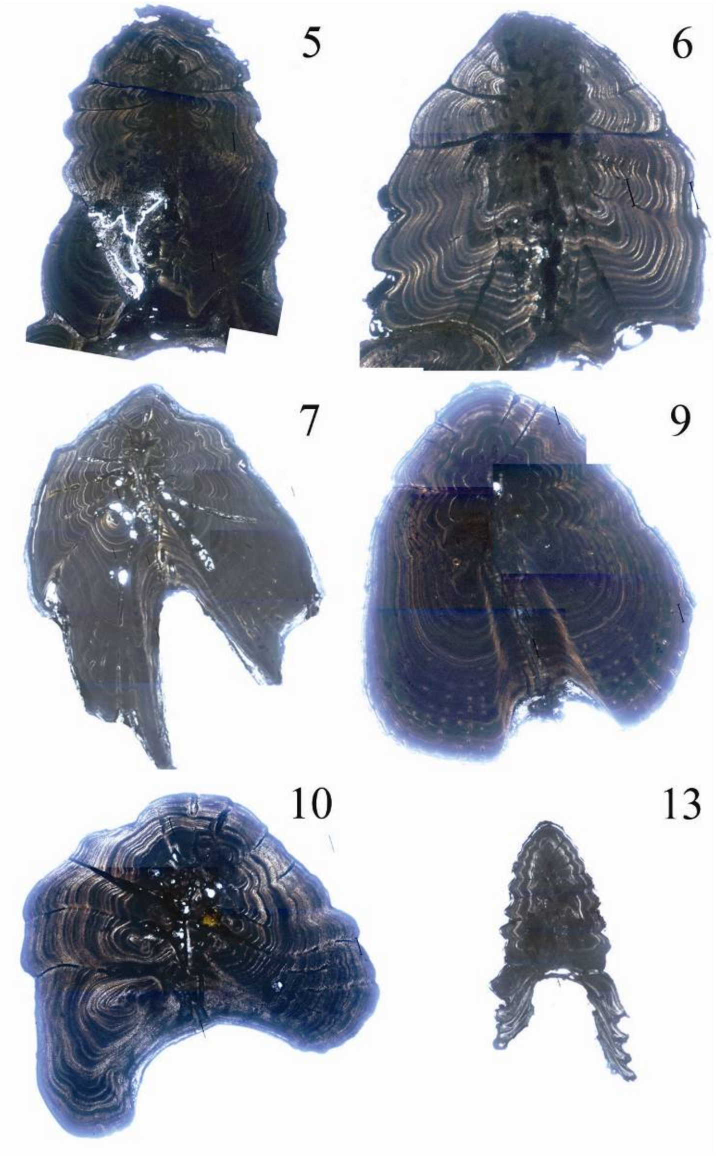


Fig. 14. Microscopic pictures of the analyzed sturgeon pectoral fin ray sections, samples 5-7, 9, 10 and 13. Sample numbers are presented in the upper right corner of each image.

The samples that were included in further analysis were four fin rays of the Russian sturgeon, three of the Stellate sturgeon, and three of the Beluga. Since the annuli of some samples proved to be hard to distinguish (Figs. 13 & 14), initial conclusions of the independent observers on the age of the studied individuals have significantly differed. Nevertheless, consensus on the age of each individual has been reached after a repeated assessment. Age of all individuals is presented in Table 8.

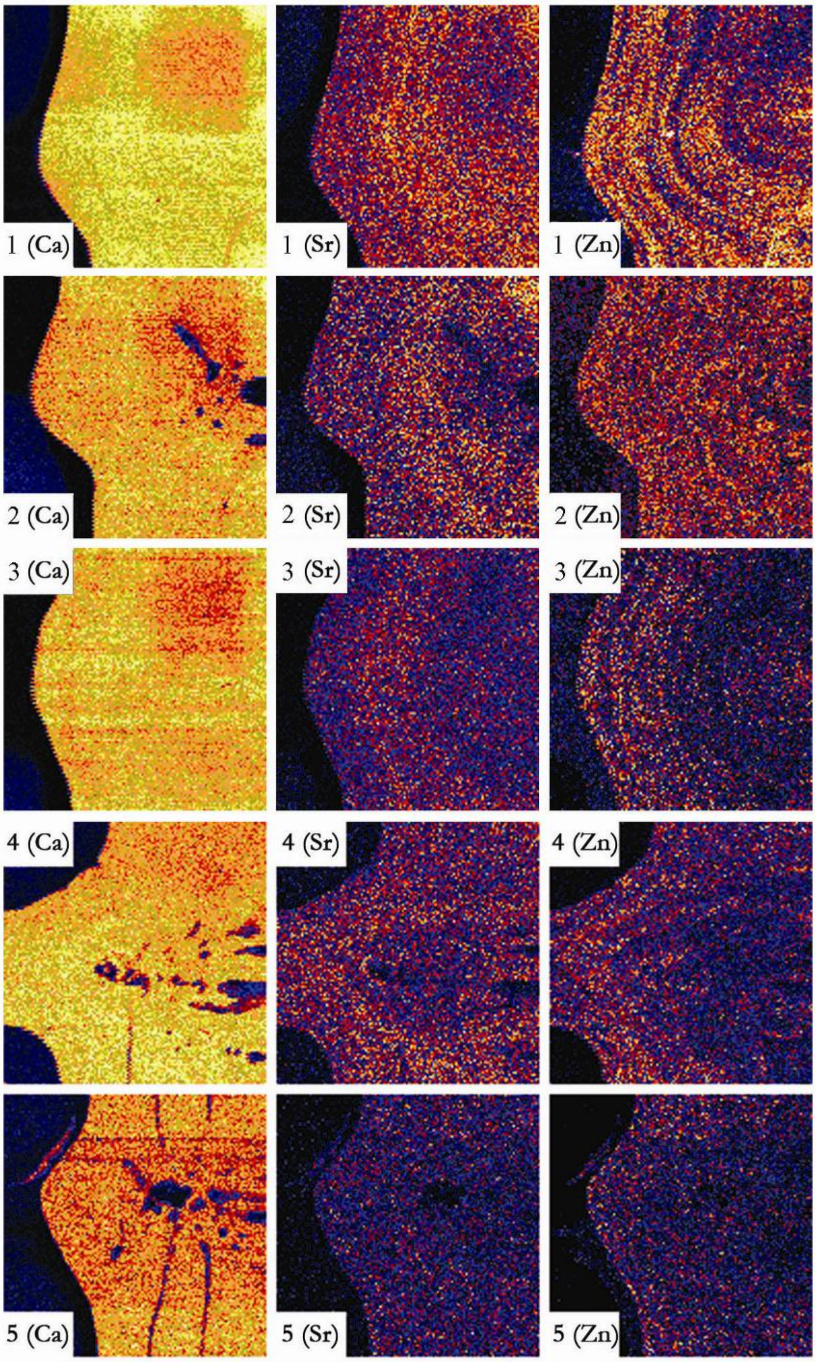


Fig. 15. Elemental maps of the sturgeon pectoral fin ray sections, samples 1-5. Sample numbers and the element that was scanned are presented in the corner of each image.

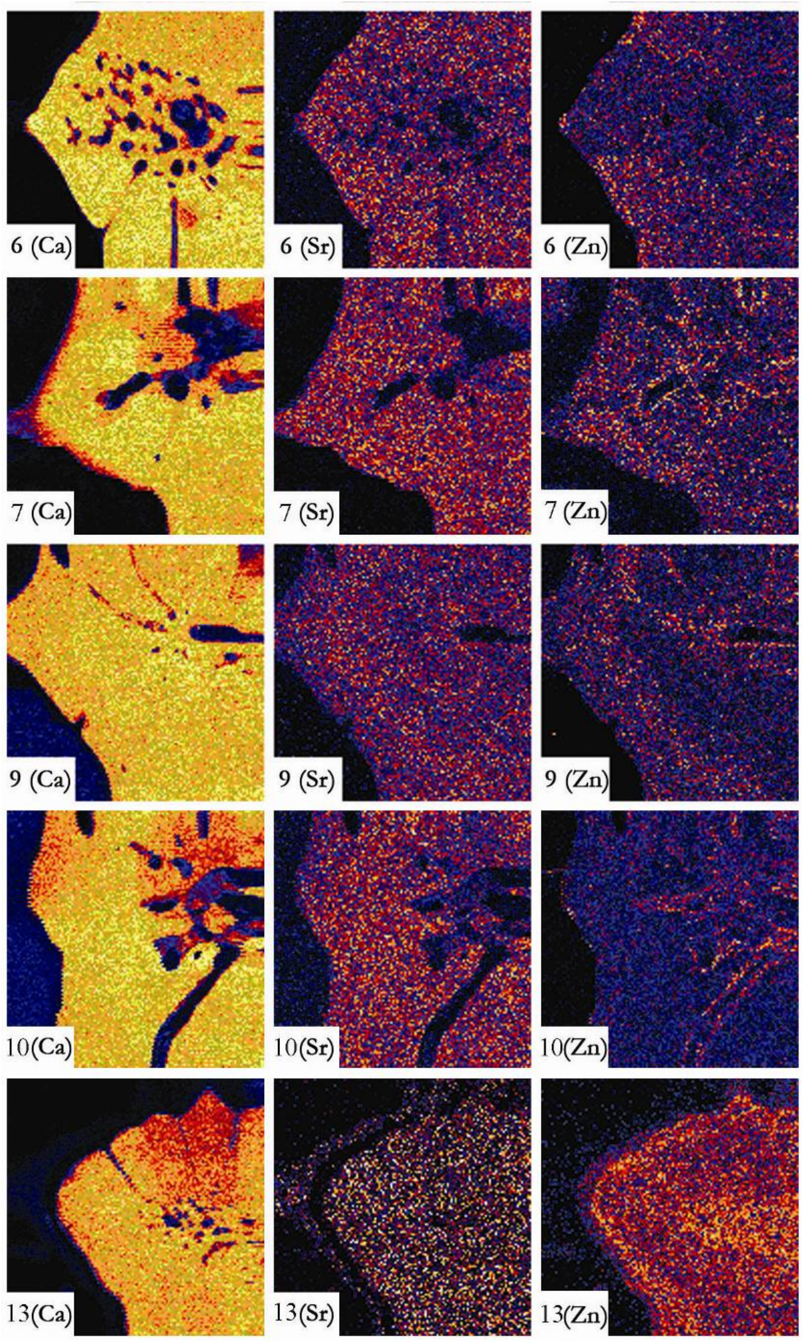


Fig. 16. Elemental maps of the sturgeon pectoral fin ray sections, samples 6, 7, 9, 10 and 13. Sample numbers and the element that was scanned are presented in the corner of each image.

Elemental maps for calcium, strontium and zinc in each of the four samples are presented in Figs. 15 & 16, and Sr:Ca ratio transect scans in Figs. 17-26. 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). Ratios of strontium to calcium along the transect scans have shown 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 8) between the transect section within the first annulus or first several annuli and the remaining annuli that were located within the transect (Figs. 17-26). This change in Sr concentration can also be noticed on the Sr elemental maps of some samples, with the weaker Sr concentrations located in the central parts of the sample (Figs. 15 & 16).

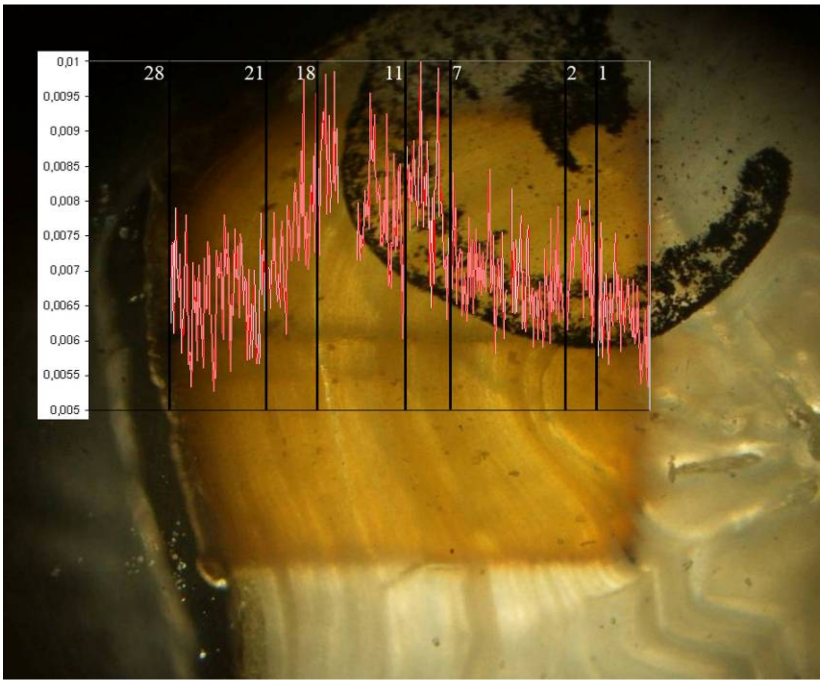


Fig. 17. Analyzed area of sample 1 (28 year old Russian sturgeon female) with the values of the Sr:Ca ratio transect scan (red line) located above the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black lines mark the end of the annuli corresponding to the age presented beside each line.

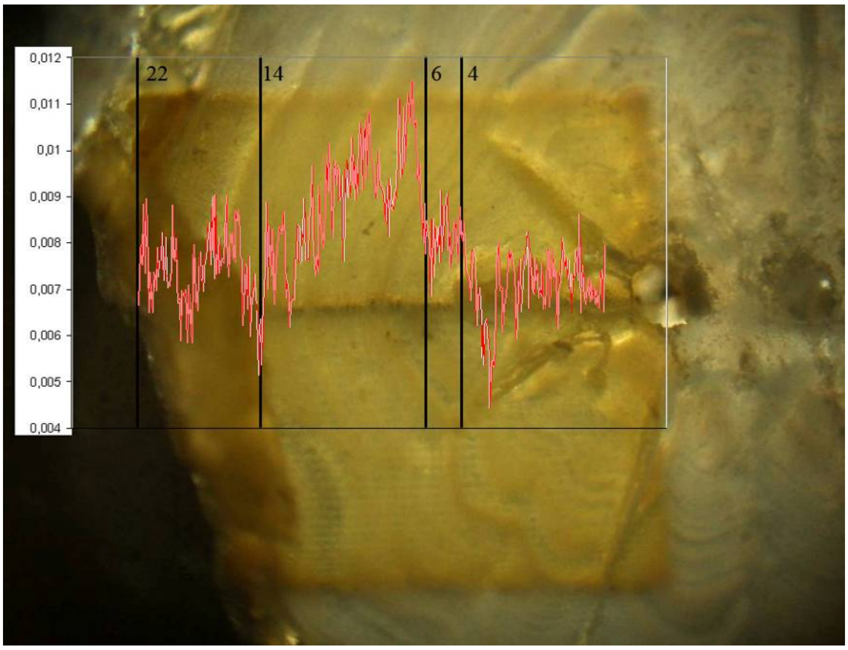


Fig. 18. Analyzed area of sample 2 (22 year old Russian sturgeon female) with the values of the Sr:Ca ratio transect scan (red line) located above the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black lines mark the end of the annuli corresponding to the age presented beside each line.

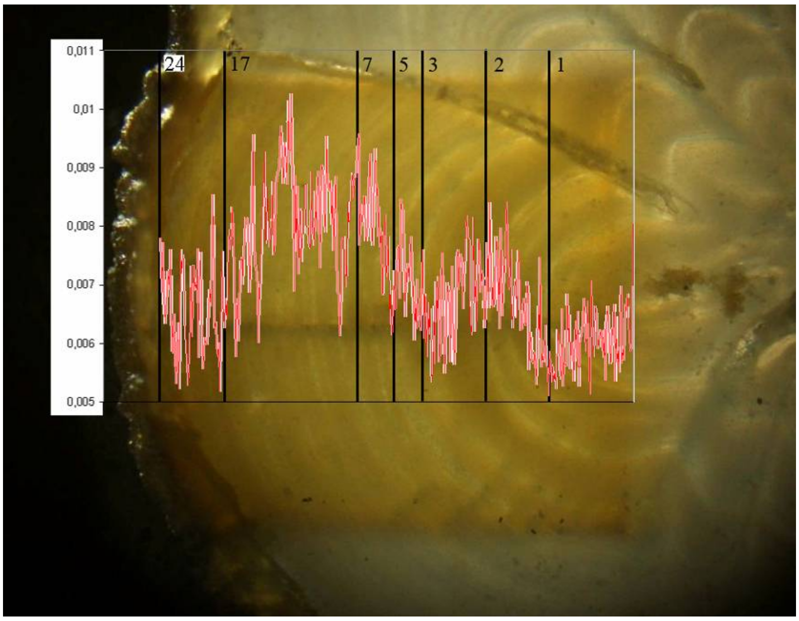


Fig. 19. Analyzed area of sample 3 (24 year old Russian sturgeon female) with the values of the Sr:Ca ratio transect scan (red line) located above the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black lines mark the end of the annuli corresponding to the age presented beside each line.

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 can be observed in samples 4 and 5 (Figs. 20 & 21), was represented by a low Sr:Ca ratio in the inner annuli (7.14×10^{-3} and 6.59×10^{-3} for samples 4 and 5, respectively) and then an increased ratio in the outer annuli (9.97×10^{-3} and 7.82×10^{-3}, respectively). 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. 22), statistical comparison between the available areas within the first annulus (7.43×10^{-3}) and the remaining annuli (7.82×10^{-3}) 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.

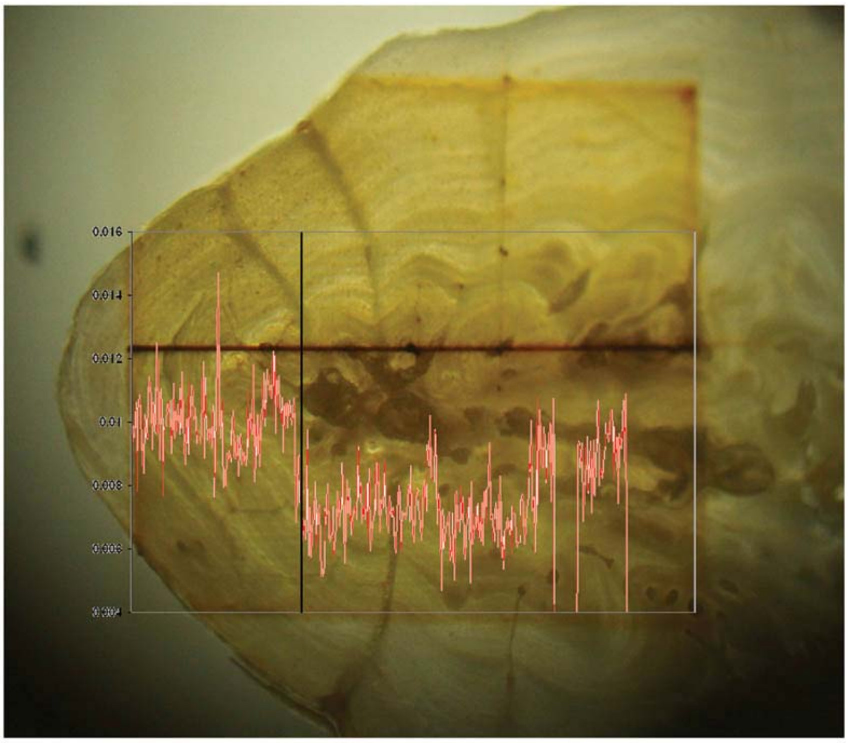


Fig. 20. Analyzed area of sample 4 (21 year old Stellate sturgeon female) with the values of the Sr:Ca ratio transect scan (red line) located below the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black line represents the edge between the first annulus (age 0-1) and the remaining annuli.

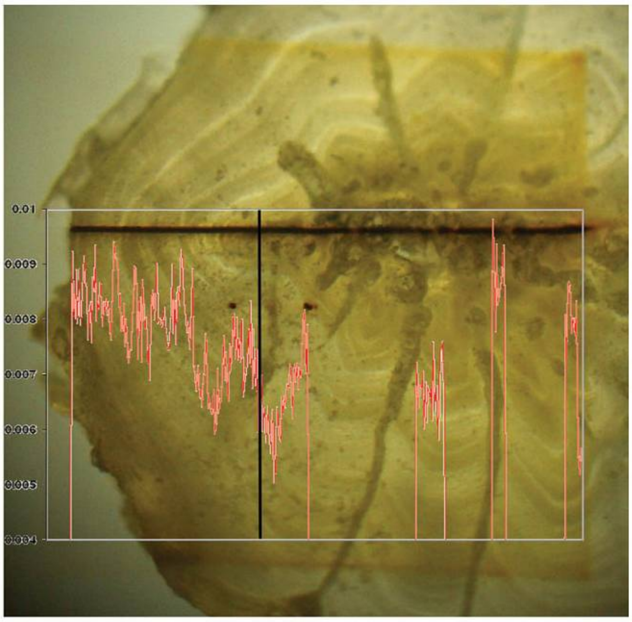


Fig. 21. Analyzed area of sample 5 (19 year old Stellate sturgeon female) with the values of the Sr:Ca ratio transect scan (red line) located below the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black line represents the edge between the first annulus (age 0-1) and the remaining annuli.

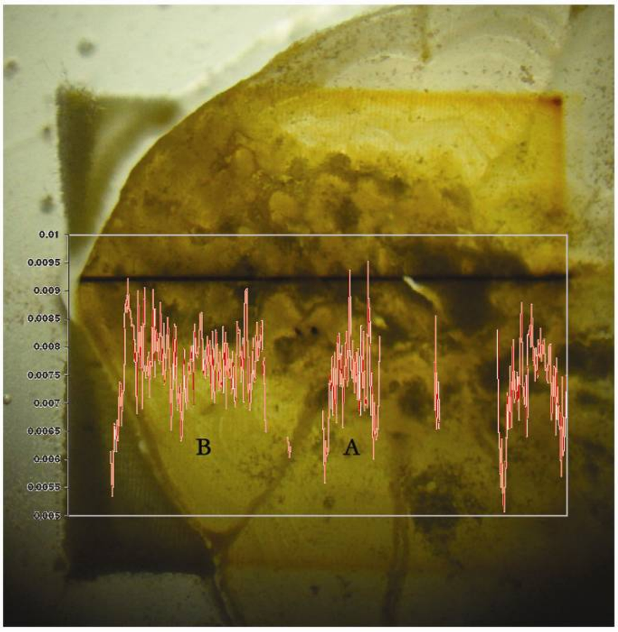


Fig. 22. Analyzed area of sample 6 (17 year old Stellate sturgeon male) with the values of the Sr:Ca ratio transect scan (red line) located below the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; A – available transect section within the first annulus (age 0-1); B – remaining part of the transect.

