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Symposium on Conservation of the Atlantic Sturgeon *Acipenser sturio* L., 1758 in Europe

(Madrid and Seville, Spain, 6-11 September 1999)

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Logo for the Symposium on Conservation of the Atlantic Sturgeon *Acipenser sturio* in Europe.
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PREFACE

'... the play, I remember, pleas'd not the million,
'twas caviary to the general.'

Hamlet's speech to the players, act 2, scene 2.

Sturgeons are an ancient group of animals, and are among the most valuable commercial fishes. Unfortunately, nowadays sturgeons the world over are greatly depleted in numbers due to overfishing, damming of rivers, and pollution.

The current scientific and technical interest in sturgeons is evident, and several meetings and books testify to this concern. Some highlights of this recent interest are the 'First International Symposium on Sturgeon' held in Bordeaux (France) in 1989, the 'International Symposium on Sturgeons' in Moscow in 1993, the 'International Conference on Sturgeon Biodiversity and Conservation' in New York City in 1994, and the '3rd International Symposium on Sturgeon' in Piacenza (Italy) in 1997. This series of meetings will continue at the next '4th International Symposium on Sturgeon' to be held in Oshkosh, Wisconsin (USA) in 2001.

The Atlantic sturgeon *Acipenser sturio* L., 1758 was formerly a commercially important species, both for its flesh and for its ripe eggs (caviar), but due to a combination of factors it is becoming increasingly uncommon. Consequently, the Atlantic sturgeon is today a rare fish; the world population of *A. sturio* is so depleted that this species is threatened with extinction. The 2000 IUCN Red List of Threatened Species considers it 'Critically Endangered - CR A2d', and it has been placed on the lists of endangered species by several European countries. The Atlantic sturgeon is also protected by international conventions, e.g. Washington, Bern, Bonn, Barcelona, and by the Habitats Directive of the European Union.

In July 1994 'The Society to Save the Sturgeon *Acipenser sturio* L. *(Gesellschaft zur Rettung des Störs* Acipenser sturio *L.*)' was founded at the Senckenberg Institute in Frankfurt. The main goals of the Society are to unite researchers and private groups involved in *A. sturio* conservation, and to develop an international collaborative effort to save this species. The Society has held annual meetings and developed several projects in its area of concern.

The international 'Symposium on Conservation of the Atlantic Sturgeon *Acipenser sturio* in Europe' held in September 6-11, 1999 in Madrid and Seville, was organised by the Department of Animal Biology I of Madrid's Complutense University and the Society to Save the Sturgeon. More than forty delegates from France, Germany, Greece, Hungary, Poland, Portugal, Romania, Russia, Slovakia, Spain, Ukraine and the USA attended and contributed to the scientific discussions during the Symposium's sessions.

The main objectives of the Symposium were to bring together those concerned with or working on conservation of *A. sturio*, and to produce a book of proceedings, including an overview of the present knowledge on the subject. It is our pleasure to now present this volume of proceedings, sponsored by the Instituto Español de Oceanografía (IEO, Spanish Institute of Oceanography), including 26 peer-reviewed articles. This collection of papers offers a valuable summary of the state of the art at the end of the 20th century.

We would like to express our sincere thanks to all the participants in the international 'Symposium on Conservation of the Atlantic Sturgeon *Acipenser sturio* in Europe', and to the authors, referees and editors of this volume of proceedings, with special thanks to all of the Symposium's sponsors.

Benigno Elvira, Symposium Convener and Contributing Editor

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CONCLUSIONS AND RECOMMENDATIONS

The present situation of the Atlantic sturgeon *Acipenser sturio* L., 1758 within its historical range can be summarised as follows:

The status of the species throughout its range is considered highly endangered.

The only identified reproducing population is the one in France's Gironde River basin. Restoration attempts have been carried out for the past 20 years.

There is serious concern about the future of the species in the wild with regard to the decrease in effective population size and genetic heterogeneity due to loss of local stocks.

The Gironde basin population is considered the only available source for future re-introduction activities. Therefore, the integrity of this population is vital.

Additional material for reproduction could be obtained from individuals representing the remains of local populations. More accurate assessments on the current situation of these relict populations are necessary to update the species's present distribution area.

Although enhancement of the species's protection under the laws currently in force is hardly possible, the regulations derived from them are considered very insufficient.

Protection measures carried out to date for the sturgeon's habitat have proved insufficient.

Due to the species's long life cycle, restoration of populations might take many years. Therefore, long-term political commitment is a necessity for its successful restoration.

For these reasons, and in keeping with our knowledge of the underlying principles, the following actions should be effectuated in order to increase protection of the species, both at the population and the individual levels:

More intensive international co-operation is essential to restore the species, involving both *ex-situ* and *in-situ* measures. Sturgeon's inclusion in international projects on migratory fish management should be encouraged.

An immediate and effective application of existing regulations to protect the species in international waters is urgently demanded (Bern

Convention). The attempt to increase the protection of the diadromous species by listing it in the Bonn Convention should be supported.

Attempts to restore *A. sturio* in Europe must be integrated into existing and planned programmes on migratory fish management, and more generally into restoration and management plans involving water, the environment, and biodiversity for drainage basins. The inclusion of *A. sturio* as an important indicator for the restoration of riverine habitat is recommended.

To effectively mediate the necessity of the species's restoration, an intensification of public awareness programmes (both locally and internationally) is considered vital to generate social acceptance and increase political pressure. Means for adequate effectuation must be outlined, and an international network be put into place.

It is considered vital for future recovery programmes to strictly avoid activities that introduce species when the historical presence of this species must be considered doubtful or the species is an exotic within the range. For the implementation of re-introduction measures with material from other drainage areas, effective analysis of the extirpation of the species in waterbodies/watersheds is considered a necessary prerequisite.

It is urgently requested to legally integrate incidental captures into an *ex-situ* stock of the species to increase the measures to effectively save the remaining genetic plasticity of the species deriving from various origins. This should be in accordance with the safeguarding measures, and, if necessary, the EIFAC/ICES Code of Practice.

Therefore, more detailed information on the genetic structure of the specimens/populations available is considered to be of utmost importance. This should lead to the development of a breeding plan to maximise the genetic heterogeneity of the brood stock and, subsequently, the stocking material.

To increase our knowledge regarding the presence of *A. sturio,* it is necessary to update the status of the species by either a rewards programme or in connection with a survey, in intensive collaboration with fishermen.

A habitat analysis, highlighting habitat during early life stages, as well as the development of a management plan for required restoration, should be undertaken.

Seville, Spain, 11 September 1999

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Past and present distribution of *Acipenser sturio* L., 1758 on the Iberian Peninsula

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ABSTRACT

The present paper reviews the ichthyological and historical literature referring to sturgeon on the Iberian Peninsula. Three different species of Acipenseridae, namely *Acipenser sturio* L., 1758, *A. naccarii* Bonaparte, 1836, and *Huso huso* (L., 1758), have, in the past, been recorded in the Iberian seas and rivers. However, examination of specimens housed in zoological collections has clearly proved that just one, the Atlantic sturgeon *A. sturio*, is native to the Iberian Peninsula. Analysis of captures and observations suggests a regression of the species in Portugal and Spain, notable from the middle of the 20th century. However, *A. sturio* is not technically extirpated, at least not in Spain, since it was fished in 1988 off the coast of Cantabria, and in 1992 near the mouth of the Guadalquivir. Consequently, two local populations are reported as still living on the Iberian littoral: one in the Bay of Biscay and the other in Cadiz Bay. This is not contradicted by historical and present data on the Iberian distribution and status of the sturgeon.

Key words: Atlantic sturgeon, Portugal, Spain, Bay of Biscay, Cadiz Bay, Mediterranean Sea.

RESUMEN

Distribución pasada y reciente de* Acipenser sturio *L., 1758 en la península Ibérica

En este trabajo se revisa la bibliografía ictiológica e histórica relativa a la presencia del esturión en la península Ibérica. En el pasado se han citado hasta tres especies distintas de Acipenseridae en los mares y ríos ibéricos: Acipenser sturio *L., 1758,* A. naccarii *Bonaparte, 1836 y* Huso huso *(L., 1758). Sin embargo, el estudio de los ejemplares conservados en colecciones zoológicas ha probado que sólo una, el esturión atlántico* A. sturio*, es nativo de la península Ibérica. El análisis de capturas y observaciones sugiere una grave regresión de la especie en Portugal y en España, muy acentuada desde mediados del siglo XX. Todavía,* A. sturio *no se puede considerar técnicamente extinguido, al menos en España, ya que ejemplares aislados fueron capturados en la costa de Cantabria en 1988 y cerca de la desembocadura del Guadalquivir en 1992. Por tanto, se reconocen dos poblaciones locales supervivientes en el litoral ibérico: una en el mar Cantábrico y otra en el golfo de Cádiz. Esto no se contradice con los datos históricos y actuales de la distribución ibérica y del estado de conservación del esturión.*

Palabras clave: *Esturión atlántico, Portugal, España, golfo de Vizcaya, golfo de Cádiz, mar Mediterráneo.*

INTRODUCTION

Three different species of sturgeon have previously been reported in the Iberian seas and rivers: the Atlantic sturgeon *Acipenser sturio* L., 1758; the Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836; and the beluga *Huso huso* (L., 1758). However, examination of specimens in zoological collections has shown that just one species, *A. sturio*, is indeed a native species of the Iberian Peninsula (Almaça, 1988; Elvira, Almodóvar and Lobón-Cerviá, 1991a).

The present paper aims to provide a general picture of the past and present status of the Atlantic sturgeon on the Iberian Peninsula (figure 1). The results of our review will be reported under three headings: Bay of Biscay and Galicia, where data mainly concerns marine captures; Atlantic rivers; and Mediterranean rivers. Some remarks on the conservation of the sturgeon in Iberian rivers will be presented thereafter.

RESULTS

Bay of Biscay and Galicia

One of the extant populations of *A. sturio* in western Europe lives in the Bay of Biscay (Lelek, 1987). Sturgeons entering the Gironde estuary belong to that population. Until recently, fishes entering the Minho and Douro Rivers probably proceeded from the same population.

Rivers of the Cantabrian slope and Galician rias (fjord-like estuaries) are too small and offer no spawning areas for the sturgeon. The presence of the sturgeon near the Cantabrian coast is proved by a few relatively recent captures:

- Bay of Biscay, Cantabria, 1914, total length (Tl) = 430 mm (Museum of the Cantabrian Sea, Santander)
- Bay of Biscay, San Sebastián (Guipúzcoa), 21 May 1975, Tl = 945 mm (Department of

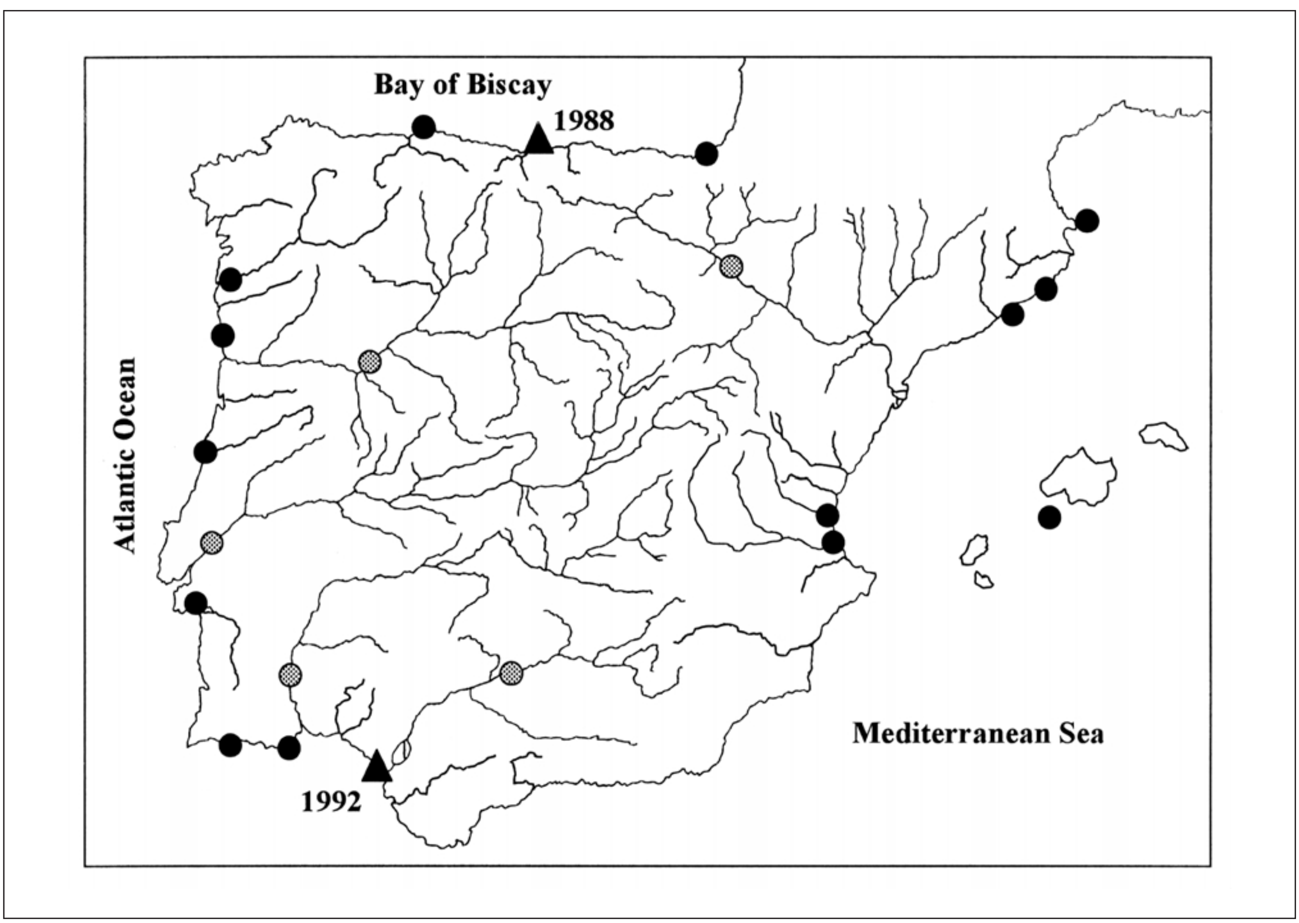

Figure 1. Distribution of *Acipenser sturio* on the Iberian Peninsula. Historical (circles) and most recent (triangles) records. Grey circles mark the known upstream migration in large rivers

Zoology and Ecology, University of Navarre, Pamplona)

– San Vicente de la Barquera (Cantabria), 10 June 1988, Tl = 1 205 mm (Museum of the Cantabrian Sea, Santander)

Other records of the species in northern Spain are reported by Elvira, Almodóvar and Lobón-Cerviá (1991a).

Atlantic rivers

In the past, sturgeon entered the larger Iberian rivers from the Minho to the Guadalquivir, although their spawning could only be proved in the Douro, Guadiana, and Guadalquivir Rivers. It still lives, at least, in the Guadalquivir (Elvira and Almodóvar, 1993), and apparently reproduced in the Douro up to the 1970s and the Guadiana in the early 1980s (Almaça, 1988). Sturgeons entering the Guadalquivir and the Guadiana Rivers proceed from the population living in Cadiz Bay, according to Lelek (1987) the other population still extant in western Europe.

We have some knowledge of the remote past situation in Portuguese rivers through the humanist André de Resende (1500-1573), whose posthumously published book *De antiquitatibus Lusitaniae* (1593) includes a chapter entirely devoted to the Portuguese rivers and the names of the sturgeon. The book has recently been translated and annotated by Rosado-Fernandes (Resende, 1996). Special comments on the sturgeon in the Guadiana and the translation of the first known document recording the presence of the species in Portugal have also been published by Rosado-Fernandes (1986).

Through Resende's book (Resende, 1996) we know that the *asturjão* or *soilho* –the old Portuguese vernacular names of the sturgeon, which gave place, respectively, to the present ones *esturjão* and *solho*– was large and very good in the Minho, but small in the Lima. In the Douro it was less common than in the Minho, and it was very rare in the Tagus. Sturgeons of moderate size entered the Guadiana from March to the summer.

So we know that, even in the remote past, the Tagus –the longest Iberian river– does not appear to have been a preferred river for the sturgeon, at least not when compared with the Douro and the Guadiana (Baldaque da Silva, 1891). There is some biogeographical consistency in this, since the two remaining sturgeon populations in western Europe are to be found in the Bay of Biscay and Cadiz Bay, as already noted. It has long been known that the most suitable spawning rivers for each one of these populations are, respectively, the Dordogne/Garonne and the Guadalquivir. It appears that the sturgeon, beyond these preferential rivers, would only by chance select a spawning river far from the more accessible suitable Iberian rivers (the Minho and the Douro for the Bay of Biscay population, and the Guadiana for the Cadiz Bay population). Nevertheless, there are reports, in addition to Resende's, both in the remote and the recent past, of sturgeon in the Tagus or near its mouth.

For example, in February 1321, King Diniz of Portugal (reigned 1279-1325) ordered the publication of a text "as a record for those who may later read this public document" reporting the capture of a sturgeon at Valada (Santarém), nearly 100 km from the mouth, which measured approximately 3.75 m in length (17 palms in the measurements of the time) and weighed some 275 kg (17.5 *arrobas*). It exhibited 30 lateral scutes "from head to tail, like shells". The size of this specimen, the rarity of the sturgeon in the Tagus, or both, justified this official document, which was transcribed by Rosado-Fernandes (1986).

Another specimen, 1.8 m long, was caught on 30 November 1940 at the mouth of the Tagus (Gonçalves, 1942), which was erroneously identified as *A. naccarii.* The characteristics of this specimen, described by Gonçalves (1942), show, however, that it was in fact *A. sturio*, a species given to great variability (Almaça, 1988). The presence of the sturgeon at the mouth of the Tagus in late November suggests the existence of a winter race of the Atlantic sturgeon, as has been suspected also in the Guadalquivir (Holčík *et al.*, 1989).

The present and recent past distribution of the sturgeon in the other Atlantic rivers are summarised below, following a north to south sequence:

– Minho River: A specimen measuring 1 750 mm (Tl) was captured in 1961. It is housed in the Vasco da Gama Aquarium, Dafundo.

– Ave River: In August and September 1893, Vieira examined the fish that was discharged daily from the boats at Póvoa de Varzim (close to the mouth of the Ave). He reports having seen two specimens of *A. sturio* (Vieira, 1893).

– Douro River: Until the river was first dammed, in 1971, the sturgeon ascended it and was considered a common species in the Tua-Barca d'Alva stretch, mainly at Pocinho and Almendra (Baldaque da Silva, 1891; Teixeira, 1925; Nobre, 1935). At these places, small specimens weighing 1-4 kg were commonly fished. Larger specimens (40-100 kg) were also captured, sometimes up to three in the same haul (Teixeira, 1925). Nevertheless, only two specimens housed in the Museum of Zoology, University of Porto (one labelled Douro River, Barca d'Alva, June 189(?), Tl = 227 mm; the other Douro River, May 1916, Tl = 1 020 mm), are known as having indeed been caught in the Douro. Three more specimens in the same collection (Tl = 1 485 mm, 485 mm, and 405 mm), and two specimens housed in the Rodrigues de Freitas School, Porto (Tl = 915 mm and 245 mm), are not labelled, but could also proceed from the Douro River. From time to time, specimens captured in the Douro were sold at Porto fish market (Nobre, 1935), being eventually bought for zoological collections.

Sturgeon reproduced in the upper reaches of the Douro until the early 1970s, since one small specimen (Tl = 264 mm), now stored in Bocage Museum (Lisboa), was collected at Freixo de Espada à Cinta, nearly 200 km from the mouth, on 12 July 1972. Hence, Lobón-Cerviá, Elvira and Rincón (1989) are wrong when they state that the sturgeon has been extirpated in the Douro since the 1950s. It is known that when the first dams were built (Carrapatelo in 1971, Régua in 1973, Valeira in 1976, and Pocinho in 1983) some adult specimens remained landlocked, having been observed and fished until 1984 (Almaça, 1988).

– Mondego River: Vieira (1898) reports one specimen collected at Buarcos (mouth of the Mondego) on 11 July 1897. The specimen is housed in the Zoological Museum of the University of Coimbra, No. 46b, Tl = 1 530 mm (Almaça, 1988).
– Sado River: Two sturgeon specimens are known as having been captured at the mouth of the Sado (Setúbal) or close to it (Sesimbra). The first was in the collection of the Museum of Natural Sciences, Madrid (Lozano-Rey, 1919); the second in the Zoological Museum of Porto (Nobre, 1904).
– Guadiana River: According to reports from fishermen, sturgeon reproduced in the Guadiana up to the early 1980s (Almaça, 1988). At the end of the 19th century, it was considered a common species in the Guadiana, mainly at Mértola, nearly 60 km from the mouth, where it probably found suitable spawning grounds (Baldaque da Silva, 1891; Pimentel, 1894). However, according to Steindachner (1866b), it ascended the Guadiana up to Mérida. This record is somewhat questionable, since upstream and not far from Mértola, the Guadiana stretches out and presents a downward slope of remarkable magnitude (Pulo do Lobo); it is hard to believe the fish could pass. Since we know that sturgeon were sent to fish markets in several towns, and that Steindachner gathered his Iberian fish collection precisely in these markets, his record of the species to Mérida becomes even more ambiguous.

  Vieira (1898) refers to a specimen housed in the Zoological Museum of Coimbra caught at Mértola, which was not found by Almaça (1988). In the Vasco da Gama Aquarium is stored a specimen from Mértola (Tl = 730 mm), fished in 1954. A sturgeon is also reported from Ayamonte on 1 February 1943 (Classen, 1944; Elvira, Almodóvar and Lobón-Cerviá, 1991a), as well as Lagos and Tavira (Anon., 1818). Fishermen reported the capture of one adult specimen (70 kg) at Mértola in the early 1970s, and the presence of small specimens (20-30 cm long) during the early 1980s in the lower Guadiana (Almaça, 1988).
– Guadalquivir River: As noted above, this is the only Atlantic river of the Iberian Peninsula where the sturgeon is not extirpated. In the recent past, the high abundance of sturgeon in the Guadalquivir was not comparable with any other Atlantic Iberian river. Elvira, Almodóvar and Lobón-Cerviá (1991b) recently compiled the available information about sturgeon in the Guadalquivir, and so it will not be repeated here. Suffice it to quote that from 1932 to 1943, a total of 1 832 specimens (76 600 kg), among which 1 484 were females (69 680 kg), were captured. A dam built at Alcalá del Río (nearly 100 km from the mouth) in 1930 reduced the spawning area of the sturgeon in the Guadalquivir. Therefore, from the 1940s onwards, a progressive reduction of the captures was noted. In any case, from 1932 to 1954 a total of 3 186 sturgeon (2 544 females) were caught. Exploitation for caviar and smoked flesh from the 1930s to the 1960s apparently ex-

hausted the population and so, from the 1970s on, the sturgeon became a rarity, even in the Guadalquivir.

The last known record of sturgeon in the area is a female (Tl = 2 100 mm) fished near the Guadalquivir River mouth on 14 September 1992 (Elvira and Almodóvar, 1993). Given the recent past history of *A. sturio* in the Guadalquivir, which proves its suitability as a spawning river, a restoration programme appears to be urgently needed for this species (Elvira, Almodóvar and Lobón-Cerviá, 1991b).

Specimens from the Guadalquivir are housed in the collections of the National Museum of Natural Sciences (Madrid), Doñana Biological Estation (Seville), Aquatic Ecology Station (University of Seville), Francisco Ibarra Collection (Seville), and Aguilar y Eslava Institute (Córdoba).

Mediterranean rivers

The only river of the Spanish Mediterranean coast where *A. sturio* was once common is the Ebro. The historical occurrence of the sturgeon in the Ebro has been recently reviewed in a volume edited by Fernández-Colomé and Farnós (1999).

Sturgeon spawned in the Ebro, where many adult and juvenile specimens are known to have been caught, and sometimes preserved in zoological collections. In historical times, the sturgeon ascended the Ebro upstream to Tudela, about 490 km from the river mouth (Farnós and Porres, 1999). However, the construction of a weir at Xerta during the 15th century cut the species distribution to about 56 km from the mouth. The literature shows many references to sturgeon's occurrence in the Ebro (Steindachner, 1866a; Gibert, 1911, 1913; Lozano-Rey, 1919). Likewise, some specimens are housed in zoological collections: National Museum of Natural Sciences (Madrid) and Zoology Museum (Barcelona) (Elvira, Almodóvar and Lobón-Cerviá, 1991a; Porres and Farnós, 1999). The regression of sturgeon in the Ebro intensified during the 20th century, and the last adults were fished in 1965 and 1966; the last juvenile was caught in 1970 (Porres and Farnós, 1999).

The occurrence of sturgeon in other Spanish Mediterranean areas is summarised below (Elvira, Almodóvar and Lobón-Cerviá, 1991a).

North to the Ebro River, Gibert (1911, 1913) reported sturgeon from Cape Creus, near the Spanish-French border. One young specimen, Tl = 1 230 mm, was fished near Blanes in early July 1949 (Arté, 1949). Likewise, Sánchez-Comendador (1904) included *A. sturio* among the fish species reported from the coasts of Barcelona.

South to the Ebro River, and after Cisternas (1877), *A. sturio* was not rare on the Valencian coasts, and occasionally occurred in the lower sections of the Turia and Júcar Rivers, near the river mouths, mainly in early summer.

Finally, Barceló y Combis (1868) and Fage (1907) reported *A. sturio* from the Balearic Islands.

Conservation

If in western Europe there are only two sturgeon populations, both off the Iberian coast, as stated by Lelek (1987), past empirical data show that preferential spawning rivers for these populations are, or were, the Dordogne/Garonne for the Bay of Biscay population and the Guadalquivir for the Cadiz Bay population. Plausibly, the Minho and the Douro could be used as alternative spawning areas for the Bay of Biscay population, as well as the Guadiana for the Cadiz Bay population. If so, the sturgeon captured at the mouths of the Mondego, Tagus, and Sado Rivers, as well as far upstream in the Tagus, were merely strays from one, the other, or both populations. Genetic markers could help us to elucidate the origins of these strays if isolation by time and distance has caused some genetic divergence between the Biscay and Cadiz populations.

Overfishing of spawning sturgeon was apparently the main cause of *A. sturio*'s present endangered status in both Iberian countries. Other factors, however, appear to have contributed to the extirpation, or near extirpation, of the sturgeon (Almaça, 1988; Elvira, 1996). Damming was crucial in the Douro River, since no suitable fish passes were included in the dams. The dam at Alcalá del Río, in the Guadalquivir, cutting down the upstream migration, reduced the sturgeon's spawning area there. In the Guadiana, damage to spawning grounds seems to have been primarily caused by sand and gravel extraction. It is possible that water quality also contributed negatively to the migration of spawners into the Guadiana.

Restoration programmes for the rivers where sturgeon spawning has been confirmed in the recent past are indispensable for the conservation of the species in western Europe. However, since the main depletive factors appear to vary from one river to another, each one must be considered individually to increase the efficiency of such restoration programmes.

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Preliminary results from characterization of the Iberian Peninsula sturgeon based on analysis of the mtDNA cytochrome *b*

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ABSTRACT

Historically, the Atlantic sturgeon *Acipenser sturio* L., 1758 was considered the only sturgeon species that inhabited rivers of the Iberian Peninsula. Nevertheless, in a recent paper, Garrido-Ramos *et al.* (1997) identified specimen EBD 8174 (museum collection of the Doñana Biological Station, Spain), from the Guadalquivir River, as the Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836, suggesting that both *A. sturio* and *A. naccarii* could be native to Spain. To test this theory, we compared partial mitochondrial cytochrome *b* (cyt *b*) gene sequences obtained from museum specimens of *A. sturio* that originated from the Iberian Peninsula, the Adriatic and the North Sea, as well as from live individuals of the Gironde River population in France. Specimens of *A. naccarii* and of the Siberian sturgeon *Acipenser baerii* Brandt, 1869 from fish farms were also included in the study. DNA from museum specimens was successfully amplified using the protocol of France and Kocher (1996) for DNA extraction from formalin-fixed and ethanol-preserved samples. Phylogenetic analysis was performed on partial cyt *b* gene sequences (over 402-bp and 245-bp). We identified specimen EBD 8174 as *A. sturio*, confirming the opinion that *A. sturio* is the only sturgeon species of the Iberian Peninsula. Further molecular analyses of museum specimens are needed for a description of the historical intraspecies genetic variation within *A. sturio*. This information is crucial for any future recovery plan for this species. Our comparison also showed interspecies sequence divergence ranging from 6.91 % (*A. sturio/A. naccarii*) to 7.43 % (*A. sturio/A. baerii*).

Key words: *Acipenser sturio, Acipenser naccarii, Acipenser baerii*, genetic variation, molecular analysis.

RESUMEN

***Resultados preliminares de la caracterización del esturión de la península Ibérica mediante el análisis del gen citocromo* b**

Históricamente, el esturión atlántico Acipenser sturio *L., 1758 ha sido considerado la única especie de esturión que vive en los ríos de la península Ibérica, siendo así demostrado por múltiples estudios morfológicos recientes. Sin embargo, un trabajo reciente identificó el ejemplar del río Guadalquivir EBD 8174 como* Acipenser naccarii *Bonaparte, 1836, sugiriendo que ambas especies de esturión podrían ser nativas de España. Para comprobar esta nueva teoría, se han analizado las secuencias del gen citocromo* b *mitocondrial en muestras de esturión atlántico preservadas en varios museos procedentes de la península Ibérica, el mar Adriático y el mar del Norte, así como material fresco del río Garona. Además, se han estudiado con fines comparativos varias muestras de* A. naccarii *y* Acipenser baerii *Brandt, 1869 procedentes de piscifactorías. Las muestras de museos fueron amplificadas con éxito usando un protocolo específico utilizado para material fijado en formol y preservado en alcohol. La muestra de esturión de España (EBD 8174) fue identificada como* A. sturio. *Este resulta-*

do confirma que A. sturio *es la única especie de esturión en la península Ibérica. De cualquier manera, antes de abordar los planes de recuperación, sería necesario realizar análisis moleculares complementarios para describir las posibles variaciones genéticas existentes entre poblaciones. La comparación de las secuencias de citocromo* b *de* A. sturio *con* A. naccarii *y* A. baerii *mostró valores de divergencia de 6,91 % y 7,43 % respectivamente.*

Palabras clave: Acipenser sturio*,* Acipenser naccarii*,* Acipenser baerii*, variación genetica, análisis molecular.*

INTRODUCTION

The Atlantic sturgeon *Acipenser sturio* L., 1758, is native to Western Europe and historically was present in many large Spanish rivers (Holčík *et al.*, 1989). Moreover, *A. sturio* was considered the Iberian Peninsula's only sturgeon (Almaça, 1988; Doadrio, Elvira and Bernat, 1991; Elvira, Almodóvar and Lobón-Cerviá, 1991a, b; Elvira and Almodóvar, 1993; Pereira, 1995). The *A. sturio* population in the Guadalquivir River was intensively exploited for caviar beginning in the 1930s, and its population dropped dramatically from the 1960s (Elvira, Almodóvar and Lobón-Cerviá, 1991a,b).

Contrary to the prevailing scientific opinion, Garrido-Ramos *et al.* (1997) recently suggested that two sturgeon species could be native to Spain: not only *A. sturio*, but also the Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836, which was formerly recognized as endemic to the Adriatic Sea basin only (Tortonese, 1989; Birstein and Bemis, 1997). In addition to a revaluation of morphological data, Garrido-Ramos *et al.* (1997) claimed that their conclusion was supported by a molecular identification of two specimens, EBD 8173 and EBD 8174, from the collection of the Doñana Biological Station (Spain), as *A. naccarii.* However, three laboratories in different countries could not confirm these results because the paper of Garrido-Ramos *et al.* (1997) did not contain a description of the molecular methods used, and, therefore, its results could not be reproduced (Doukakis *et al.*, 2000). Doukakis *et al.* (2000) suggested that Garrido-Ramos *et al.* (1997) might have worked not with the authentic extracted DNA, but with a contaminant. Furthermore, new morphological studies of these two specimens, and a comparison of them with many museum sturgeon specimens from rivers of the Iberian Peninsula, are in disagreement with the conclusions of Garrido-Ramos *et al.* (1997) (Elvira and Almodóvar, 1999, 2000; Rincón, 2000).

In the meantime, data concerning the molecular taxonomy of *A. sturio* are scarce (Wirgin, Stabile and Waldman, 1997; Birstein, Betts and DeSalle, 1998; Birstein and DeSalle, 1998; Ludwig and Kirschbaum, 1998). A molecular study of all available *A. sturio* specimens, including those kept in different museums, is crucial to any recovery programme for this species (Birstein and Doukakis, 2000; Ludwig *et al.*, 2000). Consequently, the goals of the present study were: (1) To elaborate a reliable method of DNA extraction from the museum (archival) specimens; (2) To identify and characterize by molecular methods the *A. sturio* specimens from the Iberian Peninsula and other European areas; (3) To determine the relationships between *A. sturio* and related sturgeon species.

MATERIALS AND METHODS

Sample collection

For DNA extraction, we used muscle tissue (from fresh, stuffed or ethanol-preserved specimens) from 7 specimens of *A. sturio*, 3 *A. baerii* and 3 *A. naccarii*, from different localities (table I). Fresh material was stored at –80 °C until DNA extraction.

DNA extraction

Homogenization after freezing of fresh tissue samples in liquid nitrogen was followed by a standard procedure of phenol-dicloromethane extraction and alcohol precipitation (Wirgin *et al.*, 1997). Tissues from stuffed samples were incubated in 1 ml of shaking TE9 buffer (500 mM Tris, 20 mM EDTA, 10 mM NaCl, pH 9.0; Shiozawa *et al.*, 1992) at room temperature for 24 hours, with one change of the buffer. The TE9 buffer was discarded and the tissue samples were then minced and incubated for 5 hours at 55 °C in TE9 buffer containing 50 µl of 20 % sodium dodecyl sulfate (SDS) and 12.5 µl of 30 mg/ml Proteinase K and incubated at 55 °C during 5 hours. Thereafter, another 12.5 µl of 20 mg/ml Proteinase K was added and the tissue

Table I. List of sturgeon samples studied and cytochrome *b* regions sequenced

Sample	Species	Collection/Museum	Location of capture or farm	Preservation	Fragment of cyt *b*
S1	*A. sturio*	Doñana Biological Station, Seville EBD 8174, May 1975	Guadalquivir River, Alcalá del Río, Seville, Spain	Stuffed	155-bp
S2	*A. sturio*	Cemagref, Bourdeaux	Gironde River, France	Fresh	402-bp
S3	*A. sturio*	Cemagref, Bourdeaux	Gironde River, France	Fresh	402-bp
S4	*A. sturio*	Cemagref, Bourdeaux	Gironde River, France	Fresh	402-bp
S5	*A. sturio*	Cemagref, Bourdeaux	Gironde River, France	Fresh	402-bp
S6	*A. sturio*	Senckenberg Museum, Frankfurt SMF 2448	Adriatic Sea, Trieste, Italy	Ethanol	155-bp
S7	*A. sturio*	Senckenberg Museum, Frankfurt SMF 7637	Eider River, Nübbel, Germany	Ethanol	155-bp
B1	*A. baerii*	Doñana Biological Station, Seville EBD 8157, September 1995	Guadalquivir River, Coria del Río, Seville, Spain (released)	Fresh	402-bp
B2	*A. baerii*	Department of Animal Biology I Complutense University, Madrid, 1996	Duratón River, Spain (released)	Fresh	402-bp
B3	*A. baerii*	Department of Animal Biology I Complutense University, Madrid, 1996	Duratón River, Spain (released)	Fresh	402-bp
N1	*A. naccarii*	Aquatic Ecology Station, Seville	Sierra Nevada fish farm, Spain	Fresh	402-bp
N2	*A. naccarii*	Aquatic Ecology Station, Seville	Sierra Nevada fish farm, Spain	Fresh	402-bp
N3	*A. naccarii*	Aquatic Ecology Station, Seville	Sierra Nevada fish farm, Spain	Fresh	402-bp

samples were incubated again overnight at 55 °C. Standard phenol-dicloromethane 1:1 extractions were used to isolate DNA. DNA was precipitated in 1.6 ml of ice-cold 100 % EtOH and 0.1 vol. of 3 M sodium acetate (pH 6.8); incubated at –20 °C overnight; centrifuged at 16 000 g for 5 min at 4 °C; and washed in cold 70 % EtOH. Samples were air-dried and resuspended in 10-30 µl of ddH_2O.

PCR amplification

The PCR 402-bp fragments of the cytochrome *b* (cyt *b*) gene were amplified using universal primers (L14841; 5´- AAAAAGCTTCCATCCAACATCT-CAGCATGATGAAA-3´, H15149; 5´-AAACTGCAG-CCCCTCAGAATGATATTTGTCCTCA-3´, Kocher *et al.*, 1989) synthesized by Boehringer Mannheim (Spain). Tissues may degrade due to the storage process, making DNA extraction from archival specimens extremely difficult (France and Kocher, 1996; Rosenbaum *et al.*, 1997; Wirgin *et al.*, 1997). Therefore, we designed a specific internal primer (L14993; 5´-ATGACTAATCCGAAATATTC-3´) to generate a 155 bp fragment in combination with H15149. This region was selected because of the high variability in it among *A. sturio*, *A. naccarii* and *A. baerii*.

Amplification was performed in 50 µl total volumes containing 1 unit of *Taq* I Polymerase (Biotools, Madrid, Spain), 5 µl of 10× reaction buffer (Biotools), 20 pmol of each primer, 2 mM $MgCl_2$, 1 µl of dNTP mix (12.5 mM stock; Biotools), and ddH_2O to volume. One microlitre of DNA was added to the PCR mix, and subjected to 35 cycles of amplification. DNA was amplified in a Perkin Elmer 9600 thermocycler using the following protocol: after a preliminary denaturation at 94 °C for 5 min, each cycle consisted of denaturation at 94 °C for 30 s, annealing at 44 °C for 30 s, and primer extension at 72 °C for 1 min. To improve the subsequent reamplification and sequencing, a final cycle was added, including an extension of 5 min at 72 °C. Negative controls (no DNA added) were run for each amplification. PCR products were electrophoretically separated in 1-1.5 % agarose gels with a molecular size ladder and stained in ethidium bromide solution to determine size and quality of fragments. Some PCR products from archived specimens were reamplified, in order to increase the quantity of DNA.

The amplification products were purified before sequencing, either with Qiaquick columns (Qiagen, Santa Clarita, CA, USA), or Biotools columns (Biotools, Madrid, Spain), following the manufacturer's instructions.

Sequencing of PCR products

After purification, sequencing was performed in both directions by dideoxy sequencing (Sanger, Nicklen and Coulson, 1977) with the primers we used for PCR amplification. Sequencing was performed in an ABI PRISM 377 automated sequencer (Applied Biosystems, Foster City, USA), using four-dye fluorescent technology.

The cyt *b* gene sequences

The following cyt *b* gene sequences from the related sturgeon species retrieved from GenBank were used for the phylogenetic study: *A. sturio* (accession no. AF006134), *A. baerii* (AF006123), *A. naccarii* (AF006150), *Acipenser sinensis* Gray, 1835 (AF006158), *Acipenser persicus* Borodin, 1897 (AF006156), *Acipenser oxyrinchus* Mitchill, 1815 (AF006154), *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 (AF006126), *Acipenser brevirostrum* LeSueur, 1818 (AF006124) and *Polyodon spathula* (Walbaum, 1792) (MTPSPCYTB).

Sequence analysis

Sequences were analysed on an ABI PRISM 377 automated sequencer and data were edited with a multiple sequence editor program (ABI 1992). The sequences are deposited in GenBank under accession numbers AF217206-AF217209.

All DNA sequences were aligned using Clustal X (Thompson, Higgins and Gibson, 1994). Sequence data obtained for the long (402 bp) and short (155 bp) cyt *b* segments were analysed for distance and character-based variation using PHYLIP (version 3.5; Felsenstein, 1993). Phylogenetic trees were generated according to the maximum parsimony, MP (Felsenstein, 1983), maximum likelihood, ML (Felsenstein, 1981), and the distance-based neighbour-joining, NJ (Saitou and Nei, 1987) algorithms. For the transition/transversion bias, pairwise distances between nucleotide sequences were computed according to Kimura's two-parameters model (Kimura, 1980). The statistical significance of branching orders was assessed by the bootstrap resampling technique (100 replicates). Trees were rooted using as an outgroup *P. spathula*, a close relative of the Acipenseridae (Bemis, Findeis and Grande, 1997). A majority-rule consensus tree was constructed using the CONSENSE program from the PHYLIP package. The Kishino-Hasegawa-Templeton test (Templeton, 1983; Kishino and Hasegawa, 1989) was used to determine branch lengths from distance and ML test analyses.

RESULTS

Characteristics of the cyt *b* gene partial sequences

The long amplified fragment (402-bp) corresponds to the 5´-end of the cyt *b* gene. The short am-

ATGGCAAACA	TCCGAAAAAC	ACACCCACTA	CTTAAAATTA	TTAATGGAGC	50
				1	
ATTCATTGAC	CTCCCCACAC	CCTCCAACAT	CTCCGTGTGA	TGAAA**Y**TTTG	100
2		3	4		
GCTCACTC**M**T	AGGCCTCTGC	CTTGT**S**ACAC	AAATC**Y**TAAC	AGGACTATTT	150
	5	6		7 8 9	
CTTGCAATAC	A**Y**TACACAGC	**Y**GACATTTCA	ACAGCCTTCT	CCTC**YR**T**Y**GC	200
	10				
CCACATCTGC	CGAGA**Y**GTAA	ATTACGGATG	ACTAATCCGA	AATATTCATG	250
	11	12 13			
CAAACGGGGC	CTC**Y**TTCTTC	TT**Y**AT**Y**TGCT	TGTACCTTCA	CGTAGCACGA	300
14 15	16 17			18 19 20	
GG**Y**AT**R**TACT	A**Y**GG**Y**TCATA	CCTCCAAAAA	GAAACCTGAA	A**Y**AT**Y**GG**R**GT	350
21 22	23		24	25 26	
RATCCT**Y**CTG	CT**Y**CTCACCA	TAATAACCGC	CTTCGTGGG**R**	TATGTA**Y**T**R**C	400
CC					402

Figure 1. Consensus sequence of a 402-bp cytochrome *b* fragment from *A. sturio*, *A. naccarii* and *A. baerii*. Numbers mark the variable positions among genotypes observed

Table II. Variable nucleotide positions of cytochrome *b* fragment sequences. Numbers refer to sequence position in figure 1

		Variable nucleotides																									
Genotypes	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
STU	S1	?	?	?	?	?	?	?	?	?	?	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
STU	S2	C	C	G	T	T	C	C	G	C	T	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
STU	S3	C	C	G	T	T	C	C	G	C	T	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
STU	S4	C	C	G	T	T	C	C	G	C	T	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
STU	S5	C	C	G	T	T	C	C	G	C	T	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
STU	S6	?	?	?	?	?	?	?	?	?	?	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
STU	S7	?	?	?	?	?	?	?	?	?	?	T	C	T	C	A	C	C	C	C	G	G	T	C	G	T	A
BAE 1	B1	T	C	C	C	C	T	T	A	T	C	C	T	C	T	G	T	T	C	C	A	A	C	T	A	C	G
BAE 2	B2	T	A	C	C	C	T	T	A	T	C	C	T	C	T	G	T	T	C	C	A	A	C	T	A	C	G
BAE 1	B3	T	C	C	C	C	T	T	A	T	C	C	T	C	T	G	T	T	C	C	A	A	C	T	A	C	G
NAC	N1	T	C	C	C	C	T	T	G	T	C	C	T	C	T	A	T	T	T	T	A	A	C	T	A	C	G
NAC	N2	T	C	C	C	C	T	T	G	T	C	C	T	C	T	A	T	T	T	T	A	A	C	T	A	C	G
NAC	N3	T	C	C	C	C	T	T	G	T	C	C	T	C	T	A	T	T	T	T	A	A	C	T	A	C	G

plified fragment (155-bp) corresponds to positions from 247 to 402 of the cyt *b* gene of the white sturgeon, *Acipenser transmontanus* Richardson, 1836 (Brown *et al.*, 1989; GenBank accession no. X14944).

Twenty-six polymorphic sites were found on the 402-bp fragment among 10 specimens of *A. sturio*, *A. baerii*, and *A. naccarii* (figure 1). They showed a single base-pair change and the sequence variation was predominantly due to transitions (n = 24). No deletions or insertions were observed. When we compared the 155-bp fragment obtained from S1, S6, and S7 with the other samples, we found 16 changes due to transitions in the sequences (table II). Both regions studied had a low G content (average 16.2 %) and almost equal A, C, and T contents (average 26.7, 29.3, and 27.8 %, respectively). The base composition of this cyt *b* region in *Acipenser* was similar to that found in teleosts (representatives of the order Perciformes) (Cantatore *et al.*, 1994).

Sequence variation and diversity of the cyt *b* genotypes

Four cyt *b* genotypes were identified in *A. sturio* (STU), *A. naccarii* (NAC), and *A. baerii* (BAE-1 and BAE-2) (table II). The sequence from the *A. sturio* specimen EBD 8174 was identical to those from sturgeon representing the Gironde River, Adriatic, and North Sea populations. However, at position 126 we found G in all *A. sturio* sequences, whereas Birstein and DeSalle (1998) identified T in the sequence from a North Sea individual. We did not observe any variable site within this fragment in *A. naccarii*, and we found a substitution (position 109) in one *A. baerii*.

Table III. Genetic distances among 245-bp sequences analysed following Kimura's two-parameter model. Cells in bold characters show the genetic distance analysed in the 155-bp sequence

	STU	BAE-1	BAE-2	NAC	*Acipenser sinensis*	*Acipenser persicus*	*Acipenser naccarii*	*Acipenser gueldenstaedtii*	*Acipenser brevirostrum*	*Acipenser oxyrinchus*	*Acipenser sturio*	*Polyodon spathula*
STU	0.00											
BAE-1	6.91	0.00										
BAE-2	7.43	0.41	0.00									
NAC	6.91	1.65	2.09	0.00								
A. sinensis	10.80	9.78	10.35	10.75	0.00							
A. persicus	6.91	1.65	2.09	1.65	10.75	0.00						
A. naccarii	7.83	2.50	2.95	0.82	11.73	2.50	0.00					
A. gueldenstaedtii	6.91	0.00	0.41	1.65	9.78	1.65	2.50	0.00				
A. brevirostrum	6.91	0.82	1.25	2.50	10.75	2.50	3.35	0.82	0.00			
A. oxyrinchus	4.23	9.67	10.23	8.73	12.73	8.73	8.73	9.67	10.62	0.00		
A. sturio	0.41	6.89	7.40	6.89	10.75	6.89	7.80	6.89	6.89	4.22	0.00	
P. spathula	12.95	12.89	13.52	12.89	8.36	12.89	13.92	12.89	13.92	14.96	12.89	0.00

Pairwise estimates of sequence divergence in the 402-bp fragment of the cyt *b* gene among haplotypes of *A. sturio*, *A. baerii* and *A. naccarii* were 0.41-7.43 % (table III). Evidently, the American Atlantic sturgeon *A. oxyrinchus* is the closest relative of *A. sturio*: we observed a 4.2 % divergence between the cyt *b* sequences of these species. *P. spathula*, which we used as an outgroup, presented the highest sequence divergence with *A. sturio* (12.9 %). *A. baerii* and *A. naccarii* showed a similar divergence from *A. sturio* (6.9 and 7.4 %, respectively).

The 402-bp fragment of the cyt *b* gene sequence was aligned with those of the related sturgeon species retrieved from GenBank. A phylogenetic analysis of this sequence data was performed to determine the position of the samples. The nucleotide sequence alignment consensus for all taxa was 245 bp long. Figure 2 shows the consensus tree built using the NJ method with a 7.17 ts:tv weighting assumption; *P. spathula* was used as an outgroup. The MP and ML analyses resulted in a similar and congruent tree after collapsing poorly

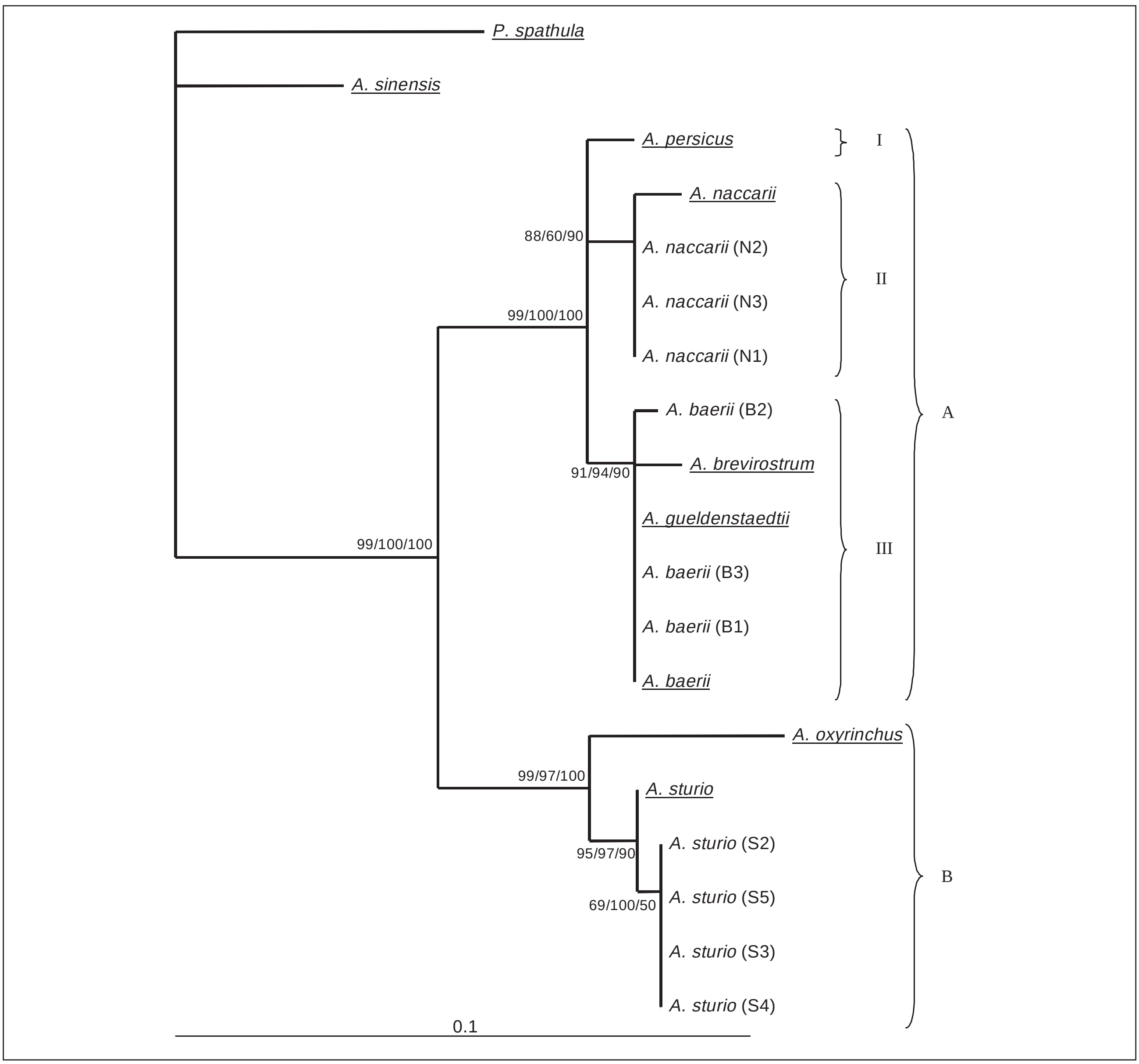

Figure 2. Estimated phylogenetic tree of 245-bp cytochrome *b* sequences. The percentages of bootstrap replicates supporting the clades are indicated at the branch points, based on (from left to right) neighbour-joining, maximum likelihood and equally-weighted parsimony methods. Bootstrap values < 50 % are not shown. Branch lengths are proportional to the estimated mean number of substitutions per site. GenBank species names are underlined

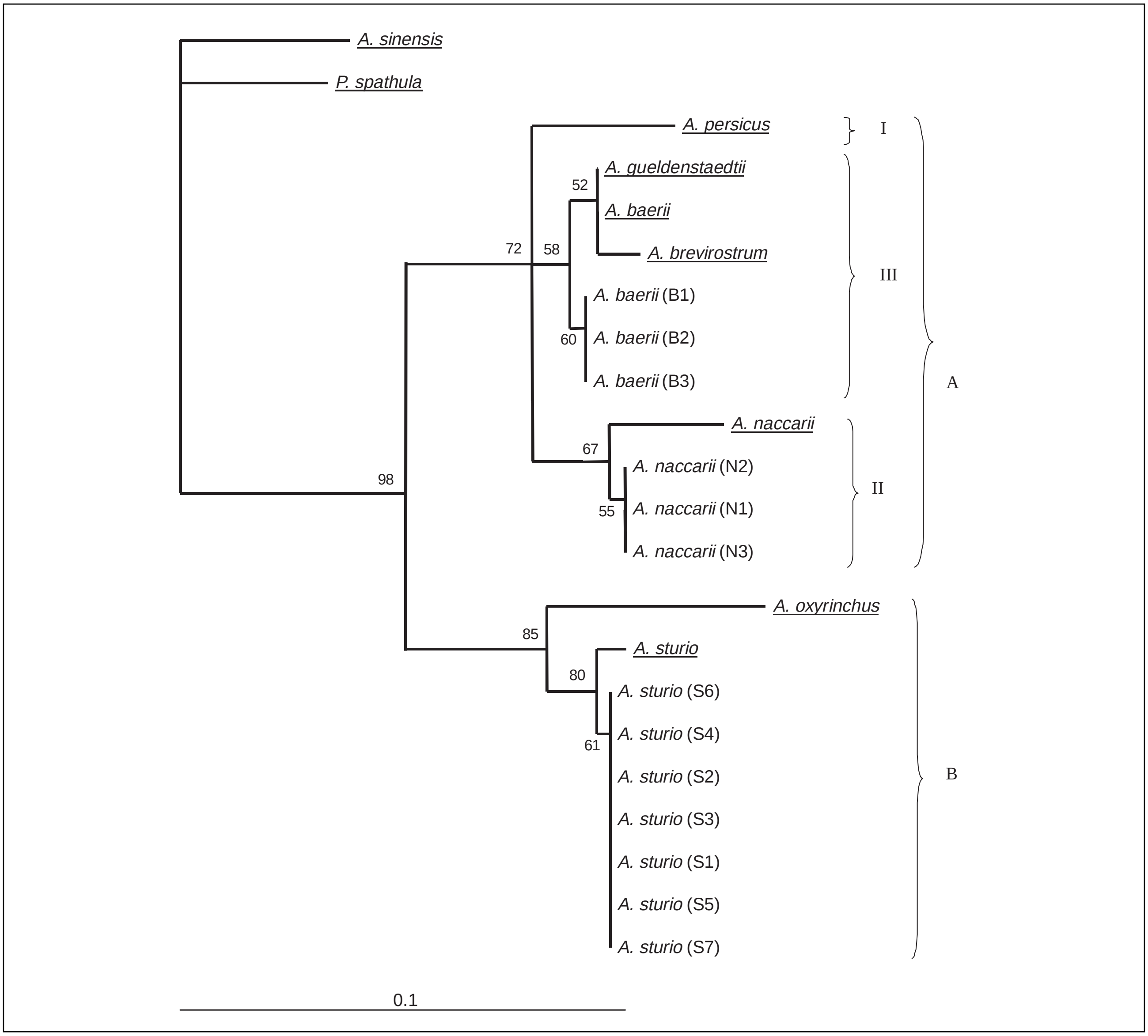

Figure 3. Estimated phylogenetic tree of 105-bp cytochrome *b* sequences according to the neighbour-joining method Kimura's distance. Bootstrap supports higher than 50 % are shown. Branch lengths are proportional to the estimated mean number of substitutions per site. GenBank species names are underlined

supported bootstrap values. The position of *A. sinensis* was not clearly resolved. Two clusters, A and B, can be clearly identified. Cluster A contained *A. persicus* (I), a clade of the *A. naccarii* specimens (II), and a clade which included *A. brevirostrum*, *A. gueldenstaedtii* and the *A. baerii* specimens (III). *A. persicus* was a sister species to (II) and (III). The clade (II) included all *A. naccarii* specimens, archival and alive. Its monophyly was well-supported (88 % for the NJ tree, 60 % ML tree, and 90 % for the MP tree). The *A. baerii* specimens analysed in the present paper, and the *A. baerii*, *A. brevirostrum*, and *A. gueldenstaedtii* specimens studied by Birstein and DeSalle (1998) (data from GenBank) also formed a well-supported clade. The second cluster, B, contained *A. oxyrinchus* and *A. sturio*.

The second phylogenetic tree was built using the 155-bp cyt *b* gene fragments obtained from *A. sturio* museum specimens originating from the Iberian Peninsula, Gironde River, North Sea, and the Adriatic (figure 3). The same overall pattern was observed for this short sequence as with the previous 245-bp segment. Sequences from all *A. sturio* samples, including that retrieved from GenBank, were clustered together, forming a monophyletic branch.

DISCUSSION

DNA from archival specimens

Currently, museum specimens are the only available material for *A. sturio* from many populations or from a geographical range of a population that has almost disappeared. Recent advances in molecular techniques offer a potential for DNA extraction from a diverse range of materials previously considered intractable (France and Kocher, 1996; Habelberg, Sykes and Hedges, 1989; Rosenbaum *et al.*, 1997; Pääbo, 1989; Wirgin *et al.*, 1997; see DeSalle and Bonwich, 1996, for a review). In our study the best yield in PCR products from archival samples was when we followed the protocol of France and Kocher (1996). However, only a short 155-bp fragment of the cyt *b* gene was successfully amplified. The tree based on the 105-bp sequences showed that a short sequence is suitable for discerning among the species studied: the nodes had good support bootstrap values (figure 3). We agree with Ludwig *et al.* (2000) that since *A. sturio* has almost disappeared in the wild, molecular analyses of specimens representing different populations in museum collections are crucial for any recovery plan for this species.

Phylogenetic implications

Clusters A and B in our trees (figures 2 and 3) generally support the phylogenetic relationships presented in Birstein and DeSalle (1998) for a longer region (650-bp) of the cyt *b* gene and for combined data for this region, plus a 350-bp fragment of the 16S rDNA and a 150-bp fragment of the 12S rDNA (figures 1 and 3 in that paper). The different position of *A. sinensis* in our tree, compared to the trees in Birstein and DeSalle (1998), was evidently caused by the high divergence of this cyt *b* gene fragment in *A. sinensis* from the other *Acipenser* species included in our study.

The relationships of species within clades of cluster A are basically congruent with the earlier phylogenetic studies. The position of the North American shortnose sturgeon of the Atlantic coast, *A. brevirostrum*, in our tree point to a close relatedness to the Russian sturgeon *A. gueldenstaedtii* inhabiting the Ponto-Caspian basin (Vlasenko *et al.*, 1989), and with the Siberian sturgeon *A. baerii*, living in Siberian rivers and Lake Baikal (Ruban, 1997). Morphologically *A. gueldenstaedtii* is similar to *A. persicus* (Vlasenko *et al.*, 1989) and *A. naccarii* (Tortonese, 1989), and it is considered to be related to *A. baerii* (Sokolov and Vasil'ev, 1989). Our results showed that *A. gueldenstaedtii* appears to be more closely related to *A. baerii* and *A. brevirostrum* than to *A. naccarii* and *A. persicus. A. persicus* is a sister species to the *A. baerii, A. gueldenstaedtii* and *A. brevirostrum* cluster, and to the *A. naccarii* clade. Conversely, Tagliavini *et al.* (1999) postulated a close relationship between *A. naccarii* and *A. gueldenstaedtii* and other clusters which includes *A. brevirostrum* and *A. baerii.*

Although Birstein and DeSalle (1998) used a much longer region of the cyt *b* gene (650 bp), their data showed that *A. gueldenstaedtii, A. persicus, A. baerii, A. naccarii,* and *A. brevirostrum* form an unresolved clade (figure 1 in their paper). They succeeded in placing *A. brevirostrum* outside this clade only when they used combined data for three genes, which contained a 1 150-bp-long sequence. In this analysis, *A. brevirostrum* was basal to the Ponto-Caspian and some Asian sturgeons (figure 3 in Birstein and DeSalle, 1998). A more detailed study of the relationships among Eurasian species of the same clade included a longer region of the cyt *b* gene (850-bp), a partial sequence of the ND5 gene (643-bp), and a fragment of the control region of mitochondrial DNA (725-bp), or approximately 2.3 kb bp (Birstein, Doukakis and DeSalle, in press). The traditionally recognised *A. gueldenstaedtii* in fact appeared to consist of two genetic forms, a "typical" and a cryptic one. The "typical" form is phylogenetically affiliated with *A. persicus* and *A. naccarii.* These data show that *A. persicus* is not a separate species; rather, it is conspecific with *A. gueldenstaedtii. A. naccarii* was diagnosed by only one G in position 164 of the control region. The cryptic form of *A. gueldenstaedtii* is very close genetically to *A. baerii*: it differed from *A. baerii* only by C-T transition at position 432 of the control sequence.

This example of complex species relationships found among a group of Eurasian sturgeons illustrates that reliable phylogenetic data can be obtained only if long DNA sequences are included in a study. Such results as those published by Tagliavini *et al.* (1999) can be considered only preliminary: these authors used very short sequences in their research and studied a small number of sturgeon species. The latter leads to an inadequate picture of species relationships due to the problem of underdiagnosis of phylogenetic lineages (Davis and Nixon, 1992). In general, the low genetic variability shown by acipenseriforms (Birstein, Hanner and DeSalle, 1997) makes a study of phylogenetic relationships within this order complicated.

A. sturio/A. oxyrinchus cluster and *A. sturio* intraspecies variability

The grouping of *A. sturio* with *A. oxyrinchus* (figures 2 and 3) is congruent with previous molecular phylogenetic results, and supports the hypothesis that these species might be direct descendents of ancestral forms of *Acipenser* (Birstein and DeSalle, 1998). The present molecular study has not detected genetic difference among specimens of *A. sturio* from the Iberian Peninsula, Gironde River, North Sea, and Adriatic. Likewise, Ludwig and Kirschbaum (1998) did not find variable sites in a 400-bp fragment of the 12S ribosomal DNA when they compared *A. sturio* specimens from the North Sea and Gironde River. However, Birstein and DeSalle (1998) obtained six nucleotide changes in a 600-bp fragment of cyt *b* in two specimens of *A. sturio*, suggesting intra-species forms of *A. sturio* (see Birstein and Doukakis, 2000, for further discussion).

The specimen EBD 8174

Garrido-Ramos *et al.* (1997) found a HindIII satellite (st) DNA in the genomes of two specimens of *A. naccarii* from a fish farm that was absent in two specimens of *A. sturio* from the Gironde River. The HindIII stDNA family was then considered a species-specific nuclear DNA marker (see Ruiz-Rejón *et al.*, 2000). Garrido-Ramos *et al.* (1997) attributed specimen EBD 8174 to *A. naccarii*, based on the presence of the HindIII sequences in the DNA extracted from this specimen. The present study enabled us to positively identify specimen EBD 8174 as *A. sturio*. Our conclusion supports the results of previous morphometric and meristic studies of this specimen (Elvira and Almodóvar, 1999, 2000; Rincón, 2000).

CONCLUSIONS

Our preliminary results did not show genetic differences between *A. sturio* individuals from the Iberian Peninsula, Gironde River, Adriatic, and North Sea. We identified specimen EBD 8174 from the Guadalquivir River as *A. sturio*. The comparison of the cyt *b* gene sequences among *A. sturio*, *A. naccarii*, and *A. baerii* showed 22-24 fixed nucleotide changes among species in that region. The analysis of our data set, together with that retrieved from GenBank, supports the hypothesis of Birstein and DeSalle (1998) that *A. oxyrinchus* is the only sturgeon species closely related to *A. sturio*. Since *A. sturio* has almost disappeared in the wild, the molecular analyses of specimens representing different populations of this species in museum collections are specially important in terms of recovery plans for this species.

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Predominance of exotic and introduced species among sturgeons captured from the Baltic and North Seas and their watersheds, 1981-1999

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ABSTRACT

Sturgeon catches (n = 256) from 1981-1999 reported mainly by commercial fishermen and anglers in German, Polish, and Dutch coastal waters and tributaries were analysed. During the study period, 20 % of catches were reported from coastal waters and 65 % from rivers and estuaries of large river systems, including the Odra, Elbe, Rhine and Weser. The data indicate that, from 1981-1993, there was a major decline in the Atlantic sturgeon *Acipenser sturio* L., 1758, and an increase in the total catches of non-indigenous sturgeon species. The Siberian sturgeon *Acipenser baerii* Brandt, 1869, the Russian sturgeon *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833, and various hybrids dominated. Occasional catches of the white sturgeon *Acipenser transmontanus* Richardson, 1836 and the sterlet *Acipenser ruthenus* L., 1758 were also reported. During the study period, significant changes in species composition and distribution of catches were observed. The predominance of non-indigenous sturgeon species is a result of the increasingly intensive sturgeon aquaculture activities in Germany, Poland, and the Netherlands. The most frequently reared species now dominate the catches. In addition to these escapees from fish farms, several intentional releases of sturgeons were reported. The results show that introduced exotic sturgeon species may thrive under certain natural conditions. Therefore, they may interfere with restoration efforts for the native *A. sturio*, competing for habitat and introducing diseases and hybridization.

Key words: *Acipenser,* aquaculture, introductions, distribution, conservation.