The second type of pattern, visible in the samples 1-3 and 9 (Figs 17-19 & 24), was represented by a lower Sr:Ca ratio in the inner part of annuli (6.77×10^{-3}, 7.08×10^{-3}, 6.52×10^{-3} and 6.72×10^{-3} for samples 1-3 and 9, respectively), followed by increased ratio in a section at the middle of the transect (7.81×10^{-3}, 8.72×10^{-3}, 8.04×10^{-3} and 7.45×10^{-3}, respectively), and then a decreased ratio in the outermost annuli (6.57×10^{-3}, 7.43×10^{-3}, 6.58×10^{-3} and 6.33×10^{-3}, respectively). While the inner and the outermost zone of transects in these samples had similar ratios and, except in the 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, can be also observed on elemental maps of strontium of these four samples (Figs. 15 & 16). All described shifts in the Sr:Ca ratio in samples 1-6 and 9 were of a magnitude that could imply a change of the salinity in the environment (Arai *et al.* 2002).

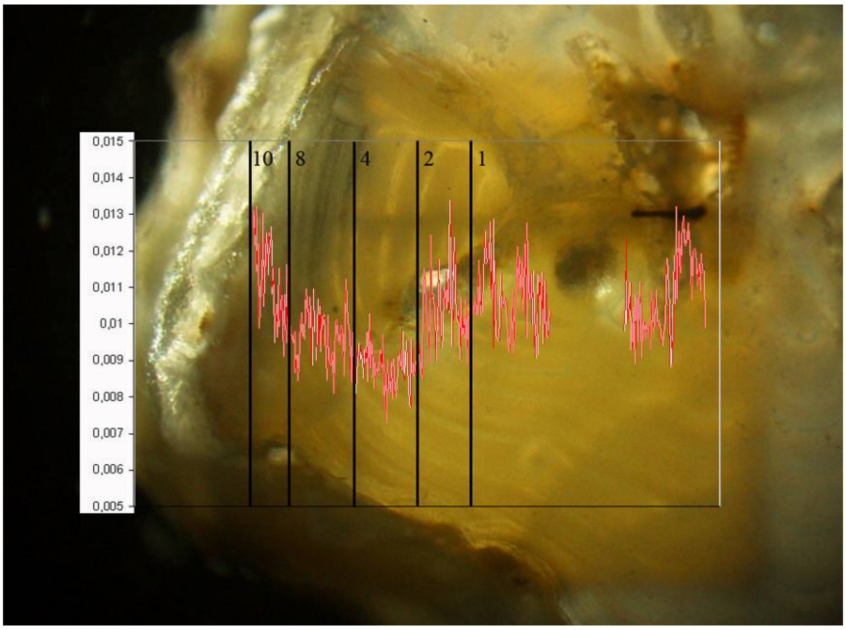


Fig. 23. Analyzed area of sample 7 (28 year old Beluga female) with the values of the Sr:Ca ratio transect scan (red line) located below the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black lines mark the end of the annuli corresponding to the age presented beside each line. Annuli corresponding to ages above 10 are missing due to the damage of the sample.

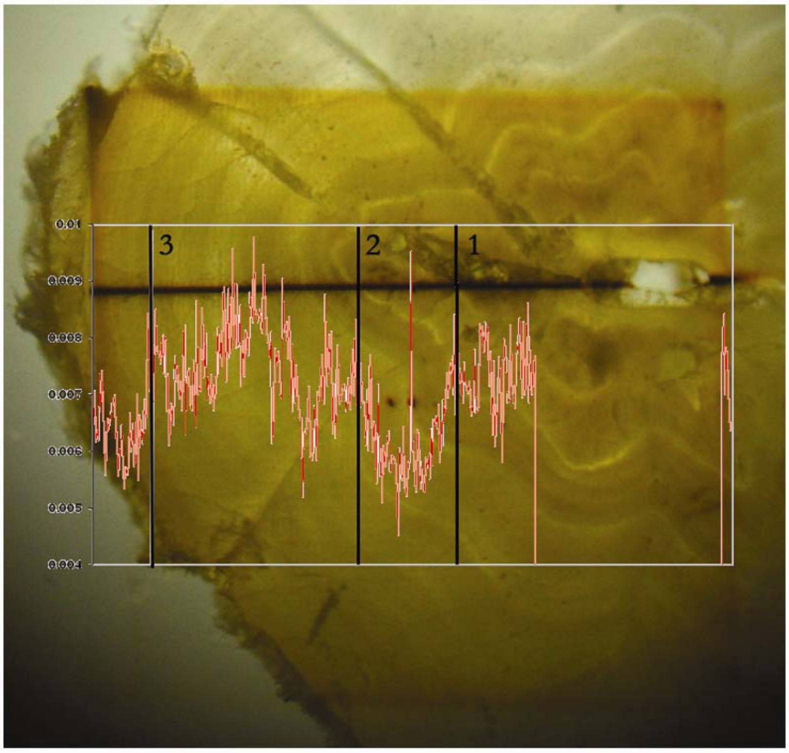


Fig. 24. Analyzed area of sample 9 (19 year old Beluga male) with the values of the Sr:Ca ratio transect scan (red line) located below the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical line 1 – border between the first annulus (age 0-1) and the second one (age 1-2); vertical line 2 – border between the second and the third annulus; vertical line 3 – border between the zone with increased Sr:Ca ratio (age 2-11) and the zone with lower ratio (age above 11).

The pattern of change in Sr:Ca ratio in sample 7 (Fig. 23) was different from other samples. The ratio in the first and second annulus (10.72 x 10^{-3} and 10.36 x 10^{-3}, respectively) was higher than in the sections corresponding to age 3-4 and 5-8 (8.80 x 10^{-3} and 9.70 x 10^{-3}, respectively). In the section with annuli corresponding to the age 9-10 there was a significant increase in the ratio (11.22 x 10^{-3}). However, since the transect was located on the damaged part of the fin and annuli after the one corresponding to age 10 were missing, it was not possible to observe further changes in the ratio. An increased Sr:Ca ratio in the first annulus in comparison to the next ones, as can be seen in samples 7 and 9, is often associated with maternally transmitted Sr (Limburg *et al.* 2001).

Changes in Sr:Ca ratio in sample 10 (Fig. 25) lacked any clear pattern. Due to less appropriate position of 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).

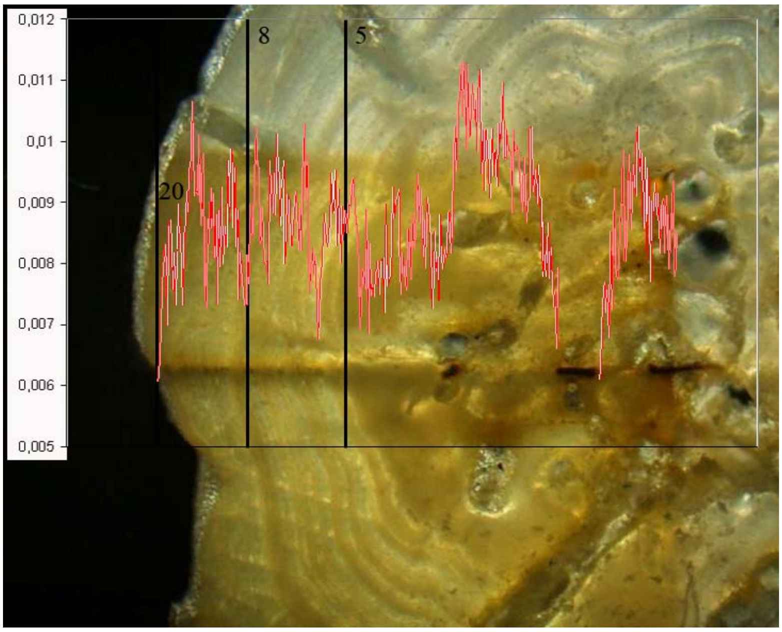


Fig. 25. Analyzed area of sample 10 (20 year old Beluga male) with the values of the Sr:Ca ratio transect scan (red line) located above the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black lines mark the end of the annuli corresponding to the age presented beside each line.

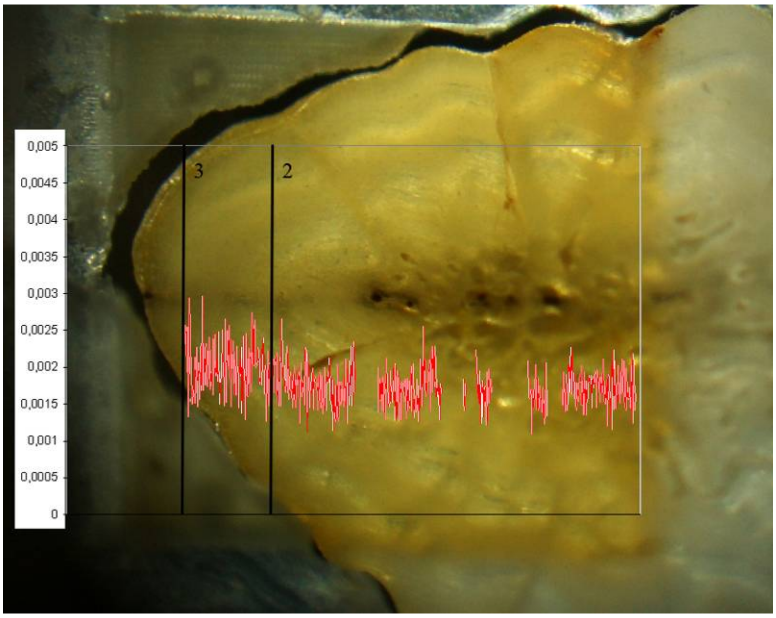


Fig. 26. Analyzed area of sample 13 (four year old Russian sturgeon juvenile male) with the values of the Sr:Ca ratio transect scan (red line) located below the scanned line (black horizontal line). *Y* axis – Sr:Ca ratio; vertical black lines mark the end of the annuli corresponding to the age presented beside each line.

Sample 13, that 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 it has 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 (1.72×10^{-3} and 2.00×10^{-3}, respectively), 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 the Table 8.

Table 8. Results of statistical comparison of the average Sr:Ca ratios within different zones in the line transects, using the Mann Whitney U Test. The Table also includes the results of the age assessment for each individual. NS – difference not statistically significant ($p>0.05$).

Sample No.	Age	Zones compared*	Average Sr:Ca ratios		Mann Whitney U Test (U value)* *	Sample size***
			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	28	Age 1-2/3-10	10.71×10^{-3}	9.83×10^{-3}	7 408	144/187
9	19	Age 2/3-11	6.34×10^{-3}	7.53×10^{-3}	1 649	79/140
10	20	Age 1-5/6-20	8.80×10^{-3}	8.58×10^{-3}	NS	219/137
13	4	Age 1-2 /3	1.72×10^{-3}	2.00×10^{-3}	17 362	469/148

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

** $p<0.001$ in all comparisons (except for sample 10).

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

After the initial increase in the Sr:Ca ratio, that probably signifies the migration of juveniles from the river to the sea, further changes in most samples were more irregular. The drop of the Sr:Ca ratio in samples 1-3 & 9 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 (see Appendix I). In sample 1, the decrease in Sr:Ca ratio however occurred somewhat later, approximately after the age 18. The beginning of the decline in ratio in sample 9 is in the annulus that corresponds to the age 11, and the minimum value is at the annulus corresponding to the age 13. These values are in accordance with the values provided by most authors for the age at maturity of Beluga males (see Appendix I). 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 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.

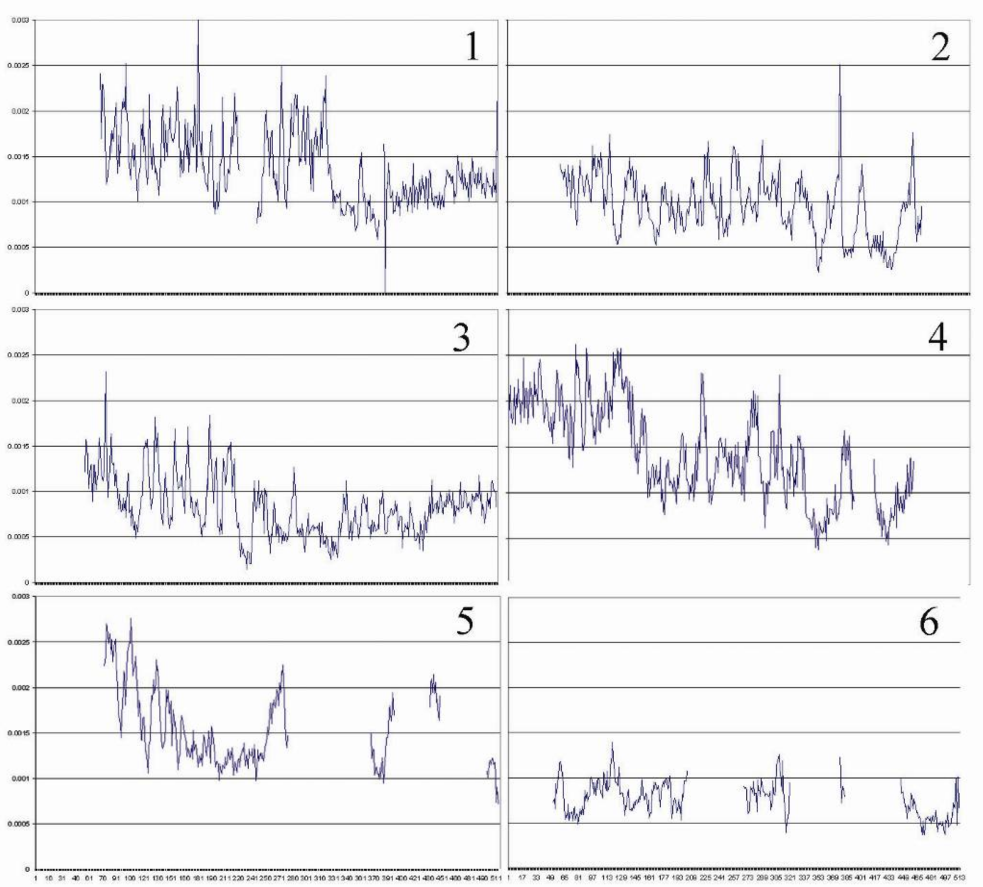


Fig. 27. Results of the Zn:Ca ratio transect scans of the sturgeon pectoral fin ray sections, samples 1-6. Sample numbers are presented in the upper right corner of each figure.

Transect scans of the zinc to calcium ratio can potentially be 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). In the 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. The Zn:Ca ratios in the transects scans had also, in general, more frequent oscillations than Sr:Ca ratios (Figs. 27 & 28). However, they were highly irregular, without a clear annual pattern, which made it difficult to associate their pattern to the transitions between different annuli.

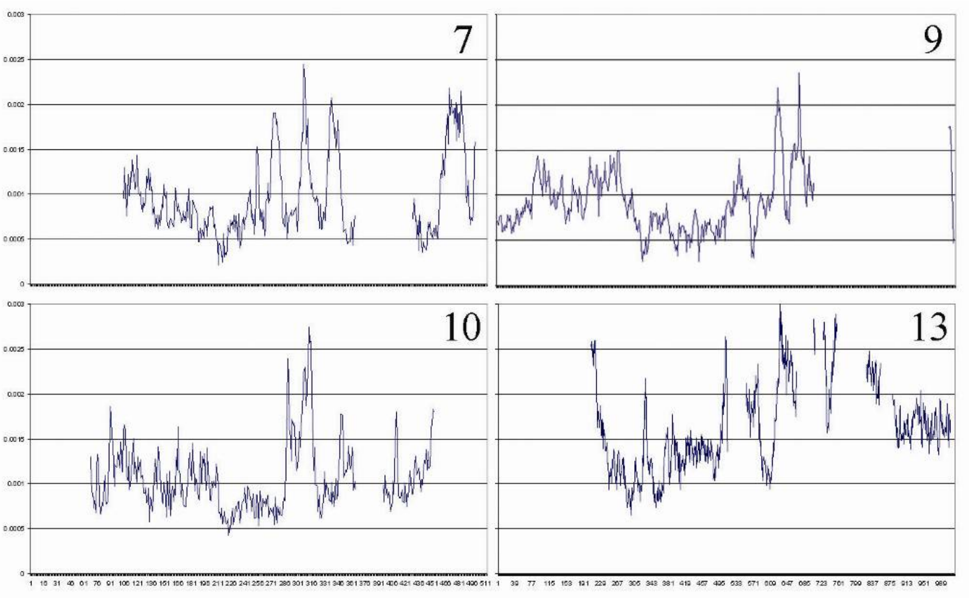


Fig. 28. Results of the Zn:Ca ratio transect scans of the four analyzed sturgeon pectoral fin ray sections, samples 7, 9, 10 and 13. Sample numbers are presented in the upper right corner of each figure.

Population viability analysis in a Vortex simulation model

Basic scenarios with the same total population size applied to all species have shown, as was expected, a high sensitivity of the model to the population size. In general, populations had a significant probability ($P<0.05$) of going extinct at the population size of 50 individuals, and the probability was close to, or even at the absolute value ($P=1.00$) for almost all species, and across the all three assessed population growth rates. In general, it was only at the population sizes above 1 000 individuals that long term viability became more likely. However, comparisons among the six studied species have shown that they substantially differed in the population viability and the other output model variables. There was an apparent pattern that the species which had the lowest proportion of the adults in a population, such as the Ship sturgeon and the Atlantic sturgeon (see Fig. 10), were also much more vulnerable than the other species at the same population size. An exception to this rule was the Sterlet, which at the same time belonged to the more vulnerable species and to those with the highest proportion of adults in a population.

In order to check the effect of the absolute number of adults in a population on the risk of extinction, basic scenarios of each species were compared again, but this time with the same number of adult females kept in each population. In such setup of the basic scenario models, the Atlantic sturgeon and the Ship sturgeon have produced results more similar to the other species, than it was in the model setup with the same total number of individuals. In general, there was a greater similarity among species, in both simulation results and their vulnerability. These results are indicating the importance of the adult individuals in the population, and the lesser usefulness of the total population size as the predictor of the population viability. However, the Sterlet has remained vulnerable in such model setup as well, which might be explained by the ecology of this species, which is very different from all other Danube sturgeons (e.g. lower offspring number, frequent spawning, and short time to maturity).

Both methods that have been applied to the assessment of the results of the sensitivity analysis, the regression analysis and the ranking technique, had a high concordance in their results. Results of both approaches are presented in the Appendix III. According to the results of the sensitivity analysis, the most influential life history parameters on the overall model behaviour were the age at which females reach the maturity, mean number of offspring, natural mortality of the 0-1 and 1-4 age groups, sex ratio, spawning frequency of females and the natural mortality of adults. The influence of the age at which females reach maturity on population persistence was higher for early maturing species than for those with delayed maturation (i.e. this parameter was the most influential for Sterlet and the least influential for Beluga).

Maximum age of reproduction and the maximum number of offspring had a low or no influence at all on the model behaviour, probably because the values that have been applied to these parameters were too high to interfere with, respectively, the longevity defined by the distribution of the age specific natural mortality and the variation in the average number of offspring. Even a substantial decrease in the maximum reproductive age was able to produce only a moderate impact on the population viability. Male-related traits, such as the age at which males reach maturity or the spawning frequency of males, had also a weak effect on the population viability.

Environmental variation in life history traits had only moderate to low influence on the model behaviour, and the environmental variation in natural mortality was the only one that had somewhat larger influence on the model. Carrying capacity and its environmental variation had significant influence on the population viability, and the impact of the environmental variation in carrying capacity was especially pronounced at the higher population growth rates (0.10 and 0.15).

Both population growth rates (deterministic and stochastic) that were assessed as model output variables were generally influenced by the same model parameters as the probability of pseudo-extinction. Since the male-related traits and environmental variation-related parameters are not included in the estimation of the deterministic population growth rate, these parameters had no influence on this output variable. The same ratio of change in the mean number of offspring, natural mortality of the 0 to 1 age group and in the sex ratio produced the same amount of change in the deterministic population growth rate, but the influence of the sex ratio on the stochastic population growth rate was always slightly weaker than the influence of the other two parameters.

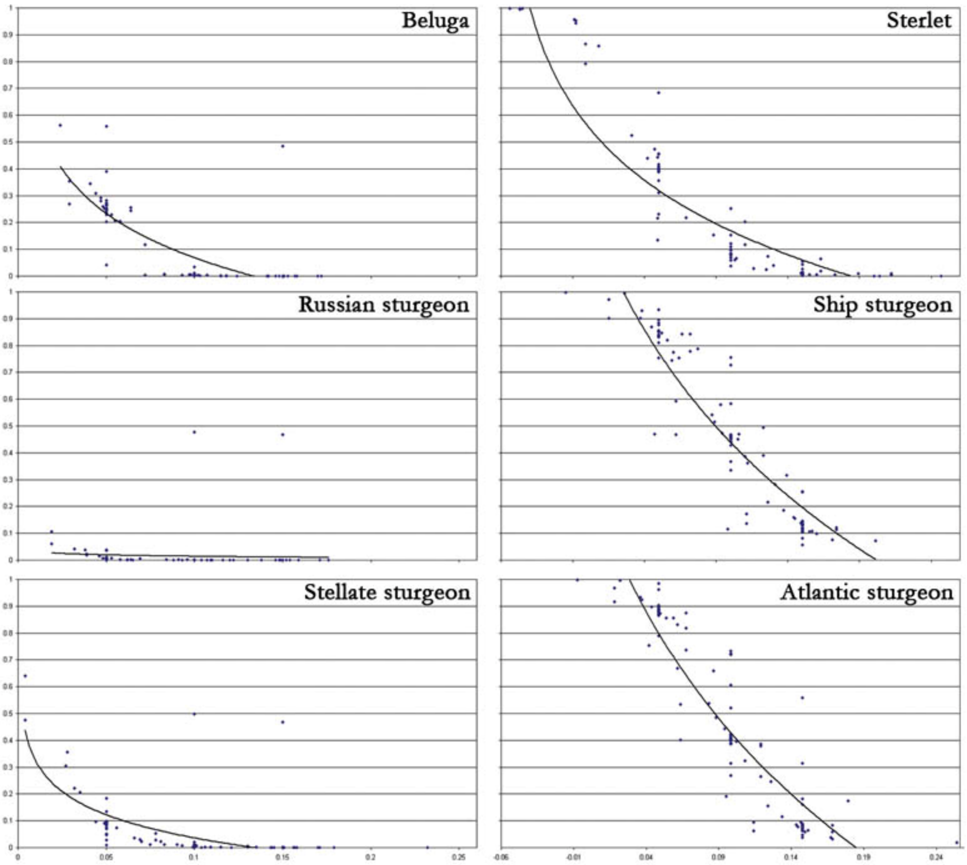


Fig. 29. Relationship between the deterministic population growth rate (*X* axis) and the probability of pseudo-extinction (*Y* axis) in the sensitivity analysis performed on the six Danube sturgeon species. Initial population size in all scenarios was set to 50 and the carrying capacity to 2 000 individuals.

The relationships between the deterministic population growth rate and the probability of pseudo-extinction for each of the studied species are presented in Fig. 29, and the relationships between the deterministic population growth rate and the expected heterozygosity in Fig. 30. As can be seen in both figures, the *r* value had a high degree of correlation with the probability of pseudo-extinction and the expected heterozygosity, but the exact relationship differed among species. Spearman's non-parametric correlation test confirmed high correlation between the *r* value and the both pseudo-extinction probability and expected heterozygosity (Table 9).