RESUMEN

Predominio de especies exóticas introducidas entre los esturiones capturados en el mar Báltico, en el Mar del Norte y en sus cuencas, 1981-1999

Se analizaron las capturas de esturiones (n = 256) entre 1981 y 1999 proporcionadas principalmente por pescadores profesionales y deportivos en las costas alemanas, polacas y holandesas, y en los sistemas fluviales que desembocan en ellas. En el periodo de estudio, el 20 % de las capturas correspondió a aguas litorales y el 65 % a ríos y estuarios de los grandes sistemas fluviales, incluidos los ríos Oder, Elba, Rin y Weser. Los datos *indican que entre 1981 y 1993 tuvo lugar el mayor declive del esturión atlántico* Acipenser sturio *L., 1758 y un incremento en las capturas totales de las especies alóctonas de esturiones. Predominaron las capturas de esturión siberiano* Acipenser baerii *Brandt, 1869, esturión ruso* Acipenser gueldenstaedtii *Brandt & Ratzeberg, 1833 y varios híbridos. También se registraron capturas ocasionales de esturión blanco* Acipenser transmontanus *Richardson, 1836 y esterlete* Acipenser ruthenus *L., 1758. En el periodo de estudio se observaron cambios significativos en la composición de especies y en la distribución de las capturas. El predominio de las especies alóctonas de esturiones es el resultado de las crecientes actividades en la acuicultura intensiva de esturión en Alemania, Polonia y Países Bajos. Las especies cultivadas más frecuentemente dominan ahora las capturas. Además de estas fugas de las piscifactorías, se han registrado varias sueltas intencionadas. Los resultados muestran que las especies de esturiones exóticas introducidas pueden prosperar en ciertas condiciones naturales. Por esta razón, pueden interferir en los esfuerzos de restauración de la especie autóctona* A. sturio*, compitiendo por el hábitat e introduciendo enfermedades e hibridación.*

Palabras clave: Acipenser, *acuicultura, introducciones, distribución, conservación.*

INTRODUCTION

The onset of the 20th century saw a major decline in the Atlantic sturgeon *Acipenser sturio* L., 1758 throughout its previous range due to anthropogenic impact (Mohr, 1952; Holčík *et al.*, 1989; Birstein, 1999). Since the 1950s, the occurrence of *A. sturio* in German, Polish, and Dutch coastal waters and tributaries has been limited to infrequent and incidental captures of single individuals (Spratte and Rosenthal, 1996).

Programmes to conserve and protect the Atlantic sturgeon are confronted not only with the scarcity or even absence of individuals, but also with the paucity of useful data on the species's biology and ecology. Moreover, the current aquaculture activities and the increasing importance of sturgeons in the pet trade since the early 1990s have contributed to a drastic increase in exotic sturgeon species in natural waters due to accidental or intentional releases by private persons or organizations. To assess this development, in 1994 the Society to Save the Sturgeon started monitoring these events (Arndt and Anders, 1997). The Society gathers data on the distribution of sturgeons, species composition of the catch, and catching techniques, in order to provide a sound database. The campaign also safeguards occasional catches of *A. sturio* in the study area, to be included in restoration attempts. The present study analyses the data collected by the Society on the 1981-1999 sturgeon catch.

MATERIALS AND METHODS

Data collection

Since the early 1990s, several research institutions have used questionnaires to uncover sturgeon catches. In 1994, a campaign was launched to obtain the collaboration of local fishermen and fisheries administrators in obtaining catch data and live specimens, featuring press releases, the distribution of information leaflets, and personal contacts. Data were collected by scientists in the respective regions, who attempted to identify sturgeon species. In instances when live fish were unavailable, fishermen were asked to provide photographs of dorsal, ventral, and side views of the whole fish. These specimens could only be confidently identified in a limited number of cases, since they were rarely photographed; in others, although fish were available, identification was not possible due to hybridization. When possible, total length and wet weight were taken.

Data analysis

Data analysis was performed after dividing the catches into two periods, period I (1981-1993) and period II (1994-1999). The cut-off between them was 1993, when the last *A. sturio* individuals were caught along the Dutch and German coasts and off Heligoland Island (Spratte and Rosenthal, 1996). To determine the temporal and spatial differences in the catches and species composition, a chi-square test (after Renner, 1981) was used.

RESULTS

A total of 256 sturgeons of various species and hybrids had been reported in the area since 1981. Differences in frequencies of catches, their distribution, and species composition were detected between periods I and II (figure 1, table I). With the exception of *A. sturio,* total sturgeon catches have increased in the study area during the 1990s. The total catches during the two periods differed significantly (n = 256; χ^2 = 5.441; d.f. = 2; p > 0.05). Peak catches of exotic sturgeons were recorded between 1994-1996 (figure 1). The catches originated from the entire observation area (figure 2). Most of the catches (85 %) were reported from coastal waters and large rivers, such as the Odra, Elbe, Weser, and Rhine. The remaining 15 % originated from canals, lakes, and other water bodies.

The origin of catches varied between the North Sea and the Baltic Sea areas. In 1981-1993, there was a significant difference between both areas in the number of recorded catches from coastal and estuary regions (n = 57; χ^2 = 7.13; d.f. = 1; p < 0.01). In the North Sea area, similar levels of catches were reported in rivers/estuaries and coastal waters. In the Baltic Sea, catches in rivers/estuaries far exceeded those from coastal waters. However, the situation was reversed during the second period, 1994-1999 (n = 162; χ^2 = 8.20; d.f. = 1; p < 0.01).

Species composition throughout the range revealed significant changes between the two periods (n = 256; χ^2 = 49.23; d.f. = 4; p < 0.001). Until 1993, *A. sturio* comprised 21 % of the catches in natural waters. The exotic species *Acipenser baerii* Brandt, 1869 and *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 were observed in similar proportions in the

Table I. Changes in total catch, species composition, and distribution of sturgeon catches in Polish, German, and Dutch coastal and inland waters, 1981-1999

	The North and Baltic Seas and tributaries		The North Sea and tributaries		The Baltic Sea and tributaries	
	1981-1993	1994-1999	1981-1993	1994-1999	1981-1993	1994-1999
Total catch (n)	62	194	42	85	20	109
A. sturio	16	2	16	2	0	0
A. baerii	11	56	11	23	0	33
A. gueldenstaedtii	8	11	1	0	7	11
A. ruthenus	1	0	1	0	0	0
A. transmontanus	0	3	0	3	0	0
Hybrids	2	12	2	9	0	3
Non-identified	24	110	11	48	13	62
Coastal waters	18	34	17	1	1	33
A. sturio	13	0	13	0	0	0
A. baerii	0	8	0	0	0	8
A. gueldenstaedtii	2	4	1	0	1	4
A. ruthenus	0	0	0	0	0	0
A. transmontanus	0	0	0	0	0	0
Hybrids	2	0	2	0	0	0
Non-identified	1	22	1	1	0	21
Rivers and estuaries	39	127	21	80	18	47
A. sturio	3	0	3	0	0	0
A. baerii	10	43	10	22	0	21
A. gueldenstaedtii	6	3	0	0	6	3
A. ruthenus	1	0	1	0	0	0
A. transmontanus	0	3	0	3	0	0
Hybrids	0	9	0	8	0	1
Non-identified	19	69	7	47	12	22
Other (lakes, canals)	5	33	4	4	1	29
A. sturio	0	2	0	2	0	0
A. baerii	1	5	1	1	0	4
A. gueldenstaedtii	0	4	0	0	0	4
A. ruthenus	0	0	0	0	0	0
A. transmontanus	0	0	0	0	0	0
Hybrids	0	3	0	1	0	2
Non-identified	4	19	3	0	1	19

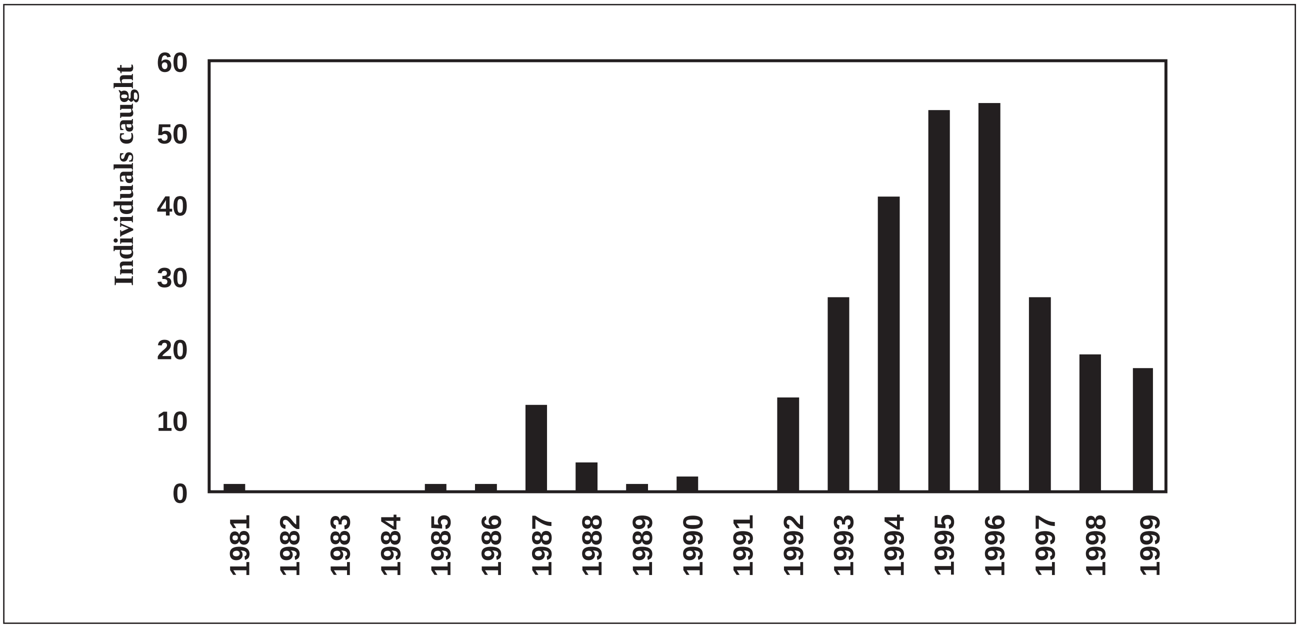

Figure 1. Development of sturgeon catches in Polish, German, and Dutch coastal and inland waters since 1981

catch during period I. In period II, *A. baerii* became dominant. Also, a significant increase was observed in the number of unidentified sturgeon species.

Species composition within specific regions also differed significantly between the two periods. In coastal waters ($n = 52$; $\chi^2 = 29.61$; d.f. = 4; $p < 0.001$), the main change was the drop in *A. sturio*. In rivers/estuaries, the main changes ($n = 166$; $\chi^2 = 18.81$; d.f. = 2; $p < 0.001$) were caused by the increase in *A. baerii* and unidentified species. In the North Sea and its tributaries, the change in species composition between the two periods was significant ($n = 127$; $\chi^2 = 35.69$; d.f. = 4; $p < 0.001$), whereas in the Baltic Sea and its tributaries, this was not the case ($n = 129$; $\chi^2 = 2.37$; d.f. = 2; $p > 0.05$).

When comparing the species composition of catches from the North Sea area to those from the Baltic Sea area for period I, significant differences ($n = 62$; $\chi^2 = 11.25$; d.f. = 2; $p < 0.01$) were observed due to the differences in catches in rivers/estuaries ($n = 39$; $\chi^2 = 9.36$; d.f. = 2; $p < 0.01$). During the second period, no significant differences were found between the two areas.

An assessment of catch by gear types was limited to period II. Earlier data had indicated that the majority of *A. sturio* were caught with active gear (i.e. trawls). For the second period, anglers (53 %) were the main source of catch reports, predominantly from rivers and estuaries. Set nets (37 %) with mesh sizes from 50-120 mm, mainly used in depths

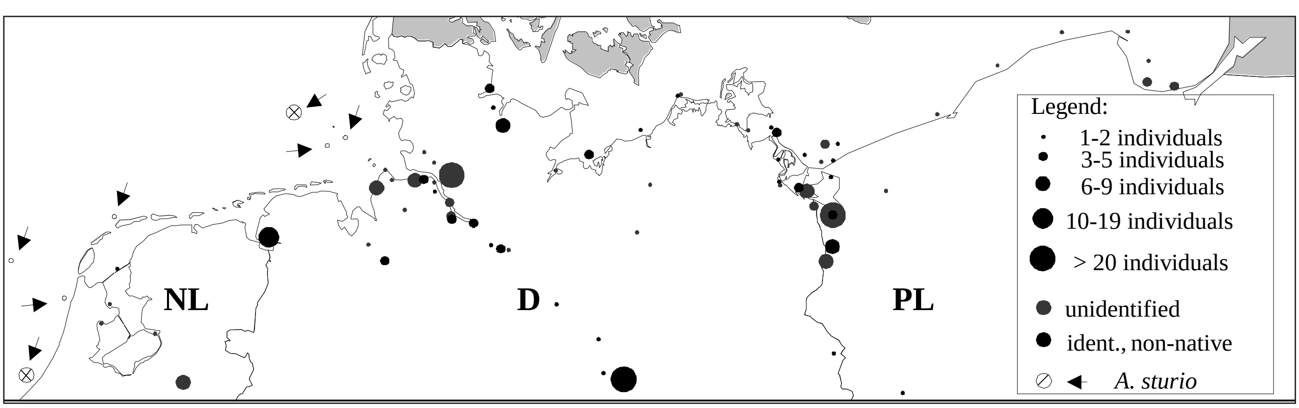

Figure 2. Distribution of sturgeon catches in Polish, German, and Dutch coastal and inland waters, 1981-1999 (modified after Gessner *et al.*, 1999)

from 2-18 m in coastal waters and estuaries, captured the second largest number of reported fish. Additional sources were water abstraction devices from nuclear power plants or factories (4 %), stow nets (4 %), fish traps (1 %), and miscellaneous others (1 %).

Also, we tried to estimate sturgeons' migration potential. The prerequisite was to include only those fish that could derive from a single mass release in a region where interference with other releases might be negligible. Accidental releases from aquaculture facilities occurred in a Polish tributary of the Odra River in 1992 and 1995, when thousands of *A. gueldenstadtii* and *A. baerii* escaped from net pens. The fish remained in the Szczecin lagoon area until they reached approximately 50 cm in length. Sturgeon specimens more than 70 cm in length were observed up to 400 km west in the Bights of Wismar, Lübeck, and Kiel. Their origin is unknown.

DISCUSSION

A. sturio was once a common fish, not only in German rivers and coastal waters, but in all major river systems of Europe, until the beginning of the 20th century (Holčík *et al.*, 1989). Since the 1950s, the species has been considered highly endangered throughout its previous range (Holčík *et al.*, 1989; Bless, Lelek and Waterstraat, 1994; Arndt, 1999).

During the study period, the occurrence of *A. sturio* in German and neighbouring waters was restricted to rare, incidental captures. The last individuals in the area were caught in Dutch and German coastal waters during the period 1981-1993 (Spratte and Rosenthal, 1996; Debus, 1997; Gessner *et al.*, 1999; table II). Contrariwise, the number of catches of non-indigenous sturgeon species increased during the 1990s (table I). This is especially alarming, because it is highly probable that only a minority of these total catches has been reported, as witnessed

Table II. Records of *A. sturio* in Polish, German, and Dutch coastal and inland waters since 1981. (1) NL: Netherlands

No.	Date	Location[1]	Length (cm)	Weight (kg)	Remarks	Author
1	Summer 1985	North Sea near Heligoland Island	140	12.80	–	Anon., 1985
2-5	Late 1980s	German Bight	–	–	Tagged	Steinert, 1990a, b
6	01 June 1989	German Bight	–	–		Lamp, 1989, 1990
7	08 January 1992	Merwede, the Rhine River delta, NL	70	–	–	Volz and De Groot, 1992
8	1992	Scheveningen, NL	98	–	–	Timmermanns and Melchers, 1994
9	25 February 1992	North Sea, Terschelling-Island, NL	135	8.50	–	Stolzenburg, 1992
10	04 May 1992	North Sea, 15 miles west of Ijmuiden, NL	125	–	–	Volz and De Groot, 1992
11	07 February 1993	Noordzeekanaal, Amdhaven, NL	61	–	–	Timmermanns and Melchers, 1994
12	May 1993	3 km off Kijkduin, NL	106	9.00	–	Timmermanns and Melchers, 1994
13	02 June 1993	Rede van Vlissingen, NL	135	8.90	–	Timmermanns and Melchers, 1994
14	12 June 1993	Noordzeekanaal zijkanal B, NL	51	–	–	Timmermanns and Melchers, 1994
15	01 September 1993	Oosten Aalsmeer, NL	35	–	–	Timmermanns and Melchers, 1994
16	26 October 1993	North Sea, south of Heligoland Island	285	142.50	–	Anon., 1993a, b
17-18	April 1996	Pond near Lake Constance	100	–	–	Originating from the Elbe River in the early 1980s

Table III. Natural range of the exotic sturgeon species caught in Polish, German, and Dutch coastal and inland waters, 1981-1993 (after Hochleithner, 1996)

Species	Natural range
A. baerii	Siberia: almost all Siberian rivers from the Ob to the Kolyma and adjacent coastal waters; Lake Baikal
A. gueldenstaedtii	Black, Azov, and Caspian Seas and their tributaries
A. ruthenus	fresh waters: tributaries of the Black, Azov, Caspian, White, and Cara Seas
A. transmontanus	Pacific coast of North America, from Alaska to California and major rivers

in several tagging experiments (Netzel, 1990; Bjordal and Skar, 1992). In addition, total captures most likely reflect only a small percentage of the population in different watersheds.

The registered catches peaked between 1994 and 1996 (figure 1). This could reflect either the increased harvest or increased reporting by fishermen, as a result of by the Society to Save the Sturgeon's public awareness campaign. The drop in reported catches from 1997 to 1999 may be attributed to the fact that fishermen became used to continuous catches and therefore lost interest in sturgeons, so that their co-operative efforts with the Society slackened.

The occurrence of exotic sturgeons is closely linked to increasingly intensive sturgeon aquaculture activities in Germany and neighbouring countries (Steffens, Jähnichen and Fredrich, 1990; Rosenthal and Gessner 1992; Arndt and Mieske, 1992, 1994; Gessner *et al.*, 1999). Catch data have revealed a rise in the number of cultured species during recent years. There were reports of sturgeons escaping from aquaculture facilities in the estuary of the Odra River in 1992 and 1995 (*A. gueldenstaedtii* and *A. baerii*), and from the Elbe River catchment area near Leipzig in 1995 (*A. baerii* × *A. ruthenus*). Apart from these escapees, additional releases by aquarists and/or anglers may have contributed to escapements, as confirmed for *A. baerii* in 1986 in the Ems River (Anon., 1987).

Non-indigenous sturgeon introductions pose a threat to ongoing or planned activities for the re-establishment of *A. sturio* within its native range (table III). A previous example of such a threat was the introduction of the Caspian beluga *Huso huso* (L., 1758) into the Sea of Azov, which led to the almost complete extinction of the endemic form (Pavlov *et al.*, 1994; Debus, 1997). Furthermore, the transfer of parasites and diseases must be considered another serious threat (Rosenthal and Gessner, 1990). Thus, a transfer of the stellate sturgeon *Acipenser stellatus* Pallas, 1771 from the Caspian into the Aral Sea resulted in a significant loss in the local population of the ship sturgeon *Acipenser nudiventris* Lovetzky, 1828 caused by the introduction of the nematode *Nitzschia sturionis* (Pavlov *et al.*, 1994; Zholdasova, 1997). Additionally, the potential interaction of non-indigenous species might interfere with natural reproduction, leading to hybridization (see Holčík, 1989 for a review). It remains unproven whether any of the introduced fish, including those introduced into the Baltic Sea during the late 1950s (Simanovskaya and Kuljusina, 1967; Otterlind, 1970; Kairov, 1975), reached sexual maturity, but the potential threat is real. Considering the fact that sturgeons tend to hybridize freely (Holčík *et al.*, 1989; Birstein, Hanner and DeSalle, 1997), there is sound reason to expect the introgression of exotic genotypes with native *A. sturio* gene pools.

Despite the fact that this development is considered detrimental, the introduction of exotic sturgeons offers the potential to investigate their migration patterns and vital rates. Such information might be beneficial when applied to re-establishment programmes. The data gathered since the escape of sturgeons from fish farms in the Odra River tributaries show that, in general, sturgeons still find niches in environments that have been drastically altered by human activities. This leads to the conclusion that the possibility of sturgeon survival cannot be neglected, as it was previously (Nellen, 1992).

In conclusion, we consider the following efforts are needed to continue monitoring the sturgeon situation in the Baltic and North Sea areas. Further research is necessary to determine the levels of incidental harvest and mortality of sturgeons in the areas under discussion. Fishery techniques should be improved to reduce sturgeon by-catch. Reports of captured sturgeons resulting from the survey carried out by the Society to Save the Sturgeon have already drawn public attention to conservation measures. Further information campaigns to increase public awareness are urgently needed to prevent anglers and aquarists from releasing fish into the rivers in naive attempts to support sturgeon stocks. Stricter safeguarding is required to avoid escapes from fish farms. Strict fish health regulations for imports, through the adoption of the ICES/EIFAC Code of Practice (Anon., 1995) are required. Improved control of the pet trade in sturgeons to avoid "littering" of the environment by exotic sturgeon species should also be considered.

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Distribution of *Acipenser sturio* L., 1758 in the Black Sea and its watershed

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ABSTRACT

The Atlantic sturgeon *Acipenser sturio* L., 1758, was always rare in the Black Sea, and the least numerous in comparison with other sturgeons. At the end of the 19th and the first half of the 20th centuries, it occurred along almost the entire Black Sea coast. It has been distributed predominantly in the eastern part of the Black Sea, especially in the region adjacent to Georgia's Inguri and Rioni rivers, with spawning grounds in the latter. It has also been recorded occasionally in the Danube River, where it occurred mostly in its delta. Earlier records even indicate its reproduction in the Danube. Recently its distribution and population density has been dramatically reduced. The distribution of this species is limited only to the eastern part of the Black Sea, and the recent total density of adults is estimated at only several hundred specimens. This situation has placed *A. sturio* on the list of critically endangered fish species. Existing data on its morphology and ecology indicate some differences between the Black Sea stock and populations from the Atlantic Ocean and the Baltic and Mediterranean Seas.

Key words: Atlantic sturgeon, Danube River, Rioni River, Inguri River, ecology, conservation.

RESUMEN

Distribución de* Acipenser sturio *L., 1758 en el mar Negro y su cuenca

El esturión atlántico Acipenser sturio *L., 1758 siempre ha sido raro en el mar Negro, y el menos numeroso en comparación con otros esturiones. A finales del siglo* XIX *y primera mitad del* XX *se encontraba a lo largo de toda la costa del mar Negro. Se distribuía predominantemente en la parte oriental del mar Negro, en especial en la región adyacente a los ríos Inguri y Rioni en Georgia, con áreas de freza en este último. También ha sido citado ocasionalmente en el río Danubio, donde ocupaba sobre todo su delta. Las citas más antiguas incluso indican su reproducción en el Danubio. Recientemente su distribución y densidad de población se han visto dramáticamente reducidas. La distribución de esta especie se limita sólo a la parte oriental del mar Negro, y la densidad total reciente de adultos se estima en sólo algunos cientos de individuos. Esta situación ha colocado a* A. sturio *en la lista de especies de peces en peligro crítico. Los datos conocidos sobre su morfología y ecología indican algunas diferencias entre la población del mar Negro y las del océano Atlántico y los mares Báltico y Mediterráneo.*

Palabras clave: *Esturión atlántico, río Danubio, río Rioni, río Inguri, ecología, conservación.*

INTRODUCTION

The Atlantic sturgeon *Acipenser sturio* L., 1758 is the rarest sturgeon species occurring in the Black Sea. Its presence in this region has been recorded quite late (Antipa, 1909), and its relative rarity was the reason why this species was not particularly studied in the countries along the western coast of the Black Sea. Sporadic records on its occurrence and catches are in papers by Antipa (1905, 1909, 1933), Bacalbașa-Dobrovici *et al.* (1984), Bănărescu (1964), Berg (1948), Borcea (1929, 1933), Drenski (1948), Elanidze (1983), Elanidze *et al.* (1970), Janković (1993, 1994, 1996), Karapetkova and Zhivkov (1995), Kasymov (1972), Kinzelbach (1997), Marinov (1966, 1978), Sal'nikov (1961), Svetovidov (1964), and Vuković and Ivanović (1973). Some of these papers also contain short notes on the biological phenomena of the Black Sea stock, but the most comprehensive information regarding these aspects can be found in Ninua (1976). See Holčík *et al.* (1989) for an extensive summary of all earlier records, including the Black Sea watershed. In the present paper, we provide a more complete picture of the occurrence, ecology and status of *A. sturio* in the Black Sea, including its tributaries.

RESULTS AND DISCUSSION

Past and present distribution of the Atlantic sturgeon in the Black Sea and its tributaries

The first record of *A. sturio*'s distribution in the Black Sea and its watershed is that of Antipa (1905, 1909). At the beginning of the 20th century, when the sturgeon populations of the Danube-Black Sea area were large, the Atlantic sturgeon was only seldom found. Specimens measuring up to 2 m in length and weighing 80 kg were caught by unbaited hooks. Reports of this species in the Danube River were also scarce. Antipa quotes fisherman, downstream of the Galati River (km 140) at the end of the 19th century, who caught only two specimens of this species during 37 years. It seems that at the time, the Atlantic sturgeon reproduced in the Danube, because Antipa (1933) recorded young-of-the-year sturgeon (5-15 cm) and even hybrids of this species with other sturgeons. He also suggested that *A. sturio* exceptionally spawns at the same time as other sturgeon species, on the sand banks off the Danube estuary. It is true that during the high waters of the Danube, in some years, the entire pre-estuary area is covered with fresh water for 2-3 months, but Borcea (1933) does not admit the Danube estuary to be the functional spawning area. Nevertheless, its spawning grounds have not been localised, and other spawning details also remain unknown.

Very accurate observations on *A. sturio* off the Romanian coast were carried out by Borcea (1929, 1933). He mentioned three specimens with a total length between 47 and 135 cm, caught with a dip net in the area off Agigea, and one specimen measuring 100 cm in the area off Constantza. Later on, Bușnitsa (1966) reported *A. sturio* as far upstream as km 400 on the Danube, but Bănărescu (1964) and Marinov (1978) raised serious doubts about its presence there and included only the Danube delta within its range (Holčík *et al.*, 1989).

Off the Bulgarian coast, the occurrence of *A. sturio* has been even rarer. It has occurred most frequently in the region of Varna and Burgas. Its migration upstream along the Danube to the Bulgarian segment of the river has not been confirmed (Marinov 1966, 1978). This species has occurred more frequently in the Struma and especially in the Maritsa Rivers (Aegean Sea watershed), where its upstream migration was recorded at Plovdiv (Drensky, 1948).

Controversial reports are those of Janković. For the Yugoslavian Danube, she (Janković in Bacalbașa-Dobrovici *et al.*, 1984; Janković, 1993, 1996) suggested that *A. sturio* would be present from Batina (river km 1422) up to the mouth of the Timok (river km 845) and also from the Djerdap (Iron Gate, river km 945). However, in one paper (Janković, 1994) she does not mention the occurrence of *A. sturio* in the Yugoslavian Danube. Vuković and Ivanović (1973), and, more recently, Simonović and Nikolić (1996), do not mention the occurrence of this species in the Yugoslavian stretch of the Danube, either. As noted by Sal'nikov (1961), the Atlantic sturgeon in the Danube is generally very rare, and practically does not occur here.

The presence of the Atlantic sturgeon along the Anatolian coast, where it was said to be the most numerous on the southern coast of the Black Sea (Pavlov, 1980) and in the Kizil-Irmak and Yesil-Irmak Rivers, as recorded by Zagorovskii (1928, after Svetovidov 1964), was not confirmed in a recent study (Kinzelbach, 1997).

On the eastern coast of the Black Sea, the Atlantic sturgeon occurred, and is still found, in the area adjacent to the Inguri and Rioni Rivers. Both rivers were entered by spawning shoals of this species up to the village of Dzhvari, some 70 km from the Inguri mouth (Pavliashvili, according to Kasymov, 1972) and regularly to Samtredia and rarely to Akhalsopeli and Bashi, i.e. 120-150 km from the mouth of the Rioni River (Ninua, 1976; Ninua and Tsepkin, 1984). The area inhabited by large juveniles of the Atlantic sturgeon adjacent to the Rioni mouth, up to 50 m in depth, has been estimated at 5 000-7 000 km^2, and the population density of the adult sturgeon at about 1 000 specimens (Ninua and Tsepkin, 1984).

Adult specimens of the Atlantic sturgeon were also recorded near Crimea, between Simferopol and Yalta, and in Karkinitskii Bay and on the western coast of the Black Sea between Odessa and the Danube River delta (Almazov, 1923, according to Svetovidov, 1964; Ninua, 1976).

During the last two decades, there have been no reliable reports of the occurrence of *A. sturio* in the western Black Sea. The probability of its catch is negligible compared with 1960; recently, we found that the number of young sturgeons caught with the dipnet method generally decreased 100-120 times. Most younger fishermen do not know about *A. sturio*. However, the occasional occurrence of *A. sturio* here cannot be rouled out. Also in the eastern Black Sea, in the Rioni River area, the density of the adult *A. sturio* population was reduced to no more than 300 specimens (Ninua and Tsepkin, 1984; Pavlov *et al.*, 1994). Kinzelbach (1997) has also reported that recently the abundance of adults in the area of the Rioni River was reduced to several hundred specimens, and the main reason for that drastic reduction was Georgia's military operations in Abkhazia.

However, the occurrence of *A. sturio* in the western part of the Black Sea is not excluded yet. People from the Fishery and Fish Farming Centre of Galati recently obtained one ripe female sturgeon in the Danube, 300 km from the river mouth. The 40 kg specimen showed some characters of *A. sturio*, but others were specific to *Acipenser gueldenstaedtii* Brandt, 1833. Unfortunately, the female did not survive transport and was sold, so positive identification is now impossible.

In summary, it can be said that the presence of *A. sturio* in the Black Sea at the end of 19th and during the first half of 20th centuries has been reported almost everywhere along the Black Sea coast. The most populated area has been that adjacent to the Rioni and Inguri Rivers on the eastern coast, with its main spawning grounds in the Rioni River. Occasional findings and even reproduction have also been recorded in the Danube River. At present, however, the distribution and also density of *A. sturio* was dramatically reduced. It seems that this species disappeared from the western Black Sea area and the Danube River, and the main –and probably the only– area inhabited by this species at present is the eastern coast of the Black Sea, including the Rioni River, its sole current spawning ground. Considering all aspects of this species's former and present distribution and population density in the Black Sea watershed, it is clear that its extinction is imminent. To save this species will be possible only as a result of the urgent and joint effort of several countries to solve problems involving its artificial reproduction. However, the most difficult task at present is to find enough specimens of parent fish in nature to carry out such work (Pavlov *et al.*, 1994).

The biology of the Atlantic sturgeon in the Black Sea

Because information on the biology of this species in the Black Sea is insufficiently known outside the former Soviet Union, and some aspects differ from those of *A. sturio* from the Atlantic Ocean, Baltic Sea, and the Mediterranean (see also Holčík *et al.*, 1989; Holčík, 2000; Elvira and Almodóvar, 2000), we decided to provide a review of the data presently available.

The first data on the biology of *A. sturio* in the Back Sea watershed were published by Marti (1939), and then used by Berg (1948), Svetovidov (1964), and Elanidze *et al.* (1970). However, the most complete information, based on much more material and observations from 1965-1975, has been published by Ninua (1976). In the following, we used her data.

This species's spawning area in the Rioni River is 120-130 km from its mouth. The lower boundary of the spawning grounds is characterised by a bottom covered with pebbles, and its upper boundary is limited by high current velocity, being 2 m s^{-1} or more. Spawning begins at the end of April and lasts until the beginning of July, peaking in May. The water temperature at that time varies from 13-18 °C. The upstream sturgeon migration speed extends to 10-15 km day^{-1}. After reaching the spawning grounds the weight of both sexes decreases by 25 %, even as much as almost 50 %, in comparison with initial migration weight. The brood stock of the Atlantic sturgeon in the Rioni River is composed of 6 age groups of males and 10 age groups of females, ranging from 7-12 and 11-20 years in age, respectively. The length of the sexually mature males varies from 96-152 cm and that of females from 110-215 cm, and their weight from 8-20 kg and 15-68 kg, respectively. The fecundity of females 141-215 cm in length and 23-68 kg in weight ranges from 0.789 to 1 815 million eggs. The autumn migration or the winter race of *A. sturio* in the Rioni River has not been observed. The downstream migration of juveniles begins in July, at an age of not less than 2-2.5 months. After reaching the river mouth their length and weight varies between 5-6 cm and 5-5.9 g, respectively. The length and weight of the young-of-the year juveniles in November ranges from 19-23 cm and 30-40 g. At least a part of the juveniles stay in the lower course and the mouth of river during the winter. The majority of the juveniles reach 18-22 cm in length by the end of their first summer. It is estimated that the area (coastal shelf up to 50 m deep) inhabited by large juveniles covers about 5 000-7 000 km^2. The diet of juveniles staying in the river mouth is mostly composed of chironomids, gammarids and pupae of various insects, while the juveniles staying in the sea feed mostly on gammarids, shrimps and fishes. The growth of males and females is almost equal, as females are negligibly longer than the males of the same age. The mean values of the back-calculated total length attained at the end of the 5th, 10th, 15th and 20th year of life is 78.0, 135.5, 183.0 and 214.5 cm, respectively. The life span of males is 12, that of females 20 years.

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Harvest and habitats of Atlantic sturgeon *Acipenser oxyrinchus* Mitchill, 1815 in the Hudson River estuary: Lessons for sturgeon conservation

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ABSTRACT

Conservation of the Hudson River population of the sturgeon *Acipenser oxyrinchus* Mitchill, 1815 has benefitted from the most intensive research programme on any population of the species. We review the history of the fishery for *A. oxyrinchus* in the Hudson River, and concisely summarise diverse research findings on its life history and habitat use. The fishery for *A. oxyrinchus* from the Hudson River had one period of very high harvest (pre-1900s), a long period (1900-1979) of minimal harvest and slow population recovery, a period (1980-1992) of restored abundance and high harvest, and finally another decline and suspension of fishing (1996). Sturgeon spawning and early juvenile development occurs in the freshwater portion of the Hudson River, whereas adult and large juvenile sturgeon occupy marine waters, and some of these fish will annually occupy low salinity sites in the Hudson River and other coastal rivers in summer. *A. oxyrinchus* of the Hudson River are genetically distinct from other populations associated with rivers along the Atlantic coast. Eight different habitats are used during the life cycle of Hudson River *A. oxyrinchus*, and these are described in physical and temporal terms. The history and biology of the Hudson River *A. oxyrinchus* suggest three lessons for sturgeon restoration: basic life history knowledge is essential, fishery management is difficult, and regular population monitoring will be needed from the start.

Key words: Fishery, life history, recovery.

RESUMEN

Explotación y hábitats del esturión atlántico* Acipenser oxyrinchus *Mitchill, 1815 en el estuario del río Hudson: lecciones para la conservación de los esturiones

La conservación de la población del río Hudson de esturión Acipenser oxyrinchus *Mitchill, 1815 se ha beneficiado del programa de investigación más intensivo de los proyectados en la preservación de cualquier otra población de la especie. Se revisa aquí la historia de la pesca de* A. oxyrinchus *en el río Hudson y se resumen concisamente diversos hallazgos de investigación sobre su historia natural y uso del hábitat. La pesca de* A. oxyrinchus *en el río Hudson tuvo un periodo de muy alta explotación (antes de 1900), un largo periodo de mínima explotación y lenta recuperación poblacional (1900-1979), un periodo de restauración de abundancia y alta explotación (1980-1992) y, finalmente, otro declive y suspensión de la pesca (1996). La puesta del esturión y el desarrollo temprano de los juveniles tienen lugar en el tramo de agua dulce del río Hudson, mientras los adultos y los juveniles grandes ocupan aguas marinas; algunos de estos peces ocuparán anualmente, en verano, lugares de baja salinidad en el Hudson y en otros ríos costeros. La población de*

A. oxyrinchus *del río Hudson es distinta genéticamente de otras poblaciones asociadas con ríos a lo largo de la costa atlántica. Durante su ciclo vital, el* A. oxyrinchus *del río Hudson utiliza ocho diferentes hábitats, que son descritos en términos físicos y temporales. La historia y la biología de esta especie del río Hudson sugieren tres lecciones para la restauración de los esturiones: es imprescindible el conocimiento básico de la historia natural, la gestión de la pesca es difícil y el seguimiento regular de la población será necesario desde el principio.*

Palabras clave: *Pesca, historia natural, recuperación.*

INTRODUCTION

Many sturgeon species throughout the world are threatened with extinction by overfishing and habitat loss (Birstein, 1993; Waldman, 1995; Birstein, Bemis and Waldman, 1997), and restoration efforts are being initiated to reverse some species declines and population losses. The sturgeon *Acipenser oxyrinchus* Mitchill, 1815 was once abundant in large rivers, estuaries, and marine waters along most of the Atlantic coast of North America. This valuable sturgeon has been greatly reduced by overfishing and alteration of essential habitat, such as the loss of spawning site access due to dams (Smith, 1985; Smith and Clugston, 1997; Waldman and Wirgin, 1998). *A. oxyrinchus* is now protected under one of the most complete and long-term management plans ever implemented for a fish species. The management goal is to restore the species to a safe level of abundance throughout its range. While the Hudson River population of *A. oxyrinchus* has been relatively abundant in the past, there is now good cause for concern about the status of this population.

The conservation of the Hudson River *A. oxyrinchus* population has benefitted from the most intensive research programme on any population of this species. Little stock-specific information was available until life history studies were performed in the 1970s and early 1980s. In the mid-1990s, a broad research effort by several university teams was funded and co-ordinated by the Hudson River Foundation for Science and Environmental Research. This research effort covered major aspects of the biology and management of Hudson River Atlantic sturgeon, including reproductive physiology, genetics, age structure and demographics, habitat use, behaviour, and fishery attributes. Our purpose in this paper is to review the history of the fishery for *A. o. oxyrinchus* in the Hudson River, and concisely summarise knowledge on its life history and habitat. This range of new and historic information is then used to identify key aspects of Atlantic sturgeon biology and management that should be considered when designing restoration efforts.

THE FISHERY

By the 1880s, the fishery for *A. oxyrinchus* had become well established in the Hudson River and along the Atlantic coast of New York and New Jersey. The total weight of sturgeon (*A. oxyrinchus* and the shortnose sturgeon *Acipenser brevirostrum* LeSueur, 1818) harvested was collected by each state for most years starting about 1880. The large size of commonly used gear (33-cm stretched mesh gill nets; Cobb, 1900) indicates that most of the harvest would have been adult *A. oxyrinchus*. The coastwide *A. oxyrinchus* fishery from 1880 through 1900 was large, with the total New York and New Jersey harvest reaching approximately 1 350 000 kg in 1890. Much of the total peak harvest for New Jersey should be attributed to the Delaware River stock (Secor and Waldman, 1999) but overall exploitation of Hudson River *A. oxyrinchus* was also high between 1880 and 1900 (table I). This large fishery for Hudson River fish apparently resulted in the removal of a major portion of the adults from the population, because the fisheries of New York, New Jersey, and all other coastal states with *A. oxyrinchus* populations collapsed after 1900 (Smith, 1985).

For eight decades after 1900, the *A. oxyrinchus* harvest was steady but very low (1 % of peak harvest) along the US Atlantic coast (table I). Fishery harvest data for the Hudson River population are lacking, but the total New York and New Jersey harvest from 1900 through 1979 suggests little effort

Table I. History of the fishery for *A. oxyrinchus* in the coastal waters of New York, New Jersey, and the Hudson River, based on statistics and plans of national and state fishery agencies. (1): Atlantic States Marine Fisheries Commission (1990). (2): The New Jersey fishery closure was not implemented until 1997, due to the time needed to administer the regulatory action. (3): Atlantic States Marine Fisheries Commission (1998)

Years	Annual weight harvested (kg)	Composition of catch by life stage	Description of fishery and management
1880-1900	900 000-1 350 000	Primarily large adults	Period of a developing fishery, peak harvest, and no fishery regulations. A substantial but unknown portion of the reported harvest includes fish from the Delaware River population and *A. brevirostrum* that occupied coastal sections of large rivers
1900-1979	6 500-13 500	Presumably adults and juveniles	Extended period of very low but stable harvest. Harvest is expected to have been largely Hudson River fish
1980-1992	10 000-125 000	Largely juveniles	Period of a redeveloping fishery focused entirely on Hudson River fish. Fishery restrictions were a minimum total length of either 107 or 122 cm (New Jersey, New York) with some gear restrictions
1993-1995	13 600-23 000	Largely juveniles	Three years of varied fishery restrictions aimed at reducing harvest to meet targets of the *A. oxyrinchus* fishery management plan(1) that had a goal of halting the continued decline of the species in US waters
1996-present	0(1)	None(2)	The Atlantic States Marine Fisheries Commission requested an emergency moratorium on harvest of *A. oxyrinchus* and New York and New Jersey(2) closed their sturgeon fisheries. Later, the fishery moratorium were changed to a long-term closure for all *A. oxyrinchus* fisheries in US waters(3)

was devoted to catching sturgeon or there were few fish available for harvest. The low catches (table I) for New York and New Jersey likely reflected a persistent but small Hudson River population, because the Delaware River would have contributed few, if any, fish to the catch. The Delaware River fishery of southern New Jersey had collapsed by 1900 (Secor and Waldman, 1999) and very few Atlantic sturgeon were being recorded from that river. Therefore, the period from 1900 through 1979 appears to be a time when Hudson River Atlantic sturgeon attracted little commercial interest, and the reported catch likely came from a variety of gears targetting many species and as bycatch landing a mix of adults and juveniles.

The first good evidence of a recovery of the Hudson River population came one century after the initial pronounced exploitation of the population. Interestingly, Secor and Waldman (1999) predicted from the analysis of the Delaware River fishery and *A. oxyrinchus* population characteristics that recovery from overexploitation would likely require a century. During the 1980s, the *A. oxyrinchus* harvest in New York and New Jersey increased to a level about 10 times the longterm harvest of the 1900s. Fishery regulation was weak, and permitted the harvest of juvenile fish (table I); again, records of harvest were limited to total weight landed. Harvest of *A. oxyrinchus* in the State of Delaware remained negligible during the 1980s, indicating that the Delaware River contributed little or nothing to the increased New York and New Jersey harvests. By the early 1990s, fishing for *A. oxyrinchus* clearly became a significant activity, and the increased harvest attracted the serious attention of fishery management agencies.

In 1990, the Atlantic States Marine Fisheries Commission (Anon., 1990) developed an *A. oxyrinchus* sturgeon fishery management plan that aimed to restore the species to a level that could support a fishery with a coastwide harvest totaling 10 % of the peak harvest, in 1890. The 10 %-of-peak goal was a management criteria, and not a product of population analyses. The Hudson River population appears to have been producing a harvest at about this 10 % level. The highest landings of the late 1980s (125 000 kg for New York and New Jersey combined, table I) approached 10 % of the peak

1890 harvest, and this regional-scale harvest is known to have included primarily Delaware River fish. To achieve the fishery management goal for all US populations, the *A. oxyrinchus* management plan specified that each state adopt one of three options: halt all *A. oxyrinchus* harvest, impose a minimum harvest size of 213 cm total length to restrict harvest to adults, or implement fishery restrictions expected to be equivalent to a 213 cm minimum total length. Both New York and New Jersey selected the latter option, and proposed fishery management restrictions aimed at controlling harvest to about 7 000 kg per year, or 229 fish for each state. All other states banned *A. oxyrinchus* fishing. In 1993, New York and New Jersey attempted to meet their harvest target with a 152 cm total length minimum size and limited fishing seasons. Harvest was reduced by these regulations in 1993, but in 1994, the New York harvest was 16 201 kg and 895 fish: more than double the intended harvest by weight and about four times the target number of fish (Anon., 1995). The New Jersey fishery harvest was similarly excessive (B. Andrews, pers. comm.) (New Jersey Department of Environmental Protection). Also, the fishermen were harvesting mostly juvenile *A. oxyrinchus*, since almost all of the catch was under 213 cm total length. Additional fishery restrictions were imposed in 1995 by both states; however that year, research findings for the Hudson River population clearly indicated that recruitment failure had occurred (Peterson, Bain and Haley, 2000).

In December 1995, the Atlantic States Marine Fisheries Commission convened a meeting of sturgeon researchers and fishery managers to assess the situation. This meeting resulted in emergency closures of Atlantic sturgeon fisheries (Anon., 1996a, b). Later, an amendment of the *A. oxyrinchus* fishery management plan (Atlantic States Marine Fisheries Commission) imposed a long-term ban on *A. oxyrinchus* harvest. The amendment stated that "The 1990 fishery management plan simply did not contain conservation measures sufficient to protect the portion of the [*A. oxyrinchus*] population and individual spawning stocks remaining at the time" (Anon., 1998). The key objective of the amended plan is to establish 20 protected year-classes of females in each spawning stock, and it was estimated that 40 years would be required to attain this objective. The actions of the Commission were made largely in response to the effect of fishing on Hudson River *A. oxyrinchus*, since this was the only population being harvested in a targeted fishery during the 1990s. The Commission's decision ended a century of marginal sturgeon fisheries, with one brief period of peak harvest that appears to have halted the recovery of the Hudson River sturgeon population.

THE HABITAT

The Hudson River

A. oxyrinchus is limited to the tidal portion of the Hudson River, between New York and the Troy Dam (figure 1). Limburg, Levin and Brandt (1989) describe the Hudson River and its estuary in detail, and selected data from their work are provided here to define the available river and estuary habitat of *A. oxyrinchus*. The average width of the river is 1 280 m (range 260 to 5 520 m), maximum depth is 35 m, and tidal flow (5 670-8 500 m^3/s) far exceeds river discharge (mean 623 m^3/s). Average water temperature is 12.3 °C, and nutrient levels are high due to sewage inputs from the New York City metropolitan area and several other significant urban areas. The salt front commonly reaches river km 100 in the summer and early autumn, and during periods of high river discharge the salt front can be as far downriver as km 32. The river channel was formed by glacial scouring, so much of the shoreline is rock and channel depths are greater than would result from typical fluvial processes.

Life cycle of Hudson River *A. oxyrinchus*

Hudson River *A. oxyrinchus* are amphidromous (diadromous fish that move between fresh and sea water for reasons beyond reproduction; McDowall, 1992), because spawning and early development occurs in the freshwater portion of the Hudson River, whereas adult and large juvenile sturgeon occupy marine waters, and some of these fish will annually occupy low salinity sites in the Hudson River and other coastal rivers in summer. After spawning in freshwater habitat, adults may go to the sea or remain in the lower river until early autumn, when they move to marine waters. Larvae are believed to remain in the freshwater portion of the river and gradually move downstream during the summer and autumn (Dovel and Berggren, 1983). This

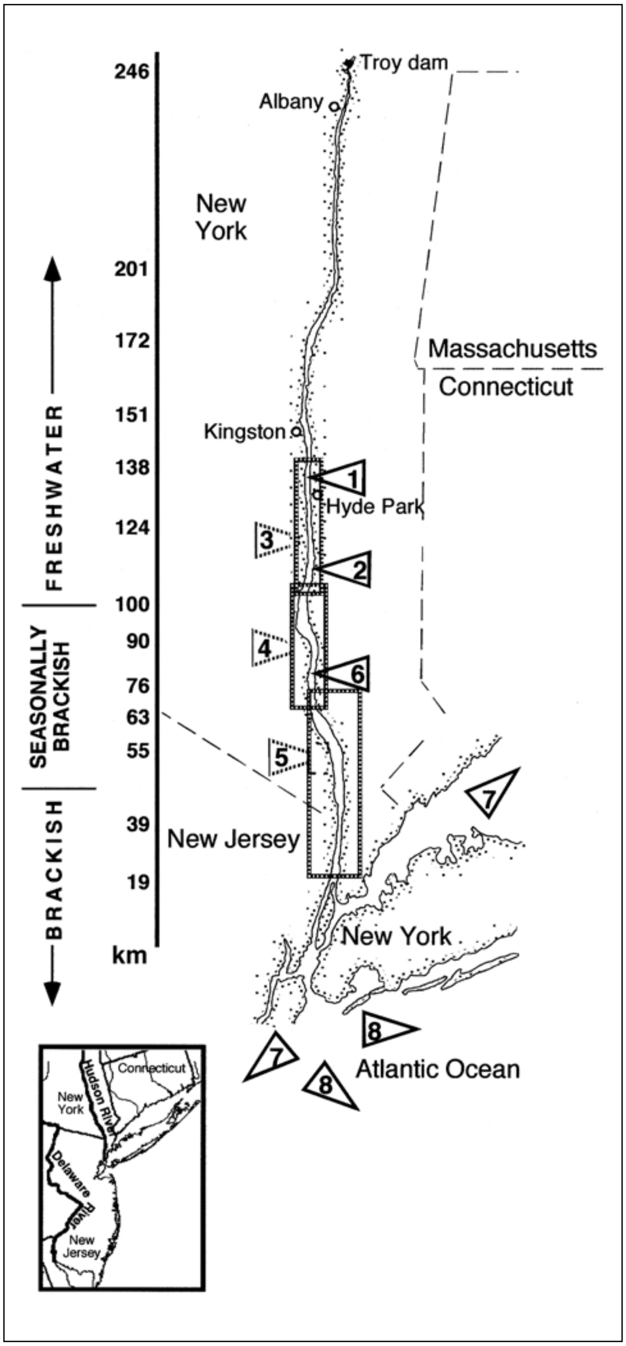

Figure 1. Map of the tidal portion of the Hudson River, showing *A. oxyrinchus* habitats. Numbers in the symbols correspond to the numbered habitats described in table II, and reviewed sequentially in the text

movement may be governed by the development of salinity tolerance in young *A. oxyrinchus*, as suggested by studies of salinity tolerance in other sturgeon species (McEnroe and Cech, 1987). Juvenile *A. oxyrinchus* grow rapidly in the Hudson River, exceeding 70 cm total length (63 cm fork length) by 3 years of age (Stevenson, 1997). Juvenile sturgeon of this size and age begin migrating to marine waters (Bain, 1997), and Dovel and Berggren (1983) concluded that all or most age 6 or younger sturgeon are marine migrants. These marine migrant juveniles have been observed to move back into coastal river systems during summer (Smith, 1985; Keiffer and Kynard, 1993), and one location in the Hudson River supports marine migrant juveniles (> 63 cm fork length; > 70 cm total length), adults, and fish that had spawned earlier in the same year (figure 1). Male and female Hudson River *A. oxyrinchus* reach maturity at 117 and 173 cm fork length (133 and 197 cm total length) and ages 12 and 14, respectively (Van Eenennaam *et al.*, 1996; Van Eenennaam and Doroshov, 1998). Using optical and chemical analyses of fin rays and otoliths, Stevenson (1997) estimated that females spawn once every four years, whereas males are thought to spawn every year (Vladykov and Greeley, 1963).

Distribution of the population

Hudson River *A. oxyrinchus* are genetically distinct from other populations associated with rivers along the US Atlantic coast (Waldman *et al.*, 1996). Waldman, Hart and Virgin (1996) used data from mitochondrial DNA restriction fragment length polymorphism analysis to estimate the relative contributions of different populations of Atlantic sturgeon to the New York Bight fishery in 1993 and 1994. The New York Bight includes coastal waters of New Jersey and New York (figure 1), and this region is in the middle of the *A. oxyrinchus* range. The results of Waldman, Hart and Wirgin (1996) indicate clearly that the sturgeon fishery in the New York Bight is supported overwhelmingly by individuals from the Hudson River population. Dovel and Berggren (1983) tagged *A. oxyrinchus* in the Hudson River, and recovery of tags indicated that the Hudson River sturgeon range extends from about just north of Cape Cod (Massachusetts) to Cape Hatteras (North Carolina). In the 1800s, sturgeon in this region included substantial numbers of fish from spawning populations in the Delaware River and the rivers of Chesapeake Bay. The nearly complete dominance of Hudson River *A. oxyrinchus* in the recent New York Bight fishery suggests that the other populations are now negligible. On a regional scale, the habitat of Hudson River *A. oxyrinchus* includes marine waters, estuaries, and rivers from Massachusetts to North Carolina, and in this region the Hudson sturgeon dominated the fishery catch.

Spawning habitat

Hudson River *A. oxyrinchus* were documented as using two spawning sites during our field studies from 1993 through 1998. The major spawning site was located in Hyde Park, New York at river km 134 (table II). This site has been known since the 1880s, when it was a major fishing location. The spawning habitat is along one side of the river (figure 1), among rock islands with irregular bedrock and substrate of silt and clay. The location is freshwater year round, with water depths ranging from 12-24 m. Mature male fish were captured in this habitat from late May through the end of June, with spawning occurring mostly in June. During 1994 and 1995, many fish were captured at the time of spawning (water temperatures 14-24 °C) with gill nets, and mostly male sturgeon were tagged with sonic transmitters.

Sonic-tagged male sturgeon revealed a second spawning site located at river km 112 (figure 1). Again, the site was located along one side of the river, in water 21-27 m deep with clay, silt, and sand substrate. The habitat was in freshwater, and used for spawning in mid-July at water temperatures around 26 °C (table II). Gill nets set at the time the habitat was occupied by sonic-tagged fish revealed ripe males and females, confirming the site as an active spawning location. No other spawning sites were indicated by sonic-tagged fish in 1994 and 1995, suggesting that these sites were the only ones being used by the Hudson River population.

Larval rearing habitat

Eggs of Atlantic sturgeon are adhesive, and the larvae remain on the bottom in deep channel habitats. Atlantic sturgeon larvae are about 7 mm total length at hatching, and in hatcheries they reach 19.9 mm total length in 20 days (Smith, Dingley and Marchette, 1980). We estimate water temperatures in habitats used for spawning would have been 19-28 °C, from observations of other life stages (table II). The transition from the larval stage to juveniles appears to occur at about 30 mm total length, based on Hudson River specimens (Bath *et al.*, 1981). *A. oxyrinchus* larvae have been recorded in the Hudson River from river km 60 through 148 (Dovel and Berggren, 1983), a range including some brackish waters (figure 1). However, larval sturgeon have limited salt tolerance, so larval habitat must be well upstream from the salt front (Van Eenennaam *et al.*, 1996). No further information is available on this stage of the *A. oxyrinchus* life cycle. Therefore, we presume that the preferred habitat for larval *A. oxyrinchus* is near the spawning habitat, with larvae extending downstream as they grow and attain the ability to endure brackish water (table II).

Early juvenile rearing habitat

Juvenile *A. oxyrinchus* less than 70 cm total length (63 cm fork length) and 6 years of age occupy summer rearing and overwintering habitats in the Hudson River. The summer growing season habitat was well described by dispersed gill-net sampling in the entire tidal river during 1995 and 1996 (Haley, 1999). From April through October, early juvenile *A. oxyrinchus* were primarily found between river km 68 and 107 at water temperatures 24-28 °C (figure 1). This is the highland gorge and wide estuary sections (Coch, 1986) of the Hudson River, where the transition from freshwater to brackish water is found. Juvenile sturgeon were most often captured in salinities ranging from 0-5 ppt (table II). Water depths associated with most captures ranged from 10-25 m, and the substrates were primarily silt and sand.

Early juvenile wintering habitat

The winter habitat of *A. oxyrinchus* has been defined by Dovel and Berggren (1983) from trawl and gill-net sampling between 1975 through 1978. When water temperature in the river reaches 9 °C, most juveniles that have not migrated to the sea congregate in a deep-water habitat between river km 19 and 74 (figure 1). Water temperatures during winter could reach 0 °C in this portion of the Hudson River. Salinity in this region ranges from 3-18 ppt, and water depths in the channel commonly range (navigation maps) from 20-40 m (table II).

Table II. Review of the life stages and habitats of Hudson River *A. oxyrinchus* from field data and observations from 1993 through 1998 and some past studies. Map code numbers refer to locations identified in figure 1.(1): Inferred from water temperature at spawning sites and the expected hatching period

Map code	Life stage	Time period	Locations (river km of notes)	Water temperature (C)	Salinity (ppt)	Water depth (m)	Bottom material	Source of information
1	Spawning	Late May and June	134	14-24	0	12-24	Rock clay silt	64 gill-net sets from late May through mid-August. 59 adult fish recorded from late May through the end of June. Historic site
2	Spawning	Mid-July	112	26	0	21-27	Clay sand silt	Site was identified from sonic telemetry of adult fish that congregated in mid-July. Two gill nets captured 6 adult fish, including a spawning female
3	Larvae	June to August	Vicinity of spawning	19-28(1)	0	–	Clay silt	Little is known about larval habitat. We assume that the habitat is close to the spawning sites
4	Early juvenile	April-October	68-107	24-28	0-5	10-25	Silt sand	166 gill-net sets captured 48 juvenile fish from June through early October. The habitat values reported are for the locations used by most (means ± 1 one standard deviation) fish
5	Early juvenile	October-March	19-74	0-9	3-18	20-40	Clay sand silt	Dovel and Berggren (1983) describe the movement of non-migratory juveniles to the lower estuary from net and trawl sampling
6	Late juvenile and adult	June-September	78	20-26	0-6	16-35	Clay silt	Site was identified from sonic telemetry of 33 adult fish that congregated in mid-July. 80 gill-net sets recorded 171 fish that were largely marine migrant juveniles and adults, including post-spawners
7	Late juvenile and adult	May-October	Coastal waters and distant rivers	Unknown	Marine to brackish	30-40 at two coastal sites	Mud at two coastal sites	Hudson Atlantic sturgeon have been recorded at several very specific locations in coastal marine waters and in the brackish water portion of rivers from New England through the Chesapeake Bay. River sites are expected to be like habitat 6 (above) in the Hudson River. Two coastal sturgeon concentrations were sampled by trawling in Long Island Sound. 60 Atlantic sturgeon were captured by trawling in July, including tagged Hudson rish. No fish were at the sites in the late autumn
8	Late juvenile and adult	October-May	Marine waters	Unknown	Marine	Unknown	Unknown	We presume Atlantic sturgeon move to deeper and coastal waters further offshore during the winter. There is reliable data that adult and juvenile sturgeon are not found in brackish estuary sites (habitat 6) or shallow coastal sites (habitat 7) used in the summer. Offshore trawling by the US National Marine Fisheries Service has recorded Atlantic sturgeon in marine waters in winter

The river-bottom sediment maps of Coch (1986) show that most juvenile sturgeon habitats hawe clay, sand, and silt substrates.

Late juvenile and adult summer habitat

Marine migrant juvenile *A. oxyrinchus* are older than 6 years of age and longer than 70 cm. These fish use marine habitats during the winter, and rivers, estuaries, and coastal marine habitats in the summer. We believe that adults display the same migratory behavior as marine migrant juveniles, because our sampling of river and coastal concentrations of large sturgeon (>70 cm total length) recorded a mix of adults and juveniles. Accounts of concentrations of *A. oxyrinchus* larger than about 150 cm have been reported from other rivers, but the literature shows no thorough characterization of the size and life-stage composition of sturgeon in these habitats.

One of the summer brackish water habitats we have studied is in the Hudson River at river km 78 near Garrison, New York (figure 1). Here, the Hudson River is very narrow and deep. Sonic-tagged spawning sturgeon revealed this location when they congregated after spawning. Gill nets set at the location captured late juveniles, adults, and post-spawn tagged adult fish in 1994 and 1995, from June through the end of September. The habitat ranged from 0-6 ppt salinity, depending on river discharge, but the fish remained in the same habitat despite salinity variations. Water temperature varied from 20-26 °C, and water depth ranged from 16-35 m. The substrate in this habitat is clay and silt, with the shoreline composed mainly of rock.

Late juvenile and adult *A. oxyrinchus* from the Hudson River are also known to occupy similar habitats in other rivers. Dovel and Berggren's (1983) map of tagged Hudson sturgeon captures shows many fish in rivers of Chesapeake Bay and the Delaware River. There are also some accounts of concentrations of late juvenile and adult *A. oxyrinchus* in coastal marine waters during the summer. We inventoried two such habitats in Long Island Sound off the Connecticut coast in the summer and autumn of 1996. Sixty from 70-200 cm fork length were captured by trawling in mid-July, and one fish was tagged in the Hudson River. The habitat was 30-40 m deep with mud substrate. Sampling in the autumn failed to capture sturgeon, so we assume that they left this habitat for their wintering areas.

Late juvenile and adult wintering habitat

No known habitats of marine migrant juvenile and adult Hudson River sturgeon support fish in the winter. Autumn captures of sturgeon leaving coastal areas and estuaries have been reported by fishermen in Chesapeake Bay, marine fishermen from New York and New Jersey, and some scientific investigations (e.g. Kieffer and Kynard, 1993). Winter trawling by the US National Marine Fisheries Service has recorded *A. oxyrinchus* in offshore marine waters, but this information has not been further investigated for its value in identifying winter habitats.

LESSONS FOR RESTORATION

Our review of the fishery and habitats of Hudson River *A. oxyrinchus* reflects the species's complex life history and the ineffective management of the population to date. Three fundamental lessons for sturgeon restoration emerge: basic life-history knowledge is essential, fishery management is difficult, and effective population monitoring will be needed. The Hudson River *A. oxyrinchus* recovered from severe overexploitation in the late 1800s and approximately reached a management goal, signalling successful restoration. This restoration was extinguished by rapid overexploitation, because management agencies and researchers were too late in understanding the situation. Future sturgeon restorations can avoid the thwarted success of the Hudson River population by implementing measures to address biology, management, and monitoring from the start.

The life cycle of *A. oxyrinchus* is complex, and a major Hudson River sturgeon research effort, involving many university and agency scientists, showed how difficult it can be to understand this species's complex behaviour and habitat use. Intensive research using expensive and labour-intensive techniques, such as telemetry and large-scale tagging, were needed to identify some key migratory behaviours and habitats. While one spawning site has been well known in the Hudson River for more than 100 years, the discovery of a second site was a surprise, or a rediscovery of a forgotten location. The summer and early autumn congregation behaviour of marine migrant juveniles and adults in the rivers and coastal waters escaped past investigators, even though evidence for this behaviour can now be seen in past research results and reported observations. Fishery biologists know the critical importance of the details of life stages and growth rates, and this information was available, and enhanced in recent research. However, knowledge of movements and habitats were equally important for managing the Hudson *A. oxyrinchus*. The susceptibility of migrating juveniles to near coastal fisheries was not realised, because no one understood that immature fish have annual migrations in and out of the Hudson River and other estuarine systems. The nearly complete dominance of the coastal fishery by Hudson River sturgeon was not appreciated, so the concentrated harvest of one population was not clear.

Despite unprecedented research by many scientists in the 1990s, some key information remains unknown, such as male repeat spawning, sex ratio at spawning, precise winter habitats, larval biology, complementarity of adult migrations with large juveniles, and age or size on first marine outmigration. Present understanding of the marine movements of the Hudson River population are based mainly on recaptures of juveniles from one period of relative abundance in the late 1970s and early 1980s. This information is important, because long movements of adults and large juveniles can lead to exploitation in distant waters not linked to a restoration effort. Another complication of long movements and the annual use of distant rivers is the misunderstanding that the presence of sturgeon in a river or estuary indicates a local population. We now believe that the only reliable information indicating a population is the capture or confirmed records of numerous small (< 70 cm total length) juveniles or adults in spawning condition. Our first lesson is that *A. oxyrinchus* conservation efforts need to place high value on understanding basic species biology. The Hudson River case shows that managers need to take a long look at what habitats are used by a population; be on the lookout for intrusions by disturbance or fishing in habitats with sturgeon concentrations; recognise that distant fisheries can impose significant harvest on a recovering population; and consider seriously the evidence for a river population when using historical observations.

A second lesson from the Hudson River is that fishery management proved more difficult than managers anticipated. We believe great caution and a protective philosophy should be fundamental to management planning. For Hudson *A. oxyrinchus*, regulations to control total weight harvested failed to protect the population. The actual harvest varied greatly in response to specific regulations, and it exceeded the intended levels. Second, fishery management was too slow, because over-intensive harvest of the recovering population occurred faster and with a major effect before fishery managers could react. The population was already greatly diminished by the time full knowledge of the situation became known through field studies. Finally, much of the harvest of the Hudson population occurred outside of the immediate river and estuary; for example, along the coast of New Jersey, where past harvests would have been attributable to other river populations. Overall, effective sturgeon population restoration will require very secure control of harvest over a large area, complete certainty in management objectives, and regulations that fully and directly protect juveniles (a high minimum size or strict gear specifications).

Our third lesson is mainly a consequence of the other two: population monitoring needs to be a central component of a restoration programme. In Waldman and Wirgin's (1998) review of options for restoration of North American *A. oxyrinchus*, they recommend that population-specific information is necessary to determine management measures. For the Hudson River *A. oxyrinchus*, managers might have been able to recognise that the population had recovered in time to protect it, if some monitoring had been underway. Also, population information at the start of the recovery could have signalled vulnerability and overharvest before overexploitation collapsed the population. In cases such as the European Atlantic sturgeon *A. sturio*, some populations have been extinct for so long that little or no retrospective information is available on population biology, habitats, and fishery harvest. For such cases, monitoring of the population under restoration will be needed to learn about the biology of and threats to the population as the restoration programme progresses. Information on the Hudson River *A. oxyrinchus* population used in concert with monitoring results regarding *A.*

sturio populations can guide managers on potentially important sources of mortality, likely population range, life history properties, and probable key habitats. Our experiences show that knowledge of the species biology and fishery is critical, and for restoring *A. sturio* this knowledge will most likely come by learning while restoring; that is, monitoring from the start.

In future restoration efforts, managers should be aware that restoration will be attempted with many unknowns and potential impediments. Clearly successful population restoration will likely require many decades, so a long-term perspective must be built into programme planning. Comparative biological information for Hudson River *A. oxyrinchus* can serve to guide initial plans. The North American fishery experiences illustrate some threats to watch out for, and to avoid. Finally, close monitoring of restoration populations will be needed to recognise successes, failures, and threats as they emerge.

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Past and present distribution of *Acipenser sturio* L., 1758 in Russia, and problems involving its restoration

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ABSTRACT

The Atlantic sturgeon *Acipenser sturio* L., 1758 has always been rare in Russia. Its main distribution was concentrated in the Gulf of Finland, from which it ascended the Neva River and entered Lake Ladoga and some of its tributaries. The landlocked population of this species was known in Lake Ladoga. In the 20th century, there are recorded catches of only 25 adult and 12 juvenile specimens. Since the last specimen was caught in 1985, the Atlantic sturgeon can be considered as extirpated in the water bodies of Russia. While recovery plans have been drawn up, the lack of living specimens is a serious obstacle to re-establishing this population.

Key words: Gulf of Finland, Lake Ladoga, landlocked population, recovery plans.