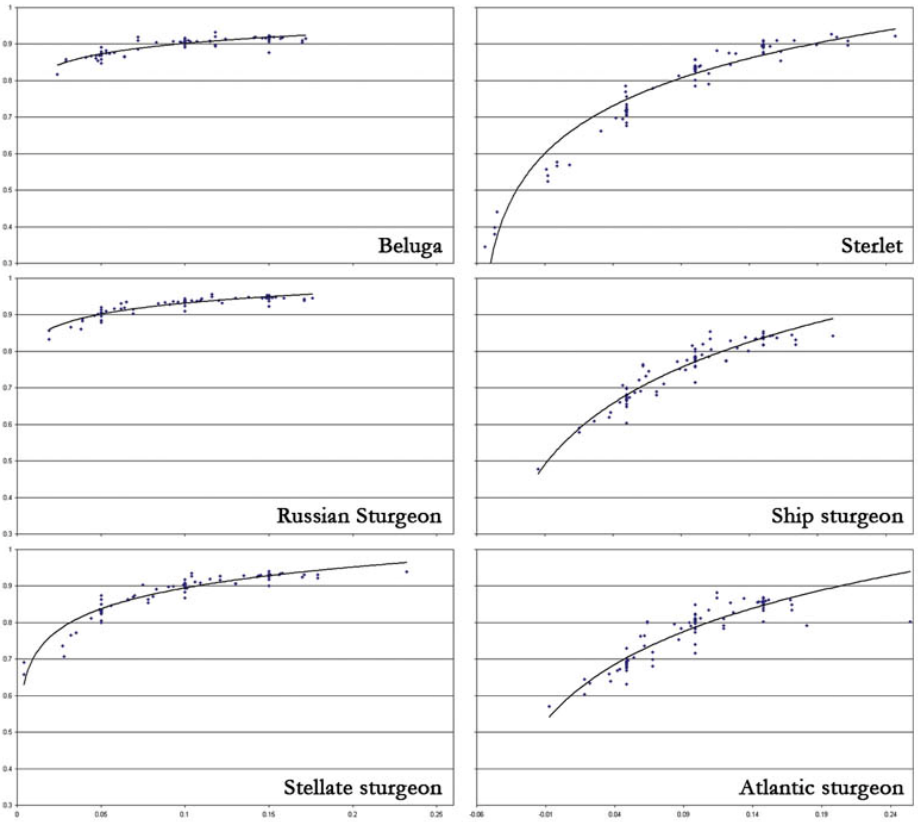
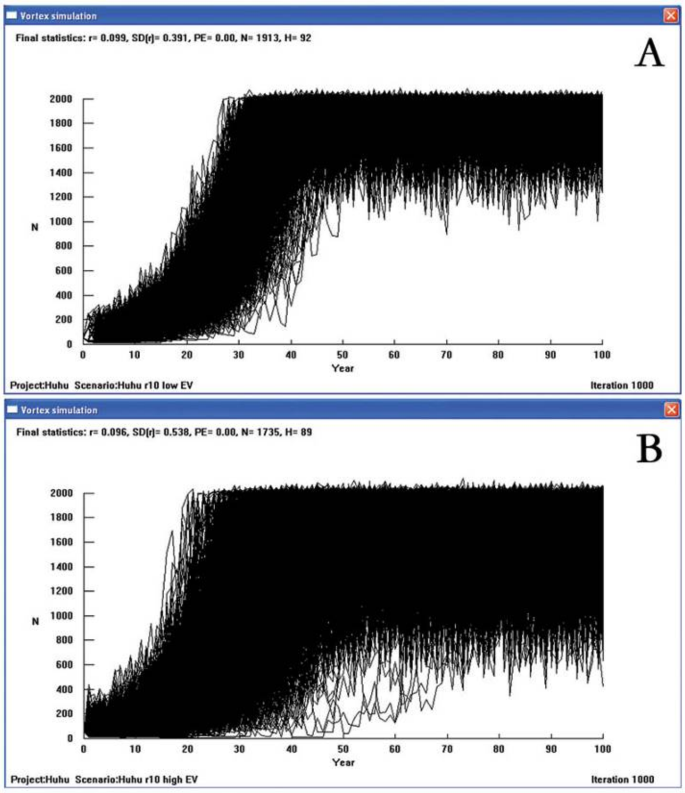


Fig. 30. Relationship between the deterministic population growth rate (*X* axis) and the expected heterozygosity (*Y* axis) in the sensitivity analysis performed on the six Danube sturgeon species. Initial population size in all scenarios was set to 50 and the carrying capacity to 2 000 individuals.

Table 9. Correlations between the deterministic growth rate, pseudo-extinction probability and heterozygosity level in the sensitivity analysis of six studied sturgeon species (Spearman's non-parametric correlation test). *r / P* – correlation between population growth rate and the pseudo-extinction probability; *r / H* – correlation between population growth rate and the level of expected heterozygosity.

	r / P		*r / H*	
	Correlation coefficient	Significance	Correlation coefficient	Significance
Beluga	-0.888	0.000	0.844	0.000
Russian sturgeon	-0.760	0.000	0.893	0.000
Stellate sturgeon	-0.891	0.000	0.937	0.000
Sterlet	-0.949	0.000	0.952	0.000
Ship sturgeon	-0.939	0.000	0.940	0.000
Atlantic sturgeon	-0.936	0.000	0.881	0.000

It is important to emphasize that many figures presented in this chapter contain only the average values among all iterations that have survived (1 000 iterations were applied in all simulations), while the individual iterations were always experiencing large variations in population size and were never able to reach such stable behaviour as the average values (e.g. as it is presented in the Fig. 33). Results shown as average values of all simulated iterations are always associated with a large stochasticity expressed in each of the single iterations. Fig. 31 shows two simulations with identical input data except in the environmental variation included in the model. As can be seen in the Figure, the actual development of population dynamics can follow many different patterns, but variations are proportional to the total amount of stochasticity included in the model. Average values can present only the most probable general direction of change, and the actual result in a particular case may diverge from this central value.



```
Final statistics: r= 0.099, SD(r)= 0.391, PE= 0.00, N= 1913, H= 92
Project:Huhu  Scenario:Huhu r10 low EV                    Iteration 1000
```

```
Final statistics: r= 0.096, SD(r)= 0.538, PE= 0.00, N= 1735, H= 89
Project:Huhu  Scenario:Huhu r10 high EV                   Iteration 1000
```

Fig. 31. Two scenarios with identical input data, except the larger amount of environmental variation that was applied in the scenario B. Images represent simulation screens from the Vortex program, with 1 000 iterations simulated in each scenario and population growth rate *r*=0.10; A) All environmental variation in the model (female spawning frequency, mean fecundity and natural mortality) reduced to zero; B) environmental variation in all three variables increased by 50 percent over those applied in basic scenarios.

Harvest scenarios showed high sensitivity of Danube sturgeon species to fishing pressure. Maximum harvest levels at which population remained viable were dependent on the population growth rate, and higher growth rates allowed higher harvest to be performed without causing the drop in population viability. Maximum harvest levels with preserved viability for each species and each population growth rate are presented in the Fig. 32.

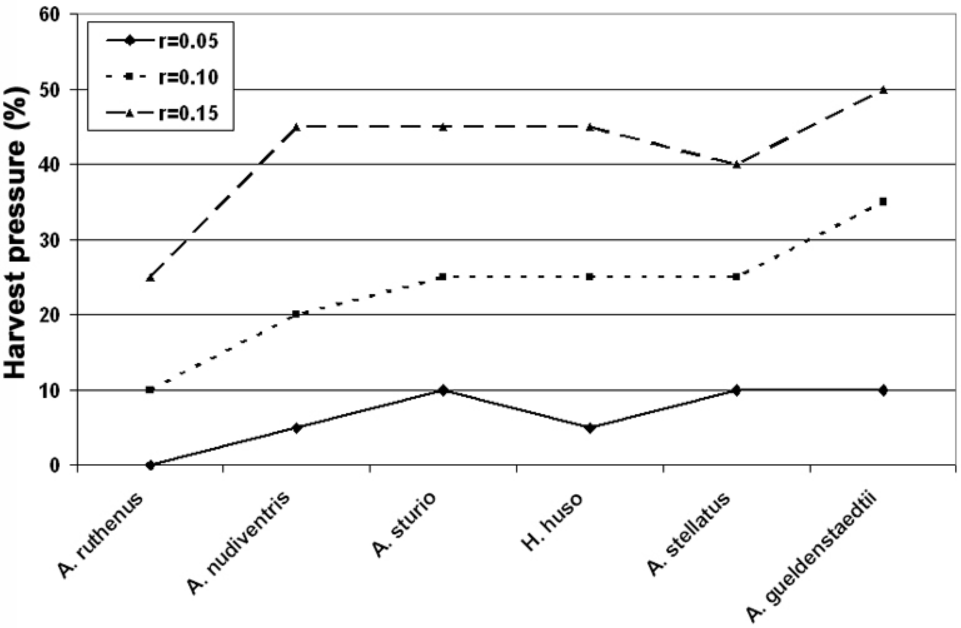


Fig. 32. Maximum harvest pressures at which the populations of six studied Danube sturgeon species remained viable (pseudo-extinction probability $P<0.05$), given three different population growth rates ($r=0.05$, 0.10 and 0.15).

At the harvest levels above those presented in the Fig. 32, populations were experiencing significant decline in population size (Fig. 33), and the extinction risk increased dramatically. A series of scenarios with different initial population sizes and carrying capacity levels was conducted, to check the effect of the carrying capacity and population size on these established thresholds of the maximum sustainable harvest levels. The results revealed that the maximum sustainable harvest level was not sensitive to the carrying capacity or the initial population size, probably since it was expressed in the percent of the adult population that is being taken, so the harvest pressure was always relative to the population size. At the unsustainable levels of harvest, population size was always reduced to half of its initial size in no more than 50 years of the simulation, whatever the size of the initial population. Populations always declined to a low population size, where the harvest pressure also became low (since it is set to be relative to the population size), and then remained at those low population numbers, but with a high risk of extinction (i.e. with a high number of iterations going extinct).

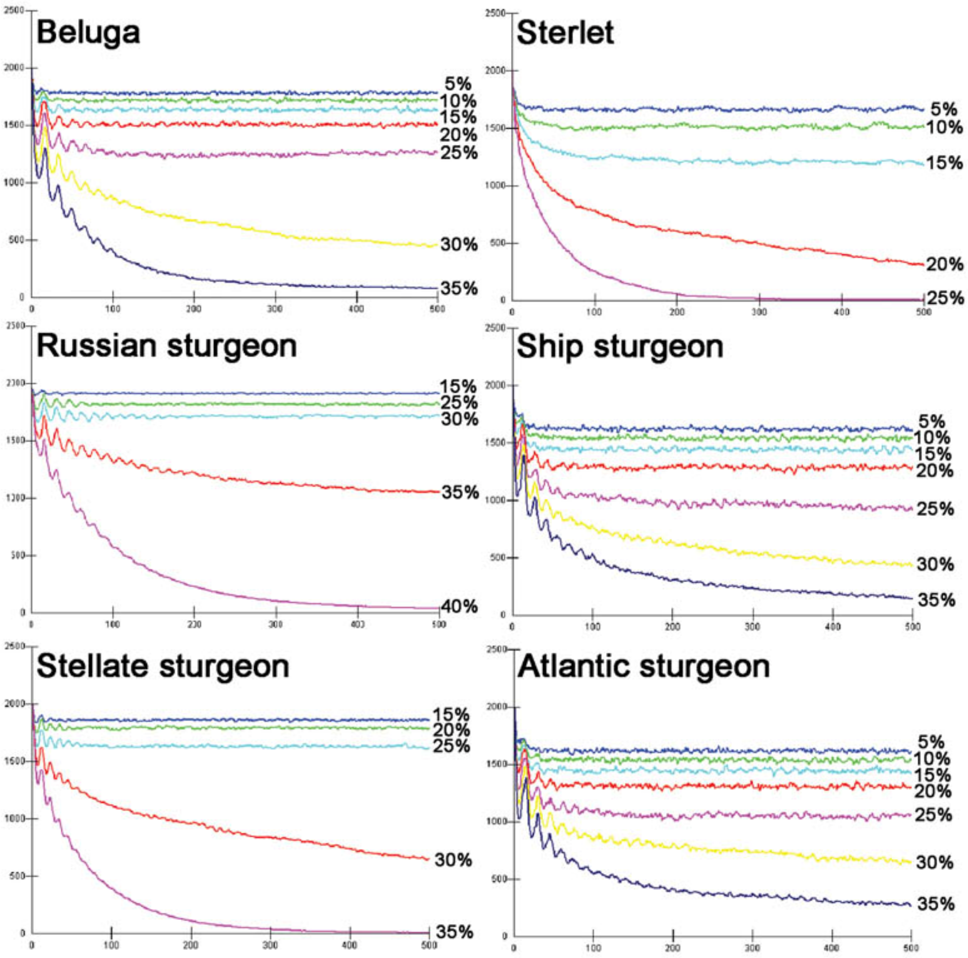


Fig. 33. Result of different harvest pressures applied on the six studied Danube sturgeon species (population growth rate r=0.10). *Y* axis – average population size among all iterations in a simulation; *X* axis – years of the simulation; percentage values represent different harvest pressures. Initial population size and carrying capacity in all scenarios were set to 2 000 individuals.

In some cases, the maximum harvest pressure at which population viability was still preserved was not the level that was at the same time ensuring the maximum total number of harvested individuals. In such cases, depending on the species, the maximum total number of harvested individuals was reached at the harvest level that was either 5 percentage units above or below the maximum viable harvest level. In other words, the maximum amount of harvested adults was reached for some species at the harvest pressure that was already above the acceptable risk of extinction (*P*>0.05, Ship sturgeon at population growth rates *r*=0.05 and 0.10, Sterlet at *r*=0.05 and 0.10, and Atlantic sturgeon at *r*=0.05), while for some species this occurred at the harvest pressure that was below the maximum viable one (Russian and Stellate sturgeon at *r*=0.10). Furthermore, in some cases the harvest level where the maximum number of harvested individuals was reached was not the same for both males and females, and in such cases the maximum number of males was harvested at 5 percent lower harvest pressure than for the females.

An unusual pattern that was noticed in some harvest scenarios was the appearance of regular oscillations of the average population size. This pattern was both appearing and then increasing in its amplitude with the increase of the harvest pressure, and then, after a certain period of years, the population stabilized at a new population size (Figs. 33 & 34). Oscillations were pronounced at high population growth rates (*r*=0.15), but almost non-existent at low growth rates (*r*=0.05), which is probably related to the shorter time needed for populations to recover at high growth rates. The frequency of the oscillations was directly related to the time that females of the species in question needed to reach maturity and to the spawning frequency – the oscillation frequency was lowest in the Beluga, and the highest but hard to distinguish in the Sterlet.

When the harvest of subadults was added to the harvest of adults, maximum viable harvest levels were dramatically reduced (Fig. 34). Even though the subadults comprised less than 50 percent of the number of adults in a population, the viability of populations when both subadults and adults were harvested was reduced two-fold, so the effect of inclusion of subadults in the harvested part of the population on the sustainability of harvesting was manifested as more than simply additive.

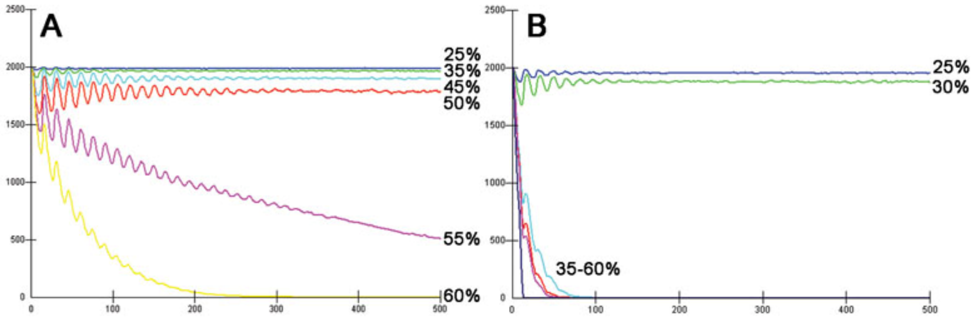


Fig. 34. Result of different harvest pressures applied on the Russian sturgeon (population growth rate *r*=0.15). A - harvest of adults only; B – harvest of both adults and the two oldest subadult age groups; *Y* axis – average population size among all iterations in a simulation; *X* axis – years of the simulation; percentage values represent different harvest pressures. Initial population size and carrying capacity in all scenarios were set to 2 000 individuals.

In stocking scenarios, the largest differences were found between scenarios with releases of juvenile and adult individuals. In general, even the release of a small number of adults (in most cases even ten individuals) was sufficient to move the population out of the high risk of extinction. On the contrary, a high number of juveniles (even above 1 000) was often necessary to ensure population survival. Effects of these two stocking strategies seem to be inversely related. For example, the highest positive effect of stocking with juveniles was manifested in the Sterlet population, while the same species also had the weakest effect of the stocking with adults. The cause probably lies in the life history of each species, mainly in relative juvenile and adult mortality, but also in the age at which females reach their maturity. The Sterlet has higher adult mortality than the other sturgeon species, which probably reduces the positive effects of the stocking with adults. On the other hand, a short time to maturity enables the larger portion of stocked Sterlet juveniles to survive to maturity and consequently increase the population size through reproduction. Due to the same reason, stocking with juveniles had smaller effect in species that need longer time to mature, since the juveniles of those species have thus less chance to survive to maturity. Examples of the effect of the stocking with the adults and juveniles are, respectively, presented in Fig. 35.

As can be seen in the Fig. 35, stocking with juveniles always produces a lag in the increase of population, when compared with the immediate effect of stocking with adult individuals. The initial population increase, caused by the release of individuals in the population, is quickly followed by a significant decline, due to the high mortality of juveniles, and the actual population recovery occurs only after a lag of a certain number of years, that is equal to the time needed for females to reach maturity.

Observed differences between scenarios with different temporal dynamics of releases were smaller than expected (Fig. 36). In general, a release of all individuals in the first year of the simulation produced a shorter time for the population to recover, in comparison to scenarios where the same total number of individuals was released during a number of years. On the other hand, multiple release events reduced the risk of extinction, in comparison to single release scenarios. However, both outcomes should probably be expected, since the earlier release of an individual means that it will have more time to reproduce and thus help the population recovery, while the annual release of individuals during the period of ten years would mean that the population cannot go extinct during that period, since new individuals are arriving in the population every year.

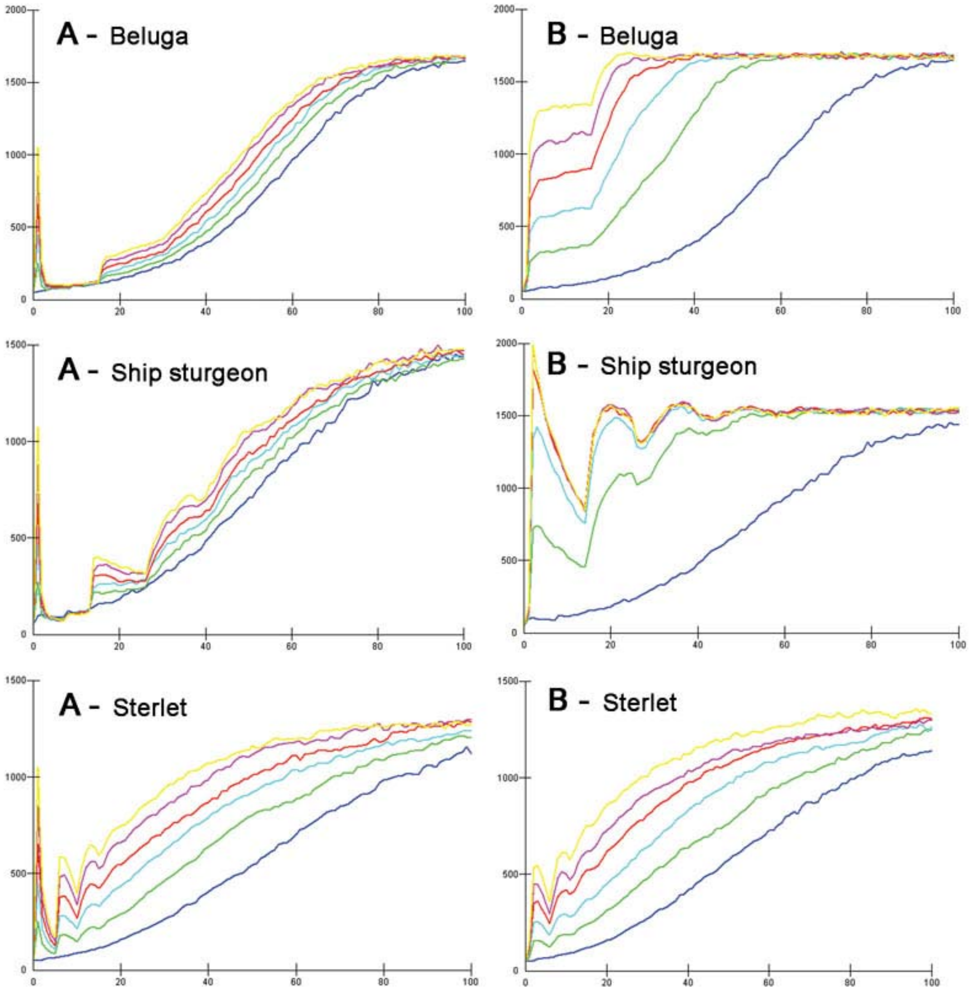


Fig. 35. Recovery of a small population of Beluga, Ship sturgeon and Sterlet with different number of juvenile (A) or adult (B) individuals released in the first year of the simulation (population growth rate *r*=0.05). From the lowest to the highest one, respectively, full lines in each graph represent: A) the basic scenario and the release of 200, 400, 600, 800 and 1 000 juvenile individuals; B) the basic scenario and the release of 20, 40, 60, 80 and 100 adult individuals; *Y* axis – average population size among iterations with surviving populations in a simulation; *X* axis – years of the simulation. Initial population size in all scenarios was set to 50 and the carrying capacity to 2 000 individuals.