RESUMEN

Distribución pasada y presente de* Acipenser sturio *L., 1758 en Rusia, y problemas relativos a su recuperación

El esturión atlántico Acipenser sturio *L., 1758 ha sido siempre raro en Rusia. Su distribución principal se concentraba en el golfo de Finlandia, desde donde ascendía por el río Neva y entraba en el lago Ladoga y en algunos de sus afluentes. Una población aislada de esta especie era conocida del lago Ladoga. En el siglo XX se recogen capturas de sólo 25 ejemplares adultos y 12 juveniles. Desde que el último ejemplar fue capturado en 1985, el esturión atlántico puede ser considerado como extinguido de las aguas de Rusia. Aunque los planes de recuperación han sido preparados, la falta de ejemplares vivos es un serio obstáculo para el restablecimiento de esta población.*

Palabras clave: *Golfo de Finlandia, lago Ladoga, población aislada, planes de recuperación.*

INTRODUCTION

In the water bodies of the Russian Federation, the Atlantic sturgeon *Acipenser sturio* L., 1758 was always rare, as documented by records of all known specimens taken from the literature. The following information summarises the past and present situation of this species in Russia, with regard to its possible recovery.

PAST OCCURRENCE

In the waters of Russia, the Atlantic sturgeon was known only from the easternmost part of the Baltic Sea, the Gulf of Finland. As can be determined from the data presented by Kessler (1864) and Berg (1948a), this species has always been rare in this region. According to Kessler (1864, p. 211): "In the Gulf of Finland the sturgeon is rather rare; however, each year in small numbers it penetrates into the Neva River and from there it goes further into Lake Ladoga and rivers entering it, such as the Volkhov, Syas, Svir', and formerly, when the Voksha River had enough water, it occurred also in the mouth of this rivulet." Kessler also mentioned the catch of a large (13 *puds*; 212.9 kg) female Atlantic sturgeon taken in the Neva River near Matisovyi Island on 16 June 1851, containing 5 *puds* (81.9 kg) of eggs.

As shown in table I, this species became extremely rare in both the Gulf of Finland and the Neva River during the 20th century. It is noteworthy that only single and exclusively adult specimens were caught. Available data also show that the Atlantic sturgeon was not part of the fish catch by the mid-1940s. Since that time, it could be considered as extirpated in the Gulf of Finland. This is also indirectly proven by the total absence of sturgeon in the fish catch of the Neva River. This phenomenon may be explained not only by the overfishing, but also by increasing pollution, especially of the Neva.

The Atlantic sturgeon was more common in Lake Ladoga, which is connected to the Gulf of Finland by the Neva River. Table II illustrates that large sturgeons were caught, mainly in the southern part of Lake Ladoga, especially in Volkhov Bay. According to Kessler (l.c.) the sturgeon entered the Volkhov River from this part of Lake Ladoga annually in June, and migrated upstream as far as the villages of Zvankoya and Duboviki. More upstream migration was blocked by rapids above Duboviki, 27 km from the mouth of the Volkhov River. Spawning was observed each year in the Volkhov River between these villages, where the river is deep, the river beds covered with pebbles, and the water has high current velocity, thus creating all of the necessary conditions for reproduction. Spawning occurred in June (Kessler, l.c.) and also in July (Berg, 1948b). Kessler (l.c.) also writes that juveniles appeared in the mouth of the Volkhov River in July and August. Because the Volkhov River is the only tributary of Lake Ladoga where the Atlantic sturgeon are known to have spawned, Kessler proposed a total ban on the fishery in this region, thereby becoming the world's first author to propose the conservation of this species.

In Lake Ladoga, sturgeons of different sizes, including juveniles, were caught (table III). Because of this, Berg (1948b) suggests that this area contained a landlocked form of this species, which did

Table I. Documented records of Atlantic sturgeon catches in the Gulf of Finland and the Neva River during the 20th century

Locality	Date	Weight (kg)	Total length (m)	Author
		Gulf of Finland		
Near Sestroreck	30 May 1934	177	2.8	Anon., 1934
–	1938	56	2.19	Berg, 1948a
Luga Bay at Lipov	July 1935	36.5	?	Berg, 1935
		Neva River		
Neva River	June 1930	"large"	?	Pravdin, 1948, 1949
–	July 1932	36	1.88	Berg, 1935
Liteinyi bridge	May 1935	96	2.29	Berg, 1948a
–	June 1935	?	2.2	Berg, 1935
Malaya Nevka mouth	June 1944	77	?	Berg, 1948b

Table II. Documented records of Atlantic sturgeon adults caught in Lake Ladoga during the 20th century

Locality	Date	Weight (kg)	Total length (m)	Author
		North-western sector		
–	December 1913	61	2.6	Jääskelainen, 1917
		North-eastern sector		
–	Autumn 1918	64	?	Berg, 1935
–	June 1947	22.5	1.63	Pravdin, 1948, 1949
Olonka River mouth	Spring 1957	Large	?	Kuderskii, 1983
		Western sector		
Burnaya River mouth	October 1925	24	?	Berg, 1935
–	June 1948	16.5	1.35	Pravdin, 1948
		South-western sector		
–	1923	37	?	Domrachev and Pravdin, 1926
–	1923	21	?	Pravdin, 1948
–	Winter 1948	Large	?	Barysheva and Bauer, 1957
		Southern sector		
–	May 1939	130	2.83	Berg, 1948
–	June 1952	73	2.22	Malashkin (pers. comm.)
–	June 1954	106	?	Vasil'ev and Reznikov, 1955
		Volkhov Bay		
–	June 1930	128	2.65	Berg, 1948
–	June 1930	Large	?	Pravdin, 1949
Volkhov River mouth	July 1957	Spent female	?	Vasil'ev and Reznikov, 1955
–	July 1969	52	?	Boyarskaya (pers. comm.)
–	June 1984	26	1.55	Podushka, 1985

not enter the Gulf of Finland. Indirect evidence supporting this hypothesis is the discovery of the remains of 60 large Atlantic sturgeon, dating from the 8th-9th century, in the Staraya Ladoga on the Volkhov River. These fossils are from sturgeons measuring 1.7-3.1 m, but mostly 2.1-2.8 m, with a mean weight of approximately 100-180 kg and an age of up to 35-36 years (Berg, 1948a, b). Nevertheless, parasitological data indicate that at least part of the sturgeons migrated to Lake Ladoga from the Baltic Sea through the Neva River (Barysheva and Bauer, 1957).

PRESENT STATUS

As shown in tables II and III, the most frequent occurrence of Atlantic sturgeon adults and juveniles in Lake Ladoga was during the periods from 1939 to 1957 and 1930 to 1935. Since that time, the catch records of this species indicate that only two adults were caught: one in 1969 and one in 1984. This means that during the past 16 years, there were no records of Atlantic sturgeon in Lake Ladoga. In the Gulf of Finland this species has been unknown since 1938, or 1944 if one specimen caught in the Neva River is considered. Thus, it can be assumed that the Atlantic sturgeon is extirpated in this region. Since 1967 there has been a ban on the fishing of this species and it is included in the Red Data Book of both the former USSR (Borodin, Bannikov and Sokolov, 1984) and Russia.

RECOVERY PLANS

To save this species, special and effective measures are needed. The restoration programme for the Atlantic sturgeon in Russia is planned, although, at present, this species is in the most diffi-

Table III. Documented records of Atlantic sturgeon juveniles in Lake Ladoga and the Volkhov River during the 20th century (modified from Kuderskii, 1983)

Locality	Date	Weight (kg)	Total length (m)	Author
North-western sector				
–	July 1910	0.325	0.43	Jääskalainen, 1917
–	July 1910	0.350	0.43	Jääskalainen, 1917
Northern sector				
–	Summer 1934	4.9	–	Persov, 1935
Eastern sector				
–	July 1933	5.0	?	Persov, 1935
–	June 1934	5.5	?	Berg, 1935
South-western sector				
–	1935	4.0	?	Persov, 1935
–	1934	8.0	?	Berg, 1935
–	1934	8.0	?	Berg, 1935
–	1934	small	?	Berg, 1935
Volkhov Bay				
–	June 1930	?	0.49	Berg, 1948
Volkhov River				
–	June 1924	4.0	?	Domrachev and Pravdin, 1926
–	Autumn 1924	?	0.25	Domrachev and Pravdin, 1926

cult situation among all endangered species of fish, because of a complete lack of living specimens. At the laboratory of the senior author, in the 1990s, the long-term Programme of Sturgeon Biodiversity Conservation was created, including the formation of live collections and a gene-pool bank of cryopreserved sperm (Barannikova, 1998; Artyukhin, Barannikova and Romanov, 1999). The next approach for sturgeon conservation is the establishment of special fish farms in different parts of Russia for rare sturgeon. Near Moscow, a new fish farm has been constructed for the central collection of rare sturgeon species. Atlantic sturgeon has been proposed for the central collection on the new Mozhaysk fish farm (Artyukhin and Romanov, 2000). In order to try and find living specimens of the Atlantic sturgeon, special leaflets containing its description were produced and distributed among fishermen in the Baltic and Ladoga basins; the necessity of the preservation of living sturgeons was stressed. In the last few years, we have received some information about catches of sturgeon in the Volkhov Bay of Lake Ladoga, but these fishes were not *A. sturio*, but rather the sterlet *Acipenser ruthenus* L., 1758 or the Russian sturgeon *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833.

Considering the present worldwide situation of the Atlantic sturgeon, in order to rescue this critically endangered species, the joint effort of experts from different countries is necessary.

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Molecular analysis of *Acipenser sturio* L., 1758 and *Acipenser oxyrinchus* Mitchill, 1815: A review

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ABSTRACT

Molecular phylogeny based on the analysis of partial sequences of mitochondrial (mt) cytochrome *b* (cyt *b*), 12S, and 16S genes supports a close relationship between the European Atlantic sturgeon *Acipenser sturio* L., 1758, and the American Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, 1815. These sister species form a separate clade within the genus *Acipenser* and appear to be the most basal clade with respect to all other species of the genus. Intraspecifically, at least two different genetic forms have been found within *A. sturio* (in the Northern and Baltic seas), but no defined subspecies exist. For *A. oxyrinchus*, fixed differences in the control region and cyt *b* gene distinguish the two subspecies, the Atlantic sturgeon *A. oxyrinchus oxyrinchus* Mitchill, 1815 and the Gulf of Mexico sturgeon *A. oxyrinchus desotoi* Vladykov, 1955. Within *A. o. oxyrinchus*, a pronounced latitudinal cline in haplotype diversity exists from north to south along the eastern coast of North America. This greater genetic diversity in southern populations is most likely a result of population continuity through the Pleistocene. Monomorphism within the two northernmost, post-Pleistocene *A. o. oxyrinchus* populations indicates a probable founder effect. *A. o. desotoi* also displays a significant geographic structuring of populations in different river systems. Because of the lack of *A. sturio* individuals necessary for population studies and the close relationship between this species and *A. oxyrinchus,* the genetic characteristics and structure of *A. oxyrinchus* can be regarded as a model for *A. sturio.* The only opportunity for future detailed molecular study of *A. sturio* resides in using tissue samples from sturgeon specimens kept in various European museums.

Key words: Atlantic sturgeon, cytochrome *b*, genetic diversity, phylogeny.

RESUMEN

Análisis molecular de* Acipenser sturio *L., 1758 y* Acipenser oxyrinchus *Mitchill, 1815: una revisión

La filogenia molecular basada en el análisis de secuencias parciales de los genes mitocondriales (mt) citocromo b *(cyt* b*), 12S y 16S apoya la próxima relación entre el esturión atlántico europeo* Acipenser sturio *L., 1758 y el esturión atlántico americano* Acipenser oxyrinchus *Mitchill, 1815. Estas especies hermanas forman un clado separado dentro del género* Acipenser *y parecen constituir el clado más basal con relación a todas las otras especies del género. Intraespecíficamente se han encontrado, al menos, dos formas genéticas diferentes dentro de* A. sturio *(en los mares del Norte y Báltico) pero no existen subespecies definidas. Para* A. oxyrinchus *las diferencias fijadas en la región control y en el gen del cyt* b *distinguen las dos subespecies: el esturión atlántico* A. oxyrinchus oxyrinchus *Mitchill, 1815 y el esturión del golfo de México* A. oxyrinchus desotoi *Vladykov, 1955. Dentro de* A. o. oxyrinchus *existe un pronunciado cline latitudinal en* *la diversidad de haplotipos desde el norte hasta el sur a lo largo de la costa este de América del Norte. Esta mayor diversidad genética en las poblaciones meridionales es, más probablemente, un resultado de la continuidad poblacional durante el Pleistoceno. El monomorfismo dentro de las dos poblaciones post-pleistocénicas más septentrionales de* A. o. oxyrinchus *indica un probable efecto fundador.* A. o. desotoi *siempre muestra una significativa estructuración geográfica de poblaciones en diferentes sistemas fluviales. Debido a la falta de individuos de* A. sturio *necesarios para los estudios poblacionales y a la próxima relación entre esta especie y* A. oxyrinchus*, las características y estructura genéticas de* A. oxyrinchus *pueden ser consideradas como modelo para* A. sturio*. La única oportunidad para un futuro estudio molecular detallado de* A. sturio *reside en el uso de muestras de tejidos de ejemplares de esturión almacenados en varios museos europeos.*

Palabras clave: *Esturión atlántico, citocromo* b*, diversidad genética, filogenia.*

INTRODUCTION

At present, the European Atlantic or Baltic sturgeon *Acipenser sturio* L., 1758 is one of the most endangered sturgeon species (Rochard, Castelnaud and Lepage, 1990; Birstein, 1993, 1999a; Lepage and Rochard, 1995; Birstein, Bemis and Waldman, 1997; Anatsky, 1999). Historically, its range included the Northeastern Atlantic Ocean, especially the shallow parts of the North and Baltic Seas and coastal areas of the Mediterranean and Pontic regions (reviews in Holčík *et al.*, 1989; Kinzelbach, 1997). Currently, only a small population of *A. sturio* exists in the Gironde River system in France (Castelnaud *et al.*, 1991; Williot *et al.*, 1997). In the spring of 1995, natural reproduction of this species was reported in this river basin for the first time since 1988 (Anon., 1995). Unfortunately, it is not known which river was used by sturgeon for spawning at that time. Analysis of the catch data of *A. sturio* provided by commercial fishermen over the period 1980-1994 indicates that juveniles from this population disperse over a very large area, from the Bay of Biscay to Scandinavia, as well as the British Isles (Rochard, Lepage and Meazé, 1997).

Until recently, another small stock of *A. sturio* was also observed in the eastern part of the Black Sea (Pavlov *et al.*, 1985, 1994). Sturgeon spawned in the lower part of the Rioni River in Georgia (Ninua, 1976). All attempts to catch *A. sturio* in that area in the 1990s have failed (E. Artuykhin and Z. Zarkua, pers. comm.)

During the last decade, several single *A. sturio* individuals were caught in the North Atlantic and the Baltic Seas. In 1985, 1989 and 1995, three sturgeon were caught in the German waters of the North Sea near the Helgoland Island (Debus, 1995, 1997; K. Busse, pers. comm.). In 1993, one *A. sturio* was captured in the North Sea near the Dutch coast (Timmermanns and Melchers, 1994) and in 1992, two juveniles were reported from a tributary of the Dutch part of the Rhine (Volz and De Groot, 1992). In May 1996, a large mature female was caught in the Estonian waters of the Baltic Sea (Paaver, 1996, 1997, 1999). Therefore, only single individuals of *A. sturio* are currently available for genetic research.

Historically, the North American Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, 1815, was considered to be the same species as *A. sturio*. Later, *A. oxyrinchus* was recognised as the separate subspecies *A. sturio oxyrinchus*, with the European Atlantic sturgeon known as *A. sturio sturio* (Smith, 1891; Vladykov and Greely, 1963). In 1963, Magnin and Beaulieu suggested elevation of these subspecies to species ranks, with the European form retaining the name *A. sturio*, and the American form recognised as *A. oxyrinchus* (Magnin and Beaulieu, 1963; Magnin, 1964). Since then, they are usually considered sister species (e.g., Birstein and Bemis, 1997; Choudhury and Dick, 1998), but recently Artyukhin and Vecsei (1999) lowered them to subspecies level again. However, this opinion contradicts current data on genetic differences between *A. sturio* and *A. oxyrinchus* (see below).

Two subspecies, the Atlantic sturgeon, *A. oxyrinchus oxyrinchus* Mitchill, 1815 and the Gulf of Mexico sturgeon, *A. oxyrinchus desotoi* Vladykov, 1955, were described within *A. oxyrinchus* (Vladykov, 1955; Vladykov and Greely, 1963). Currently, the Atlantic subspecies occurs from the Hamilton Inlet of Labrador in Canada to the St. Johns River in eastern Florida, while the Gulf sturgeon inhabits most river systems of the northern Gulf of Mexico from the mouth of the Mississippi River to the Suwannee River and coastal waters up to Florida Bay, Florida (Smith and Clugston, 1997).

In contrast to the European Atlantic sturgeon, different populations of *A. oxyrinchus* have been studied intensively and numerous genetic data for this species exist in the literature.

In the present paper, we review all of the genetic data known for both *A. sturio* and *A. oxyrinchus*.

RESULTS AND DISCUSSION

General genetic characteristics and the position of *A. sturio-A. oxyrinchus* within the Acipenseridae

The chromosome number in *A. sturio* is 116 ± 4 (Fontana and Colombo, 1974; Tagliavini *et al.*, 1999). The *A. sturio* karyotype consists of 35 pairs of meta- and submetacentric large and medium-sized chromosomes, two pairs of small acrocentrics and about 20 pairs of microchromosomes (Fontana and Colombo, 1974; Tagliavini *et al.*, 1999). The DNA content in *A. sturio* is 3.6 pg/nuclei (Fontana, 1976), which is characteristic of sturgeons with 120 chromosomes (Birstein, Hanner and DeSalle, 1997). For *A. o. oxyrinchus* the DNA content was reported as 4.55 pg/nuclei (Blacklidge and Bidwell, 1993), but the difference in the DNA content between these two species appears to be due to different methods of evaluation (microdensitometry for *A. sturio* and flow cytometry for *A. oxyrinchus*). For *A. oxyrinchus*, the number of chromosomes in *A. o. desotoi* cultured cardiac cells was 99-112 (Li *et al.*, 1985).

All of this evidence confirms that both *A. oxyrinchus* and *A. sturio* belong to the 120-chromosome group of sturgeons and, therefore, *A. sturio* belongs to the group of 120-chromosome sturgeon species; members of the other group of species have 240 chromosomes (for detailed discussion of the problem of ploidy in sturgeons, see Birstein, Hanner and DeSalle, 1997; Birstein and DeSalle, 1998; Vasil'ev, 1999). On the basis of karyotypic structure, a high number of nucleoli in nuclei, and a high level of duplicated loci, the 120-chromosome sturgeon species are usually considered tetraploids (Ohno *et al.*, 1969; Birstein, Hanner and DeSalle, 1997). The American paddlefish *Polyodon spathula* (Walbaum, 1792), a representative of the second acipenseriform family Polyodontidae, also has 120 chromosomes and similar karyotypic characteristics to those of the 120-chromosome sturgeons (Dingercus and Howell, 1976). In *P. spathula*, the duplicated loci were found for insulin, glucagon, glucagon-like peptide, and proopiomelanocortin (Nguen *et al.*, 1994; Danielson *et al.*, 1999). Since karyotypes of the shovelnose sturgeon *Scaphirhynchus platorynchus* (Rafinesque, 1820) (which belongs to presumably the oldest sturgeon subfamily Scaphirhynchinae – see Mayden and Kuhajda, 1996) and of *P. spathula* are similar, the tetraploid karyotype of 120 chromosomes is regarded as ancestral for acipenseriforms (reviewed in Birstein, Hanner and DeSalle, 1997; Vasil'ev, 1999). However, some authors describe the 120-chromosome sturgeon species, including *A. sturio*, as diploids (Fontana *et al.*, 1996; Tagliavini *et al.*, 1999).

A molecular phylogenetic study of all sturgeon species showed that *A. sturio* and *A. oxyrinchus* are clustered together in one clade, and that this clade is the sister-group to the other main clades of sturgeon species (Birstein and DeSalle, 1998). This conclusion was based on combined analysis of data for fragments of three mitochondrial (mt) genes, the 12S, 16S, and cytochrome *b* (cyt *b*) (150-bp, 350-bp, and 650-bp, respectively). The position of this clade in the phylogenetic tree supports the hypothesis that *A. sturio* (together with *A. oxyrinchus*) is probably a descendant of ancestral forms of *Acipenser* (Nesov and Kaznyshkin, 1983). Based on the fact that the main geological changes in the North Atlantic Ocean occurred during the Lower (135-95 My ago) and Upper (95-65 My ago) Cretaceous (Smith, Smith and Funnell, 1994), it is assumed that the *A. sturio-A. oxyrinchus* lineage originated during the Middle Cretaceous, ca. 90 My ago (Birstein and DeSalle, 1998).

Molecular difference between *A. sturio* and *A. oxyrinchus*

Wirgin, Stabile and Waldman (1997) compared a partial sequence (203-bp) of the control region of the mtDNA of *A. sturio* (an individual from the Gironde River) to sequences from 159 individuals representing both *A. o. oxyrinchus* and *A. o. desotoi*. They found a minimum of 31 and maximum of 33 nucleotide changes between *A. sturio* and *A. oxyrinchus* individuals. There were also three sites of insertions/deletions recognised: a CA insertion in positions 35-35, and deletions of GC in positions 58-59 and A in position 119 of *A. oxyrinchus* relative to *A. sturio*. The number of nucleotide changes in pairwise comparisons between the two subspecies of *A. oxyrinchus* ranged between 5 and 8, with no insertions and/or deletions, and is therefore much lower than between *A. sturio* and *A. oxyrinchus*. With the exclusion of insertions and deletions, nucleotide divergence between these two species was much higher (about 15 %) than between the two subspecies of *A. oxyrinchus* (maximum 3.5 %; Ong *et al.*, 1996). Also, we found 7 nucleotide changes in the 295-bp cyt *b* gene regions between *A. sturio* and *A. oxyrinchus* (figure 1; see also Birstein and DeSalle, 1998). We observed even more changes in a 642-bp fragment of the ND5 gene (table I).

Wirgin, Stabile and Waldman (1997, p. 387) concluded that “the level of [molecular] differentiation observed argues strongly for full species status of each of the western and eastern Atlantic sturgeons.” Therefore, molecular data support the traditional recognition of *A. sturio* and *A. oxyrinchus* as separate species (Magnin and Beaulieu, 1963; Magnin, 1964) and not subspecies, as Artyukhin and Vecsei (1999) suggested recently.

Are there intraspecies forms within *A. sturio*?

A comparison of two fragments of the cyt *b* gene in *A. sturio* individuals from different locations showed considerable intraspecific genetic differentiation (Birstein, Betts and DeSalle, 1998). Four *A. sturio* individuals were used for this analysis: two from the Gironde River in France, one caught in the North Sea near the Dutch coast (Timmermanns and Melchers, 1994), and one captured in the Baltic Sea in Estonian waters (Paaver, 1996, 1997). The sequences from two individuals from the Gironde River were practically identical (GR1 and GR 2) and differed in only a few changes from the sequence of the individual from the North Sea (GR1, GR 2, and NS, respectively, in figure 1 and table II). The partial cyt *b* sequence from the Baltic Sea individual (BS) differed slightly from that of the NS (6 changes) and GS1/ GS 2 (4 changes) (figure 1, table II). Ludwig *et al.* (2000) also described a difference in the distribution of microsatellite allelles in the museum specimens of *A. sturio* from the populations in the North and Baltic Seas. They found some alleles in the North Sea individuals only (alleles 121 and 124 of the microsatellite Afu-19; 147 of Afu-34; 120 of Afu-39 and 152 of Afu-68), while two alleles (130 of the microsatellite Afu-19 and 180 of Afu-180) were observed exclusively in the Baltic Sea specimens. All these data point to the possibility of intraspecies differentiation within *A. sturio*.

Earlier, two forms differing in meristic characters were described within *A. sturio* (Marti, 1939; Ninua, 1976). Marti (1939) showed that the number of dorsal and lateral scutes in the Baltic Sea *A. sturio* is lower than in the Black Sea *A. sturio* (9.6 and 14.3, and 27.7 and 32.8, respectively). Later it was found

Table I. Nucleotide changes in a partial sequence (642-bp) of the ND5 gene of *A. sturio* from the Gironde River and *A. oxyrinchus desotoi*[1]. ([1]): Data from Doukakis, Birstein and DeSalle (unpublished). Tissue samples from the same individuals GR1 and AD as in figure 1 and table II were used. ([2]): Position numbers refer to the ND 5/6 primer of Bembo *et al.* (1995)

Species	Positions of nucleotides[2]									
	39	50	68	70	103	128	154	164	173	193
A. sturio	A	C	C	G	T	A	G	C	C	C
A. oxyrinchus desotoi	G	T	T	A	C	T	A	G	T	T
	196	216	238	259	319	337	346	352	382	385
A. sturio	T	T	T	C	T	C	A	G	C	A
A. oxyrinchus desotoi	C	C	C	T	C	T	G	A	T	G
	388	398	412	445	466	469	478	517	532	544
A. sturio	T	T	G	A	A	A	G	C	T	T
A. oxyrinchus desotoi	C	C	A	G	G	G	A	T	C	C
	550	571								
A. sturio	T	A								
A. oxyrinchus desotoi	C	G								

```
Cytochrome b — a part of the region 1
NSa   CCGCCTTCCCATACATCGGCGACACACTAGTGCAATGAATCTGAGGCGGCTT
BSa   ··································G·················
GR1a  ····················································
GR2a  ····················································
ADa   ·························G····GT··G·················
AOa   ·························G····GT··G·················

Cytochrome b — a part of the region 2
NSb   GAATCATACTTTCTCTTTGCCTACGCCATCCTCCGATCTATTCCGAACAAACTAGGCGG
BSb   ····G···T··C···············································
GR1b  ····G······················································
GR2b  ····G······················································
ADb   ····G···T··C···············································
AOb   ····G···T··C···············································

NSb   AGTACTGGCCCTTCTATTCTCCATCCTAGTCCTAATATTGGTACCAGTCCTCCACACCT
BSb   ·····················T·····································
GR1b  ···························································
GR2b  ···························································
ADb   ···························································
AOb   ···························································

NSb   TCCAAACAACGGGGAAATACATTTCGGCCCCTCTCCCAAATCCTATTTTGAGCCCTAGT
BSb   ···········A···············································
GR1b  ···········A··············A·······················GA····G··
GR2b  ··························A·······················GA····G··
ADb   ···········A···············································
AOb   ···········A···············································
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Figure 1. Variable areas in the regions 1 (384-435 nucleotides) and 2 (811-986) of the cytochrome *b* gene in *A. sturio* and *A. oxyrinchus*. NS is the *A. sturio* specimen from the North Sea (Dutch coast): NSa corresponds to a partial sequence of the sequence submitted to GenBank under No. AF006145 (372-494 nucleotides); NSb is a partial sequence of a sequence under No. AF006176. BS is the *A. sturio* specimen from the Baltic Sea (Estonia): BSa and BSb regions correspond to NSa and NSb, respectively. AG1 and AG2 are two *A. sturio* specimens from the Gironde River (Cemagref, France). AD is a specimen of *A. oxyrinchus desotoi* (caught in the Pearl River, Louisiana, USA): ADa corresponds to NSa (accession No. AF006153) and ADb corresponds to NSb (accession No. AF006164). AO is a specimen of *A. oxyrinchus oxyrinchus* (caught in the St. Lawrence River, Quebec, Canada): AOa (accession No. AF006140) and AOb (accession No. AF006163) correspond to NSa and NSb, respectively. The description of specimens NS, BS, GR1, and GR2 was given in Birstein, Betts and DeSalle (1998), and of AD and AO, in Birstein and DeSalle (1998). Nucleotide positions correspond to positions in the cytochrome *b* gene sequence of the white sturgeon, *A. transmontanus* (Brown *et al.*, 1989; GenBank accession no. X14944)

that sturgeon from the Atlantic Ocean (Gironde River), the Mediterranean, and the Black Sea have similar numbers of scutes: for the dorsal scutes 12.7, 13.0, and 13.5, respectively; and the lateral scutes, 35.1, 33.4, and 32.0, respectively (Magnin, 1963; Ninua, 1976). Based on these data, Holčík *et al.* (1989, p. 371) suggested that the Baltic Sea population of *A. sturio* "is probably separated from the remaining ones and may be a distinct subspecies."

Studies on the molecular difference between the Baltic and North Sea sturgeons, as well as the Gironde River individuals, support this hypothesis concerning the possible existence of at least two forms of *A. sturio*. Although more in-depth studies are needed to confirm this hypothesis, such studies may be impossible, since live Atlantic sturgeon are caught very rarely.

Table II. Nucleotide changes in a combined sequence (295-bp) of the cytochrome *b* gene of *A. sturio* from the Gironde River (two specimens, GR1 and 2), North Sea (NR), and Baltic Sea (BS)[1]. ([1]): Data from Birstein, Betts and DeSalle (1998). Two regions of the cyt *b* gene cover nucleotides (372-494) and (811-1018) (see figure 1). ([2]): Numbers correspond to positions in the sequence of the cytochrome *b* gene of the white sturgeon, *A. transmontanus* (Brown *et al.*, 1989)

Fish sample	Positions of nucleotides[2]									
	478	815	819	822	892	939	955	979	980	985
NS	A	C	C	T	C	G	G	A	G	A
BS	G	G	T	C	T	A	G	A	G	A
GR1	A	G	C	T	C	A	A	G	A	G
GR2	A	G	C	T	C	G	A	G	A	G

Was *A. sturio* the only native sturgeon species in the Guadalquivir River in Spain?

In 1997, a group of Spanish authors suggested that two specimens from the collection of the Doñana Biological Station in Seville (EBD 8173 and 8174) were not *A. sturio*, but were rather the Adriatic (Italian) sturgeon, *Acipenser naccarii* Bonaparte, 1836 (Garrido-Ramos *et al.*, 1997). This theory completely contradicted the commonly accepted opinion that *A. sturio* was the Iberian Peninsula's only native sturgeon (Almaça, 1988; Elvira, Almodóvar and Lobón-Cerviá, 1991a, b; Elvira and Almodóvar, 1993). *A. naccarii* is an endangered species which lives in the Adriatic Sea and spawns mainly in the Po River basin in Italy (Tortonese, 1989; Rossi *et al.*, 1991). Recently, a second population of this species was found in Albanian waters of the Adriatic (Ludwig and Kirschbaum, 1998). This species is closely related to the Russian *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 and Siberian *Acipenser baerii* Brandt, 1869 sturgeons (Birstein, 1999b; Birstein, Doukakis and DeSalle, 2000), but not to *A. sturio*.

In addition to completing a morphological study, Garrido-Ramos *et al.* (1997) presented the results of a molecular study of one of the two specimens under discussion. Using a hypothesized "species-specific" nuclear satellite DNA (stDNA) Hind III from identified tissue samples of vouched specimens of *A. naccarii*, the authors claimed that they were able to hybridize this stDNA to the DNA extracted from the questioned *A. sturio* specimen. The presence of the "species-specific" satellite in the museum specimen and in extant *A. naccarii*, and the absence of this stDNA in extant *A. sturio*, led Garrido-Ramos *et al.* (1997) to conclude that "the museum specimen EBD 8173 captured in the Guadalquivir River corresponds to *A. naccarii*." Unfortunately, the authors did not describe their method of DNA extraction and, therefore, their experiments could not be reproduced.

Later the same authors showed that this HindIII stDNA is not "species-specific", since it is present in at least two other sturgeon species, *A. baerii* and *Huso huso* (L., 1758) (Ruiz-Rejón *et al.*, 2000). Previously a similar or a closely-related family of stDNA was also found in the Russian sturgeon *A. gueldenstaedtii*, sterlet *Acipenser ruthenus* L., 1758, and stellate sturgeon *Acipenser stellatus* Pallas, 1771 (Mikhailova *et al.*, 1995). Evidently, additional proof is needed to support the conclusion of Garrido-Ramos *et al.* (1997) and Ruiz-Rejón *et al.* (2000) that *A. sturio* does not contain the HindIII or a closely related stDNA. Also, the possibility that in the case of the specimen EBD 8173, Garrido-Ramos *et al.* (1997) extracted an authentic and not a contaminant DNA, should be eliminated.

Three laboratories in different countries (Molecular Laboratory, American Museum of Natural History, New York; Institute of Freshwater Ecology and Inland Fisheries, Berlin; Department of Biodiversity and Evolutionary Biology, National Museum of Natural Sciences, Madrid) tried to obtain additional molecular information on the two specimens under study. Sequencing of the following mitochondrial genes was employed for comparison of *A. sturio* with *A. naccarii* and for testing the questioned specimens: cyt *b*, NADH 5, control region, and 12S rRNA. It had been shown previously that the 389-bp fragment of the 12S gene in the two species differed by 7 nucleotide changes (table III) and that *A. sturio* and *A. naccarii* could, therefore, easily be discriminated using this gene (Ludwig and Kirschbaum, 1998).

None of the three laboratories could extract authentic DNA from the specimens in question, even though different DNA extraction methods were

Table III. Nucleotide changes in a partial sequence of the 12S ribosomal gene of *A. sturio* and *A. naccarii*[1]. (1): Data from Ludwig and Kirschbaum (1998). (2): Position numbers refer to the primer L1091 (Kocher *et al.*, 1989)

Species	Positions of nucleotides[2]						
	38	203	298	309	325	383	388
A. sturio	C	C	C	T	A	G	C
A. naccarii	G	T	T	C	G	A	G

used in each laboratory (Doukakis *et al.*, 2000). Moreover, Almodóvar, Machordom and Suárez (2000) later succeded in obtaining and sequencing a 152-bp fragment of the cyt *b* gene from the specimen EBD 8174. These authors demonstrated the similarity of this sequence and those from two other archival museum specimens of *A. sturio* to the ones already published, and generated by themselves the cyt *b* gene sequences from the recently collected *A. sturio* individuals. Further detailed morphological studies of the two specimens under discussion also failed to support the Garrido-Ramos *et al.* (1997) conclusion (Elvira and Almodóvar, 1999; Rincón, 2000). Therefore, until further proof using molecular methods is provided, these two specimens should be considered *A. sturio*. However, Garrido-Ramos and his colleagues still insist that the specimen EBD 8173 is *A. naccarii* (Ruiz-Rejón *et al.*, 2000) and not *A. sturio*.

Currently, there is no convincing evidence that *A. naccarii* historically lived in Spanish waters, and future conservation efforts should respect this finding. Also, although several Siberian sturgeon, *A. baerii*, have been caught in the rivers of Spain, these individuals were typical escapes from fish farms (Birstein, Betts and DeSalle, 1998; Elvira and Almodóvar, 1999). Therefore, *A. baerii* should not be considered endemic to this system, either.

A. oxyrinchus as a model for possible genetic traits in *A. sturio*

General structure of the mtDNA

In contrast to the length variation observed in the species of the Pacific coast (white sturgeon, *Acipenser transmontanus* Richardson, 1836, and green sturgeon *Acipenser medirostris* Ayres, 1854), no length variation in mtDNA molecules was observed among many specimens of *A. oxyrinchus* (specifically *A. o. oxyrinchus*) (Brown *et al.*, 1996; Waldman *et al.*, 1996; Ludwig *et al.*, 2000). Brown *et al.* (1996) discovered only one individual with longer mtDNA. This was a case of heteroplasmy, i.e. a difference in the length of the D-loop region due to variation in number of repeated segments (see below): this specimen of *A. o. oxyrinchus* had three repeats in the D-loop instead of one. However, Miracle and Campton (1995) observed heteroplasmy in 18.5 % (31) of the individuals out of 168 *A. o. desotoi* studied. The heteroplasmic individuals displayed 1-4 copies of tandemly repeated segments. These data need more detailed confirmation. Ludwig *et al.* (2000) found only one individual of *A. sturio* with three repeats in the D-loop among 38 museum and 28 live specimens studied.

The general organization of the control region in the mtDNA of *A. oxyrinchus* is similar to that in other sturgeon species such as *A. transmontanus* (Buroker *et al.*, 1990). There are, however, some specific organizational details which differ in *A. oxyrinchus*. Within an approximately 1-1.1 kb segment between the 3' end of the cyt *b* gene and the 5' end of the conserved sequence block (CSB) of *A. oxyrinchus*, the following elements exist: the genes for $tRNA^{Thr}$ and $tRNA^{Pro}$, an RTX segment (which has limited homology to the repeated sequence, RS), a 3'-flanking segment with 95 % sequence homology to the repeated sequence RS, an RS, a 5'-flanking segment with 60-70 % sequence homology to the RS, and then a region of a unique sequence of the control region (Brown *et al.*, 1996). Brown *et al.* (1996) determined the RS length in *A. oxyrinchus* as 80 bp, while Ludwig and Jenneckens (2000) considered it to be 79 bp in *A. o. oxyrinchus* and 80 bp in *A. sturio*. The RTX segment, which is characteristic of the *A. oxyrinchus* mtDNA, has not been found in the mtDNA of any other sturgeon species studied so far (Brown *et al.*, 1996).

Discrimination between A. o. oxyrinchus *and* A. oxyrinchus desotoi

Ong *et al.* (1996) used direct sequencing of a variable 203-bp region within the control region for comparison of *A. o. oxyrinchus* and *A. o. desotoi.* This 203-bp segment corresponds to positions from 3 to 237 of the control region of *A. transmontanus* (Buroker *et al.*, 1990). Three fixed differences at positions 104, 199, and 200 were found among 15 polymorphic sites within this region between the two subspecies of *A. o. oxyrinchus* (Ong *et al.*, 1996). The other polymorphic sites were scattered throughout the repeated sequences beginning at position 87 and ending at position 231. These data confirm the difference between the two subspecies. It has been hypothesised that the phylogenetic separation of the two subspecies might have been initiated by population separation in the late Pliocene or Pleistocene (Bowen and Avise, 1990; Avise, 1992). This disjunction could have occurred with changes in the size of the Florida Peninsula associated with the sea level and climatic changes during this period.

The evolutionary history of *A. sturio* was probably similar. During the last glaciation, the Baltic Sea was covered with ice (Forsström and Punkari, 1997; Klimanov, 1997), so the Baltic Sea form of *A. sturio* must have originated during the post-glacial time, i.e. during the last 11 000 years. The colonization or recolonization of *A. sturio* in the Baltic Sea occurred from the southwestern part of the North Sea, which was not covered with ice.

However, a post-glacial founder effect is not the only possible explanation of the facts. Ludwig *et al.* (2000) considered another potential scenario: the decrease in population size in *A. sturio* and *A. o. oxyrinchus* caused by overfishing could result in a loss of genetic variability during the 19th-20th centuries. If so, the decrease in population size could lead to a lineage sorting effect.

Stock distribution of A. oxyrinchus *along the North American East coast*

The data on the structure of the *A. oxyrinchus* stocks may give an idea of possible structure and history of the former *A. sturio* populations. RFLP analysis of mtDNA using four diagnostic restriction enzymes (*Bgl* I, *Msp* I, *EcoR* V, *Hinf* I, and *Hinc* II) (Waldman, Hart and Wirgin, 1996; Waldman *et al.*, 1996) and sequencing of the 203-bp fragment of the control region mentioned above (Wirgin *et al.*, 2000) was used to characterise the stock structure of *A. o. oxyrinchus* along the Atlantic coast. Using sturgeon samples from 11 river systems, chi-square analysis showed that populations could be grouped into three main highly differentiated stocks (with four subgroups in the southeastern stock):

(1) Canadian: St. Lawrence and St. John Rivers;
(2) Hudson River;
(3) Southeastern:
- (a) Albermarle Sound;
- (b) Cape Fear River;
- (c) Edisto River;
- (d) Savannah, Ogeechee, and Altamaha Rivers.

A profound latitudinal cline in the number of composite mtDNA haplotypes and in haplotypic diversity was observed, which increased from north to south (Wirgin *et al.*, 2000). The number of haplotypes in each population ranged from one in each of the two most northern populations to 17 in the Savannah River. Haplotypic diversity (Nei and Tajima, 1981) among populations ranged from complete monomorphism (0.0, St. John River) in the two Canadian populations to considerable polymorphism in southeastern populations (0.90, the Savannah River). Previously, Brown *et al.* (1996) also observed a very low level of mtDNA sequence diversity in Atlantic sturgeon from the St. Lawrence River. These results, combined with the monomorphism of the two Canadian populations, suggest a strong post-glacial founder effect (but see above). Three haplotypes unique to northern populations were probably the result of nucleotide substitutions that occurred during post-glacial times, i.e. within the last 10 000 years. The greater genetic diversity in the southern populations is most likely due to continuity of these populations through the Pleistocene: all river systems with populations of sturgeons characterised by a high level of haplotypic diversity are in an area which was not glaciated (Swift *et al.*, 1986). Possibly, the second factor of high genetic diversity was faster mutation rates in these sturgeon because of their shorter generation times (Wirgin *et al.*, 2000). Twenty-five (64 %) of the 39 composite mtDNA haplotypes found were unique to sturgeon from a particular population.

A similar analysis of RFLP data (*Bgl* I, *Msp* I, *EcoR* V, and *Hinf* I) and sequencing of a 203-bp variable fragment of the control region (the same as in Ong *et al.*, 1996) allowed Stabile *et al.* (1996) to identify 5 regional or river-specific stocks of *A. o. desotoi* from west to east, as follows:

(1) Lake Ponchartrain and Pearl River (Louisiana);
(2) Pasacagoula River (Mississippi);
(3) Escambia and Yellow rivers (Florida);
(4) Choctawhatchee River (Florida);
(5) Apalachicola, Ochlockonee, and Suwannee rivers (Florida).

These data point to a strong reproductive isolation of *A. o. desotoi* stocks, a point which is reinforced by the low gene flow estimates between populations (details in Stabile *et al.*, 1996 and Wirgin, Stabile and Waldman, 1997). Haplotypic diversity (the RFLP data) ranged from 0.09 between the western and Choctawhatchee River stocks to 0.66 between the western and Escambia River-Yellow River stocks. Stabile *et al.* (1996) discussed two possible explanations for these results. First, these data indicate the possibility of strong homing fidelity of individuals within *A. o. desotoi*. Second, the homing fidelity may be reinforced by metabolic, temperature-dependent constraints: the sturgeon return to the same rivers from the Gulf of Mexico to summer near cold water springs in the cool water refuges (Clugston, Foster and Carr, 1995; Foster and Clugston, 1997).

Molecular data and conservation projects

Since *A. sturio* is internationally listed as critically endangered (Anon., 1996), there is a serious concern in the European scientific community regarding this species. The Gironde River population of *A. sturio* has been studied for 20 years and a project for its restoration has been elaborated by French experts (Elie, 1997). A search for sturgeons from the Gironde (France) and Rioni (Georgia) Rivers, in an effort to create an artificial aquaculture of *A. sturio* in northern Germany, was recently suggested (Debus, 1995). In 1994, the Society to Save the Sturgeon *Acipenser sturio* was organised in Europe (Elvira and Gessner, 1996). The importance of genetic and molecular data illustrated in this paper stresses the necessity to base all practical efforts on the results of genetic research. Individuals caught in the historic area of *A. sturio*, especially young sturgeon, can be easily misidentified because of their morphological similarity to the Russian sturgeon, *A. gueldenstaedtii*, Siberian sturgeon, *A. baerii*, or Italian sturgeon, *A. naccarii*. The confusion with the last species is extremely important in the Adriatic Sea, where both *A. sturio* and *A. naccarii* historically spawned in the same rivers and local fishermen did not distinguish between them (Holčík *et al.*, 1989; Tortonese, 1989).

Currently, the problem of discrimination between *A. sturio* and *A. naccarii* is especially relevant due to the discussion of a project to introduce *A. naccarii* from an aquaculture stock developed in Italy into the Guadalquivir River in Spain (Ruiz-Rejón, Hernando and Domezain, 1998; Domezain, 1999). The implementation of this project should be postponed until convincing genetic data can prove that *A. naccarii* in fact historically existed in Spain. A species introduction must not be based on the poorly supported opinion of only one laboratory that one of many scores of sturgeon specimens kept in museum collections of Spain was not *A. sturio*, but *A. naccarii*.

The IUCN Red List classifies *A. o. oxyrinchus* as near threatened and *A. o. desotoi* as vulnerable (Anon., 1996). Recently, the US Fish and Wildlife Service and the US National Marine Fisheries Services were petitioned to list and protect both subspecies under the Endangered Species Act (Waldman and Wirgin, 1998). In 1994 and 1996, several thousand *A. o. oxyrinchus* from an artificially cultured Hudson River broodstock were released into the Hudson River and Chesapeake Bay (Secor, 1996; Waldman and Wirgin, 1998; Secor and Waldman, 1999). In the latter case, within 8 months the released fish had spread through the Chesapeake Bay from Baltimore Harbor to the lower James River in Virginia and even to neighbouring North Carolina (St. Pierre, 1999). However, Waldman and Wirgin (1998) recommended avoiding interstock transfer of *A. oxyrinchus* in future stocking to prevent disintegration of locally adapted gene pools. Another concern is the possibility of inbreeding depression of *A. oxyrinchus* due to a low number of artificially bred individuals used in hatchery-based stocking projects (St. Pierre, 1996, 1999).

Concluding remarks: The need to study museum specimens

Genetic data on the current structure of *A. oxyrinchus* stocks enabled Wirgin, Stabile and Waldman (1997) and Wirgin *et al.* (2000) to reconstruct the history of this species. Also, genetic experiments on *A. oxyrinchus* demonstrated that genetic data are crucial to any conservation project. The lack of experimental material is the limiting factor of genetic study of *A. sturio,* since only several live *A. sturio* were caught during the last decade; the only solution is to study museum specimens. Since new DNA extraction for formalin-preserved museum specimens has recently been developed (France and Kocher, 1996; Vachot and Monnerot, 1996; Jiang, Wu and Lin, 1997; Wirgin *et al.*, 1997), such a study on *A. sturio* is more realistic than ever. The first results using microsatellite DNA extracted from museum specimens of *A. sturio* from the North and Baltic Seas showed a high genetic similarity between these populations in the past, and a possible decline in genetic variability in both populations from 1823 to 1992 (Ludwig *et al.*, 2000).

Future molecular experiments should be performed in conjunction with morphological study of the same specimens. This will allow researchers to describe intraspecific forms within *A. sturio*, if they in fact existed. All of the main European museum collections holding *A. sturio* specimens should be involved in this work and it should be coordinated by a group of experts.

The evolutionary history of *A. sturio* is practically unknown. Below are some of the main outstanding questions:

(1) Was the Gironde River basin a refugium from which colonization of other populations in the North and Baltic seas occurred?
(2) How were the populations of the Gironde River, Iberian Peninsula, Mediterranean and Adriatic Sea genetically interrelated?
(3) Finally, did the Black Sea population originate from the sturgeons of the Adriatic-Marmora Seas after the Black Sea became connected with the Mediterranean approximately 8 000 years ago (Ryan *et al.*, 1997), or is this population older?

Only results of a detailed genetic research can yield answers to these questions.

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First results on the diet of the young Atlantic sturgeon *Acipenser sturio* L., 1758 in the Gironde estuary

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ABSTRACT

Very little is known about the diet of the European sturgeon *Acipenser sturio* L., 1758 in its natural environment. For juveniles, improved knowledge in this field could lead towards the determination of the species's major feeding habitats in the estuary, in order to then improve their preservation or protection. From May 1998 to March 1999, the stomach contents of 61 juveniles were collected by gastric lavage. The fish were caught during monthly trawling campaigns to monitor sturgeon migration in the Gironde estuary (southwestern France). Specimens were mainly caught during spring and summer in two areas of increased abundance. The gastric lavage method used had been previously tested on Siberian sturgeon *Acipenser baerii* Brandt, 1869 in captivity. Twelve taxa of prey were found. The highest proportions in number identified consisted of polychaetes, mainly represented by *Heteromastus filiformis* (Claparede, 1864) in zone 7 and *Polydora* Bosc, 1802 sp. in zone 1. Crustaceans were the second most abundant group of prey.

Key words: Feeding, juvenile, France, invertebrate fauna.

RESUMEN

Primeros resultados sobre la dieta de los jóvenes esturiones atlánticos* Acipenser sturio *L., 1758 en el estuario del Gironda

Se conoce muy poco sobre la dieta del esturión atlántico Acipenser sturio *L., 1758 en su medio natural. Para los juveniles, un conocimiento mejorado en este campo podía conducir hacia la determinación de los principales hábitats alimentarios de la especie en el estuario, como preámbulo para su mejor preservación y conservación. Entre mayo de 1998 y marzo de 1999 fueron recogidos los contenidos estomacales de 61 juveniles por lavado gástrico. Los peces fueron capturados durante las campañas mensuales de pesca de arrastre para el seguimiento de la migración del esturión en el estuario del Gironda (suroeste de Francia). Los ejemplares fueron capturados principalmente en primavera y verano en dos áreas de alta abundancia. El método de lavado gástrico utilizado fue probado previamente en cautividad con esturión siberiano* Acipenser baerii *Brandt, 1869. Se encontraron presas de doce taxones. Las proporciones más altas en número correspondieron a poliquetos, principalmente representados por* Heteromastus filiformis *(Claparede, 1864) en la zona 7 y* Polydora *Bosc, 1802 sp. en la zona 1. Los crustáceos fueron el segundo grupo de presas más abundante.*

Palabras clave: *Alimentación, juveniles, Francia, fauna de invertebrados.*

INTRODUCTION

Knowledge about the diet of *Acipenser sturio* L., 1758 in its natural environment is very scarce. For juveniles, a better understanding in this field is considered a step towards the assessment of the species's major foraging areas in the estuary. These data would make it possible to protect them from adverse human impact. Additionally, the results could lead to an improvement in the efficiency of the alimentation of *A. sturio* in captivity.

Acipenser oxyrinchus Mitchill, 1815, between 106 and 213 cm (total length, Tl) mainly feed on polychaetes and isopods (Johnson *et al.*, 1997), while subadults of *A. oxyrinchus desotoi* Vladykov, 1955 forage mainly on arthropods, annelids and molluscs (Mason and Clugston, 1993).

The diet of the white sturgeon *Acipenser transmontanus* Richardson, 1836, is based on *Corophium* Latreille, 1806 (crustacean) and *Corbicula fluminea* (Muller, 1774) (mollusc) for individuals less than 80 cm long (McCabe, Emmett and Hinton, 1993; Muir, Emmett and McConnell, 1998). Zolotarev, Shlyakhov and Akselev (1996) show that juvenile Russian sturgeon *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 predominantly feed on molluscs, whereas stellate sturgeon *Acipenser stellatus* Pallas, 1771 consume mainly polychaetes. The diet of the juvenile lake sturgeon *Acipenser fulvescens* Rafinesque, 1817 essentially comprises crustaceans and insect larvae (Nilo, 1996; Beamish, David and Rossiter, 1998) as in *Acipenser brevirostrum* LeSueur, 1818 (Dadswell, 1979; Carlson and Simpson, 1986).

These results show that young sturgeons are benthic feeders that prefer small, soft-bodied, benthic prey organisms.

Some preliminary research was conducted on the feeding habits of juvenile *A. sturio* in the Gironde estuary by Magnin (1962). He found that fishes caught in the upper part of the estuary predominantly feed on shrimps *Crangon crangon* (L., 1758) and gammarids. Fishes from the lower part of the estuary were described as mainly consuming polychaetes and mysids. However, those results are very incomplete, and present only few details about the occurrence and proportions of each of the items found in the stomach contents. Therefore, we decided to study more precisely the diet of juvenile *A. sturio* during their initial stay in the Gironde estuary.

MATERIALS AND METHODS

From May 1998 to March 1999, the stomach contents of 61 juveniles were collected by gastric lavage during monthly trawling campaigns primarily aimed at monitoring the spatial distribution of juvenile sturgeons (Rochard *et al.*, 1998). Each trawl lasted for 30 minutes and took place in 12 predefined zones of the estuary (figure 1).

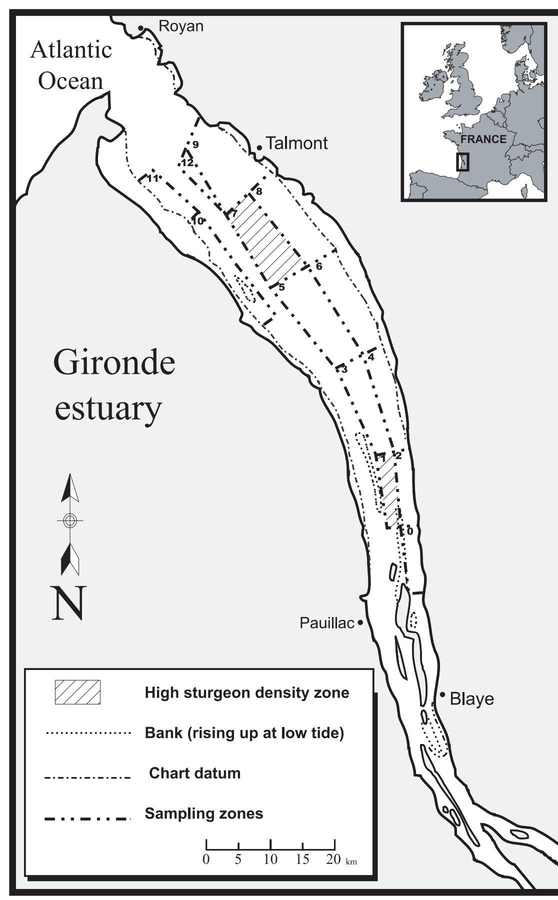

Figure 1. The Gironde estuary, study areas and sampling zones

The gastric lavage method we used is adapted from Nilo (1996). This method has been tested on the Siberian sturgeon *Acipenser baerii* Brandt, 1869 in captivity, and we concluded that it is easy to use, non-traumatic, and permits a good recovery rate of feed-particles. Average recovery rate in *A. baerii* was determined to be between 47 % and 83 %, depending on the prey's shape (Brosse *et al.*, in preparation). Upon their capture, specimens were measured and weighed (two length classes: 63-86 cm and 87-116 cm) before the stomach flushing took place. The gastric lavage device was composed of a garden sprayer equipped with a soft tube (Ø6 mm ext.) for the injection of the water inserted into a bigger one (Ø12 mm ext.) designed to collect the stomach contents (figure 2). Sturgeons were not anaesthetised during this operation because of uncertainties with regard to the efficiency and potential harmful side effects of the clove oil anaesthetic under brackish water conditions for this rare species.

Data were processed using discriminate analysis and the Kruskall-Wallis test to compare for spatial and seasonal variation in the diet. Using the same tests, we checked for differences in numbers or in proportions among the different groups of prey found in the contents.

RESULTS

A. sturio specimens in the Gironde estuary were mainly caught during spring (n = 41) and summer (n = 15) in eight of the 12 sampled zones. However, most of them were collected in two zones (no. 1 and 7, figure 1) of higher sturgeon density (figure 3).

Fish measured between 63 and 116 cm Tl and weighed 1-8 kg. All of the sturgeon captured were in good condition and were released into the estuary after stomach flushing. A total of 12 prey taxa were found (table I).

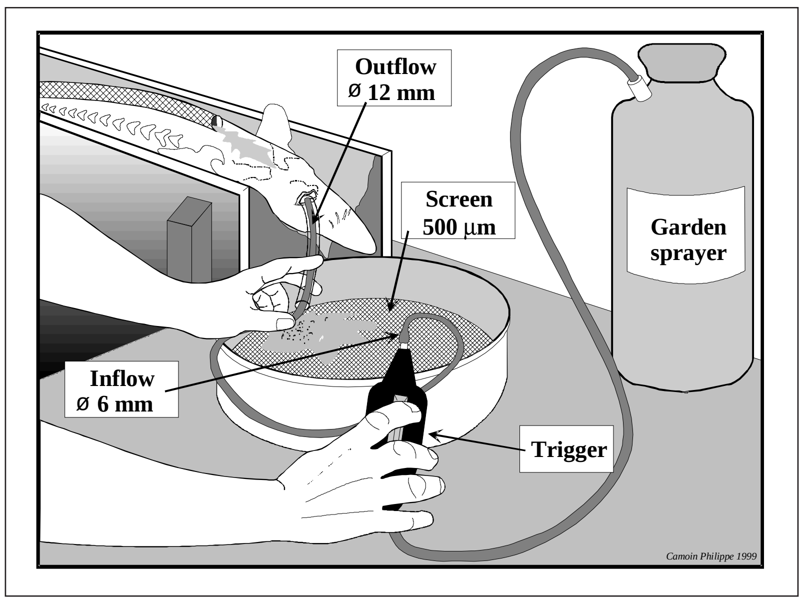

Figure 2. The stomach flushing disposal in place

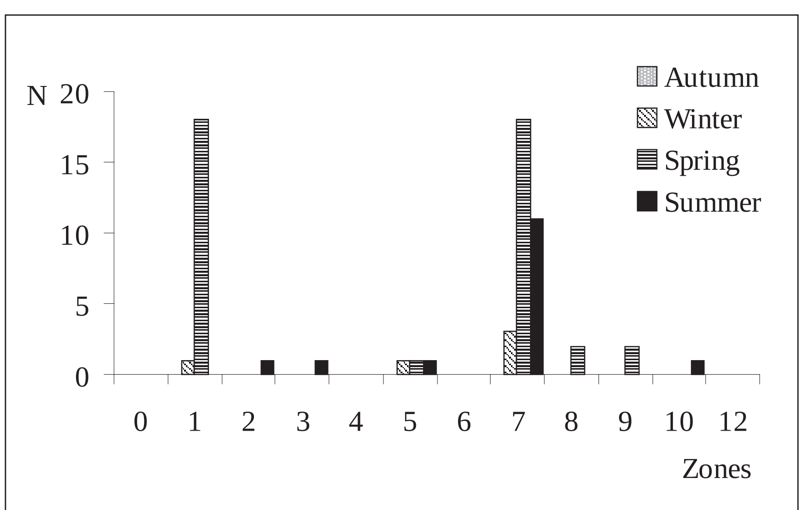

Figure 3. Number (N) of stomach contents of *A. sturio* analysed in the different zones of the esturary during one year (N_{total} = 61)

Table I. Mean number (N), standard deviation (s), and proportion (%), for each taxa encountered in the stomach contents for all the zones and seasons where stomachs were analysed

	Winter		Spring		Summer	
Preys	N(s)	%	N(s)	%	N(s)	%
Amphipoda						
Bathyporeia pelagica Bate, 1857					0.5 (2.0)	0.1
Corophium volutator Pallas, 1766					9.5 (35.7)	1.1
Gammarus sp.	0.6 (0.8)	0.3	12.9 (52.9)	1.2		
Isopoda						
Cyathura carinata (Krøyer, 1847)	3.8 (2.8)	2.0	2.6 (4.5)	0.2	0.5 (0.7)	0.1
Syntidotea sp.	4.2 (4.8)	2.2	0.3 (1.9)	0.0	0.4 (1.0)	0.0
Mysidacean						
Mysids			1.8 (3.9)	0.2	3.3 (9.6)	0.4
Decapoda						
Carcinus maenas (L., 1758)	0.2 (0.4)	0.1				
Crangon crangon (L., 1758)	7.2 (10.6)	3.8	0.5 (1.1)	0.1	2.4 (4.2)	0.3
Polychaeta						
Heteromastus filiformis (Claparede, 1864)	170.4 (184.7)	90.7	721.6 (1 522.9)	67.9	878.8 (1 166.2)	98.0
Nereide sp.	1.4 (1.0)	0.7	7.8 (28.5)	0.7	0.8 (1.2)	0.1
Polydora sp.			314.4 (669.5)	29.6		
Pisces						
Pomatoschistus minutus (Pallas, 1770)			0.0 (0.2)	0.0	0.3 (1.0)	0.0

Table I (continued)

	Zone 1		Zone 2		Zone 3		Zone 5		Zone 7		Zone 8		Zone 9		Zone 10	