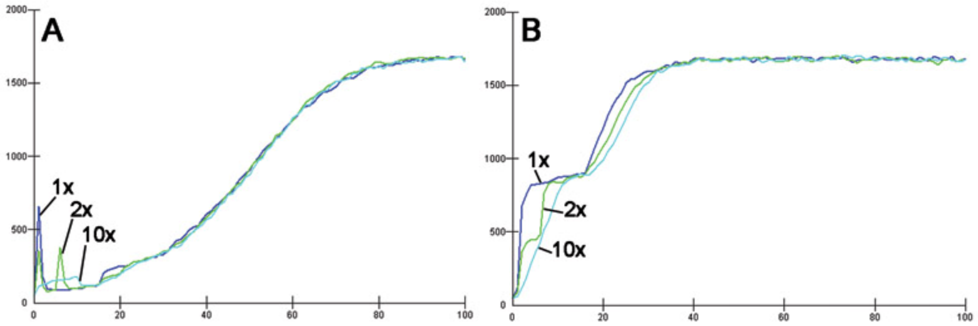


Fig. 36. Recovery of a small population of Beluga, with the same number of juvenile (A) or adult (B) individuals released in three different patterns (population growth rate r=0.05). 1x - all individuals released at once in the first year of the simulation, 2x – equal portions of individuals released two times, in the first and sixth year of the simulation, 3x – equal portions of individuals released each year during the initial ten years of the simulation; Y axis – average population size among iterations with surviving populations in a simulation; X axis – years of the simulation. Initial population size in all scenarios was set to 50 and the carrying capacity to 2 000 individuals.

Discussion

The population viability analysis that was performed in the present study has shown in practice that the individual-based models, such as the Vortex simulation program, can be applied to species with life histories similar to those of the six studied Danube sturgeon species. It is important to emphasize that Vortex is a computer package developed primarily for the simulation of small populations of mammals, reptiles and birds (Miller & Lacy 2005), and that fish species have been so far rarely modelled in this program. Species with large populations and a life history similar to those of sturgeons (e.g. large fecundity) are not ideal objects of IBMs, since it is demanding for a computer to simulate such a large number of individuals. The limitations of the model do not allow simulation of the actual population sizes of sturgeons (measured in hundreds of thousands of individuals), so only theoretical populations could be simulated (e.g. 2 000 individuals).

On the other hand, there are also several advantages in using a model such as Vortex for the species in question. Some aspects of population dynamics, such as stochasticity, and the inclusion of genetic considerations have rarely been addressed in PVAs of sturgeon species so far, and Vortex represents a tool with the ability to deal with these issues and thus provide new insights in sturgeon ecology. Furthermore, while the real population sizes could not be included in the model, absolute prediction was never a goal of this study, so the model was still expected to be capable to provide meaningful comparisons of relative differences among scenarios. The only situation where this study could provide certain power in the absolute projections is the case if sturgeons would drop to such small population sizes, in other words when the simulated population size would reflect the actual one, which may be the case for the Atlantic and Ship sturgeon. Finally, this study could be a valuable case study for improvement of our knowledge and understanding of Vortex as a PVA model, since this is probably one of the rare cases when this widely used model has been applied to species with such a specific ecology and life history.

There are also some other PVA software packages, such as RAMAS, that might also be successfully applied to sturgeons. As it was stated by Brook *et al.* (1997), RAMAS software can handle very large population sizes, which is an advantage when it is applied to species with high fecundities, such as fish. It is also suitable for cases when there are spatial and habitat suitability factors that should be incorporated in the model. On the other hand, RAMAS is not an IBM, but an age or stage structured model that therefore lacks genetic considerations and more complex demographic patterns. In the end, none of the existing PVA software packages can be recognized as a superior one, since each one has its advantages and drawbacks, and studies that involve comparison of efficiency of different models are common (Brook *et al.* 1997, 1999, 2000; Lindenmayer *et al.* 1995; Mills *et al.* 1996).

Most sturgeon PVAs have been based on age-structured models (Beamesderfer *et al.* 1995; Pine *et al.* 2001; Bajer & Wildhaber 2007; Heppell 2007; Kennedy & Sutton 2007). Only Henriëtte I. Jager and her co-authors have applied individual-based models in sturgeon PVAs (Jager *et al.* 2000, 2001; Jager 2001, 2005, 2006:1, 2006:2). Except for these few examples of IBM applications, sturgeon PVAs have mostly lacked considerations of demographic and environmental stochasticity and genetics. The majority of models applied to sturgeons so far have been developed specifically for the purpose, and the only software packages used were MOCPOP, an age-structured population model (Beamesderfer *et al.* 1995; Pine *et al.* 2001; Kennedy & Sutton 2007), and the Beverton-Holt equilibrium yield model within the software package Fishery Analyses and Simulation Tools (FAST) (Quist *et al.* 2002; Colombo *et al.* 2007). Software packages for IBMs, such as Vortex, have not been used previous to this study.

This study has revealed considerable sensitivity of sturgeon populations to changes in a number of life history parameters. Population viability was most strongly influenced by changes in the age at which females reach maturity, in the mean fecundity, egg to age 1 natural mortality and juvenile mortality (age groups 1-4), as well as by sex ratio, spawning frequency and adult mortality. These results are in accordance with PVAs performed on North American sturgeon species (Pine *et al.* 2001; Beamesderfer *et al.* 2007; Kennedy & Sutton 2007), that have found that the most influential parameters were egg mortality, age at maturity of females, spawning frequency and the overall natural mortality. Kennedy & Sutton (2007) estimated that the egg mortality was the single most influential population parameter, and an increase of 0.0001 percentage units in mortality rate (from 0.9998 as the average value) caused as much as a 95 percent decline in population abundance and biomass. Bajer & Wildhaber (2007) have found that the egg, juvenile and young adult mortality were the most influential parameters on the population growth rate, while the fecundity was a less sensitive parameter. This differs from the findings in the present study, where the fecundity had the same effect on population persistence as the egg to age 1 mortality and belonged to the most sensitive parameters. However, it is important to note that these two life history parameters have been combined in this study (see subchapter Model parameterization in Material and methods), so it was not really possible to make distinction between their effects.

It was determined in both the present study and by Kennedy & Sutton (2007) that the mortality of eggs and the youngest age classes were much more influential than the mortality of adult individuals. On the other hand, Pine *et al.*

(2001) have established that the population of *A. oxyrinchus desotoi* was sensitive to even small changes in the adult mortality, and Boreman (1997) and Heppell (2007) have claimed that a small reduction in mortality of the adults could balance a relatively large increase in egg to age 1 mortality. Boreman (1997) supported this claim with the explanation that the fish are exposed to a high mortality rate of age 0 to 1 during only one year of their life, while the mortality in adults encompasses many age groups. While I recognize the importance of the adult mortality on population viability, I have to disagree with such an explanation. High influence of the egg to age 1 mortality on the population persistence is caused by the fact that this age group comprises the major part of the population, so any change in their mortality will have an impact on a large number of individuals. The adult mortality indeed extends its impact on many age groups, but there are few individuals, so the total number of fish that are impacted by adult mortality will not be so large. In sturgeon populations, adults comprise only a small part of the total population. Beamesderfer *et al.* (2007) have estimated that the adults of *A. medirostris* have represented only 12 percent of the total population at equilibrium. In the present study, the proportion of adults in populations of different sturgeon species at stable age distribution varied between 2.7 and 15.1 percent, with the average value between 7.5 and 8.7 percent, depending on the population growth rate (Fig. 10).

However, the importance of adults in a population has been confirmed in this study as well. It was indicated by the behaviour of scenarios where the six studied species were first compared at the same total population size and then at the same number of adults, with the latter approach leading to a greater similarity between species in result parameters. Since there are so few adults in a population, each adult individual is important to the population persistence. Importance of adult individuals is further pronounced by their high survival rate, which enables them to reach older age and have multiple spawning events. It is generally considered that populations of long-lived organisms are sensitive to adult survivorship (Boyce 1992; Beissinger 2002). According to Colombo *et al.* (2007), harvested populations are likely to collapse due to the removal of reproductively viable adults, and age-specific maternal effects on offspring survival may emphasize the importance of maintaining large, old females in the population (Heppell 2007). However, the influence of adult mortality in the present study was found to be inversely related to the age at which individuals reach maturity, and not strongly related to the longevity of the species. In other words, species that matured early, such as the Sterlet and Stellate sturgeon, were more influenced by the adult mortality than those species that mature late, such as the Beluga, even though the Beluga has much greater longevity and thus has significantly greater number of adult age groups. The reason lies in the fact that species such as the Sterlet have larger relative number of adult age groups than species with great longevity – i.e. the proportion of age classes that belong to adults, not their absolute number, is larger.

Life history parameters related to males have weak influence on the population viability, as long as there are enough males to fertilize the eggs in each spawning event. Indeed, due to this fact, many sturgeon PVA models have been restricted only to females (Bajer & Wildhaber 2007; Kennedy & Sutton 2007).

The life history parameters that are most influential on population viability are usually proposed for the highest management or research priority (Mills & Lindberg 2002). However, as it was stated by Mills & Lindberg (2002), parameters that are influential can often have low level of actual variation, so this issue has also to be addressed with certain caution.

The present study has confirmed a high correlation between the population growth rate (deterministic and stochastic) and the population viability and rate of genetic loss. This confirms the value of the population growth rate as the overall predictor or indicator of the population state. Population growth rate has been used in many studies as one of the main simulation parameters for the assessment of the population viability (Pine *et al.* 2001; Bajer & Wildhaber 2007). However, any such use of the population growth rate should be conducted with the awareness that this parameter does not consider certain aspects, such as stochasticity, genetically linked demographic traits and the male-related traits, and that it is thus unable to register changes in these traits that might impact population viability.

What is the real value of population growth rate in the Danube populations? Surprisingly, this demographic parameter seems to be rarely touched upon or discussed in existing literature on the demography of sturgeons, and estimates of the rate were presented in few sources. Sturgeon PVAs generally produce the conclusion that slow physical growth and discontinuous spawning of sturgeons result in low intrinsic population growth rates (Heppell 2007), but the actual value of this parameter is seldom determined or presented. According to the scarce literature that is available, it can range from 0.05 (Bruch 2008) to 0.10 (Secor & Niklitschek 2002), or vary between 0.05 and 0.15 (Balnath *et al.* 2008). However, the present study has confirmed that the model behaviour and its sensitivity to changes in vital rates were particularly dependent on the population growth rate. The importance of this parameter was revealed by both the comparison of the scenarios with the three different population growth rates employed (0.05, 0.10 and 0.15), and by a strong correlation of the population growth rate with the population viability and the amount of genetic loss. As it was shown in harvest scenarios, maximum harvest pressure (expressed as relative to population size) was dependent on population growth rate and not on the initial population size. The apparent lack of understanding of the real population growth rate in the six studied species may significantly hinder development of the effective management in the future, so the assessment of the intrinsic population growth rate of the Danube sturgeon populations should represent one of the highest research priorities. However, it is important to emphasize that, while the population growth rate is indeed an important population parameter, there are other parameters as well that might be used instead as good indicators of population state, which are at the same time more simple to measure, such as the population age structure.

Comparisons of the PVA results for the six studied sturgeon species have revealed that the Sterlet population model was showing large differences compared to the other five species. This was shown through the different relative sensitivity to the life history parameters, lower general viability of the population in both types of basic scenarios (that were comparing species through the same population size and the same number of adult females), and by the extreme sensitivity to harvesting at low population growth rates (r=0.05). Such model behaviour seems to be counterintuitive when compared to the actual situation in the field where, despite the high harvest pressure, this species has remained the least affected one – this is the only sturgeon species in the Danube River that is still an object of the commercial fishery. According to the IUCN Red List of Threatened Species (IUCN 2008), the Sterlet is globally categorized as a Vulnerable species, while the Stellate, Russian, Ship sturgeon and Beluga are categorized as Endangered and the Atlantic sturgeon as a Critically Endangered species. Based on these facts, it seems likely that the Sterlet actually has a higher intrinsic population growth rate than the other five Danube sturgeon species. At higher population growth rates the model of this species behaves like the other five species at lower growth rates (e.g. if the population growth rate r=0.10 is applied to the Sterlet and r=0.05 to the other five species). This difference can also be noticed at the maximum viable harvest levels in Fig. 32. Furthermore, while most sturgeon species resemble to a certain extent the K-selected type of species (Pianka 1970) - great longevity, large body size, delayed reproduction, infrequent spawning (the exception to this is their high fecundity), the Sterlet is less evolved in this direction than the other sturgeons and more towards the r-selected type of species – significantly shorter longevity, earlier maturation, frequent spawning and smaller body size. One of the common distinctions between those two types of selection is the larger population growth rate of the r-selected species. However, the question of the actual intrinsic population growth rate of the Sterlet and other Danube sturgeons will be only resolved through future research efforts.

The present study has confirmed that the natural mortality rates of Danube sturgeon species, beside their intrinsic population growth rate, should probably represent the single most important object of any future study on Danube sturgeon populations. While the data on the actual natural mortality rates of sturgeons is almost non-existent, it is even more so regarding the Danube populations. Although it is generally acknowledged that it is probably the most difficult life history parameter to determine (Boyce 1992; Beissinger & Westphal 1998), it is at the same time one of the key parameters for any PVA scenario building, so it is likely that the lack of data on the sturgeon natural mortality will represent one of the main hindering factors for future development of Danube sturgeon PVAs.

Another issue that has been poorly addressed within the present literature on sturgeon ecology is the existence of senescence in sturgeons, the maximum reproductive age that occurs before the maximum longevity has been reached. In most of the studies performed so far, as well as in review studies dealing with the sturgeon ecology, this issue has not been raised or touched upon, not even regarding the North American sturgeon species that are generally considered as well studied. The only available reference that confirms existence of senescence in sturgeons is that of Lagutov & Lagutov (2008). A study by Williot *et al.* (2005) registered senescence in aquaculture populations, and it was hypothesized by Patrick Williot (Williot pers. comm.) that it should be also present in the nature. On the other hand, most of the interviewed sturgeon experts were either uncertain whether senescence exists in sturgeons, or they were even certain that sturgeons can spawn throughout their whole life. Sulak & Randall (2002) have argued against the widely accepted paradigm in sturgeon ecology that sturgeons can commonly attain those reported large body sizes and long age, and claimed that such large individuals are rare and their contribution to the population only minor. The sensitivity analysis has indicated that the existence of senescence would not have large influence on population viability, except if the maximum reproductive age is reached a long time before their maximum reported longevity. This issue, however, needs to be studied further.

All models that have been developed within the present study have dealt with a single small population of each species. This could correspond to an isolated subpopulation in a fragmented ecosystem, or a depleted population within a large distribution area. For some of the species this may be a realistic scenario at present, for other species the models are used to assess minimum viable population sizes, although the populations today are larger. The scenarios employed do not capture the case of several subpopulations partly linked through gene flow between them, but such scenarios should be an important object of further studies.

All studied sturgeon species have shown a relatively high susceptibility to harvest pressure. While it differed among different species and among different population growth rates applied (Fig. 32), the general pattern was that, at population growth rate of *r*=0.05, the maximum viable harvest pressure was within the range of 0.05-0.10 of the adult population size, at *r*=0.10 it ranged from 0.20 to 0.30, and at *r*=0.15 from 0.40 to 0.50. As already discussed, the only exception was the Sterlet, whose maximum harvest pressure was always more similar to that of other species at the lower population growth rate (e.g. the threshold harvest pressure at *r*=0.10 was similar to the harvest threshold of other species at *r*=0.05). These threshold levels fall within those determined by other PVAs, performed mostly on North American sturgeon species – reported threshold values were below 0.10 (Quist *et al.* 2002), 0.05-0.15 (Beamesderfer *et al.* 1995), 0.15-0.20 (Colombo *et al.* 2007), 0.35 (Kennedy & Sutton 2007). Although these PVAs have not presented the growth rates of simulated populations, comparison of these threshold values with those obtained in the present study shows that they are mostly similar to the threshold ranges obtained at *r*=0.05 and 0.10, which are also the more realistic population growth rates for sturgeons (as was previously discussed). It is important to note, however, that the harvest threshold levels that are expressed in such relative terms (i.e. in percentage of adults taken from the population) are difficult to be implemented in practice, since they would require a good knowledge of the actual population size, thus demanding constant efforts of making population censuses, that would be certainly both time and money consuming.

In harvest assessment scenarios developed by Kennedy & Sutton (2007), the model was not significantly influenced by the initial population size, and the harvest pressures, expressed as ratios, had the same influence on different sizes of population. The model in the present study had the same behaviour, and whatever initial population size was applied, the harvest threshold levels remained the same. At unsustainable harvest rates, populations in general needed less than 50 years to be reduced to the half of their initial size. If compared with results of harvest scenarios obtained in the present study, the actual rate of decline of the total sturgeon catch in Romania (Fig. 5) would correspond to harvest pressures that are far above the threshold levels (Fig. 33).

Cases of harvest pressure that ensures maximum total yield being above the maximum viable harvest pressure (within the simulated period of 500 years), that were observed in the present study, represent additional problem in reaching the management goal of maximum sustained yield. Since the exploitation is always directed at levels that maximize yield on short term, in such cases they might tend to pass over the viable harvest thresholds, and reach a level with an unacceptable risk of extinction (*P*>0.05). According to Ludwig & Walters (2002), maximum sustained yields are an inefficient and rarely achieved goal, which belongs more to the convenient political fiction than to the actual science. Botsford *et al.* (1997) have stated that the two main reasons for the failure of the approach of maximum sustained yield are the lack of political will to implement it and the imprecise predictions of harvest levels, due to an intrinsic uncertainty of the system. Kennedy & Sutton (2007) have proposed that the optimum harvest levels should always be 0.10 below the value that represents the maximum viable harvest level, in order to allow natural variation in life history parameters such as the age at maturity. Such an approach would reduce the potential risk that harvest becomes a selection pressure towards the maturation at younger age and smaller body size (Kuparinen *et al.* 2008).

Fishing pressure that is at the same time directed at both adult and subadult age groups was shown to significantly reduce population viability, as well as the threshold of maximum viable harvest. The large impact of subadult harvest most likely originates from the fact that the subadult individuals that are taken from the population have thus failed to have at least one successful reproduction and in that way to compensate for the individuals that have been harvested. Currently, the Sterlet population in Serbia is still considered to be under significant pressure of commercial fishery that is, due to illegal fishing practices, also comprised of subadults (Lenhardt *et al.* 2006:1), which is likely to further reduce the population viability.

While this study has not tested the efficiency of such fishing regulations as the length or slot limits, the formulation of such management strategies has been one of the main objectives of many sturgeon PVAs (Quist *et al.* 2002; Bajer & Wildhaber 2007; Kennedy & Sutton 2007; Colombo *et al.* 2007; Heppell 2007). Length limits represent the prohibition of catching the fish that is below certain length, while the slot limits represent introduction of both minimum and maximum allowed length for fishery. Kennedy & Sutton (2007) have determined the exact value of minimum length limits for fisheries that would offer a balance between the optimum levels of protection and harvest in studied population. The minimum length limit should be at least several cm longer than the length at which individuals reach maturity, in order to enable at least one or two spawning events prior to harvest. The conservation policy for some sturgeon species has introduced slot limits instead of just minimum length limits (Heppell 2007). Slot limits can provide greater exploitation rates than strictly yield based fishery that is focused on adults, and overexploitation can occur only if slot limits are too wide, or if the exploitation rate within the slot limit is too great (Beamesderfer *et al.* 2007). On the other hand, a model by Kennedy & Sutton (2007) has indicated that the upper bound of the slot limit for studied species was unnecessary, because it did not result in significant increase of population viability. Use of the minimum length limits represents one of the key management strategies in the Danube sturgeon fisheries

(Lehardt *et al.* 2005), so future PVAs of the Danube sturgeons should also tackle such management scenarios.

Stocking scenarios have shown significantly higher relative effect of stocking with adults, when compared to juveniles. This issue was the object of many debates in the field of conservation ichthyology, and there are many pros and cons for both approaches. The majority of sturgeons used for stocking worldwide are fingerlings (Chebanov & Billard 2001; Shahifar 2006). It is important to note that the juveniles released in stocking scenarios in the present study (Fig. 35) consisted of one year old individuals, while the most commonly released individuals in real cases are often only a few weeks or one month old. Since only a small number of fingerlings would survive to age 1, each released fish of age 1 would correspond to an actual release of several hundreds of fingerlings. Therefore, if the rough relationship between the effect of one released adult and the release of one year old fish is more than 1 : 10, the relationship between stocked adults and fingerlings would be then more than 1 : 1 000 or even 1 : 10 000. However, although stocked juveniles have a low survival when released in the natural environment (Chebanov & Billard 2001), stocking with adults comprises a number of other problems, such as the domestication and other selection processes caused by aquaculture conditions, as well as substantially reduced fitness when compared with fish from natural populations (Reisenbichler & Rubin 1999). Rearing individuals up to their maturity is both time and money consuming, so it is usually not practiced, except in the commercial aquaculture for caviar production and in the case of permanent brood-stock. The only exception is probably the Sterlet, due to its early maturity, and some countries like Austria have been also releasing Sterlet adults (Williot *et al.* 2002). The pattern of lower effectiveness of juvenile stocking for late maturing species, that is caused by the lower chances of survival of stocked individuals if they need longer time to reach maturity and reproduce, indicates that this aspect of life history has to be always taken into consideration when deciding on the amount of juvenile individuals that should be released.

Another important pattern that was revealed in juvenile stocking scenarios is the delayed effect of stocking on the population recovery, proportional to the time of maturity (Fig. 35). This lag, caused by the time that released individuals need to wait before they start reproducing, has to be included in management planning. For instance, the release of large numbers of Beluga juveniles will produce effect on population dynamics and recovery only after 15 years, which is the average time that females of this species need to reach the maturity.

The stocking scenarios within the present study have not dealt with the important question of the effect of supportive stocking on the inbreeding (and outbreeding) depression (Ludwig 2006), and there is a need for wider use of genetic models which could provide information on the costs and benefits of supplementing fish populations with hatchery-reared stock (Jager *et al.* 2000). As claimed by Hedrick (2002), effective population size of fish species with a high number of offspring can be surprisingly small, since there is a large variance in contribution to the next generation, which is even more visible in supportive stocking, when few adults are used to produce a large number of stocked individuals. It is a common practice to use no more than ten sturgeon individuals of each sex to produce hundreds of thousands of offspring that will be released (Patriche pers. comm.).

So far, I have been unable to find an unambiguous explanation for the unusual pattern of regular oscillations in the average population size, which was noticed in the harvest scenarios (Figs. 33 & 34). It is apparent that it emerges with an increase in harvest pressure, and that it has a larger amplitude of oscillations at higher population growth rates (r=0.15). The oscillation frequency seems to be proportional to the time that a species needs to reach maturity. The interesting coincidence is that these oscillations strongly resemble the ones that were determined by Lenhardt *et al.* (2006:2) on catch data of Beluga and Russian sturgeon in Serbia (Fig. 37). Both populations (the simulated and the actual one) were under the high harvest pressure when the oscillations were observed. Although it was not discussed by authors, on one of the graphs with the results of the model developed by Pine *et al.* (2001), there are similar oscillations in the total population size at high harvest pressures. Furthermore, similar oscillations also appear at high harvest pressures in the model by Kennedy & Sutton (2007), in a graph presenting changes in average body length of adults. However, whether these oscillations represent the effect of unsustainable harvest or only an artefact of the model simulation can not be answered at this point.

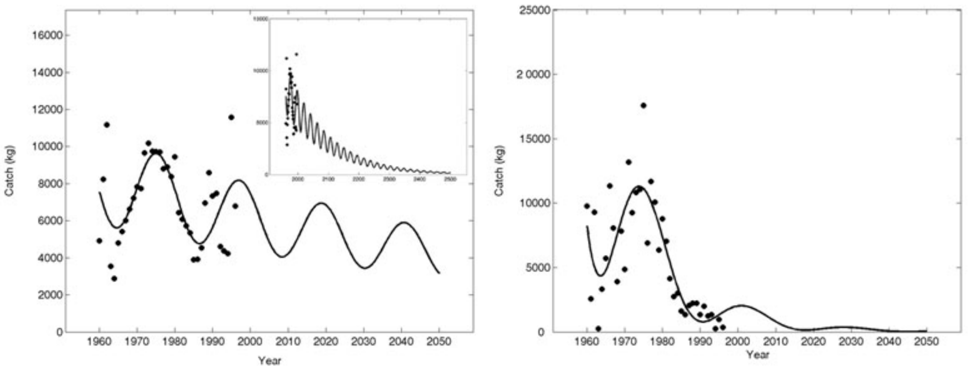


Fig. 37. Catch data (circles) for Beluga (left figure) and Russian sturgeon (right figure) in Serbia (1960-1996), fitted to a sinusoidal oscillation model (solid curves) with exponential extinction. Small plot in the left figure shows projection of the model up to the year 2500 (Lenhardt *et al.* 2006:2).

Ratios of concentrations of strontium to calcium in annuli of ten analyzed sturgeon pectoral fin rays, which were assessed by the use of NMP technique, have not shown as clear patterns as were registered by Limburg *et al.* (2001) in fish otoliths. There was however a statistically significant increase in concentrations at the end of the first or several first years of age, which should imply transition to an environment with higher water salinity. 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 8). Arai *et al.* (2002) have found changes of similar magnitude in pectoral fins of the Russian sturgeon from the Caspian Sea (average increase 2×10^{-3}, maximum increase $4\text{-}5 \times 10^{-3}$). However, observed ratios in the innermost annuli (on average 4.3×10^{-3}, range $3.4\text{-}5.5 \times 10^{-3}$) in their study were lower than those found in the present study ($6.34\text{-}7.43 \times 10^{-3}$). According to Arai *et al.* (2002), Sr:Ca ratios of 4×10^{-3} and $6\text{-}12 \times 10^{-3}$ were, respectively, indicating residence in the fresh and brackish water environment. Most of the studied sturgeons (samples 1-7 and 9) 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 have 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.

Due to its isolation from the global ocean and a large influx of freshwater from numerous rivers, the Black Sea has so 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, that was observed in this study, was caused by a small difference in salinity between the sea and freshwater. Furthermore, as can be seen in the Fig. 38, the lowest water salinity is located in the north-western part of the Black sea, where the Danube River mouth is situated. As the Figure shows, these areas of low water salinity are strongly overlapping with the sturgeon feeding areas in the sea, so this might imply that the sturgeons in the Black Sea actually never leave the low salinity areas during their life. Further studies should be made to assess the relationship between the Sr:Ca concentrations in the Danube and Black Sea environment and in sturgeon pectoral fins. As Arai *et al.* (2002) have proposed, this could be most effectively performed through studies that would involve a tagging and recapture approach.

Analysis of Sr:Ca ratio in the fin ray of the juvenile Russian sturgeon caught below the Djerdap II dam 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 & Holčik 1997), or as a specimen escaped from some aquaculture facility. The latter might be also an explanation for the significantly lower Sr:Ca ratio in the fin ray of this individual, when compared with the other studied individuals.

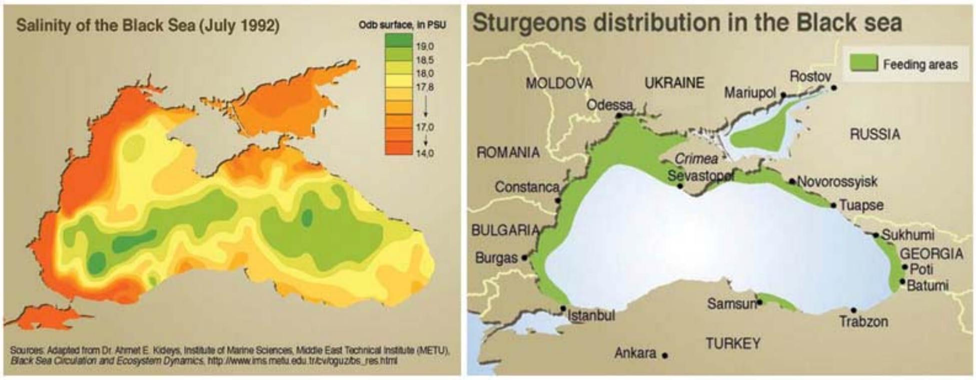


Fig. 38. Maps of the Black Sea, showing water salinity (left image) and sturgeon feeding areas (right image, green colour) (source: http://maps.grida.no).

According to the results of all methods based on sighting records that have been applied, the Atlantic sturgeon in the Danube River is extinct, with the extinction most likely having occurred in 1966-1967, and at the latest in 1970 (Fig. 39).

For the Ship sturgeon, different equations provided different probabilities for the presence of the species, with an average value of 0.392. Although none of the given values were significant, they are certainly alarming. In the absence of new observations in the years to follow, the extinction probability would reach significant levels in two or three decades; even with a few new observations this critical period would not be dramatically extended. According to the method for extinction time estimation (Solow 2005), the absence of new observations in forthcoming years would imply that the Ship sturgeon in Danube would become extinct somewhere around 2009, or at the latest in 2018.

Why is there such a dramatic difference in the rate of decline between the Atlantic sturgeon and the Ship sturgeon, and why are they both doing worse than the other four sturgeon species in the Danube River? According to historical data, the Atlantic sturgeon and Ship sturgeon have never been abundant in the Danube River, and thus never gained a status of high economic importance (Bacalbaşa-Dobrovici & Holčik 2000; Reinartz 2002). Population size per se is often considered as the major determinant of population persistence (Reed *et al.* 2003), and rare species are often much more vulnerable to extinction (Roberts & Hawkins 1999). Thus, it is possible that a smaller population size of these two species has been the cause of their lower resistance to all anthropogenic pressures and changes in habitat, making them the first to disappear from the Danube River region.

Fig. 39. A rare Atlantic sturgeon specimen from the now probably extinct Danube population, exhibited in the National Museum of Natural History in Sofia, Bulgaria (Photo: I. Jarić, December 2008).

Studies by McInerny *et al.* (2006) and Robbirt *et al.* (2006) found significant correlations between estimations based on the probabilistic models used in this study and the ranked IUCN Red list categories. As in McCarthy (1998) and Regan *et al.* (2000), they have suggested that such indices may not only be used for estimating probability of species extinction, but also to infer threat and decline in species considered to be still extant. As such, they should be included in the evaluation criteria for all IUCN categories (Robbirt *et al.* 2006).

As Diamond (1987) suggested, the burden of proof should not be placed on proving that a species is extinct; on the contrary, a species must be presumed extinct or endangered unless shown to be extant and secure. On the other hand, there have been many cases where a species has been recorded as extinct and later been rediscovered (Regan *et al.* 2000; Roberts & Kitchener 2006), and species that become increasingly rare before their final extinction may continue to exist unseen for many years (Roberts & Solow 2003). One example could be the reappearance of the Atlantic sturgeon in Estonian waters almost 30 years after the last catch (Paaver 1999). Reinartz (2002) states that a search for Ship sturgeon in the Danube River system should be one of the priorities within sturgeon conservation activities in this region. Grogan & Boreman (1998), who applied some of the methods used in this study on two sturgeon species in US, state that the final determination of extirpation status should be based on the combined use of indirect methods and directed surveys, with the addition of all available ancillary information. According to IUCN (2001), a taxon is presumed extinct when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), and throughout its historic range have failed to record an individual. Little experience exists in applying this approach to species with the life history of Acipenseriformes, thus due caution in interpreting results is necessary. This approach was therefore meant to be more scenario-building, rather than providing a final conclusion on the presence or extinction of the studied species.

One of the aims of this study was also to present an alternative method for the assessment of extinction probability, which is based on sighting records that show trends in sighting intervals. Although more advanced assessment methods, such as PVA, are certainly a more reliable approach to assess extinction threats to a species (Akçakaya & Sjögren-Gulve 2000), sighting records are in certain cases the only available data for quantitative assessment. In such cases, these models can represent the only available quantitative approach, which could provide strength and reliability to extinction assessments.

As is commonly proposed (McCarthy 1998; Robbirt *et al.* 2006), the greatest reliability in the use of these methods could be achieved if a number of complementary models are applied, since each can be sensitive to different characteristics of the collection/sighting records. Likewise, the new method presented in this paper would provide the greatest reliability if it is used together with other models, namely, those published by Solow (1993:1, 1993:2, 2005), Solow & Roberts (2003), Burgman *et al.* (1995) and McInerny *et al.* (2006). Their combination should enhance the overall ability to detect extinction (McCarthy 1998).

Conclusions

The population viability analysis performed in the present study has revealed a large sensitivity of the Danube sturgeon populations to changes in life history parameters, especially in the natural mortality, fecundity, age at maturity and spawning frequency, so any anthropogenic impact that has an influence on these vital rates can also significantly increase their risk of extinction. It was also confirmed that the sturgeons are highly susceptible to even moderate levels of commercial fishery, and that the pressures that are above the thresholds of population viability can reduce a population to less then half of its initial size in only a few decades. Slow recovery of sturgeon populations, observed in no-impact scenarios, confirms the claim of Lenhardt *et al.* (2006:1) that the recovery of sturgeons, especially of those long-lived species like the Beluga, is a multi-decadal affair. This fact is important to take into consideration for any management planning in the present situation of established moratoria on sturgeon fishery in the Danube.

Stocking will probably remain one of the main conservation measures in the Danube basin. While the present study has shown that the stocking with adult individuals produces considerably greater effect on population persistence than the stocking with juveniles, the latter approach can be still considered as preferable, since it avoids many inherent problems of aquaculture cultivation. The significant lag in positive effects of stocking on population persistence and recovery, which was revealed in juvenile stocking scenarios and especially in slow maturing species, has to be considered in future conservation and management policy planning activities.

The methods used in this study that infer extinction based on sighting records indicate that the Atlantic sturgeon can be considered as extinct in the Danube River, with extinction having occurred somewhere between 1966 and 1970. The ship sturgeon is probably still present in the Danube basin, but its extinction could probably occur within the next few decades. In any case, given the current absence of knowledge on their presence in the Danube River basin, the best advice to management authorities on protection of these two species could be to apply all feasible protection measures developed for the other four Danube sturgeon species. The Action Plan for the Conservation of Sturgeons (Acipenseridae) in the Danube River, adopted in 2005 by the Standing Committee of the Bern Convention, has defined a specific set of actions designed to be relevant simultaneously for the conservation of all Danube sturgeons (Bloesch *et al.* 2006). As in the case of "umbrella species", measures aimed at the other four sturgeon species could at the same time provide protection for these two species, if they are not already extinct or beyond recovery. As stipulated in the Action Plan, ex situ-measures, mainly through aquaculture and restocking programs, could provide additional help for recovery of these species, or even represent a last resort in case of their extinction.

This study has also presented a new sighting trend model, an assessment of its sensitivity and an illustration of its application. It is crucial, however, to apply further testing of this method on different data sets, in order to improve our knowledge on its potential and adequate application in the field of conservation biology. The main limitation of this model is its inability to distinguish distribution of the changes in trend within the observation period (e.g. whether the change in frequency has appeared at the beginning or at the end of the observation period), so future research should be oriented on the improvement of the method that would overcome this problem.

Danube sturgeon populations have experienced such serious decline over the past decades, that there is urgent need to implement efficient conservation strategies. This is hindered by the present lack of knowledge on the status of the populations, extinction risks and on the impact of different endangering factors and efficiency of different management scenarios. So far, these questions were answered mostly by "rule of thumb" or through transfer of experiences from well studied populations. Decision makers in the Danube River basin have so far been often forced to act with limited resources for management activities which, combined with the absence of clear assessments of the possible outcomes of different management alternatives, have made them reluctant to make choices. In such a state of affairs, PVA could prove to be a valuable tool.

Development of PVA models for Danube sturgeons can be slowed to an extent by lack of exact data on sturgeon life history parameters. There is a present need for extensive studies on sturgeon ecology and life history that would provide better estimation of their life history parameters. Based on the results of this study, future research efforts should be oriented towards the estimation of the inherent population growth rate of each Danube sturgeon species, natural mortality rates of the each age group, and better assessment of the average fecundity, age at maturity and spawning frequency of females. The use of nuclear microprobe and PIXE methods on strontium to calcium 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 the future research efforts to determine some life history parameters, such as the age at maturity and spawning frequency.

However, due to an expressed need for urgent conservation measures, the development of PVA should not wait for better data. On the contrary, PVA can provide insights regarding the parameters that have the strongest impact on population persistence, and thus endow researchers with directions to research activities of greatest importance. The availability of PVA program packages like Vortex, which have already proved their usefulness and reliability (Brook *et al.* 2000), could diminish the necessity for experience in programming and developing PVA models. If used with ample precaution and thoroughness and taking into account present uncertainties, PVAs could significantly improve our knowledge about these remarkable species and possible ways of their protection.

Future development of PVAs on sturgeon populations in the Danube River, beside the assessment of population viability and future extinction risks, should be oriented towards three main issues: 1) the estimation of impacts of different life history parameters on population persistence, and consequently the identification of key life history parameters as future research priorities; 2) the likely effects of different management scenarios on population persistence and recovery, for the estimation of management measures with the greatest potential; 3) implementation of adaptive management approach, with the PVA as an integral evaluation and planning component.

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Jarić, I., Lenhardt, M., Cvijanović, G. & Ebenhard, T. 2009. *Acipenser sturio* and *Acipenser nudiventris* in the Danube – extant or extinct? *Journal of Applied Ichthyology* 25:137-141.

Jarić, I., Lenhardt, M., Cvijanović, G. & Ebenhard, T. 2009. Population viability analysis and potential of its application to Danube sturgeons. *Archives of Biological Sciences* 61(1):123-128.

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While these publications have a number of co-authors who have all made a contribution to this research, I confirm that the major part of the research was done by me.

References

Akçakaya, H.R. 2000. Population viability analyses with demographically and spatially structured models. *Ecological Bulletins* 48:23-38.

Akçakaya, H.R. & Raphael, M.G. 1998. Assessing human impact despite uncertainty: viability of the northern spotted owl metapopulation in the northwestern USA. *Biodiversity and Conservation* 7:875-894.

Akçakaya, H.R. & Sjögren-Gulve, P. 2000. Population viability analyses in conservation planning: an overview. *Ecological Bulletins* 49:9-21.

Allendorf, F.W. & Ryman, N. 2002. The role of genetics in population viability analysis. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 50-85. The University of Chicago Press, Chicago & London.

Arai, T., Levin, A.V., Boltunov, A.N. & Miyazaki, N. 2002. Migratory history of the Russian sturgeon *Acipenser guldenstadti* in the Caspian Sea, as revealed by pectoral fin spine Sr:Ca ratios. *Marine Biology* 141:315-319.

Bacalbaşa-Dobrovici, N. 1991. Statut des differentes especes d'esturgeons dans le Danube Roumain: problemes lies a leur maintenance. In Williot, P. (ed.) *Acipenser*, 185-192. Cemagref-Dicova, Bordeaux.

Bacalbaşa-Dobrovici, N. 1997. Endangered migratory sturgeons of the lower Danube River and its delta. *Environmental Biology of Fishes* 48:201-207.

Bacalbasa-Dobrovici, N. & Patriche, N. 1999. Environmental studies and recovery actions for sturgeons in the Lower Danube River system. *Journal of Applied Ichthyology* 15:114-115.

Bacalbaşa-Dobrovici, N. & Holčik, J. 2000. Distribution of *Acipenser sturio* L., 1758 in the Black Sea and its watershed. Boletín. *Instituto Español de Oceanografía* 16(1-4):37-41.

Bajer, P.G. & Wildhaber, M.L. 2007. Population viability analysis of Lower Missouri River shovelnose sturgeon with initial application to the pallid sturgeon. *Journal of Applied Ichthyology* 23:457–464.

Balnath, C., Gessner, J. & Rosenthal, H. 2008. Sturgeon conservation and aquaculture. XXXII Scientific Conference on Fisheries and Aquaculture; Proceedings of the International Workshop on Sturgeon Conservation and Breeding, Szarvas, Hungary, 15-16 May 2008 [http://hakinapok.haki.hu – Accessed on 15th April 2009].

Beamesderfer, R.C.P., Rein, T.A. & Nigro, A.A. 1995. Differences in the Dynamics and Potential Production of Impounded and Unimpounded White Sturgeon Populations in the Lower Columbia River. *Transactions of the American Fisheries Society* 124:857-872.

Beamesderfer, R.C.P., Simpson, M.L. & Kopp, G.J. 2007. Use of life history information in a population model for Sacramento green sturgeon. *Environmental Biology of Fishes* 79:315–337.

Beissinger, S.R. 2002. Population viability analysis: past, present, future. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 5-17. The University of Chicago Press, Chicago & London.

Beissinger, S.R. & Westphal, M.I. 1998. On the use of demographic models of population viability in endangered species management. *The Journal of Wildlife Management* 62(3):821-841.

Bemis, W.E., Findeis, E.K. & Grande, L. 1997. An overview of Acipenseriformes. *Environmental Biology of Fishes* 48:25–71.

Birstein, V.J. 1993. Sturgeons and paddlefishes: threatened fishes in need of conservation. *Conservation Biology* 7(4):773-787.

Birstein, V.J., Bemis, W.E. & Waldman, J.R. 1997. The threatened status of acipenseriform species: a summary. *Environmental Biology of Fishes* 48:427–435.

Bloesch, J., Jones, T., Reinartz, R. & Striebel, B. (eds.) 2006. Action Plan for the conservation of sturgeons (Acipenseridae) in the Danube River Basin. *Nature and Environment* 144:1-121.

Boreman, J. 1997. Sensitivity of North American sturgeons and paddlefish to fishing mortality. *Environmental Biology of Fishes* 48:399-405.

Botsford, L.W., Castilla, J.C. & Peterson, C.H. 1997. The management of fisheries and marine ecosystems. *Science* 277:509-515.

Boyce, M.S. 1992. Population viability analysis. *Annual Review of Ecology and Systematics* 23:481-506.

Boyce, M.S. 2002. Reconciling the small-population and declining-population paradigms. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 41-49. The University of Chicago Press, Chicago & London.

Brook, B.W., Lim, L., Harden, R. & Frankham, R. 1997. Does population viability analysis software predict the behaviour of real populations? A retrospective study on the Lord Howe Island woodhen *Tricholimnas sylvestris* (Sclater). *Biological Conservation* 82:119-128.

Brook, B.W., Cannon, J.R., Lacy, R.C., Mirande, C. & Frankham, R. 1999. Comparison of the population viability analysis packages GAPPS, INMAT, RAMAS and VORTEX for the whooping crane (*Grus americana*). *Animal Conservation* 2:23-31.

Brook, B.W., O'Grady, J.J., Chapman, A.P., Burgman, M.A., Akçakaya, H.R. & Frankham, R. 2000. Predictive accuracy of population viability analysis in conservation biology. *Nature* 404:385-387.

Bruch, R.M. 2008. *Modelling the population dynamics and sustainability of lake sturgeon in the Winnebago system, Wisconsin.* PhD thesis, University of Wisconsin-Milwaukee, USA.

Burgman, M.A., Akçakaya, H.R. & Loew, S.S. 1988. The use of extinction models for species conservation. *Biological Conservation* 43:9-25.

Burgman, M.A., Grimson, R.C. & Ferson, S. 1995. Inferring threat from scientific collections. *Conservation Biology* 9(4):923-928.

Carpaneto, G.M., Mazziotta, A. & Valerio, L. 2007. Inferring species decline from collection records: roller dung beetles in Italy (Coleoptera, Scarabaeidae). *Diversity and Distributions* 13:903-919.

Chambers, R.C. 1993. Phenotypic variability in fish populations and its representation in individual-based models. *Transactions of the American Fisheries Society* 122:404-414.

Chebanov, M. & Billard, R. 2001. The culture of sturgeons in Russia: production of juveniles for stocking and meat for human consumption. *Aquatic Living Resources* 14:375-381.

Ciolac, A. & Patriche, N. 2005. Biological aspects of main marine migratory sturgeons in Romanian Danube River. Migration of fishes in Romanian Danube River, No 4. *Applied Ecology and Environmental Research* 3(2):101-106.

Colombo, R.E., Garvey, J.E., Jackson, N.D., Brooks, R., Herzog, D.P., Hrabik, R.A. & Spier, T.W. 2007. Harvest of Mississippi River sturgeon drives abundance and reproductive success: a harbinger of collapse? *Journal of Applied Ichthyology* 23:444–451.

De Groot, S.J. 2002. A review of the past and present status of anadromous fish species in the Netherlands: is restocking the Rhine feasible? *Hydrobiologia* 478:205-218.

Diamond, J.M. 1987. Extant unless proven extinct? Or, extinct unless proven extant? *Conservation Biology* 1(1):77-79.

Dulvy, N.K., Ellis, J.R., Goodwin, N.B., Grant, A., Reynolds, J.D. & Jennings, S. 2004. Methods of assessing extinction risk in marine fishes. *Fish and Fisheries* 5:255–276.

Ebenhard, T. 2000. Population viability analyses in endangered species management: the wolf, otter and peregrine falcon in Sweden. *Ecological Bulletins* 48:143-163.

Elfman, M., Limburg, K.E., Kristiansson, P., Malmqvist, K. & Pallon, J. 1999. Application of micro-PIXE to fish life history analyses: trace element analysis of otoliths. *Nuclear Instruments and Methods in Physics Research B* 150:272-276.

Elfman, M., Limburg, K.E., Kristiansson, P., Svedäng, H., Westin, L., Wickström, H., Malmqvist, K. & Pallon, J. 2000. Complex life histories of fishes revealed through natural information storage devices: case studies of diadromous events as recorded by otoliths. *Nuclear Instruments and Methods in Physics Research B* 161-163:877-881.

Elfman, M., Pallon, J., Auzelyte, V., Kristiansson, P., Malmqvist, K., Nilsson, C., Shariff, A. & Wegdén, M. 2005. The Lund Nuclear Microprobe sub-micron set-up. Part III: Sample stage, optical imaging and detector configuration in the experimental chamber. *Nuclear Instruments and Methods in Physics Research B* 231:14-20.

FISAT II 2000. FAO-ICLARM Fish Stock Assessment Tools. Version 1.2.2. FAO, Rome, Italy.

Gessner, J., Van Eenennaam, J.P. & Doroshov, S.I. 2007. North American green and European Atlantic sturgeon: comparisons of life histories and human impacts. *Environmental Biology of Fishes* 79:397-411.

Grogan, C.S. & Boreman, J. 1998. Estimating the Probability That Historical Populations of Fish Species Are Extirpated. *North American Journal of Fisheries Management* 18:522–529.