Preys	N(s)	%	N(s)	%	N(s)	%	N(s)	%	N(s)	%	N(s)	%	N(s)	%	N(s)	%
Amphipoda																
Bathyporeia pelagica															8.0	16.7
Corophium volutator							47.7 (67.4)	58.6								
Gammarus sp.	0.5 (1.8)	0.1					18.3 (25.9)	22.5	0.3 (1.1)	0.0	1 (0.0)	0.1	227 (87.0)	96.2		
Isopoda																
Cyathura carinata	4.6 (5.9)	0.7	1.0	25.0	1.0	10.0	1.3 (25.9)	1.6	1.2 (1.8)	0.1	1.5 (0.5)	0.2				
Syntidotea sp	0.1 (0.2)	0.0					4.3 (3.7)	5.3	0.4 (1.9)	0.0			6 (6.0)	2.5		
Mysidacean																
Mysids	0.1 (0.3)	0.0	3.0	75.0			4.7 (6.6)	5.7	1.8 (3.8)	0.1	2 (1.0)	0.2	2.5 (2.5)	1.1	39.0	81.3
Decapoda																
Carcinus maenas	0.1 (0.2)	0.0														
Crangon crangon	1.6 (6.2)	0.2			6.0	60.0	3.3 (2.9)	4.1	1.4 (3.0)	0.1	1 (1.0)	0.1				
Polychaeta																
Heteromastus filiformis					3.0	30.0			1 304.8 (1 692.0)	98.8	932 (676.0)	99.4	0.5 (0.5)	0.2		
Nereide sp.	0.2 (0.5)	0.0					0.3 (0.5)	0.4	10.4 (31.8)	0.8	0.5 (0.5)	0.1			1.0	2.1
Polydora sp.	678.4 (848.6)	99.0														
Pisces																
Pomatoschistus minutus	0.1 (0.2)	0.0					1.3 (1.9)	1.6	0.0 (0.2)	0.0						

The rate of empty stomachs was very low (2.5 % in spring and 0 % in summer and winter). The gut contents mainly consisted of polychaetes, with a strong dominance in number. The species present in the gut contents were dominated by *Polydora* Bosc, 1802 sp. (Spionidae) in zone 1 and *Heteromastus filiformis* (Claparede, 1864) (Capitellidae) in zone 7.

The juvenile sturgeon diet varied according to sampling season (spring compared to summer: $H = 4.767$; $p = 0.0052$) and sampling zone (zone 1 vs. 7: $H = 9.803$; $p < 0.001$) but not according to fish length ($H = 1.591$; $p = 0.126$).

DISCUSSION

The diet of the *A. sturio* juveniles mainly consisted of polychaetes and benthic crustaceans (table I). All of these items are small, and seem to have a limited ability to escape. The strong dominance of the polychaetes in the diet may be due to the very high densities of these organisms in the substrate (Bachelet, Bouchet and Lissalde, 1981).

There are some differences between our results and those from Magnin (1962), mainly concerning the diet of sturgeons caught in the upper part of the estuary. Magnin found that sturgeons eat primarily shrimps and gammarids, while we found that they mainly consume polychaetes. Magnin only indicated what he occasionally observed in the gut contents of sturgeons, but he did not perform a special study on this aspect.

Our differences could be explained using two hypotheses:

- The sturgeons in Magnin's study were younger than those used in ours and it is possible that their diet changed during their stay in the estuary.
- The benthic fauna in this part of the estuary has significantly changed between the two studies, and the sturgeons have modified their diet in order to stay in this part of the estuary.

Both studies describe young *A. sturio* as benthic feeders, which consume small soft-bodied prey organisms. Compared to data on other sturgeon species, it is apparent that all are feeding on invertebrates, mainly arthropods and annelids (Dadswell, 1979; Carlson and Simpson, 1986; Mason and Clugston, 1993; McCabe, Emmett and Hinton, 1993; Zolotarev, Shlyakhov and Akselev, 1996; Nilo, 1996; Johnson *et al.*, 1997; Beamish, David and Rossiter, 1998, and Muir, Emmett and MacConnell, 1998).

Sturgeons are mainly bottom feeders, as can be concluded from the ventral position of their mouth and the way they collect their food using a projection of their oropharyngian cavity. Therefore, all of the sturgeons did not consume exactly the same taxa. There are three main groups of prey: arthropods (insect larvae and crustaceans), annelids (oligochaetes and polychaetes) and molluscs (bivalves and gastropods). *A. sturio* mainly feeds on annelids and arthropods, as does *A. oxyrinchus* (Mason and Clugston, 1993; Johnson *et al.,* 1997) and *A. stellatus* (Zolotarev, Shlyakhov and Akselev, 1996). Some, such as *A. fulvescens,* (Nilo, 1996; Beamish, David and Rossiter, 1998) or *A. brevirostum* (Dadswell, 1979; Carlson and Simpson, 1986), mainly feed on arthropods. Others, such as *A. gueldenstaedtii* (Zolotarev, Shlyakhov and Akselev, 1996), essentially consume molluscs. The differences in predominant food may be linked to the main fauna of the habitats where these sturgeons live. For example, the benthic fauna in the rivers is dominated by arthropods and annelids, while it is mainly composed of annelids, molluscs, and arthropods in the estuaries.

The temporal variation in the young sturgeons' diet may be linked with their seasonal migrations, as described previously (Rochard *et al.*, 1998). However, the fact that there were no sturgeons caught in the zone 1 during summer may be sufficient to influence the results of our statistical tests.

Thus, the migrations observed may be due to changes in the benthic fauna composition. We can neither accept nor reject this theory at present, because we lack information on the composition and spatio-temporal distribution of the benthic fauna in the estuary channels.

Because of these uncertainties, we cannot draw conclusions on this temporal variation in sturgeon abundance until more information on the benthic fauna (repartition, dynamics, etc.) becomes available.

The spatial differences in food composition are stronger than the temporal ones. These differences essentially result from the predominant consumption of two different polychaete taxa (*Polydora* sp. and *Heteromastus filiformis*) in the main upstream and downstream capture zones. We can formulate two hypotheses to explain this segregation between those two species:

- These two polychaetes have different ecological requirements and do not live under the same conditions. Therefore, it should be realistic to think that *Polydora* sp. is present in high densities in zone 1 and *Heteromastus filiformis* in zone 7. In that case, young sturgeons would consume the most abundant prey and have an opportunistic feeding behaviour.
- The two polychaetes are found in high densities throughout the entire estuary because of their wide ecological spectrum. This leads to the conclusion that young sturgeons are selecting *Polydora* sp. in the upper estuary and *Heteromastus filiformis* in the downstream area of the estuary, indicating a specialist feeder behaviour.

Because we have no quantitative data on the benthic fauna found in the channels of the

Gironde estuary, future studies and subsequent comparisons with dietary changes in *A. sturio* are to be carried out.

This work has collected quantitative and qualitative data on the *A. sturio*'s diet, and its spatial and temporal variability. The present study shows that there are dietary differences among sturgeon species living in different water bodies, such as rivers and estuaries.

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Distribution and conservation of *Acipenser sturio* L., 1758 and related species in Greek waters

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ABSTRACT

Four sturgeon species have been reported to exist in Greek waters: the Atlantic sturgeon, the great sturgeon, the stellate sturgeon and the Adriatic sturgeon. The Atlantic sturgeon *Acipenser sturio* L., 1758 has an almost regular presence in the Evros River (Aegean Sea), and is rather rare in the Pinios and Acheloos Rivers. The great sturgeon or beluga *Huso huso* L., 1758 is suspected to be an occasional visitor in Greek waters, or it is a case of undocumented information. The presence of the stellate sturgeon *Acipenser stellatus* Pallas, 1771 has been recently verified by a specimen caught close to the Thracian shore. The Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836 is supposed to have its southern distribution limits in the waters around the island of Corfu, although this information has never been confirmed. The distribution of these species is obviously related to salinity, probably prohibiting the extension of other sturgeon species to the Mediterranean, except for *A. sturio.* The discontinuous distribution of *H. huso* and *A. stellatus* may be a result of sea-level changes, while salinity played some role in the last period of glaciation. In the marine area of the Evros River *A. sturio* has been economically significant until 1975, supporting a small black caviar industry. Since then, the species has become rare mainly because of overfishing, and the reduction and pollution of the Evros River, in the upstream of which some important sturgeon spawning sites have been located.

Key words: *Acipenser naccarii, Acipenser stellatus, Huso huso,* production, conservation status, Greece.

RESUMEN

Distribución y conservación de* Acipenser sturio *L., 1758 y especies próximas en aguas griegas

Cuatro especies de esturiones han sido citadas en las aguas griegas: el esturión atlántico Acipenser sturio *L., 1758, el beluga* Huso huso *(L., 1758), el esturión estrellado* Acipenser stellatus *Pallas, 1771 y el esturión del Adriático* Acipenser naccarii *Bonaparte, 1836. El esturión atlántico* A. sturio *tiene una casi regular presencia en el río Evros (mar Egeo), y es bastante raro en los ríos Pinios y Acheloos. El beluga* H. huso *se sospecha que es un visitante ocasional de las aguas griegas, o es un caso de información indocumentada. La presencia de esturión estrellado* A. stellatus *ha sido recientemente verificada con un ejemplar capturado cerca de la costa tracia. El esturión del Adriático* A. naccarii *se supone que tiene sus límites meridionales de distribución en las aguas alrededor de la isla de Corfú, aunque esta información nunca ha sido confirmada. La distribución de estas especies está obviamente relacionada con la salinidad, impidiendo, probablemente, la extensión de otras especies de esturiones en el Mediterráneo, con excepción de* A. sturio. *La distribución discontinua de* H. huso *y* A. stellatus *puede ser el resultado de cambios en el nivel del mar, mientras que la salinidad desempeñó algún papel en el último periodo glacial. En el área marina del río Evros,* A. sturio *ha sido económicamente significativo hasta 1975, soportando una pequeña industria de* *caviar. Desde entonces, la especie ha llegado a ser rara, principalmente a causa de la sobrepesca y la reducción y contaminación del río Evros, en cuya parte superior se localizaban importantes lugares de freza para el esturión.*

Palabras clave: Acipenser naccarii, Acipenser stellatus, Huso huso, *producción, estado de conservación, Grecia.*

INTRODUCTION

It is generally accepted (Berg, 1948) that the watersheds of the Black and Caspian Seas comprise the most important areas in the world for the speciation of sturgeon. Few of these species, especially those unable to tolerate the higher salinities of seas such as the Mediterranean, have the ability to move far away from their dispersion centres. *Acipenser sturio* L., 1758 is obviously such a species, which apparently attracts the greatest attention in the many European countries where it is encountered. However, other sturgeon species are also found in some eastern European areas, and they are also in need of study and conservation measures. Additionally, because of the great confusion in the determination of many historical specimens and their citation, it is necessary to clarify this matter before any major study can proceed. Therefore, an overview for Greece is useful, because there is a contact with the main dispersal zone of the different sturgeon species, four of which have been reported in Grecian waters.

Since early historical times, a plethora of information on sturgeon has appeared in the books of many classical Greek authors (Thompson, 1947). This became even more frequent when ancient Greek colonies became involved in a number of important economic activities around the Black Sea shore, which continued up to the late Byzantine period, even during the Ottoman Empire. Information about species of these fishes in the Greek seas, such as the Aegean and Ionian, has been rather poor. Avoiding any literate approach, the information given by Atheneus (2nd-3rd century A.D.) concerning a much valued fish, for which Rhodes was celebrated, seems to be interesting. This was "a kind of short sized and large snout *akkipisios* (sturgeon) with a rather triangular shape". This description led Cuvier to think of the little sterlet *Acipenser ruthenus* L., 1758 which has not, however, entered the Mediterranean (Thompson, 1947). Furthermore, as was pointed out by Georgacas (1978), many products, especially around the Black Sea, derived from the treatment of fish ovaries have kept names of Greek origin (e.g. caviar and botargo) to the present.

For the present study we have used previous original but contemporary references, usually poorly documented, as well as some new material.

MATERIALS AND METHODS

Some morphometric characteristics of three *A. sturio* specimens, collected by Economidis (1974) from the Evros River (Aegean Sea) and stored in the fish collection of the School of Biology of the Aristotle University, have been used. The morphology and measurements of *Acipenser stellatus* Pallas, 1771 presented below are based on a rather immature male specimen caught recently close to the Thracian shores and deposited in the fish collection of the Fisheries Research Institute of Nea Peramos (Kavala). All measurements and counts are made according to those proposed by Holčík, Banarescu and Evans (1989) for sturgeon taxonomy. Standard length (Sl), fork length (Fl), predorsal distance (pD), head length (lc) and body depth (H) are calculated as % of the total length (Tl, mm), while head depth at nape (hc), head depth at center of eye (hco), distance between tip of snout and mouth (s-m), distance between tip of snout and cartilaginous arch of mouth (s-mc), distance between base of barbels and cartilaginous arch of mouth (b-mc), length of barbel (lb) and width of mouth (lam), as well as width of snout at base of barbels (lab) are calculated as % of the head length (lc). Dorsal (SD), lateral (Sl) and ventral (SV) scutes, dorsal (D) and anal (A) fins and branchial spines (Sp.br) are counted separately. The rest of the material included in this paper comes from literature collected for historical overview purposes.

RESULTS

Species records

The sturgeon species have been known in the Greek language from ancient times under several names: *akkipisios* (ακκιπήσιος, ancient name), *xyryhi* (ξυρύχι, ancient name: οξύρυγχος), *mourouna* (μουρούνα), *stourioni* (στουριόνι). According to Economidis *et al.* (2000), three sturgeon species, *Acipenser baerii* Brandt, 1869, *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 and *Acipenser ruthenus*, have been introduced in Greek waters (mainly Lake Pamvotis), while the four species discussed below are reported as native.

Acipenser sturio L., 1758

As is well known, the species has the largest distribution among all the other species of Acipenseridae in the European watersheds (Holčík *et al.*, 1989). In the Greek seas and freshwater catchment, the species was reported off Corfu (Apostolidis, 1883, 1907); in the rivers flowing into the Thracian Sea, such as Evros (table I), both on the Greek and the Bulgarian sides (Kovatchev, 1921; Konsuloff and Drensky, 1943; Drensky, 1948, 1951; Berg, 1948; Belloc, 1948; Stancovic, 1960; Economidis, 1973, 1974); Strymon (Drensky, 1948, 1951; Belloc, 1948; Stancovic, 1960); and in the estuaries of the Pinios River, in Thessaly (Panagiotopoulos, 1916; Athanassopoulos, 1917). Belloc (1948), in his monograph on fishery research in Greek waters, also mentions the presence of the species in the Gulf of Euboikos, in Peloponnesia, on the western coasts of Greece and off Corfu. Furthermore, the presence of the species in the estuaries of the Acheloos River has been confirmed by material deposited in the zoological collections of the University of Patras. It also has a rather regular occurrence along the Turkish coasts of the Aegean Sea (Geldiay and Balik, 1988) and in the Adriatic and Albanian watersheds, mainly in Lake Skadar (Poljakov, Filipi and Basho, 1958; Vuković and Ivanović, 1971; Ivanović, 1973; Rakaj, 1995).

Acipenser naccarii Bonaparte, 1836

The presence of this species in Greek waters, along its northwestern shoreline (mainly the island of Corfu), still remains undocumented. The first record for Corfu is from Heldreich (1878), who was not an ichthyologist, but a botanist. Some years later Apostolidis (1883) claimed that *A. sturio* lived off Corfu, a piece of information he repeated later (Apostolidis, 1907). None of these two authors' reports were based on actual fish material. Carus (1893), perhaps following Heldreich, listed the species as present off Corfu. The same is also true for Berg (1933), and more recently for Tortonese (1989). Despite the fact that the species could appear around the waters of Corfu, mainly close to the nearby estuaries of the Thyamis River, it seems that all of the above references are undocumented, because they are not based on actual material, and the confusion with *A. sturio* is very probable. Nevertheless, although rarely (Svedovidov, 1984; Tortonese, 1989), the species is present in the Adriatic Sea (Berg, 1933; Vukovic and Ivanovic, 1971), mainly on the eastern shore (Tortonese, 1989), and especially in Albanian waters, around Lake Skadar (Poljakov, Filipi and Blasho, 1958; Rakaj, 1995). Rakaj (1995) reports two specimens from Buna-Skadar.

Acipenser stellatus Pallas, 1771

The most recent approach (Shubina, Popova and Vasiliev, 1989) considers the species as having a regular distribution in the Black and the Caspian Seas, and on the Adriatic coast at Zadar, while its presence in the watershed of the Aegean Sea is disputable. This is based on older references, mainly from Berg (1933, 1948). Concerning the Aegean Sea area, Berg (1948) claims that "individual specimens some-

Table I. Measurements (in mm) of the three Atlantic sturgeon specimens *Acipenser sturio* (after Economidis, 1974). Fish collection of the School of Biology of Aristotle University (Thessaloniki)

Total length Tl	Dorsal Du	Anal Au	Branchial spines Sp.br.	Dorsal scutes SD	Lateral scutes SL	Ventral scutes SV
410-485	36-43	23-26	18-20	13-14	34-38	11-12

times penetrate the Maritza (Evros)". Drensky (1948, 1951) notes that the species is present in the Maritza River, where it does not enter so far (up to Svilengrad?). The species has been reported by Stanković (1960) as also living in the Evros and Strymon Rivers, while Geldiay and Balik (1988) have traced it on almost all the Turkish coasts of the Aegean Sea. On the other hand, the species seems to have a certified presence in the Albanian watershed of the Adriatic, since Rakaj (1995) mentions three specimens from the Matit River.

All of this confusion has been definitely clarified by the confirmation of the presence of the species in the Thracian Sea (North Aegean Sea) after the catch on 20 March 1999 of one specimen at a depth of about 1-2 m, near the village of Fanari (figure 1). This specimen was a rather immature male of 476 mm Tl, with a snout 60 % larger than its head length (figure 2). It is deposited in the fish collection of the Fisheries Research Institute of Kavala. Measurements are given in table II.

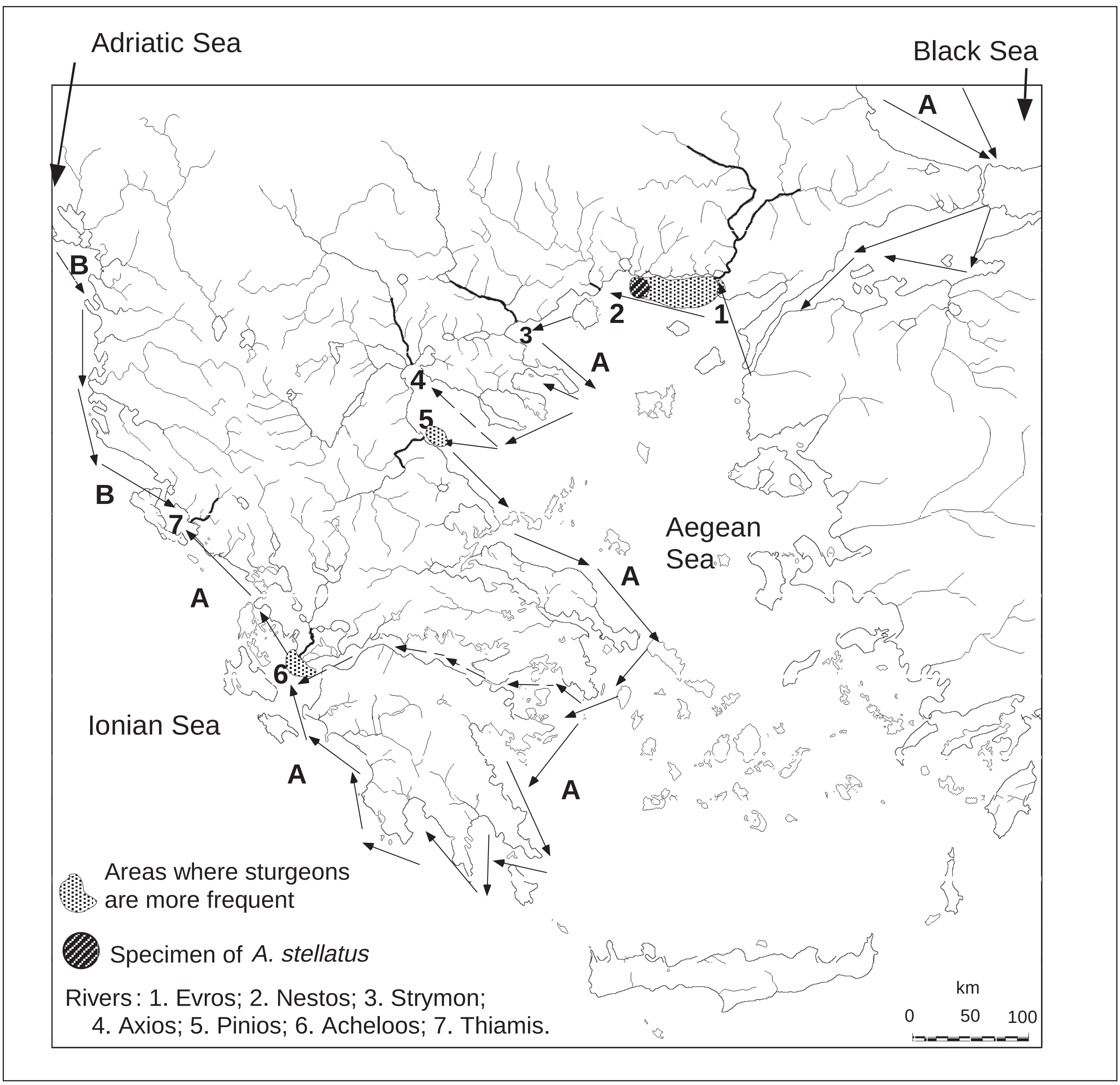

Figure 1. Dotted areas indicate where sturgeon are more frequent, black hatched circles mark sites where *Acipenser stellatus* was caught. Arrows mark putative migration route: (A): of *Acipenser sturio* and eventually *A. stellatus* and *Huso huso*; and (B): of *Acipenser naccarii*

Table II. Measurements (in mm) of a stellate sturgeon *Acipenser stellatus* specimen caught in the North Aegean Sea (after Holčík, Banarescu and Evans, 1989). (*): Fish collection of Fisheries Research Institute of Nea Peramos (Kavala)

	Total length Tl	Standard length Sl	Fork distance Fl	Dorsal scutes SD	Lateral scutes SL	Ventral scutes SV	Predorsal length pD	Head length lc	Body depth H	Head depth hc	Head depth hco	Premouth length s-m	Premandible length s-m	Prebarbel length s-b	Postbarbel length b-mc	Barbel length lb	Mandible length lam	Barbel zone width lab
Immature rater male specimen*	476	404	415	13	33-34	11	319	115	45	29	24	81	77	53	24	14	18	24

Huso huso (L., 1758)

According to Pirogovskii, Sokolov and Vasiliev (1989), the species is distributed in the drainage of the Caspian and Black seas and in the Adriatic, as well. This discontinuous distribution is quite peculiar, mainly because the Aegean and Ionian are inserted between the Black and the Adriatic Seas, making the communication between them completely free. It could thus be supposed that the species is an occasional visitor, passing through Greek waters; it cannot stay permanently, because of the higher water temperatures and salinities, as well as the lack of large estuaries. If this were true, it is obvious that such a large fish should be observed rather frequently in the area, and even fished. However, Banarescu (1964) is the only author who regards the presence of the species in the Aegean Sea as probable. Additionally, on the Ladiges and Vogt (1965, 1979) distribution map the species is traced (in black) with no documentation along the shoreline of Greece. Concerning the distribution of the species in the Adriatic, there are many records of its presence along the coast (Berg, 1933, 1948; Vuković and Ivanović, 1971; Svedovidov, 1984; Pirogovskii, Sokolov and Vasiliev, 1989; Rakaj, 1995). Rakaj (1995) reports two specimens from Albanian waters (Drin River at Lezhâ), while Geldiay and Balik (1988) do not trace the distribution of the species along the Aegean shoreline of Turkey. All of this information strongly suggests that the species probably has a discontinuous distribution between the Black and the Adriatic Seas, as is also the case of some other euryhaline fish.

Several records of sturgeon catches in Greek waters have appeared in newspapers or market reports. Apparently, they cannot be validated because they confuse the species. Two such cases are, for instance, the catch of a specimen weighing about 40 kg near the Acheloos River estuary (6 February 1984), and another very recent catch, inside the Evros River delta, of a non-preserved or examined specimen, weighing about 3 kg (29 June 1999).

Figure 2. Specimen of *Acipenser stellatus* caught on the Thracian shore (north Aegean Sea)

Production

Sturgeon have high market demand for consumption as fresh meat and for the production of black caviar. Regarding the latter, it is noteworthy that in the past a number of artisanal enterprises producing small quantities of black caviar operated in several areas of the Aegean Sea. According to Potamianos (1965) and Marsellos (1973) the main centres of such production were the estuaries of the Evros River in Thrace (Alexandroupolis) and the estuaries of the Pinios River in Thessaly (Tsagezi). Belloc (1948) reports that before World War II the annual production of sturgeon in Greece was about 10 t, and the production of black caviar about 2-2.5 t. After the war, production declined dramatically due to irrigation works in the rivers (e.g. dams and channels), overfishing, and pollution of the water, mainly in the Evros River, which is the most important area for sturgeon in the Aegean Sea. The watershed of this river is shared by three countries (Greece, Bulgaria, Turkey); consequently, any activity in it is practically out of control. According to Georgacas (1978), the last registered catches of sturgeon and black caviar production were as shown in table III.

Conservation status

Sturgeon are protected by the Greek State by a set of restrictions and conservation measures. With Royal Decree No. 1/1970 "on the protection of sturgeon species", fishing and selling of any sturgeon were prohibited for a period of three years. In Presidential Decree No. 67/1981 "on the protection of wild flora and fauna and the determination of procedures for the coordination and control of research", the species *A. sturio* and *A. naccarii* are listed among those of the fauna of Greece that are fully protected. Greece has also ratified the Bern Convention on the conservation of European wildlife and natural habitats, where *A. sturio* and *A. naccarii* are also included. Due to the continuous decline in the *A. sturio* population, the IUCN Red List of Threatened Animals considers it as "critically endangered". It has also been placed in the lists of the CITES (1973), the European Red List of Globally Threatened Animals and Plants, and Annex II of the 92/43/EEC Habitats Directive, which was also ratified by Greece (December 1998).

It seems that this set of measures was quite effective, because sturgeon began to appear more frequently in the Greek seas, especially in their main distribution area, the Evros River and its estuary. Consequently, it is necessary for these appearances to be registered, and for any caught specimens to be kept alive for further study.

DISCUSSION

The species *A. sturio* and *A. stellatus* are frequent in Greek waters, the former historically mostly around the estuaries of the country's larger rivers, the latter at the moment only in the Thracian Sea (North Aegean), close to the estuary of the Evros River. The distribution of *H. huso* and *A. naccarii* in Greek territorial waters still remains doubtful. In the case of the latter species, it should be noted that no serious reasons exist preventing its presence south of the Greek coasts along the Ionian Sea. This, however, could only be proved by catching some live specimens. The only probable restriction is likely to be related to the species's salinity tolerance, which, as noted by Tortonese (1989), remains unknown. On the contrary, this environmental factor seems to play an important role in the distribution pattern of other sturgeon species. As pointed out by Holčík *et al.* (1989), *A. sturio* appears to be more resistant to high salinity than other sturgeon species. This ability could probably explain its wide distribution along the north coastal zone of the Mediterranean and the Atlantic, in-

Table III. Annual fish catch and caviar production of sturgeons in Alexandroupolis, a town close to the Evros River estuaries (after Georgacas, 1978)

Years	Individuals	Fresh caviar (kg)	Processed and canned (kg)
Up to 1969	12 individuals, weight up to 40 kg per fish	up to 150	up to 100
1970-1972	Sturgeon fishing was prohibited	0	0
1973	5 individuals up to 35 kg per fish	up to 50	up to 35
1974	8 individuals up to 40 kg per fish	up to 90	up to 60
1975	9 individuals up to 45 kg per fish	up to 120	up to 90

cluding the Black and Baltic Seas. Regarding the other species considered here, Pirogovskii, Sokolov and Vasiliev (1989) note that *H. huso* survives at salinities as high as 22 ‰, and Shubina, Popova and Vasiliev (1989), based on studies done in the Black and Caspian Seas, underline that *A. stellatus* is not dependent on any particular salinity, and both adults and juveniles are encountered in a wide range of salinities from 0.1-13.5 ‰. It can thus be explained that, apart from *A. sturio,* which can survive and migrate easily to high- or low-salinity areas, the other species obviously have difficulties in controlling their osmoregulation when confronting the Mediterranean waters. Apart from such factors as the very dense traffic in large ships to and from the Black Sea across the straits of the Dardanelles and Bosphorus, the pollution in many watersheds of the Black and North Aegean Seas, and overfishing, the only other species which can migrate from one sea to the other is perhaps *A. stellatus.* If such movements are presently possible, they should follow the Greek shoreline, mainly near estuaries (figure 1). Nevertheless, it would be more convenient to accept the view that the Adriatic stocks of *H. huso* and *A. stellatus* have been separated from those of the same species of the closely-related Black Sea stocks since the end of the last glaciation. During this time, the two stocks were isolated because of the progressive salinity increase in the southern parts of the Mediterranean, combined with the lack of major estuaries and the elevation of the sea level. If this is true, then any genetic approach to these stocks could essentially contribute to our knowledge. The population of *A. stellatus* in the north Aegean Sea may be an interesting intermediate one.

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Further observations on the morphological characters of *Acipenser sturio* L., 1758 from the Iberian Peninsula: A comparison with North and Adriatic Sea populations

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ABSTRACT

Forty-two *Acipenser sturio* L., 1758 from Spain and Portugal were morphologically analysed. These Iberian sturgeons were collected in the Bay of Biscay, mouths of the Mondego and Tagus, and the Douro, Guadalquivir and Ebro Rivers. Specimens captured in the North and Adriatic Seas were compared with these Iberian specimens. In addition, morphological characters of various specimens of *Acipenser naccarii* Bonaparte, 1836 were included in this comparative analysis. Various morphological features, as well as 39 morphometric and 12 meristic characters, were determined and compared among specimens. Principal Component Analysis (PCA), using a correlation matrix, was used to assess meristic and shape variations. This technique revealed morphological differences between *A. sturio* and *A. naccarii*, and grouped all *A. sturio* from the three geographical areas considered. Nonetheless, univariate analysis revealed significant differences among North Sea specimens, and both Iberian and Adriatic *A. sturio*. Intra-specific variation of morphological patterns in *A. sturio* warrants further comparative research, adding specimens from other European regions, particularly from the Gironde estuary and from the Black and Baltic Seas.

Key words: Biometrics, conservation, distribution, fish, morphometry.

RESUMEN

Datos complementarios sobre la morfología de* Acipenser sturio *L., 1758 de la península Ibérica: comparación con las poblaciones del Mar del Norte y del Adriático

Se ha estudiado la morfología de cuarenta y dos ejemplares de Acipenser sturio *L., 1758 conservados en colecciones de España y Portugal. Estos esturiones ibéricos proceden del mar Cantábrico, de las desembocaduras del Mondego y del Tajo, y de los ríos Duero, Guadalquivir y Ebro. Asimismo, ejemplares de* A. sturio *del Mar del Norte y del Adriático fueron comparados con los de la península Ibérica. Los caracteres morfológicos de algunos ejemplares de* Acipenser naccarii *Bonaparte, 1836 también fueron incluidos en el análisis. Se estudiaron algunos caracteres morfológicos cualitativos, así como 39 caracteres morfométricos y 12 merísticos. Para evaluar la variación merística y de la forma utilizamos el análisis de componentes principales (ACP), a partir de una matriz de correlación. Este análisis multivariado mostró diferencias morfológicas entre los ejemplares de* A. sturio *y los de* A. naccarii, *pero no marcó diferencias apreciables entre grupos para los ejemplares de* A. sturio *de las tres áreas investigadas. Sin embargo, cuando aplicamos un análisis univariado, encontramos algunas diferencias significativas de los ejemplares de* A. sturio *del Mar del Norte frente a los ibéricos y adriáticos. Estas variaciones intraespecíficas del patrón morfológico deben ser contrastadas incluyendo muestras adicionales de otras regiones europeas, principalmente del estuario del Gironda y de los mares Báltico y Negro.*

Palabras clave: *Biometría, conservación, distribución, peces, morfometría.*

INTRODUCTION

Acipenser sturio L., 1758 is the only sturgeon native to the Iberian Peninsula (Doadrio, Elvira and Bernat, 1991; Elvira, Almodóvar and Lobón-Cerviá, 1991a, b; Elvira and Almodóvar, 1993; Pereira, 1995; Almaça and Elvira, 2000). In contrast to this generalised opinion, Garrido-Ramos *et al.* (1997) considered that the Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836 is also native to Iberian waters. Though this hypothesis has recently been refuted, based on morphological (Elvira and Almodóvar, 1999; Rincón, 2000a, b) and molecular (Doukakis *et al.*, 2000; Almodóvar, Machordom and Suárez, 2000) approaches, Hernando *et al.* (1999) extended this issue, stating that *A. naccarii* and the beluga *Huso huso* (L., 1758) are also native to Iberian waters. Therefore, this study attempts to elucidate the occurrence of *A. naccarii* and *H. huso* in Spain and Portugal. For this purpose we examined preserved Iberian sturgeons available in collections, including the specimens previously studied by Garrido-Ramos *et al.* (1997) and Hernando *et al.* (1999).