Guti, G. 2006. Past and present status of sturgeons in Hungary. Proceedings 36th International Conference of IAD, 143-147. Austrian Committee Danube Research / IAD, Vienna.

Hanski, I. 2002. Metapopulation of animals in highly fragmented landscapes and population viability analysis. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 86-108. The University of Chicago Press, Chicago & London.

Harka, A. & Sallai, Z. 2004. *Magyarorszag halfaunaja* [Fish fauna of Hungary]. Nimfea Termeszetvedelmi Egyesulet, Szarvas (In Hungarian).

Hedrick, P.W. 2002. Application of molecular genetics to conservation: new issues and examples. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 367-387. The University of Chicago Press, Chicago & London.

Hensel, K. & Holčik, J. 1997. Past and current status of sturgeons in the upper and middle Danube River. *Environmental Biology of Fishes* 48:185-200.

Heppell, S.S. 2007. Elasticity analysis of green sturgeon life history. *Environmental Biology of Fishes* 79:357–368.

Holčik, J., Klindová, A., Masár, J. & Mészáros, J. 2006. Sturgeons in the Slovakian rivers of the Danube River basin: an overview of their current status and proposal for their conservation and restoration. *Journal of Applied Ichthyology* 22(Suppl. 1):17-22.

IUCN 2001. *IUCN Red List Categories and Criteria.* Version 3.1. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK.

IUCN 2008. *2008 IUCN Red List of Threatened Species.* International Union for Conservation of Nature and Natural Resources [www.iucnredlist.org - Accessed on 9th April 2009].

Jackson, N.D., Garvey, J.E. & Colombo, R.E. 2007. Comparing aging precision of calcified structures in shovelnose sturgeon. *Journal of Applied Ichthyology* 23:525-528.

Jager, H.I. 2001. Individual variation in life history characteristics can influence extinction risk. Ecological Modelling 144:61–76.

Jager, H.I. 2005. Genetic and demographic implications of aquaculture in white sturgeon (*Acipenser transmontanus*) conservation. *Canadian Journal of Fisheries and Aquatic Sciences* 62:1733-1745.

Jager, H.I. 2006:1. Chutes and ladders and other games we play with rivers. I. Simulated effects of upstream passage on white sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences* 63:165-175.

Jager, H.I. 2006:2. Chutes and ladders and other games we play with rivers. II. Simulated effects of translocation on white sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences* 63:176-185.

Jager, H.I., Lepla, K., Chandler, J., Bates, P. & Van Winkle, W. 2000. Population viability analysis of white sturgeon and other riverine fishes. *Environmental Science & Policy* 3:S483-S489.

Jager, H.I., Chandler, J.A., Lepla, K.B. & Van Winkle, W. 2001. A theoretical study of river fragmentation by dams and its effects on white sturgeon populations. *Environmental Biology of Fishes* 60:347-361.

Kálmán, I. 1961. A 23 és fél kilós tok. *Magyar Horgász* 15(7):1-2 (In Hungarian).

Kennedy, A.J. & Sutton, T.M. 2007. Effects of harvest and length limits on shovelnose sturgeon in the upper Wabash River, Indiana. *Journal of Applied Ichthyology* 23:465–475.

Killgore, K.J., Hoover, J.J., Kirk, J.P., George, S.G., Lewis, B.R. & Murphy, C.E. 2007. Age and growth of pallid sturgeon in the free-flowing Mississippi River. *Journal of Applied Ichthyology* 23:452-456.

Kolarević, J. 2004. *Population - ecological study of sterlet (Acipenser ruthenus L.) in waters of Danube on territory of Belgrade*. MSc thesis, University of Belgrade, Serbia (In Serbian with English Summary).

Kuparinen, A., O'Hara, R.B. & Merilä, J. 2008. The role of growth history in determining age and size at maturation in exploited fish populations. *Fish and Fisheries* 9:201-207.

Kynard, B., Suciu, R. & Horgan, M. 2002. Migration and habitats of diadromous Danube River sturgeons in Romania: 1998-2000. *Journal of Applied Ichthyology* 18:529-535.

Lacy, R.C. 1993. VORTEX: a computer simulation model for population viability analysis. *Wildlife Research* 20:45-65.

Lacy, R.C. 2000. Structure of the VORTEX simulation model for population viability analysis. *Ecological Bulletins* 48:191-203.

Lagutov, V. & Lagutov, V. 2008. The Ural River sturgeons: population dynamics, catch, reasons for decline and restoration strategies. In Lagutov, V. (ed.) *Rescue of sturgeon species in the Ural River Basin*, 193-276. Springer Science.

Lande, R. 2002. Incorporating stochasticity in population viability analysis. In Beissinger, R. & McCullough D.R. (eds) *Population Viability Analysis*, 18-40. The University of Chicago Press, Chicago & London.

Lenhardt, M., Hegediš, A. & Jarić, I. 2005. *Action plan for sturgeon species management in fishery waters of Republic Serbia*. Institute for Biological Research "Siniša Stanković", Developed for the Ministry of Science and Environmental Protection of Republic Serbia.

Lenhardt, M., Jarić, I., Bojović, D., Cvijanović, G. & Gačić, Z. 2006:1. Past and current status of sturgeon in the Serbian part of the Danube River. Proceedings 36th International Conference of IAD, 148-151. Austrian Committee Danube Research / IAD, Vienna.

Lenhardt, M., Jaric, I., Kalauzi, A. & Cvijanovic, G. 2006:2. Assessment of extinction risk and reasons for decline in sturgeon. *Biodiversity and Conservation* 15:1967-1976.

Limburg, K.E., Landergren, P., Westin, L., Elfman, M. & Kristiansson, P. 2001. Flexible modes of anadromy in Baltic sea trout: making the most of marginal spawning streams. *Journal of Fish Biology* 59:682-695.

Lindenmayer, D.B., Burgman, M.A., Akçakaya, H.R., Lacy, R.C. & Possingham, H.P. 1995. A review of the generic computer programs ALEX, RAMAS/space and VORTEX for modelling the viability of wildlife metapopulations. *Ecological Modelling* 82:161-174.

Ludwig, A. 2006. A sturgeon view on conservation genetics. *European Journal of Wildlife Research* 52:3-8.

Ludwig, A., Lippold, S., Debus, L. & Reinartz, R. 2009. First evidence of hybridization between endangered sterlets (*Acipenser ruthenus*) and exotic Siberian sturgeons (*Acipenser baerii*) in the Danube River. *Biological Invasions* 11:753-760.

Ludwig, D. 1999. Is it meaningful to estimate a probability of extinction? *Ecology* 80(1):298-310.

Ludwig, D. & Walters, C.J. 2002. Fitting population viability analysis into adaptive management. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 511-520. The University of Chicago Press, Chicago & London.

Major, C.O., Goldstein, S.L., Ryan, W.B.F., Lericolais, G., Piotrowski, A.M. & Hajdas, I. 2006. The co-evolution of Black Sea level and composition through the last deglaciation and its paleoclimatic significance. *Quaternary Science Reviews* 25:2031-2047.

Manea, G. 1980. *Sturgeons; Taxonomy, biology, sturgeon rearing and sturgeon rearing ponds*. Ceres, Bucuresti (In Romanian).

McCarthy, M.A. 1998. Identifying declining and threatened species with museum data. *Biological Conservation* 83(1):9-17.

McDowall, R.M. 1999. Different kinds of diadromy: Different kinds of conservation problems. *ICES Journal of Marine Science* 56:410–413.

McInerny, G.J., Roberts, D.L., Davy, A.J. & Cribb, P.J. 2006. Significance of sighting rate in inferring extinction and threat. *Conservation Biology* 20(2):562-567.

Miller, P.S. & Lacy, R.S. 2005. VORTEX: A stochastic simulation of the extinction process. Version 9.50 user's manual. Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, MN, USA.

Mills, L.S., Hayes, S.G., Baldwin, C., Wisdom, M.J., Citta, J., Mattson, D.J. & Murphy, K. 1996. Factors leading to different viability predictions for a grizzly bear data set. *Conservation Biology* 10(3):863-873.

Mills, L.S. & Lindberg, M.S. 2002. Sensitivity analysis to evaluate the consequences of conservation actions. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 338-366. The University of Chicago Press, Chicago & London.

Navodaru, I., Staras, M. & Banks, R. 1999. Management of the sturgeon stocks of the Lower Danube River system. In Stiuca, R. & Nuchersu, I. (eds) *The Delta's: State-of-the-art protection and management*, 229-237. Conference Proceedings, Tulcea, Romania.

Nikcevic, M., Lenhardt, M., Cakic, P., Mickovic, B., Kolarevic, J. & Jaric, I. 2004. Historical review and new initiatives for sturgeon fisheries, aquaculture and caviar production in Serbia and Montenegro. In Oddmund, O. (ed.) *Releasing development potentials at the Eastern Adriatic.* Norwegian University of Science and Technology (NTNU), Trondheim, Norway [www.easternadriatic.com – Accessed on 15th April 2009].

Ognjanović, D., Nikolić, V. & Simonović, P. 2008. Morphometrics of two morphs of sterlet, *Acipenser ruthenus* L., in the middle course of the Danube River (Serbia). *Journal of Applied Ichthyology* 24:126-130.

Paaver, T. 1999. Historic and recent records of native and exotic sturgeon species in Estonia. *Journal of Applied Ichthyology* 15:129-132.

Paragamian, V.L. & Beamesderfer, R.C. 2003. Growth estimates from tagged white sturgeon suggest that ages from fin rays underestimate true age in the Kootenai River, USA and Canada. *Transactions of the American Fisheries Society* 132:895-903.

Patriche, N., Pecheanu, C. & Billard, R. 1999. Sturgeon fisheries, caviar production in Romania and possible measures of rehabilitation in the Lower Danube. *Rivista Italiana di Acquacoltura* 34:83-91.

Petrović, M. 1998. *Ribarstvo.* [Fisheries]. Zavod za udzbenike i nastavna sredstva, Belgrade, Serbia (In Serbian).

Pianka, E.R. 1970. On r- and K-selection. *The American Naturalist* 104(940):592-597.

Pikitch, E.K., Doukakis, P., Lauck, L., Chakrabarty, P. & Erickson, D.L. 2005. Status, trends and management of sturgeon and paddlefish fisheries. *Fish and Fisheries* 6:233-265.

Pine, W.E.III, Allen, M.S. & Dreitz, V.J. 2001. Population Viability of the Gulf of Mexico Sturgeon: Inferences from Capture–Recapture and Age-Structured Models. *Transactions of the American Fisheries Society* 130:1164–1174.

Pintér, K. 1991. Sturgeons in Hungary, past and present situation. In Williot, P. (ed.) *Acipenser*, 173-178. Cemagref-Dicova, Bordeaux.

Poleksic, V., Lenhardt, M., Jaric, I., Djordjevic, D., Gacic, Z., Cvijanovic, G. & Raskovic, B. 2009. Liver, gills and skin histopathology and heavy metal content of the Danube sterlet (*Acipenser ruthenus* Linnaeus, 1758). *Environmental Toxicology & Chemistry* (in press).

Quist, M.C., Guy, C.S., Pegg, M.A., Braaten, P.J., Pierce, C.L. & Travnichek, V.H. 2002. Potential influence of harvest on shovelnose sturgeon populations in the Missouri River system. *North American Journal of Fisheries Management* 22:537-549.

Reed, D.H., O'Grady, J.J., Brook, B.W., Ballou, J.D. & Frankham, R. 2003. Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation* 113:23-34.

Reed, J.M. 1996. Using statistical probability to increase confidence of inferring species extinction. *Conservation Biology* 10(4):1283-1285.

Reed, J.M., Mills, L.S., Dunning Jr., J.B., Menges, E.S., McKelvey, K.S., Frye, R., Beissinger, S.R., Anstett, M.-C. & Miller, P. 2002. Emerging issues in Population Viability Analysis. *Conservation Biology* 16(1):7-19.

Regan, H.M., Colyvan, M. & Burgman, M.A. 2000. A proposal for fuzzy International Union for the Conservation of Nature (IUCN) categories and criteria. *Biological Conservation* 92:101-108.

Reinartz, R. 2002. *Sturgeons in the Danube River. Biology, Status, Conservation. Literature study*. International Association for Danube Research (IAD), Bezirk Oberpfalz, Landesfischereiverband Bayern, e.V.

Reisenbichler, R.R. & Rubin, S.P. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. *ICES Journal of Marine Science* 56:459–466.

Rien, T.A. & Beamesderfer, R.C. 1994. Accuracy and precision of white sturgeon age estimates from pectoral fin rays. *Transactions of the American Fisheries Society* 123:255-265.

Ristić, M.Đ. 1963. Ribarsko biološka studija. Acipenseridae-a jugoslavenskog dela donjega Dunava. [Fishery biological study. Acipenserids of Yugoslavian part of the Lower Danube.] *Ribarstvo Jugoslavije* 2:48-58 (In Serbian).

Robbirt, K.M., Roberts, D.L. & Hawkins, J.A. 2006. Comparing IUCN and probabilistic assessments of threat: do IUCN red list criteria conflate rarity and threat? *Biodiversity and Conservation* 15:1903-1912.

Roberts, C.M. & Hawkins, J.P. 1999. Extinction risk in the sea. *Trends in Ecology & Evolution* 14(6):241-246.

Roberts, D.L. & Kitchener, A.C. 2006. Inferring extinction from biological records: were we too quick to write off Miss Waldron's Red Colobus Monkey (*Piliocolobus badius waldronae*)? *Biological Conservation* 128:285-287.

Roberts, D.L. & Solow, A.R. 2003. When did the dodo become extinct? *Nature* 426:245.

Rochard, E., Williot, P., Castelnaud, G. & Lepage, M. 1991. Elements de systematique et de biologie des populations sauvages d'esturgeons. In Williot, P. (ed.) *Acipenser*, 475-507. Cemagref-Dicova, Bordeaux (In French with English summary).

Secor, D.H. & Niklitschek, E.J. 2002. Sensitivity if sturgeons to environmental hypoxia: a review of physiological and ecological evidence. In Thurston, R.V. (ed.) *Fish Physiology, Toxicology and Water Quality*, 61-78. Symposium Proceedings, La Paz, Mexico, EPA/600/R-02/097.

Shaffer, M.L. 1990. Population Viability Analysis. *Conservation Biology* 4(1):39-40.

Shaffer, M., Watchman, L.H., Snape III, W.J. & Latchis, I.K. 2002. Population viability analysis and conservation policy. In Beissinger, R. & McCullough, D.R. (eds) *Population Viability Analysis*, 123-142. The University of Chicago Press, Chicago & London.

Shahifar, R. 2006. Economic comparison between Sturgeon fishery yield and releasing efforts of fingerlings in the Southern Caspian Sea (1972-2003). *Journal of Applied Ichthyology* 22(Suppl. 1):119-124.

Shariff, A., Auzelyte, V., Elfman, M., Kristiansson, P., Malmqvist, K., Nilsson, C., Pallon, J. & Wegdén, M. 2005:1. The Lund Nuclear Microprobe sub-micron set-up. Part I: Ion optics calculation. *Nuclear Instruments and Methods in Physics Research B* 231:1-6.

Shariff, A., Nilsson, C., Auzelyte, V., Elfman, M., Kristiansson, P., Malmqvist, K., Pallon, J. & Wegdén, M. 2005:2. The Lund Nuclear Microprobe sub-micron set-up. Part II: Beam line, focusing system and scanning. *Nuclear Instruments and Methods in Physics Research B* 231:7-13.

Simonović, P. 2001. *Fishes of Serbia.* NNK International, Institute of Nature Protection of Republic Serbia, Biological Faculty of the Belgrade University, Belgrade, Serbia.

Simonović, P., Budakov, Lj., Nikolić, V. & Marić, S. 2005. Recent record of the ship sturgeon *Acipenser nudiventris* in the middle Danube (Serbia). *Biologia* 60(2):231-233.

Sjögren-Gulve, P. & Hanski, I. 2000. Metapopulation viability analysis using occupancy models. *Ecological Bulletins* 48:53-71.

Solow, A.R. 1993:1. Inferring extinction from sighting data. *Ecology* 74:962–964.

Solow, A.R. 1993:2. Inferring extinction in a declining population. *Journal of Mathematical Biology* 32:79-82.

Solow, A.R. 2005. Inferring threat from a sighting record. *Mathematical Biosciences* 195:47-55.

Solow, A.R. & Roberts, D.L. 2003. A nonparametric test for extinction based on a sighting record. *Ecology* 84(5):1329-1332.

Stevenson, J.T. & Secor, D.H. 1999. Age determination and growth of Hudson River Atlantic sturgeon, *Acipenser oxyrinchus*. *Fishery Bulletin* 97:153-166.

Suciu, R. 2008. Present situation and perspectives of sturgeon conservation and aquaculture in Romania, with special regard to the sterlet. XXXII Scientific Conference on Fisheries and Aquaculture; Proceedings of the International Workshop on Sturgeon Conservation and Breeding, Szarvas, Hungary, 15-16 May 2008, 21-24.

Sulak, K.J. & Randall, M. 2002. Understanding sturgeon life history: Enigmas, myths, and insights from scientific studies. *Journal of Applied Ichthyology* 18:519-528.

Uchmański, J. 1999. What promotes persistence of a single population: an individual-based model. *Ecological Modelling* 115:227-241.

Vásárhely, I. 1957. Felső-tiszai színtokok – és a Tiszalöki vízilépcsö. [Ship sturgeon catches in the upper Tisza River and the Tiszalok water step] *Halászat* 4:105 (In Hungarian).

Vassilev, M. 2006. Lower Danube – the last refuge for surviving of sturgeon fishes in the Black Sea Region. In Hubert, P. (ed.) *Water Observation and Information System for Decision Support.* Conference Proceedings, Balwois, Ohrid, Macedonia [http://balwois.org – Accessed on 15th April 2009].

Veinott, G., Northcote, T., Rosenau, M. & Evans, R.D. 1999. Concentrations of strontium in the pectoral fin rays of the white sturgeon (*Acipenser transmontanus*) by laser ablation sampling – inductively coupled plasma – mass spectrometry as an indicator of marine migrations. *Canadian Journal of Fisheries and Aquatic Sciences* 56:1981-1990.

Williot, P., Rochard, E., Castelnaud, G., Rouault, T., Brun, R., Lepage, M. & Elie, P. 1997. Biological characteristics of European Atlantic sturgeon, *Acipenser sturio*, as the basis for a restoration program in France. *Environmental Biology of Fishes* 48:359-370.

Williot, P., Arlati, G., Chebanov, M., Gulyas, T., Kasimov, R., Kirschbaum, F., Patriche, N., Pavlovskaya, L.P., Poliakova, L., Pourkazemi, M., Kim, Y., Zhuang, P. & Zholdasova, I.M. 2002. Status and management of Eurasian sturgeon: an overview. *International Reviews in Hydrobiology* 87(5–6):483–506.

Williot, P., Brun, R., Rouault, T., Pelard, M., Mercier, D. & Ludwig, A. 2005. Artificial spawning in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites. *Aquaculture* 246:263-273.

Appendix I – Input data sheets for basic scenarios in Vortex simulations

General input data that was used in basic scenarios for all species:
Number of iterations: 1 000
Number of years: 500
Extinction definition: $N < 10$
Number of populations: 1
Inbreeding depression: no
Concordance of environmental variation in reproduction and survival: yes
No of types of catastrophes: 0
No of population state variables: 0
Dispersal: off
Age distribution: stable age distribution
Initial population size and Carrying capacity (K) tested for 6 different values in 6 scenarios: N and K = 50, 100, 200, 500, 1 000 and 2 000
SD in K due to environmental variation: 0
Future change in K: no
Harvest: no
Population supplemented: no

Huso huso – basic scenario input parameters

Reproductive system: polygynous
Age of first offspring for females: 15
(13-15 *Bloesch et al. 2006; Reinartz 2002*; 14-26 *Bacalbaşa-Dobrovici 1991;* 16-18 (17) *Ciolac & Patriche 2005; Ristić 1963*; 16-22 *Lenhardt et al. 2005*; 16-23 *Vassilev 2006*)
Age of first offspring for males: 11
(10-13 *Bloesch et al. 2006; Reinartz 2002*; 11 years *Ristić 1963*; 11–14 *Ciolac & Patriche 2005*; 12-14 years *Bacalbaşa-Dobrovici 1991*; 12-16 *Lenhardt et al. 2005*; 14-18 years *Vassilev 2006*)
Maximum age of reproduction: 100
(>50 years *Ristić 1963*; 100 historic *Pikitch et al. 2005*; >100 years *Vassilev 2006; Reinartz 2002; Bloesch et al. 2006*; 118 years *Lenhardt et al. 2005*)
Maximum number of progeny per year: 360 (900 000 * 0.0004)
(964 800 *Reinartz 2002*; 3 200 000 (frequently 900 000) *Ciolac & Patriche 2005*; 0.0004 is the maximum natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Sex ratio at birth – in % males: 50

Density dependent reproduction: no
Percent adult females breeding: 17%
Environmental variation in % adult females breeding: 1.5 percentage units
(each 5-7 years *Lenhardt et al. 2005*)
Distribution of offspring per female per year: normal distribution approximation
Mean number of offspring per year: 108 (540 000 * 0.0002)
Standard deviation in number of offspring per year: 30
(506 000 *Ciolac & Patriche 2005*; 574 400 *Reinartz 2002*; 0.0002 is the average natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Percent males in breeding pool: 20%
(Each 4-7 years *Lenhardt et al. 2005*)

Natural mortality fitted to the three different values of intrinsic population growth rate:

Natural mortality of females (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	80.0 ± 10.0	70.0 ± 15.0	50.0 ± 20.0
age 2 to 3	70.0 ± 15.0	50.0 ± 20.0	30.0 ± 12.0
age 3 to 4	50.0 ± 12.5	30.0 ± 7.5	15.0 ± 3.75
age 4 to 5	20.0 ± 5.0	14.0 ± 3.5	9.0 ± 2.25
age 5 to 15	5.6 ± 1.4	5.6 ± 1.4	5.6 ± 1.4
age 15+	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1

Natural mortality of males (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	80.0 ± 10.0	70.0 ± 15.0	50.0 ± 20.0
age 2 to 3	70.0 ± 15.0	50.0 ± 20.0	30.0 ± 12.0
age 3 to 4	50.0 ± 12.5	30.0 ± 7.5	15.0 ± 3.75
age 4 to 5	20.0 ± 5.0	14.0 ± 3.5	9.0 ± 2.25
age 5 to 11	5.6 ± 1.4	5.6 ± 1.4	5.6 ± 1.4
age 11+	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1

* Age 0 to 1 natural mortality is incorporated in the mean and maximum number of offspring; see subchapter Model parameterization in Material and methods for clarification.

Acipenser gueldenstaedtii **– basic scenario input parameters**

Reproductive system: polygynous
Age of first offspring for females: 14
(12-13 *Vassilev 2006*; 13-15 *Bacalbaşa-Dobrovici 1991; Ciolac & Patriche 2005*; 12-16 *Reinartz 2002; Bloesch et al. 2006; Lenhardt et al. 2005*)
Age of first offspring for males: 12
(7-8 *Vassilev 2006*; 8-12 *Bacalbaşa-Dobrovici 1991; Ciolac & Patriche 2005*; 11-13 *Reinartz 2002; Bloesch et al. 2006; Lenhardt et al. 2005*)
Maximum age of reproduction: 50
(46 *Lenhardt et al. 2005*; 48 *Birstein 1993*; 50 *Vassilev 2006*; >50 *Reinartz 2002; Pikitch et al. 2005; Manea 1966 citt. in Rochard et al. 1991*)
Maximum number of progeny per year: 160 (400 000 * 0.0004)
(400 000 eggs *Ciolac & Patriche 2005*; 406 800 *Reinartz 2002; Bloesch et al. 2006*; 0.0004 is the maximum natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Sex ratio at birth – in % males: 50

Density dependent reproduction: no
Percent adult females breeding: 21%
Environmental variation in % adult females breeding: 2 percentage units
(about 20% *Reinartz 2002*; each 3-5 years (sex?) *Vassilev 2006*; 4 *Pikitch et al. 2005*; 5-6 *Lenhardt et al. 2005; Reinartz 2002*; >6 years *Vlasenko et al. 1989 citt. in Rochard et al. 1991*)
Distribution of offspring per female per year: normal distribution approximation
Mean number of offspring per year: 34 (170 000 * 0.0002)
Standard deviation in number of offspring per year: 9
(125 000-135 000 *Ristić 1963*; 29 500-406 800 (range) *Reinartz 2002*; 140 000 *Ciolac & Patriche 2005*; 0.0002 is the average natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Percent males in breeding pool: 25%
(about 20 % *Reinartz 2002*; each 3-5 years (sex?) *Vassilev 2006*)

Natural mortality fitted to the three different values of intrinsic population growth rate:

Natural mortality of females (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	70.0 ± 15.0	50.0 ± 20.0	20.0 ± 8.0
age 2 to 3	45.0 ± 18.0	20.0 ± 8.0	9.0 ± 3.6
age 3 to 4	20.0 ± 5.0	11.0 ± 2.75	6.7 ± 1.68
age 4 to 5	10.0 ± 2.5	6.7 ± 1.68	6.7 ± 1.68
age 5 to 14	6.7 ± 1.68	6.7 ± 1.68	6.7 ± 1.68
age 14+	6.7 ± 0.67	6.7 ± 0.67	1.0 ± 0.1

Natural mortality of males (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	70.0 ± 15.0	50.0 ± 20.0	20.0 ± 8.0
age 2 to 3	45.0 ± 18.0	20.0 ± 8.0	9.0 ± 3.6
age 3 to 4	20.0 ± 5.0	11.0 ± 2.75	6.7 ± 1.68
age 4 to 5	10.0 ± 2.5	6.7 ± 1.68	6.7 ± 1.68
age 5 to 12	6.7 ± 1.68	6.7 ± 1.68	6.7 ± 1.68
age 12+	6.7 ± 0.67	6.7 ± 0.67	1.0 ± 0.1

* Age 0 to 1 natural mortality is incorporated in the mean and maximum number of offspring; see subchapter Model parameterization in Material and methods for clarification.

Acipenser stellatus – basic scenario input parameters

Reproductive system: polygynous

Age of first offspring for females: 10

(7 *Bacalbaşa-Dobrovici 1991*; 7-10 *Reinartz 2002; Bloesch et al. 2006;* 8–10 *Ciolac & Patriche 2005*; 8-12 years *Vassilev 2006*; average 10 *Lenhardt et al. 2005; Pikitch et al. 2005; Ristić 1963*)

Age of first offspring for males: 6

(5 *Bacalbaşa-Dobrovici 1991*; 4-7 *Lenhardt et al. 2005; Ciolac & Patriche 2005;* 5-6 *Reinartz 2002; Bloesch et al. 2006*; 5-8 *Vassilev 2006*; 6 *Ristić 1963*)

Maximum age of reproduction: 35

(27 *Lenhardt et al. 2005; Birstein 1993*; 35 *Reinartz 2002; Bloesch et al. 2006*; historic 41 *Pikitch et al. 2005*)

Maximum number of progeny per year: 160 (400 000 * 0.0004)

(180 000 *Ciolac & Patriche 2005*; 430 000 *Reinartz 2002; Bloesch et al. 2006*; 0.0004 is the maximum natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)

Sex ratio at birth – in % males: 50

(*Ciolac & Patriche 2005*)

Density dependent reproduction: no
Percent adult females breeding: 25%
Environmental variation in % adult females breeding: 2.5 percentage units

(not less than 3-4 year *Lenhardt et al. 2005*; Sea of Azov *Reinartz 2002*; 3 times in life Ural River *Lagutov & Lagutov 2008*; Sea of Azov *Reinartz 2002*)

Distribution of offspring per female per year: normal distribution approximation
Mean number of offspring per year: 30 (150 000 * 0.0002)
Standard deviation in number of offspring per year: 9

(80 000-180 000, 150 000 *Reinartz 2002*; 145 000-160 000 *Ristić 1963*; 0.0002 is the average natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)

Percent males in breeding pool: 29%

(not less than 3-4 year *Lenhardt et al. 2005*; Sea of Azov *Reinartz 2002*; 3 times in life Ural River *Lagutov & Lagutov 2008*; Sea of Azov *Reinartz 2002*)

Natural mortality fitted to the three different values of intrinsic population growth rate:

Natural mortality of females (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	60.0 ± 20.0	45.0 ± 18.0	27.0 ± 10.8
age 2 to 3	40.0 ± 16.0	25.0 ± 10.0	20.0 ± 8.0
age 3 to 4	25.0 ± 6.25	14.0 ± 3.5	13.0 ± 3.25
age 4 to 5	15.0 ± 3.75	13.0 ± 3.25	13.0 ± 3.25
age 5 to 10	13.0 ± 3.25	13.0 ± 3.25	13.0 ± 3.25
age 10+	13.0 ± 1.3	13.0 ± 1.3	5.0 ± 0.5

Natural mortality of males (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	60.0 ± 20.0	45.0 ± 18.0	27.0 ± 10.8
age 2 to 3	40.0 ± 16.0	25.0 ± 10.0	20.0 ± 8.0
age 3 to 4	25.0 ± 6.25	14.0 ± 3.5	13.0 ± 3.25
age 4 to 5	15.0 ± 3.75	13.0 ± 3.25	13.0 ± 3.25
age 5 to 6	13.0 ± 3.25	13.0 ± 3.25	13.0 ± 3.25
age 6+	13.0 ± 1.3	13.0 ± 1.3	5.0 ± 0.5

* Age 0 to 1 natural mortality is incorporated in the mean and maximum number of offspring; see subchapter Model parameterization in Material and methods for clarification.

Acipenser ruthenus – basic scenario input parameters

Reproductive system: polygynous
Age of first offspring for females: 5
(*Bacalbaşa-Dobrovici 1991*; 4-7 *Reinartz 2002; Bloesch et al. 2006; Lenhardt et al. 2005*; 7 *Vassilev 2006*)
Age of first offspring for males: 4
(3-4 *Bacalbaşa-Dobrovici 1991*; 3-5 *Reinartz 2002; Bloesch et al. 2006; Lenhardt et al. 2005*; 3-7 *Manea 1966 citt. in Rochard et al. 1991*; 4 *Vassilev 2006*)
Maximum age of reproduction: 26
(24 *Reinartz 2002; Bloesch et al. 2006*; 25 *Vassilev 2006; Simonović 2001; Kolarević 2004*; 26 *Lenhardt et al. 2005*; 26 (archaeological remains) *Reinartz 2002*)
Maximum number of progeny per year: 43 (108 000 * 0.0004)
(108 000 *Reinartz 2002*; *Kolarević 2004; Bloesch et al. 2006*; 0.0004 is the maximum natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Sex ratio at birth – in % males: 50
(*Reinartz 2002*)

Density dependent reproduction: no
Percent adult females breeding: 70% (33.33-100%)
Environmental variation in % adult females breeding: 15 percentage units
(every year *Manea 1966 citt. in Rochard et al. 1991*; 1-2 *Jankovic 1958 citt. in Rochard et al. 1991*; *Bacalbaşa-Dobrovici 1991; Reinartz 2002*; 1-3 *Vassilev 2006*)
Distribution of offspring per female per year: normal distribution approximation
Mean number of offspring per year: 12 (58 000 * 0.0002)
Standard deviation in number of offspring per year: 5
(7 000–108 000 *Reinartz 2002; Kolarević 2004; Bloesch et al. 2006*; 0.0002 is the average natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Percent males in breeding pool: 75%
(every year in Danube *Reinartz 2002; Manea 1966 citt. in Rochard et al. 1991*; 1-2 (sex?) *Bacalbaşa-Dobrovici 1991*; 1-3 (sex?) *Vassilev 2006*)

Natural mortality fitted to the three different values of intrinsic population growth rate:

Natural mortality of females (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	62.0 ± 19.0	50.0 ± 20.0	40.0 ± 16.0
age 2 to 3	39.0 ± 15.6	35.6 ± 14.24	31.7 ± 12.68
age 3 to 4	32.5 ± 8.13	31.7 ± 7.93	31.7 ± 7.93
age 4 to 5	31.7 ± 7.93	31.7 ± 7.93	31.7 ± 7.93
age 5+	31.7 ± 3.17	31.7 ± 3.17	28.0 ± 2.8

Natural mortality of males (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	62.0 ± 19.0	50.0 ± 20.0	40.0 ± 16.0
age 2 to 3	39.0 ± 15.6	35.6 ± 14.24	31.7 ± 12.68
age 3 to 4	32.5 ± 8.13	31.7 ± 7.93	31.7 ± 7.93
age 4+	31.7 ± 3.17	31.7 ± 3.17	28.0 ± 2.8

* Age 0 to 1 natural mortality is incorporated in the mean and maximum number of offspring; see subchapter Model parameterization in Material and methods for clarification.

Acipenser nudiventris **– basic scenario input parameters**

Reproductive system: polygynous
Age of first offspring for females: 13
(12-14 *Rochard et al. 1991*; in Kura River *Reinartz 2002*; in Kura River *Bloesch et al. 2006; Lenhardt et al. 2005*; Average 17 *Pikitch et al. 2005*)
Age of first offspring for males: 8
(6-9 *Rochard et al. 1991*; in Kura River Reinartz 2002; in Kura River *Bloesch et al. 2006; Lenhardt et al. 2005*)
Maximum age of reproduction: 36
(36 years *Vassilev 2006*; in Ural River and Aral Sea *Reinartz 2002*; in Ural River *Bloesch et al. 2006; Lenhardt et al. 2005*)
Maximum number of progeny per year: 520 (1 300 000 * 0.0004)
(1 300 000 *Reinartz 2002; Bloesch et al. 2006*; 0.0004 is the maximum natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)
Sex ratio at birth – in % males: 50

Density dependent reproduction: no
Percent adult females breeding: 42%
Environmental variation in % adult females breeding: 4 percentage units
(2-3 *Lenhardt et al. 2005; Reinartz 2002*; averagely every 3 years (global data) *Pikitch et al. 2005*)

Distribution of offspring per female per year: normal distribution approximation
Mean number of offspring per year: 150 (40-260) (750 000 * 0.0002)
Standard deviation in number of offspring per year: 50

(200 000–1 300 000 *Reinartz 2002; Bloesch et al. 2006*; 0.0002 is the average natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)

Percent males in breeding pool: 50%

(*(2) Reinartz 2002*)

Natural mortality fitted to the three different values of intrinsic population growth rate:

Natural mortality of females (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	86.0 ± 7.0	80.0 ± 10.0	70.0 ± 15.0
age 2 to 3	70.0 ± 15.0	60.0 ± 20.0	50.0 ± 20.0
age 3 to 4	50.0 ± 12.5	40.0 ± 10.0	35.0 ± 8.75
age 4 to 5	26.0 ± 6.5	20.0 ± 5.0	18.0 ± 4.5
age 5 to 13	8.0 ± 2.0	8.0 ± 2.0	8.0 ± 2.0
age 13+	8.0 ± 0.8	8.0 ± 0.8	5.0 ± 0.5

Natural mortality of males (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	86.0 ± 7.0	80.0 ± 10.0	70.0 ± 15.0
age 2 to 3	70.0 ± 15.0	60.0 ± 20.0	50.0 ± 20.0
age 3 to 4	50.0 ± 12.5	40.0 ± 10.0	35.0 ± 8.75
age 4 to 5	26.0 ± 6.5	20.0 ± 5.0	18.0 ± 4.5
age 5 to 8	8.0 ± 2.0	8.0 ± 2.0	8.0 ± 2.0
age 8+	8.0 ± 0.8	8.0 ± 0.8	5.0 ± 0.5

* Age 0 to 1 natural mortality is incorporated in the mean and maximum number of offspring; see subchapter Model parameterization in Material and methods for clarification.

Acipenser sturio – basic scenario input parameters

Reproductive system: polygynous
Age of first offspring for females: 14

(8-12 in Po River *Holcik et al. 1989 citt. in Gessner et al. 2007*; 8-14 in Rioni River *Elanidze et al. 1970 citt. in Rochard et al. 1991*; in Rioni River *Ninua 1976 citt. in Gessner et al. 2007*; In Rioni River *Reinartz 2002*; In Rioni River *Bloesch et al. 2006*; 8-20 *Birstein 1993*; 14 In Rhine River *De Groot 2002*; 14-15 in Guadalquivir River *Classen 1944 citt. in Gessner et al. 2007*; 15 Gironde *Williot et al. 1997*; 16-18 in Gironde *Magnin 1962 citt. in Rochard et al. 1991*; 16-

20 in Gironde *Magnin 1963 citt. in Gessner et al. 2007*; southern populations mature 2-6 years earlier than in Gironde River *Holcik et al. 1989 citt. in Gessner et al. 2007*)

Age of first offspring for males: 12

(7-9 in Rioni River *Ninua 1976 citt. in Gessner et al. 2007*; in Rioni River *Reinartz 2002*; in Rioni River *Bloesch et al. 2006*; 7-10 in Po River *Holcik et al. 1989 citt. in Gessner et al. 2007*; 7-12 in Rioni *Elanidze et al. 1970 citt. in Rochard et al. 1991*; 7-15 Birstein 1993; 8 in Rhine River *De Groot 2002* ; 10 Gironde *Williot et al. 1997*; 10-11 in Guadalquivir River *Classen 1944 citt. in Gessner et al. 2007*; 13-15 in Gironde *Magnin 1963 citt. in Gessner et al. 2007*; 14-18 in Gironde *Magnin 1962 citt. in Rochard et al. 1991*)

Maximum age of reproduction: 48

(48 *Reinartz 2002*; in Gironde *Holcik et al. 1989 citt. in Gessner et al. 2007*; *Bloesch et al. 2006*; 100 in Gironde Estuary *Holcik et al. 1989 citt. in Gessner et al. 2007*; in 8th and 9th century 35-36, in Gironde 42 *Reinartz 2002*; life span of males is 12, that of females 20 *Bacalbaşa-Dobrovici & Holčik 2000*)

Maximum number of progeny per year: 560 (1 400 000 * 0.0004)

(1 412 000 *Holcik et al. 1989 citt. in Gessner et al. 2007*; 1 820 000 in Rioni River *Reinartz 2002*; in Rioni River *Bloesch et al. 2006*; 0.0004 is the maximum natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)

Sex ratio at birth – in % males: 50

Density dependent reproduction: no

Percent adult females breeding: 33.3%

(Every 3 years *Williot pers. comm.*)

Environmental variation in % adult females breeding: 4 percentage units

Distribution of offspring per female per year: normal distribution approximation

Mean number of offspring per year: 170 (851 000 * 0.0002)

Standard deviation in number of offspring per year: 50

(851 000 *Holcik et al. 1989 citt. in Gessner et al. 2007*; 0.0002 is the average natural survival of age 0 to 1 *Pine et al. 2001; Jager 2005; Jager 2006a; Bajer & Wildhaber 2007; Kennedy & Sutton 2007*)

Percent males in breeding pool: 50%

(Every 2 years *Williot pers. comm.*)

Natural mortality fitted to the three different values of intrinsic population growth rate:

Natural mortality of females (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	86.0 ± 7.0	80.0 ± 10.0	74.0 ± 13.0
age 2 to 3	70.0 ± 15.0	60.0 ± 20.0	51.0 ± 20.4
age 3 to 4	50.0 ± 12.5	38.0 ± 9.5	30.0 ± 7.5
age 4 to 5	28.0 ± 7.0	16.0 ± 4.0	10.0 ± 2.5
age 5 to 14	6.7 ± 1.68	6.7 ± 1.68	6.7 ± 1.68
age 14+	6.7 ± 0.67	6.7 ± 0.67	1.0 ± 0.1

Natural mortality of males (age specific mortality as % ± SD due to environmental variation):

	r=0.05	*r*=0.10	*r*=0.15
age 0 to 1	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
age 1 to 2	86.0 ± 7.0	80.0 ± 10.0	74.0 ± 13.0
age 2 to 3	70.0 ± 15.0	60.0 ± 20.0	51.0 ± 20.4
age 3 to 4	50.0 ± 12.5	38.0 ± 9.5	30.0 ± 7.5
age 4 to 5	28.0 ± 7.0	16.0 ± 4.0	10.0 ± 2.5
age 5 to 12	6.7 ± 1.68	6.7 ± 1.68	6.7 ± 1.68
age 12+	6.7 ± 0.67	6.7 ± 0.67	1.0 ± 0.1

* Age 0 to 1 natural mortality is incorporated in the mean and maximum number of offspring; see subchapter Model parameterization in Material and methods for clarification.

Appendix II – Input values used in the sensitivity analysis scenarios in Vortex

This appendix contains values for different parameters that were applied in the sensitivity analysis. They are represented by either a range of values (value placed in bold was the starting value, that was used in basic scenarios), or by a percentage by which the original value was decreased or increased. All simulations that were part of the sensitivity analysis were run for 100 years and at the carrying capacity set at 2 000. Initial population size was basically set to *N*=50, but the effect of a change in this parameter was also tested in the sensitivity analysis (see values provided in the table).

Parameter	*Huso huso*	*Acipenser gueldenst.*	*Acipenser stellatus*	*Acipenser ruthenus*	*Acipenser nudiventris*	*Acipenser sturio*
Age of first offspring (♀)	13/15/18	12/14/16	7/10/12	4/5/7	10/13/17	8/14/20
Age of first offspring (♂)	10/11/16	7/12/13	4/6/8	3/4/5	6/8/10	7/12/18
Max. age of reproduction	60/80/100	40/50/60	30/35/40	16/26/30	30/36/42	30/48/100
Max. No. of progeny / year*	180/360/540	80/160/240	80/160/240	22/43/65	260/520/780	280/560/840
Sex ratio (in percent of ♂)	25/50/75	25/50/75	25/50/75	25/50/75	25/50/75	25/50/75
Percent of ♀ breeding / year	14.29/17/20	16.6/21/25	20/25/33.3	33.3/70/100	33.3/42/50	25/33.3/50
Environmental variation in percent of ♀ breeding	0.5/1.5/2.5	1/2/3	1.5/2.5/3.5	7.5/15/22.5	2/4/6	2/4/6
Mean No. of progeny / year*	54/108/162	17/34/51	15/30/45	6/12/18	75/150/225	85/170/255
Environmental variation in mean No. of progeny	15/30/45	4.5/9/13.5	4.5/9/13.5	2.5/5/7.5	25/50/75	25/50/75
Percent of ♂ breeding / year	14.29/20/25	20/25/33.3	25/29/33.3	50/75/100	33.3/50/70	33.3/50/70
Adult mortality (r=0.05)	0.5/1/1.5	3.35/6.7/10.05	6.5/13/19.5	15.85/31.7/47.55	4/8/12	3.35/6.7/10.05
Adult mortality (r=0.10)	0.5/1/1.5	3.35/6.7/10.05	6.5/13/19.5	15.85/31.7/47.55	4/8/12	3.35/6.7/10.05
Adult mortality (r=0.15)	0.5/1/1.5	0.5/1/1.5	2.5/5/7.5	14/28/42	2.5/5/7.5	0.5/1/1.5

Age 1-2, 2-3 and 3-4 mortality	± 10%**	± 10%	± 10%	± 10%	± 10%	± 10%
Mortality of age groups 4 to adult	± 10%	± 10%	± 10%	± 10%	± 10%	± 10%
Environmental variation in mortality for all age groups	± 50%	± 50%	± 50%	± 50%	± 50%	± 50%
Environmental variation in carrying capacity	0/400/800	0/400/800	0/400/800	0/400/800	0/400/800	0/400/800
Initial population size	40/50/60	40/50/60	40/50/60	40/50/60	40/50/60	40/50/60

**Through the mean and maximum number of progeny / year input variables, sensitivity analysis of both the age 0 to 1 natural mortality and the mean and maximum fecundity were performed, since the values for these two input variables were derived through combination of both life history parameters (for the explanation how the age 0 to 1 natural mortality was incorporated in the model, see the subchapter Model parameterization in Material and methods).*

***I.e. if the basic value was 20, ± 10% would mean variation from 18 to 22.*

Appendix III – Results of the sensitivity analysis scenarios in Vortex

Assessment of the results of sensitivity analysis by the use of ranking technique

Following four tables include results of the ranking preformed on the outcome of the sensitivity analysis in Vortex simulation model. They present the influence of each life history parameters through the total rank value (where higher value signifies higher influence) for each of five resulting parameters – pseudo-extinction probability, heterozygosity level; deterministic population growth rate, stochastic population growth rate and the population recovery time. Parameters are sorted in each table according to the values in the column with the total ranking value, from the highest to the lowest one.

Legend: fem age – time needed for females to reach maturity; 1-4 mort – natural mortality of year 1-4 age groups; N – initial population size; fem breed – female spawning frequency; ad mort – natural mortality of adult individuals; mean offs – mean number of offspring (also represents egg to age 1 natural mortality); mort EV – environmental variation in natural mortality of all age groups; K EV – environmental variation in carrying capacity; max age – maximum reproductive age; male age – time needed for males to reach maturity; fem breed EV – environmental variation in female spawning frequency; mean offs EV – environmental variation in mean number of offspring; 4-ad mort – natural mortality of year 4 to adulthood age groups; male breed – male spawning frequency; max offs – maximum fecundity.

Pseudo-extinction probability

Extinction probability	Russian sturgeon	Ship sturgeon	Sterlet	Stellate sturgeon	Atlantic sturgeon	Beluga	Total
fem age	121	157	165.5	148.5	173	111.5	876.5
1-4 mort	108	166.5	130.5	115	161.5	121.5	803
N	126.5	152.5	117	130.5	131.5	127.5	785.5
fem breed	107	124.5	164	137	137	103	772.5
ad mort	129.5	112.5	158	130.5	94.5	103.5	728.5
mean offs	105.5	125.5	153.5	126	104	98	712.5
mort EV	105	125.5	137.5	103.5	110.5	109.5	691.5
K EV	112.5	94.5	63.5	96	117	80.5	564
sex ratio	104.5	76	134	106.5	47	91	559
max age	78.5	110	41.5	69.5	101.5	102	503
male age	74.5	65	50	60	124.5	86.5	460.5
fem breed EV	79	49.5	98	62	68.5	97	454
mean offs EV	86	65	67	79	52	73.5	422.5
4-ad mort	102.5	67	9	88	58.5	92	417
male breed	66.5	35	58	76	53.5	99	388
max offs	77.5	58	37	56	49.5	88	366

Heterozygosity level

Heterozygosity level	Russian sturgeon	Ship sturgeon	Sterlet	Stellate sturgeon	Atlantic sturgeon	Beluga	Total
N	169	142	118.5	146	136	175	886.5
mean offs	143	139.5	154	140.5	133.5	174	884.5
fem age	127	150	169	167	151	96	860
ad mort	138	144	165	161	122	113	843
sex ratio	112	123	131	112	129	145	752
1-4 mort	85	167	137.5	93.5	146	118	747
mort EV	91.5	143	121	88	120	144.5	708
fem breed	114	79	167	115	110	59.5	644.5
K EV	112	91.5	44	120	143	86.5	597
male age	92	43.5	77	83.5	104.5	90	490.5
4-ad mort	88.5	86.5	9	103.5	82	67.5	437
max age	76.5	85.5	42	72	66.5	77.5	420
mean offs EV	68	54.5	73.5	57	58	68	379
male breed	61	46	67	59.5	24.5	65.5	323.5
fem breed EV	46.5	53	62.5	25	29	59	275
max offs	60	36	46	40.5	29	45	256.5

Deterministic population growth rate

Character	Russian sturgeon	Ship sturgeon	Sterlet	Stellate sturgeon	Atlantic sturgeon	Beluga	Total
fem age	159.5	177	176.5	180	184	163	1 040
mean offs	182	166.5	168	175	162.5	177	1 031
sex ratio	182	166.5	168	175	162.5	177	1 031
1-4 mort	140	174	129	127.5	171	163.5	905
fem breed	144	135	166.5	144.5	144.5	144.5	879
ad mort	138	135	146	148	123.5	119	809.5
4-ad mort	125	117	9	121	119	127	618
max age	73.5	89	81	81	93	73	490.5
N	55	53	61	54	53	55	331
mort EV	55	53	61	54	53	55	331
K EV	55	53	61	54	53	55	331
male age	55	53	61	54	53	55	331
fem breed EV	55	53	61	54	53	55	331
mean offs EV	55	53	61	54	53	55	331
male breed	55	53	61	54	53	55	331
max offs	55	53	61	54	53	55	331

Stochastic population growth rate

Character	Russian sturgeon	Ship sturgeon	Sterlet	Stellate sturgeon	Atlantic sturgeon	Beluga	Total
fem age	161.5	181.5	180	180.5	186	165.5	1 055
mean offs	185	169	168	177.5	163	180	1 042.5
sex ratio	176	158	156.5	169	147.5	169.5	976.5
fem breed	144.5	141	168	145	154	143.5	896
1-4 mort	142	159	129	128.5	170	163.5	892
ad mort	134	140.5	152.5	151.5	117	109	804.5
4-ad mort	124.5	109.5	9	118.5	104.5	119	585
mort EV	74	84.5	117	86.5	86	92.5	540.5
N	57	77.5	71.5	60	66.5	89.5	422
max age	57.5	77.5	47	71.5	55	72	380.5
K EV	57	61.5	67	59.5	70.5	47.5	363
fem breed EV	57.5	49	104	49.5	46.5	48.5	355
male breed	66.5	32.5	71.5	59.5	37.5	50	317.5
male age	41.5	49.5	41	42	95.5	42.5	312
max offs	57.5	51	48	43	40.5	44	284
mean offs EV	48	42.5	54	42	44	47.5	278

Population recovery time

Character	Russian sturgeon	Ship sturgeon	Sterlet	Stellate sturgeon	Atlantic sturgeon	Beluga	Total
fem age	164.5	172	169.5	179	183	168	1 036
mean offs	181.5	168.5	162	172	160.5	181	1 025.5
sex ratio	155.5	145.5	153.5	155	136.5	156	902
fem breed	146.5	140.5	162.5	147.5	152	145	894
1-4 mort	129.5	165	141	128	156	149	868.5
ad mort	117	123	156.5	140	102.5	85	724
N	118.5	87.5	84	113.5	69.5	131.5	604.5
K EV	91	105.5	68.5	91.5	134.5	67.5	558.5
4-ad mort	93	83	9	111	75.5	82.5	454
mort EV	61.5	73.5	112.5	72	56.5	76.5	452.5
max age	55	70.5	51	51	70	70.5	368
fem breed EV	56.5	46	87	46	52	55	342.5
mean offs EV	64	53.5	61	51	45	65.5	340
male age	40	38.5	59	45.5	97.5	48	328.5
male breed	55	69.5	55.5	40.5	45	55	320.5
max offs	55	42	51.5	40.5	48	48	285

Assessment of the results of sensitivity analysis by the use of regression analysis

Following two tables include results of the regression analysis preformed on the outcome of the sensitivity analysis in Vortex simulation model. They present the most influential life history parameters, for each resulting parameter (left column) and each simulated population growth rate (r=0.05, 0.10 and 0.15). Only up to four most important parameters were included per each simulated population growth rate, and only those regressions with statistical significance (p<0.05). The most influential parameters are bolded, and each two life history parameters that produced the same effect on the resulting parameters are placed in italics (e.g. as it was often the case for the mean number of offspring and the sex ratio).

Legend: *P* – pseudo-extinction probability; *r* det – deterministic population growth rate; *r* stoch – stochastic population growth rate; *H* – heterozygosity level; RT – population recovery time; N – initial population size; 1-4 – natural mortality for year 1-4 age group; mort EV – environmental variation in natural mortality of all age groups; fem age – time needed for females to reach maturity; mean offs – mean number of offspring (also represents egg to age 1 natural mortality); adult mort – natural mortality of adult individuals; fem breed – spawning frequency of females; K EV – environmental variation in carrying capacity; male age - time needed for males to reach maturity.

	Beluga	Russian sturgeon	Stellate sturgeon	Sterlet	Ship sturgeon	Atlantic sturgeon
P	0.05: N 1-4 mort EV fem age 0.10: N 0.15: K EV	0.05: mean offs sex ratio 0.10: K EV 0.15: K EV	0.05: mean offs sex ratio adult mort 0.10: K EV 0.15: K EV	0.05: fem breed fem age adult mort mean offs 0.10: fem age fem breed mean offs sex ratio 0.15: fem age fem breed	0.05: 1-4 max age fem age 0.10: fem age 1-4 N K EV 0.15: fem age N mort EV 1-4	0.05: 1-4 fem age fem breed mean offs 0.10: fem age 1-4 K EV male age 0.15: K EV fem age male age

r det	0.05: 1-4 mean offs sex ratio fem age 0.10: mean offs sex ratio 1-4 fem age 0.15: mean offs sex ratio fem age fem breed	0.05: sex ratio mean offs 1-4 adult mort 0.10: sex ratio mean offs fem age fem breed 0.15: sex ratio mean offs fem age fem breed	0.05: fem age mean offs sex ratio adult mort 0.10: fem age mean offs sex ratio adult mort 0.15: fem age mean offs sex ratio fem breed	0.05: fem age fem breed mean offs sex ratio 0.10: fem age fem breed mean offs sex ratio 0.15: fem age fem breed mean offs sex ratio	0.05: 1-4 mean offs sex ratio fem age 0.10: 1-4 fem age mean offs sex ratio 0.15: fem age mean offs sex ratio 1-4	0.05: 1-4 fem age mean offs sex ratio 0.10: fem age 1-4 mean offs sex ratio 0.15: fem age mean offs sex ratio 1-4
r stoch	0.05: 1-4 mean offs sex ratio fem age 0.10: mean offs sex ratio fem age 1-4 0.15: mean offs fem age sex ratio fem breed	0.05: mean offs sex ratio 1-4 fem age 0.10: mean offs sex ratio fem age adult mort 0.15: mean offs sex ratio fem age fem breed	0.05: mean offs fem age sex ratio adult mort 0.10: fem age mean offs sex ratio adult mort 0.15: fem age mean offs sex ratio fem breed	0.05: fem age fem breed mean offs adult mort 0.10: fem age fem breed mean offs sex ratio 0.15: fem age fem breed mean offs sex ratio	0.05: fem age mean offs adult mort 1-4 0.10: fem age 1-4 mean offs sex ratio 0.15: fem age mean offs sex ratio 1-4	0.05: 1-4 fem age mean offs fem breed 0.10: fem age 1-4 mean offs sex ratio 0.15: fem age mean offs sex ratio 1-4

	Huhu	Acgu	Acsl	Acru	Acnu	Acst
H	0.05: 1-4 N 0.10: mean offs N	0.05: mean offs adult mort 1-4 sex ratio 0.10: K EV N	0.05: mean offs adult mort sex ratio fem age 0.10: adult mort fem age	0.05: fem breed mean offs fem age sex ratio 0.10: fem age fem breed	0.05: 1-4 adult mort fem age sex ratio 0.10: 1-4 K EV	0.05: 1-4 adult mort mean offs fem age 0.10: K EV 1-4

	mort EV sex ratio	adult mort	mean offs K EV	mean offs sex ratio	fem age adult mort	N adult mort
	0.15: K EV mean offs N	0.15: K EV mean offs N	0.15: K EV N fem age mean offs	0.15: fem age fem breed mean offs	0.15: mean offs N mort EV fem age	0.15: K EV mean offs fem age
RT	0.05: 1-4 mean offs sex ratio fem age	0.05: mean offs sex ratio 1-4 adult mort	0.05: fem age mean offs adult mort sex ratio	0.05: adult mort mean offs fem breed sex ratio	0.05: 1-4 fem age mean offs sex ratio	0.05: fem age 1-4 mean offs fem breed
	0.10: mean offs fem age 1-4 sex ratio	0.10: K EV mean offs	0.10: K EV mean offs fem age	0.10: fem age fem breed mean offs sex ratio	0.10: K EV fem age mean offs 1-4	0.10: K EV fem age mean offs 1-4
	0.15: mean offs fem age K EV	0.15: mean offs fem age fem breed	0.15: fem age mean offs sex ratio fem breed	0.15: fem age fem breed mean offs sex ratio	0.15: fem age mean offs 1-4 sex ratio	0.15: fem age mean offs K EV fem breed

Appendix IV – Additional information on Danube sturgeon species

General information on morphology and ecology of each species was taken from Lenhardt *et al.* (2005), except for the Atlantic sturgeon, which was taken from Reinartz (2002).

Beluga – *Huso huso*

Species description:
Body is massive, narrowing towards the tail. Mouth is big with semicircular shape; its biggest width exceeds half of the head width at that spot. Nine to 17 dorsal scutes, 37 to 53 lateral scutes, seven to 14 ventral scutes. Branchiostegal membranes are joined to one another and separated from the isthmus.

Taxonomy:
Possibility of existence of two forms or races, spring and winter - one form migrates in summer and autumn and spawns next year in spring, and the other migrates at the end of winter and in early spring and spawns in the same year. Can hybridize with other sturgeon species, in the Danube with the Sterlet, Ship sturgeon, Russian sturgeon and Stellate sturgeon (Reinartz 2002).

Range (global):

Fig. 40. Global range of the Beluga; green area represents the countries where it is still present and the red area where it is considered to be extinct (source: www.cites.org).

Ecology:
Anadromous species. Inhabits pelagic zone in the sea, and during migrations occupies bottom zone of the river, occasionally moving to the surface. Spawning occurs at deep water at rocky or gravelly bottoms. Juveniles feed

mostly on benthic invertebrates, and the adults are piscivorous predators, but during migrations feed also on crabs and molluscs.

IUCN Category: Endangered (EN, A2d)
Listing in CITES Appendices: Appendix II

Species specific threats: unsustainable fishery, habitat fragmentation, pollution, loss of spawning grounds.

Russian sturgeon – *Acipenser gueldenstaedtii*

Species description:
Body is spindle-shaped and proportionally wide. Differs from other members of the genus Acipenser by short snout with rounded tip, and by centrally interrupted lower lip. Barbells are short, curved and not fringed, situated near the top of the snout. Eight to 18 dorsal scutes, 24 to 50 lateral scutes, six to 13 ventral scutes. Branchiostegal membranes are attached to the isthmus.

Taxonomy:
Possibility of existence of two varieties – the Azov sturgeon (*Acipenser gueldenstaedtii var. tanaica*) and the Black Sea – Caucasian variety (*Acipenser gueldenstaedtii var colchica*), with the latter often elevated to the level of subspecies (*Acipenser gueldenstaedtii colchicus*) (Reinartz 2002). Frequently interbreeds with the Sterlet, and more rarely with Beluga and Stellate sturgeon (Reinartz 2002). Possibility of existence of a resident, potamodromous form (Hensel & Holčik 1997).

Range (global):

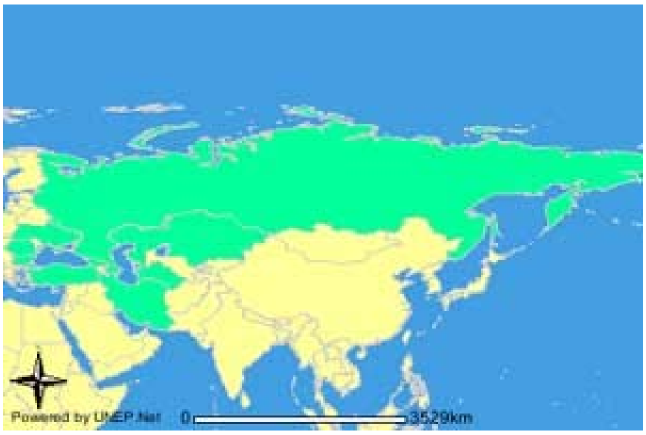

Fig. 41. Global range of the Russian sturgeon; green area represents the countries where it is present (source: www.cites.org).

Ecology:
Anadromous species. In the sea, it inhabits shallow water near the shore, and dwells mostly in brackish waters. During migrations in the river, dwells on the depth from two to 30 m. Beside gravelly bottom, reportedly also spawns in flood zones along river bank and on sandy area at the river mouth. Benthic feeder, diet mostly based on snails, shellfish, crabs, insect larvae and smaller fish specimens (predominantly members of the family Gobiidae and genera *Engraulis* and *Sprattus*).

IUCN Category: Endangered (EN, A2d)
Listing in CITES Appendices: Appendix II

Species specific threats: unsustainable fishery, habitat fragmentation, pollution, loss of spawning grounds.

Stellate sturgeon – *Acipenser stellatus*

Species description:
Body is elongated, snout is long, narrow and dorso-ventrally flattened, and its length exceeds 50 percent of the total head length. Lower lip centrally interrupted, barbells short and not fringed. Nine to 16 dorsal scutes, 26 to 43 lateral scutes, nine to 14 ventral scutes. Lateral scutes significantly remote one from the other.

Taxonomy:
Possibility of existence of two forms or races – spring and winter, that differ on the time of spawning, where one spends winter in the river prior to spawning (Reinartz 2002). Can hybridize with other sturgeon species, in the Danube with the Sterlet, Ship sturgeon, Russian sturgeon and Beluga (Reinartz 2002).

Range (global):

Fig. 42. Global range of the Stellate sturgeon; green area represents the countries where it is present (source: www.cites.org).

Ecology:
Anadromous species. In the sea, it inhabits inshore locations in the middle and upper water strata during the spring and summer, and deeper offshore locations during the winter. During migration in rivers moves through the main river flow, and resides in the shallow water with weak water current. During migrations in the river, dwells on the depth from two to 30 m. Spawns later then the other sturgeon species and prefers gravelly, rocky or stone bottom. Mostly feeds on molluscs, invertebrates and smaller fish.

IUCN Category: Endangered (EN, A2d)
Listing in CITES Appendices: Appendix II

Species specific threats: unsustainable fishery, habitat fragmentation, pollution, loss of spawning grounds.

Sterlet – *Acipenser ruthenus*

Species description:
The smallest sturgeon species in the Danube River. Differs from the Ship sturgeon by centrally interrupted lower lip and from other sturgeon species by fimbriate barbells and larger number of lateral scutes. Rostrum length varies, but usually comprises 37-49 percent of the total head length. Eleven to 18 dorsal scutes, 56 to 71 lateral scutes, 10 to 20 ventral scutes.

Taxonomy:
Possibility of existence of two sympatric morphs – pointed-snout and blunt-snout (Ognjanović *et al.* 2008). Can hybridize with other sturgeon species in the

Danube, most frequently with the Russian sturgeon, but also with the Beluga, Ship sturgeon and the Stellate sturgeon and Beluga (Reinartz 2002), as well as with the exotic Siberian sturgeon (*Acipenser baerii*) (Ludwig *et al.* 2009).

Range (global):

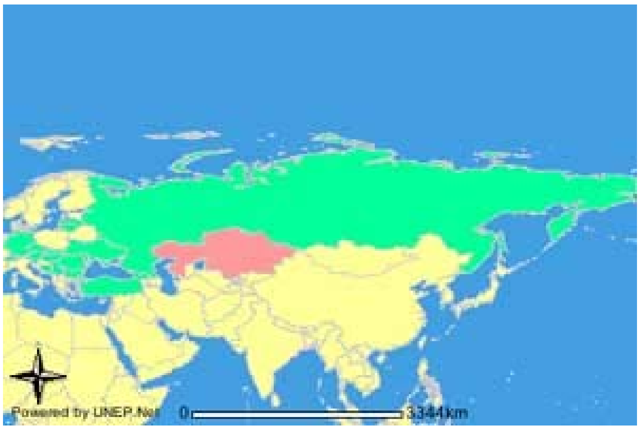

Fig. 43. Global range of the Sterlet; green area represents the countries where it is still present and the red area where it is considered to be extinct (source: www.cites.org).

Ecology:
Potamodromous species. Inhabits lowland river areas, mainly resides in the mainstream of river flow, in the deeper depressions of river bottom. During spring floods moves to flood zones for foraging. Spawns on gravelly ground on the river bottom, as well as in flood zones. Makes upstream spawning migrations within the river. Mostly feeds on smaller benthic organisms, such as insect larvae, molluscs and benthic invertebrates.

IUCN Category: Vulnerable (VU, A1c + 2d)
Listing in CITES Appendices: Appendix II

Species specific threats: unsustainable fishery, habitat fragmentation, pollution, loss of spawning grounds, hybridization with exotic sturgeon species, such as *A. baerii* (Ludwig *et al.* 2009).

Ship sturgeon – *Acipenser nudiventris*

Species description:
Distinguished from all other Danube sturgeons by the continuous, uninterrupted lower lip. Barbells are fimbriated and rostrum rounded and conical. Eleven to 17 dorsal scutes, 49 to 74 lateral scutes, and 11-17 ventral scutes which can be also partially or completely absent. First dorsal scute stands under the inclination in regard to the head profile.

Taxonomy:
Can hybridize with the Sterlet, Russian sturgeon, Beluga and more often with the Stellate sturgeon (Reinartz 2002).

Range (global):

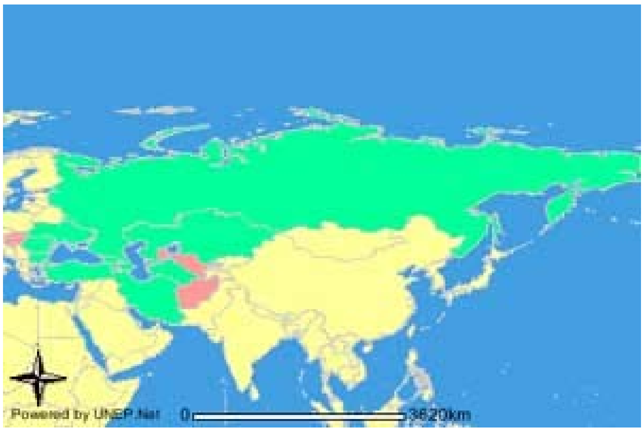

Fig. 44. Global range of the Ship sturgeon; green area represents the countries where it is still present and the red area where it is considered to be extinct (source: www.cites.org).

Ecology:
While the Danube is inhabited only by potamodromous form, there are also anadromous populations in other regions. Dwells mostly in shallow water above muddy bottom. Spawns on gravelly, rocky or stone bottom. Diet mostly based on insect larvae, molluscs, crabs, as well as the fish.

IUCN Category: Endangered (EN, A1acde + 2d)
Listing in CITES Appendices: Appendix II

Species specific threats: unsustainable fishery, habitat fragmentation, pollution, loss of spawning grounds.

Atlantic sturgeon – *Acipenser sturio*

Species description:
The body is elongated and low, snout broad, slightly turned up and more pointed than in the Russian sturgeon. Lower lip is centrally interrupted. Nine to 16 dorsal scutes, 24 to 39 lateral scutes, and nine to 14 ventral scutes. There are many patches of rhombic denticles between the dorsal and lateral scute rows (Reinartz 2002).

Taxonomy:

There are no subspecies described, but genetic studies imply high degree of relationship between the *A. sturio* and the *A. oxyrinchus* (Reinartz 2002). Reportedly interbreeds with the Russian sturgeon, but rarely (Reinartz 2002).

Range (global):

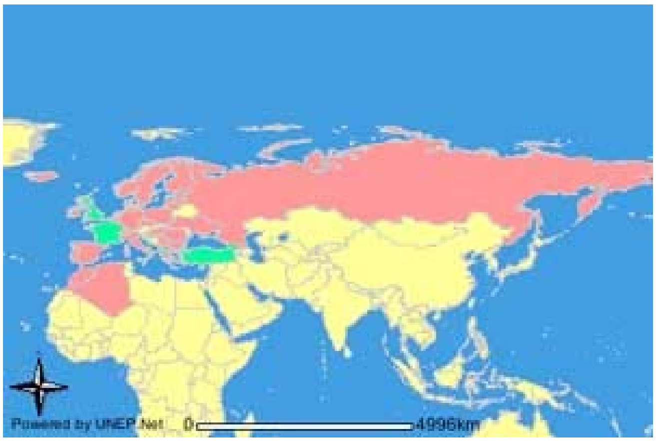

Fig. 45. Global range of the Atlantic sturgeon; green area represents the countries where it is still present and the red area where it is considered to be extinct (source: www.cites.org).

Ecology:
Anadromous species. In the sea, it inhabits littoral zones, mainly estuaries. Younger specimens usually stay no further than 100 km from the river mouth. Spawns on a rocky or pebble bottom. While their diet is usually mostly based on molluscs, Polychaete worms, Ispods, shrimps and small fish, in the Black Sea they feed mainly on fishes, predominantly on the European anchovy (*Engraulis encrasicholus*) (Reinartz 2002).

IUCN Category: Critically Endangered (CR, A2d)
Listing in CITES Appendices: Appendix I and II

Species specific threats: unsustainable fishery, habitat fragmentation, pollution, loss of spawning grounds.