Taxonomical and regional variability of *A. sturio* within the distribution area have recently been revised by Birstein, Betts and DeSalle (1998); Ludwig and Kirschbaum (1998); Artyukhin and Vecsei (1999); Debus (1999), and Holčík (2000). These studies reported marked morphological and molecular variability in contrasting stocks throughout the European seas. Therefore, our study is also a contribution to broaden the knowledge of the morphological variability of Atlantic sturgeon.

A. sturio is on the verge of extinction, and has been catalogued as “Critically Endangered (CR A2d)” by the IUCN (Anon., 2000) (Birstein, 1997, 1999; Birstein, Bemis and Waldman, 1997; Williot *et al.*, 1997; Elvira (ed.), 1999, 2000). Understanding local variations and their taxonomic implications is especially relevant for the implementation of recovery programmes (Birstein, Betts and DeSalle, 1998; Doukakis *et al.*, 2000, Holčík, 2000), specially if stocking becomes mandatory.

MATERIALS AND METHODS

Details on the material examined are included in appendix I. This includes information on specimens from 21 Spanish and Portuguese collections, and from Senckenbergische Museum, Frankfurt (Germany), and the Trieste Museum of Natural History (Italy). Institutional abbreviations used follow Leviton *et al.* (1985), and Leviton and Gibbs (1988), unless otherwise stated. Morphometric analysis included 42 preserved specimens (27 stuffed and 15 preserved in alcohol) from the Iberian Peninsula. Twenty-five specimens were of known locality, whereas other specimens were only presumably collected in Spanish or Portuguese waters. Comparative material included 21 preserved *A. sturio* specimens, 11 specimens from the North Sea, and 10 specimens from the Adriatic Sea. Moreover, 16 specimens of *A. naccarii* were analysed for comparative purposes.

Thirty-nine morphometric and 12 meristic characters were determined, according to Holčík, Banarescu and Evans (1989) (Elvira and Almodóvar, 2000). Measurements and meristic characters are listed in table I. Multivariate data analyses included principal component analysis (PCA) of all metric and meristic characters. Before computation, all these characters were standardised (Sokal, 1961) to pool information from different characters into a comparable scale. Morphometric characters were divided by total length, and meristic characters were log transformed. The morphological measurements were tested for allometry using the *log*-transformed relation $log\,M = log\,(a + b) \cdot log\,Tl$, where M is the character measurement and Tl, the total length. To explore differences in body shape among specimens, the metric measurements were adjusted for differences in body length according to: $M_C = M \cdot (Tl/Tl_M)^b$, where M_C is the adjusted measurement, M, the original measurement, Tl, total length, Tl_M, grand mean total length, and b, the regression coefficient of the $log\,M - log\,Tl$ relationship. A PCA for the allometrically adjusted measurements, factoring the correlation matrix, was also performed. This approach was preferred by Reist (1985, 1986) to other methods of size correction, and was used by Rincón (2000a, b).

Differences between *A. sturio* populations for metric and meristic characters were studied using univariate analysis. Owing to the controversy on the use of ratios to adjust size variations (Mayden and Kuhajda, 1996), an ANCOVA for total length as covariate was conducted to test for significant differences among those metric characters. Group means were then compared by a Tukey test (Zar, 1999). Differences between populations for meristic characters were identified using the non-parametric Kruskal-Wallis test. Multiple comparisons were carried out using Dunn’s procedure (Zar, 1999).

For multivariate and univariate analyses, we employed STATISTICA (Anon., 1996) and NTSYSpc (Rohlf, 1998) packages. Unless otherwise indicated, statistical significance is $p < 0.01$.

RESULTS

A PCA was carried out factoring the correlation matrix of the metric and meristic data, whose fac-

Table I. Synopsis of the metric and meristic characters of 55 *A. sturio* specimens from the Iberian Peninsula, Adriatic Sea and North Sea

Character	n	Minimum	Maximum	Mean	sd
Tl	55	80.0	2 020.0	737.1	554.34
Fl	55	71.5	1 880.0	674.0	511.34
Sl	55	66.5	1 840.0	645.4	494.64
lc	55	24.0	370.0	152.4	102.49
prO	55	12.9	165.0	66.9	40.75
Oh	55	2.6	48.0	15.7	10.78
poO	55	8.5	187.0	69.7	53.77
pD	55	52.0	1 405.0	511.9	394.76
pP	55	25.4	420.0	165.6	115.31
pV	55	47.3	1 165.0	435.6	331.37
pA	55	53.5	1 485.0	541.9	418.34
lpcd	55	7.7	225.0	79.7	59.02
lpc	55	5.2	220.0	72.4	54.73
P-V	55	20.5	747.0	246.7	198.32
P-A	55	26.7	1 060.0	354.0	285.96
V-A	55	5.5	260.0	82.8	70.35
lD	55	5.3	168.8	59.4	46.20
hD	55	7.4	136.9	53.3	38.29
lPbs	55	3.4	101.5	27.3	21.52
lP	55	11.0	195.0	78.1	53.30
lVbs	55	2.3	75.0	27.1	20.70
lV	55	7.0	115.0	44.7	33.44
lA	55	5.2	108.5	33.7	26.52
hA	55	5.0	153.2	54.9	42.54
H	55	7.9	265.0	86.7	65.96
h	55	2.4	68.5	24.1	18.16
hco	55	5.1	118.0	44.9	33.53
hc	55	8.2	200.0	73.0	57.43
lam	54	4.5	110.5	34.7	25.71
laim	54	4.1	84.5	26.7	19.14
lac	55	10.0	200.0	74.9	56.69
io	55	6.2	130.0	50.3	36.79
lasa	54	8.0	166.0	62.4	45.46
lab	54	6.8	102.0	35.0	23.34
lb	53	3.6	45.0	18.3	10.16
s-m	53	14.2	190.0	81.2	50.98
s-mc	53	14.0	185.0	76.7	46.59
s-b	54	4.1	110.0	40.5	23.74
b-mc	53	5.5	93.5	35.4	24.06
Du	54	35	51	40.7	3.90
Pu	54	23	46	36.8	4.18
Vu	54	21	32	27.1	2.61
Au	54	22	38	27.2	2.94
Cu	43	69	110	85.9	9.25
Fu	45	18	31	25.2	3.21
Sp.br.	28	14	32	18.3	3.62
SD	55	9	14	11.7	1.20
SL-left	55	25	41	33.1	3.16
SL-right	55	24	40	33.2	3.26
SV-left	55	7	14	10.9	1.26
SV-right	55	6	14	10.9	1.41

Table II. Factor loadings for the first three principal components for *A. sturio* and *A. naccarii* specimens

	PC1	PC2	PC3
Fl	–0.622	0.267	0.571
Sl	–0.771	0.138	0.461
lc	0.678	0.621	0.211
Oh	0.468	0.096	–0.072
poO	0.185	0.084	0.608
pD	–0.644	0.599	0.341
pP	0.628	0.652	0.195
pV	–0.518	0.686	0.377
pA	–0.767	0.308	0.435
lpcd	–0.177	–0.446	–0.027
lpc	0.034	–0.738	–0.087
P–V	–0.929	0.043	0.141
P–A	–0.928	–0.154	0.223
V–A	–0.615	–0.422	0.253
lD	–0.092	–0.618	0.277
hD	0.736	–0.051	–0.210
lPbs	0.330	–0.236	0.464
lP	0.752	0.267	–0.037
lVbs	–0.184	–0.265	0.254
lV	0.746	0.117	0.015
lA	0.117	–0.028	0.497
hA	0.494	–0.109	–0.137
H	–0.153	0.157	0.333
h	–0.132	–0.070	0.605
hco	0.547	0.097	0.366
hc	–0.185	0.057	0.672
lam	0.569	–0.578	0.443
laim	0.617	–0.593	0.376
lac	0.320	–0.099	0.386
io	0.556	–0.110	0.551
lasa	0.476	–0.408	0.519
lab	0.660	–0.482	0.243
lb	0.791	0.114	0.010
s–m	0.712	0.628	0.110
s–mc	0.712	0.620	0.106
b–mc	0.605	0.594	0.204
Du	–0.028	–0.107	0.099
Pu	0.413	–0.075	–0.046
Vu	–0.081	–0.069	0.003
Au	0.108	0.130	–0.087
Cu	–0.123	–0.036	–0.334
Fu	0.268	–0.342	–0.063
Sp.br.	0.019	–0.584	–0.129
SD	0.211	0.534	0.096
SL–left	0.206	–0.613	0.246
SL–right	0.301	–0.604	0.219
SV–left	–0.293	0.124	–0.011
SV–right	–0.268	0.017	–0.094
Explained variance (%)	12.33	7.23	4.78

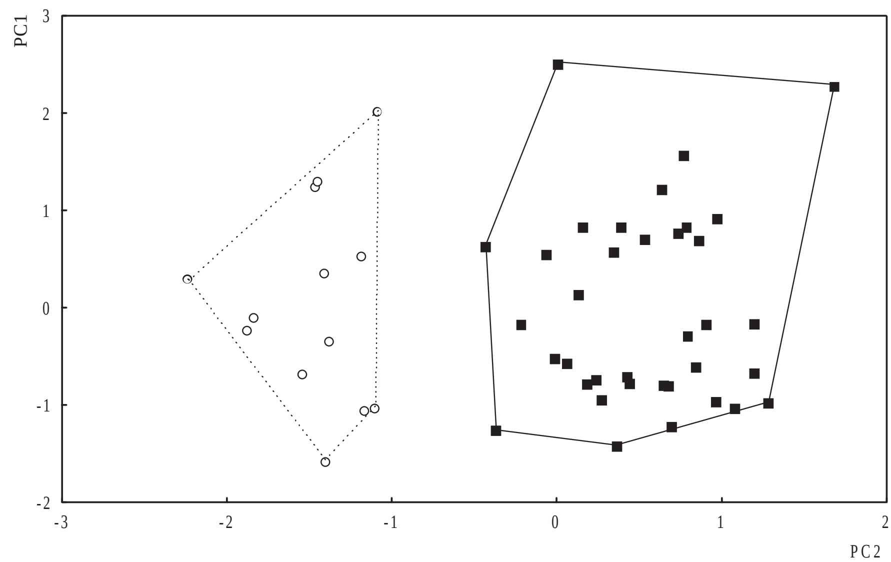

Figure 1. Plot of the factor scores for PC1 and PC2 of all metric and meristic characters for 36 *A. sturio* (■) and 13 *A. naccarii* (○).

tor loadings are shown in table II. The variance explained by the first two components was 19.6 %. The first factor was mainly defined by measurements of size (Sl), head shape (lc, lb, s-m, s-mc), and by the position (pA, P-V, P-A) and size of fins (hD, lP, lV). The second component was mainly correlated with meristic characters (Sp.br., SL), metric measurements related to fin position (pD, pP, pV), and length of caudal peduncle (lpc).

Visual inspection of the plots for the first and second components easily distinguished two major groups (figure 1). The first factor of the PCA did not separate species, but the second component resulted in an evident separation of the two species. Unlike *A. naccarii*, *A. sturio* is characterised by lower numbers of gill rakers and lateral scutes, and by shorter mean caudal peduncles, mean dorsal fin lengths and mean mouth widths. Moreover, larger

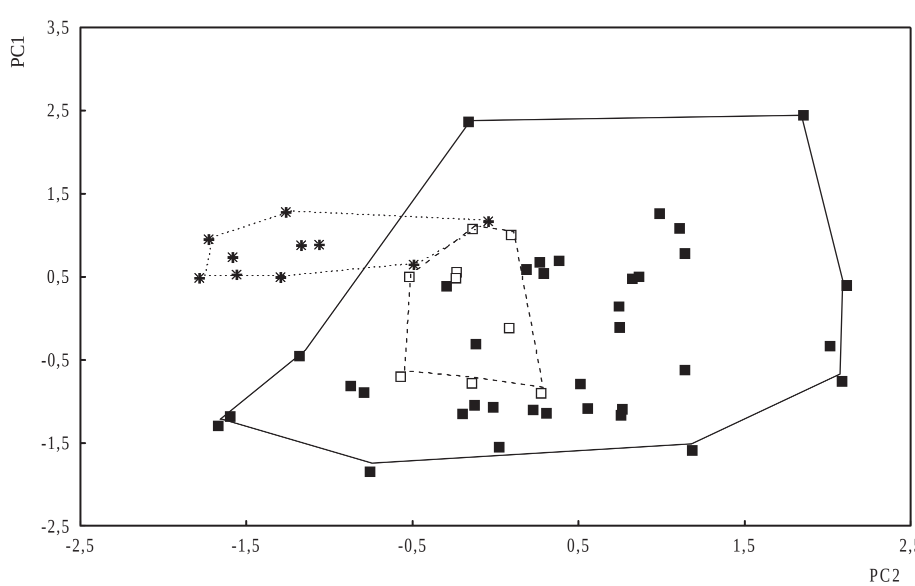

Figure 2. Plot of the factor scores for PC1 and PC2 of all metric and meristic characters for 55 specimens of *A. sturio*: Iberian Peninsula (n = 36) (■), Adriatic Sea (n = 9) (□) and North Sea (n = 10) (*).

mean pre-dorsal, pre-pectoral and pre-ventral distances, larger mean head length, and longer snout identified *A. sturio* specimens. In addition, PCA's allometry adjusted metric measurements together with *log*-transformed meristic characters resulted in similar relationships between *A. naccarii* and *A. sturio*.

A second PCA for all of these characters was performed, including the dataset for *A. sturio* from the Iberian Peninsula, North Sea and Adriatic Sea (factor loadings in table III). Nevertheless, the plot of the factor scores for the first and second principal components showed no obvious separation among the three populations (figure 2). Further differentiation was not possible using the successive principal components.

Conversely, ANCOVAs for metric characters among *A. sturio* populations with total length as covariate revealed significant differences ($p < 0.01$) in nine out of 38 metric characters (table IV). Moreover, subsequent comparisons of means values revealed significant differences between the North Sea and both Adriatic and Iberian Peninsula sturgeons in eight out of the nine former characters (Tukey test, $p < 0.01$), while the length of ventral fin was significantly different (Tukey test, $p < 0.01$) among the three sturgeon populations. Adjusted means for the *log*-transformed metric characters indicated that sturgeons from the North Sea have longer caudal peduncles, shorter fins, narrower mouths and lower interorbital distances.

We also found significant differences (Kruskal-Wallis test, $p < 0.01$) in five out of 12 meristic characters among the three *A. sturio* populations (table V). A posterior comparison among mean values showed that these differences occurred between the North Sea and the other two populations (Dunn test, $p < 0.01$). Sturgeons from the North Sea had significantly higher numbers of unbranched rays in the pectoral and ventral fins, and lower numbers of fulcrae and lateral scutes.

Table III. Factor loadings for the first three principal components for Iberian Peninsula, Adriatic Sea and North Sea *A. sturio* specimens

	PC1	PC2	PC3
Fl	–0.575	0.570	0.384
Sl	–0.726	0.413	0.380
lc	0.737	0.403	0.331
Oh	0.533	–0.095	0.099
poO	–0.010	0.628	0.189
pD	–0.730	0.450	0.343
pP	0.711	0.362	0.419
pV	–0.569	0.504	0.520
pA	–0.769	0.484	0.335
lpcd	0.119	–0.330	0.357
lpc	0.136	–0.456	0.153
P–V	–0.935	0.179	0.062
P–A	–0.939	0.244	0.043
V–A	–0.801	0.296	0.033
lD	–0.298	0.529	–0.551
hD	0.411	0.366	–0.050
lPbs	0.373	0.226	0.233
lP	0.636	0.302	–0.177
lVbs	–0.109	0.144	–0.013
lV	0.543	0.374	–0.358
lA	0.084	0.453	0.016
hA	0.344	0.114	–0.441
H	–0.124	0.332	0.096
h	–0.223	0.593	–0.128
hco	0.505	0.417	–0.016
hc	–0.394	0.621	0.177
lam	0.577	0.465	–0.073
laim	0.701	0.471	–0.138
lac	0.198	0.392	0.097
io	0.374	0.649	0.040
lasa	0.467	0.638	0.148
lab	0.798	0.231	–0.025
lb	0.850	0.026	0.007
s-m	0.827	0.192	0.326
s-mc	0.834	0.175	0.327
b-mc	0.613	0.390	0.264
Du	0.130	0.135	–0.322
Pu	0.534	–0.185	0.233
Vu	0.234	–0.421	0.280
Au	0.363	–0.333	–0.026
Cu	0.124	–0.510	0.044
Fu	–0.084	0.265	–0.585
Sp.br.	0.149	–0.173	–0.155
SD	0.011	0.455	–0.372
SL-left	–0.094	0.406	–0.616
SL-right	–0.110	0.457	–0.694
SV-left	–0.278	–0.040	–0.236
SV-right	–0.205	–0.217	–0.172
Explained variance (%)	12.79	7.36	4.08

DISCUSSION

Garrido-Ramos *et al.* (1997) reported four Iberian sturgeons preserved in Portuguese and Spanish collections as *A. naccarii*, namely specimens labelled EBD-8173, EBD-8174, UC-46b, and UC-uncat. In a later study, these same authors (Hernando *et al.*, 1999) accepted the taxonomical position determined for three specimens (EBD-8173, EBD-8174 and UC-uncat.) as *A. naccarii*. However, by applying skin patterns as diagnostic characters previously validated by Artyukhin and Vecsei (1999) and Debus (1999) to differentiate *A. sturio* from closely-related

Table IV. Results of the ANCOVA using total length as covariate (only for characters that became significantly different) for metric characters among *A. sturio* populations: Iberian Peninsula (IP), Adriatic Sea (AS), and North Sea (NS)

Character	$F_{(2,33)}$	p	Tukey test
Length of caudal peduncle from dorsal fin	7.60	0.0019	IP, AS < NS
Length of caudal peduncle from anal fin	9.20	0.0007	IP, AS < NS
Length of dorsal fin	15.42	< 0.0001	IP, AS > NS
Length of pectoral fin	8.37	0.0012	IP, AS > NS
Length of ventral fin	13.52	0.0001	IP > AS > NS
Depth of anal fin	5.81	0.0069	IP, AS > NS
Width of mouth	9.60	0.0005	IP, AS > NS
Internal width of mouth	8.38	0.0011	IP, AS > NS
Interorbital distance	6.54	0.0041	IP, AS > NS

taxa, specimens EBD-8173 and EBD-8174 (labelled in the Guadalquivir River) were assigned to *A. sturio* by Elvira and Almodóvar (1999, 2000). Portuguese specimens UC-uncat. (labelled in Lisbon) and UC-46b (labelled in Buarcos), included in our present study, resulted in clustering of these with other *A. sturio*. Likewise, the skin patterns of these two specimens are typical of the species. Therefore, we conclude that there is no reason to consider native sturgeon specimens from Iberian waters as members of *A. naccarii*.

Hernando *et al.* (1999) determined the specimen UP-1 as *H. huso*, whilst they considered the specimen UP-84 as a hybrid *H. huso* × *A. sturio*. Specimen UP-1 (unrecorded locality, no date; Portugal) is mounted, and its poor preservation conditions do not permit an appropriate morphological assessment: its mouth was re-constructed with plasticine by the taxidermist (but it is not crescent), and barbels are four pieces of cord (!). In fact, we were unable to detect unequivocal external diagnostic characters to assign this specimen as *H. huso*. In contrast, the specimen UP-84 (Douro River) is well preserved in alcohol, and showed only one uncommon meristic character for *A. sturio*: 32 gill rakers. However, we found no character attributable to *H. huso*: this specimen showed an isthmus, the mouth was not crescent, the gill rakers were not rod-shaped, the barbels were not laterally compressed, and did not bear foliate appendages. Consequently, we considered this specimen a young *A. sturio* with a peculiarly high number of gill rakers. In conclusion, by no means could native sturgeons collected in Iberian waters be considered *H. huso*.

All native sturgeons studied from Spain and Portugal were assigned as *A. sturio*. The putative occurrence of *A. naccarii* and *H. huso* in these waters could not be confirmed. Our findings agree with those previously presented by us and other research groups (Elvira and Almodóvar 1999, 2000; Doukakis *et al.*, 2000; Almodóvar, Machordom and Suárez, 2000; Rincón 2000a, b), and further reinforce the idea that the only sturgeon species native to the Iberian Peninsula is *A. sturio*.

With regard to the three sturgeon populations analysed, we detected significant differences between sturgeon from the North Sea versus the Iberian Peninsula and Adriatic Sea. Even considering the small sample available for this study, differences detected are significant enough to infer that the currently accepted species *A. sturio* may consist of several different separate taxonomical units, i.e. species (?) (Almaça, 1988; Holčík *et al.*, 1989; Artyukhin and Vecsei, 1999; Debus, 1999; Almaça and Elvira, 2000; Holčík, 2000). In any case, we believe further analyses ought to be undertaken to unequivocally demonstrate the taxonomical value of these differences. Meanwhile, cautionary and reversibility principles must be considered by

Table V. Results of the Kruskal-Wallis test (only for characters that became significantly different) for meristic characters among *A. sturio* populations: Iberian Peninsula (IP), Adriatic Sea (AS), and North Sea (NS)

Character	H	p	Dunn test
Unbranched rays of pectoral fin	10.60	0.0050	IP, AS < NS
Unbranched rays of ventral fin	9.83	0.0073	IP, AS < NS
Fulcrae	12.83	0.0016	IP, AS > NS
Lateral scutes (left)	15.78	0.0004	IP, AS > NS
Lateral scutes (right)	22.90	< 0.0001	IP, AS > NS

decision-makers on transfers or translocations of specimens among these different areas.

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APPENDIX I

Sturgeon material examined. Specimens marked with (*) are preserved in poor condition and were not included in the multivariate analysis. Unless otherwise indicated, size is expressed as standard length

Acipenser sturio (63 specimens)

Atlantic sturgeons from the Iberian Peninsula (42 specimens)

MNCN uncat. (IFIE 7bis); Ebro River, Amposta, Tarragona; 9 December 1951; 66.5 mm; alcohol

MNCN 1579-1581 (3 ex.); Ebro R., Amposta, Tarragona; 226, 303 and 325 mm; alcohol

MNCN 1582; Guadalquivir R.; 208 mm; alcohol

MNCN 1583; Ebro R., Tortosa, Tarragona; 242 mm; alcohol

MNCN 44145; Mediterranean Sea, Spain; 755 mm; stuffed

VBCM uncat.; no data, Spain?; 455 mm; stuffed

Doñana Biological Station, Seville, EBD 8173 (*); Guadalquivir R., Alcalá del Río, Seville; 12 April 1974; Tl = 1 755 mm; alcohol

EBD 8174 (*); Guadalquivir R., Alcalá del Río, Seville; 11 May 1975; Tl = 1 520 mm; stuffed

EBD 8401; Guadalquivir R., Coria del Río, Seville; Winter 1981; 1 450 mm; alcohol

Aguilar y Eslava Institute, Cabra, Córdoba, IAEC uncat.; Guadalquivir R.; 1880; 1 150 mm; stuffed

J. M. Pascual Collection, Seville, CJMP uncat.; Guadalquivir R.; 1965-1967; 288 mm; alcohol

F. Ibarra Collection, Seville, CFI uncat.; Guadalquivir R.; 268 mm; stuffed

Department of Animal Biology, University of Badajoz, DABUBA uncat.; no data, Spain?; 195 mm; stuffed

Department of Animal Biology, University of Seville, DABUS uncat. (3 ex.) (*); Cádiz; 189, 277 and 300 mm; stuffed

Cantabrian Museum, Santander, Cantabria, MMCS 3/Vc/171; Bay of Biscay, Cantabria; 1914; 430 mm; alcohol

MMCS 3/Vc/1210; San Vicente de la Barquera, Cantabria; 10 June 1988; 1 205 mm; stuffed

Zoology Museum, Barcelona, MZB 82-5337; Ebro Delta, Tarragona; 1 285 mm; stuffed

MZB 82-5340; no data, Spain?; 460 mm; stuffed

MZB 82-5342; no data, Spain?; 730 mm; stuffed

MZB 95-0105; no data, Spain?; 835 mm; stuffed

Department of Animal Biology, University of Barcelona, DABUB uncat.; no data, Spain?; 680 mm; alcohol

Department of Animal Biology, University of Granada, DABUG uncat. (*); no data, Spain?; Tl = 1 600 mm aprox.; stuffed

DABUG; no data, Spain?; 675 mm; stuffed

Capuchins Ethnographic-Missions Museum, Barcelona, MEMCCB uncat.; no data, Spain?; 970 mm; stuffed

Sagrada Familia Institute, Puerto de Santa María, Cadiz, ISFPSM uncat.; no data, Spain?; 1 640 mm; stuffed

Cardenal Cisneros Institute, Madrid, ICCM uncat.; no data, Spain?; 1 640 mm; stuffed

Luis Iglesias Museum, Santiago de Compostela, A Coruña, MLISC uncat.; no data, Spain?; 1865-1870; 1 430 mm; stuffed

P. P. Paúles Museum, Villafranca del Bierzo, León, MPVB uncat.; no data, Spain?; 1 460 mm; stuffed

Department of Zoology and Ecology, University of Navarre, Pamplona, DZEUN uncat.; Bay of Biscay, San Sebastián, Guipúzcoa; 21 May 1975; 945 mm; alcohol

University of Porto, UP 1; Portugal?; 1 340 mm; stuffed

UP 2; Portugal; 447 mm; stuffed

UP 3; Douro R.; May 1916; 920 mm; stuffed

UP 84; Douro R., Barca d'Alva; June 189(?); 183.5 mm; alcohol

UP 85; no data, Portugal?; 360 mm; alcohol

Rodrigues de Freitas Institute, Porto, LRFP 88; no data, Portugal?; 915 mm; stuffed

LRFP uncat.; no data, Portugal?; 245 mm; alcohol

University of Coimbra, UC 46b; Buarcos; 11 July 1897; 1 310 mm; stuffed

UC uncat.; Lisbon; 1890; 1 840 mm; stuffed

Atlantic sturgeons from the Adriatic Sea
(10 specimens)

Trieste Museum of Natural History, MCSNT 97; Adriatic Sea; 265 mm; alcohol

MCSNT 300; Adriatic Sea; 335 mm; alcohol

MCSNT 302; Adriatic Sea; 430 mm; alcohol

MCSNT uncat.; Adriatic Sea; 1 320 mm; stuffed

MCSNT uncat.; Adriatic Sea; 1 415 mm; stuffed

MCSNT uncat.; Adriatic Sea; 505 mm; stuffed

Senckerbergische Museum, Frankfurt, SMF 794; Adriatic Sea; 1831; 258 mm; alcohol

SMF 2397; Adriatic Sea, Trieste; 1902; 395 mm; alcohol

SMF 2448; Adriatic Sea, Trieste; 1902; 393 mm; alcohol

SMF 7647 (*); Adriatic Sea, Neapel (?); 1827; 470 mm; stuffed

Atlantic sturgeons from the North Sea
(11 specimens)

SMF 158; North Sea; 1830; 333 mm; alcohol

SMF 792 (2 ex.); North Sea; 1830; 169 and 155 mm; alcohol

SMF 1341; Lower Elbe, Kraütsand; 1907; 142 mm; alcohol

SMF 4640; North Sea, Deutsche Bucht; 20 October 1958; 203 mm; alcohol

SMF 7636; Elbe R., Hamburg; 1913, 225 mm; alcohol

SMF 7637-7640 (4 ex.); Eider R., Nübbel; April 1913; 313, 208, 366 and 190 mm; alcohol

SMF 7651 (*); North Sea, south of Helgoland; 1960; 525 mm; alcohol

Acipenser naccarii (16 specimens)

MCSNT uncat.; Adriatic Sea; 860 mm; stuffed

MCSNT uncat.; Adriatic Sea; 830 mm; stuffed

MCSNT uncat.; Adriatic Sea; 875 mm; stuffed

SMF 7634; Neapel (?); 21 July 1924; 485 mm; alcohol

SMF uncat.; Saxenstör 94; 6 June 1995; 156 mm; alcohol

VBCM uncat. (3 ex.) (*); fish farm; 75, 75 and 100 mm; alcohol

EEAUS uncat. (2 ex.); fish farm; 177 and 190 mm; alcohol

EEAUS uncat. (2 ex.); fish farm; 650 and 690 mm; frozen

EBD uncat. (4 ex.); fish farm; 405, 1 120, 1 120 and 1 190 mm; frozen

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Acipenser sturio L., 1758 conservation resources on the World Wide Web

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ABSTRACT

This paper presents a comprehensive review of the information currently available on the World Wide Web regarding *Acipenser sturio* L., 1758, featuring 113 selected sites concerning its conservation, biology, and related subjects. Web sites with additional information on other sturgeon species' conservation are also included. A Web page of links to the main sources of news on *A. sturio* and the conservation of other sturgeons can be accessed at *http://www.ucm.es/info/zoo/Vertebrados/elvira.htm*.

Key words: Atlantic sturgeon, information sources, protection, www.

RESUMEN

***Recursos sobre conservación de* Acipenser sturio *L., 1758 en la* World Wide Web**

Este trabajo comprende un análisis detenido de la información disponible sobre conservación y otros temas relacionados de Acipenser sturio *L., 1758 localizada en la* World Wide Web. *Se seleccionaron 113 páginas con información relevante sobre la biología y conservación del esturión atlántico. Asimismo, se incluyen las direcciones* Web *con información complementaria sobre conservación de esturiones. Una página* Web *que contacta con las principales fuentes de noticias sobre conservación de* A. sturio *y otros esturiones se encuentra disponible en* http://www.ucm.es/info/zoo/Vertebrados/elvira.htm.

Palabras clave: *Esturión atlántico, fuentes de información, protección,* www.

INTRODUCTION

The Atlantic sturgeon *Acipenser sturio* L., 1758 is a highly threatened species (Lepage and Rochard, 1995; Birstein, Bemis and Waldman, 1997), currently on the verge of extinction and catalogued worldwide as "Critically Endangered – CR A2d" (Anon., 2000). For this reason, several recent efforts have been aimed at recovering this formerly common species (Elvira and Gessner, 1996; Williot *et al.*, 1997). A consequence of this interest was the recent organization of the first scientific symposium devoted exclusively to the conservation of *A. sturio* (Elvira (ed.), 1999, 2000).

The phenomenal growth of the Internet has occurred because of the creation of the World Wide Web (WWW). The Web stores a huge amount of all sorts of biological information. It poses unlimited opportunities for people to access information. Furthermore, the Web has grown from being a resource centre for a select group of scientists to a large database of information which is available to both professionals and the public. The Web is considered to be a viable tool for scientific research, and several investigators have already made use of it in their studies. Ubiquity, global reach, interactivity, a decentralized, hyperlinked structure, and multimedia format characterise the WWW. The present paper is aimed at facilitating the access of people concerned with sturgeon conservation to this wealth of online information.

MATERIALS AND METHODS

Searches were undertaken from March 1999 to September 1999 (the last, updated search was conducted in October 2000 during proofs correction) using nearly 40 search engines. More than 200 Web documents were visited and evaluated, providing a final selection of 113 addresses.

The top 25 search engines (alphabetical order) providing the highest number of positive results for *Acipenser sturio* are as follows:

- Alta Vista: *http://www.altavista.com/*
- America On Line: *http://search.aol.com/*
- CNET Search: *http://www.search.com/*
- C4: *http://www.c4.com/*
- EuroSeek: *http://www.euroseek.com/*
- Excite: *http://www.excite.com/*
- Fast Search: *http://www.ussc.alltheweb.com/*
- Go: *http://www.go.com/*
- Google: *http://www.google.com/*
- Goto: *http://www.goto.com/*
- Hotbot: *http://www.hotbot.com/*
- Infospace: *http://www.infospace.com/*
- LookSmart: *http://www.looksmart.com/*
- Lycospro: *http://lycospro.lycos.com/*
- Magellan: *http://magellan.excite.com/*
- MetaCrawler: *http://www.metacrawler.com/*
- MSN: *http://search.msn.com/*
- NBCI: *http:// home.nbci.com/*
- Netscape Search: *http://search.netscape.com/*
- Northern Light: *http://www.northernlight.com/*
- Raging Search: *http://ragingsearch.altavista.com/*
- Scrub The Web: *http://www.scrubtheweb.com/*
- Search Hound: *http://www.searchhound.com/*
- WebTop: *http://www.webtop.com/*
- Yahoo: *http://www.yahoo.com/*

RESULTS

One hundred and thirteen Web URLs on *A. sturio* were selected, and are presented below. Likewise, four Web pages with valuable information on sturgeon conservation are also listed below. All of our results are listed on the Web page *http://www.ucm.es/info/zoo/Vertebrados/elvira.htm*, where links with the main sources of information on *A. sturio* and the conservation of other sturgeons are available. This page provides the opportunity of visiting administrative departments and bureaus, museums, universities, and private locations with primary information on the subject. All of these files are available 24 h/day, 7 days/week with no online charges.

The URL addresses are listed in alphabetical order, each followed, in brackets, by the page name, the species involved (only *A. sturio* or several Acipenseridae), the country where the page was produced, the language, and the main content subject.

Acipenser sturio biology and conservation

- *http://alun.uio.no/zoomus/fisk/fiskeliste.html* (Zoologisk museum - Fiskeavdelingen / *A. sturio* / Norway / Norwegian / faunal lists)
- *http://base.dux.ru/eco/kniga/bfish.htm#r1* (Kniga / *A. sturio* / Russia / Russian / general)
- *http://fish-news.teia.org/osetr.htm* (Gigantic fish of Russia. Sturgeon. / *A. sturio* / Russia / Russian / general)
- *http://free.imd.it/Colapesce/Pescitalia/Pagine Pesci/Acipenseriformi/storione.htm* (Storione / *A. sturio* / Italy / Italian / general)
- *http://freeweb.aspide.it/freeweb/FishingTaverne/storione.htm* (Storione / *A. sturio* / Italy / Italian / general)
- *http://home.online.no/~ka-ei/eurfish/sp002.htm* (Acipenser sturio / *A. sturio* / Norway / English / general)
- *http://home.wxs.nl/~hwdenie/extinct.htm* (Extinct fish species - uitgestorven vissoorten / *A. sturio* / Netherlands / Dutch / general)
- *http://ibs.uel.ac.uk/fish-bin/fishgen.pl?speccode=2066* (FishBase WWW:Taxonomy / *A. sturio* / UK / English / general)
- *http://ibss.iuf.net/blacksea/species/freelife/pisces.html* (IBSS NASU. Black Sea. Pisces

(Fish) / Acipenseridae / Ukraine / English / faunal lists)
- *http://ligam.cg24.fr/SITEWEB/PAGES/EnCeMom/Sturio/Sturio.htm* (Esturgeon accueil / *A. sturio* / France / French / general)
- *http://linnaeus.nrm.se/zool/fish/P00094.html.en* (NRM Ichthyology Collection: NRM 94 / *A. sturio* / Sweden / English / general)
- *http://members.es.tripod.de/solopeces/fichas/esturionc.htm* (Esturión común / *A. sturio* / Spain / Spanish / general)
- *http://members.magnet.at/aquaculture/sturgeon.htm* (Gesellschaft zur Rettung des Störs / *A. sturio* / Austria / English / general)
- *http://members.xoom.it/piasal/pescare/storione_comune.htm* (Storione Comune / *A. sturio* / Italy / Italian / general)
- *http://mitglied.tripod.de/MiksHomepage/fischpage/Fische/Stoer.html* (Stör Störs / *A. sturio* / Germany / German / general)
- *http://ourworld.compuserve.com/homepages/BMLSS/sturgeon.htm* (Sturgeon Acipenser sturio / *A. sturio* / UK / English / general)
- *http://ourworld.compuserve.com/homepages/bmlss/sussex.html#Sturgeon* (Rare Fishes of Sussex (Marine) / *A. sturio* / UK / English / general)
- *http://perso.libertysurf.fr/pechepassion/page/poisson/esturgeon.htm* (esturgeon / *A. sturio* / France / French / general)
- *http://rdb.eaurmc.fr/c_peche/html/poi_mi4.html* (L'Esturgeon / *A. sturio* / France / French / general)
- *http://salix.fme.vutbr.cz/ENCYKLOP/CERVKNIK/2CKOHRO.HTM* (Èervená kniha 2 podle ohrození / *A. sturio* / Czech Republic / Czech / faunal lists)
- *http://server.parliament.ge/GOVERNANCE/GOV/enviro/manual/Tevzi.html#5* (Tevzi / *A. sturio* / Georgia / English / images)
- *http://space.tin.it/scienza/cjbur/sch_stor_comune.htm* (Storione comune - scheda / *A. sturio* / Italy / Italian / general)
- *http://space.tin.it/scienza/psoria/specie.html* (Le specie ittiche presenti in provincia di Pavia / *A. sturio* / Italy / Italian / faunal lists)
- *http://sunsite.ee/animals/Kalad/kalalist2.htm* (Systematic list of Estonian fishes / *A. sturio* / Estonia / English / faunal lists)
- *http://sunsite.ee/loomad/Kalad/ACISTU.htm* (Tuur - täiendav info / *A. sturio* / Estonia / Estonian / general)
- *http://sunsite.ee/loomad/Kalad/ACISTU2.htm* (Tuur - liigikirjeldus / *A. sturio* / Estonia / Estonian / general)
- *http://utenti.tripod.it/husohuso/storioni.htm* (Storioni / Acipenseridae / Italy / Italian / general)
- *http://vissen.huisdier.net/0053.htm* (Acipenser sturio / *A. sturio* / Netherlands / Dutch / general)
- *http://www.ac-bordeaux.fr/Etablissement/ADaquitaine/estuaire/poissons.htm* (Les poissons de l'estuaire' / *A. sturio* / France / French / general)
- *http://www.ac-nancy-metz.fr/ia57/lindrebasse/Site%20phil/texte%20html/esturgeon.htm* (esturgeon / *A. sturio* / France / French / general)
- *http://www.anglersdirectory.net/Sturgeon.htm* (Sturgeon / *A. sturio* / UK / English / general)
- *http://www.aquaclub.de/rotliste.htm* (Aqua Quality Club - Artenschutzliste / *A. sturio* / Germany / German / faunal lists)
- *http://www.arconet.es/users/marta/Esturion.htm* (Esturion / *A. sturio* / Spain / Spanish / images)
- *http://www.arkive.org.uk/public/search1/?species_uid=1* ("Untitled" / *A. sturio* / UK / English / general)
- *http://www.BerlinOnline.de/wissen/wissenschafts-archiv/980429/.html/berlin1.html* (BerlinOnline: Wissenschaft / *A. sturio* / Germany / German / news)
- *http://www.bianchi.ch/warenkunde/suesswasser/34.htm* (Bianchi: Warenkundelexicon / *A. sturio* / Switzerland / German / general)
- *http://www.biokotor.org/projects/check/ribe.htm* (Check liste ribe - Zavod za biologiju mora, Kotor / *A. sturio* / Yugoslavia / Serbian / faunal lists)
- *http://www.birkhauser.com/cgi-win/ISBN/3-7643-5321-X* (Birkhauser - Conservation of Endangered Freshwater Fish / *A. sturio* / Switzerland / English / references)
- *http://www.bordeaux.cemagref.fr/doc/Esturgeon.pdf* (Cemagref Bordeaux: Publications récentes / Acipenseridae / France / French / references)
- *http://www.bordeaux.cemagref.fr/doc/rabx97.html* (Cemagref Bordeaux: Unité RAC, publica-

tions 1997 / Acipenseridae / France / French / references)

- *http://www.bretagne-online.com/telegram/htdocs/archive/1998/19980315/article/3426666.htm* (Un esturgeon dans l'estuaire de l'Odet / *A. sturio* / France / French / news)
- *http://www.bretagne-online.com/telegram/htdocs/archive/1999/19991126/MORBIHAN/article/art_010A0B0000_389755.htm* (Un plan de sauvegarde pour l'esturgeon européen / *A. sturio* / France / French / news)
- *http://www.bretagne-online.com/telegram/htdocs/archive/2000/20000207/29_LOCALES_SUD/article/art_0105080601_686253.htm#INTER1* (Esturgeons dans la baie: et de deux! / *A. sturio* / France / French / news)
- *http://www.bretagne-online.com/telegram/htdocs/archive/2000/20000205/29_LOCALES_SUD/article/art_0105080601_679266.htm#INTER1* (Un esturgeon pêché par un plaisancier dans la baie / *A. sturio* / France / French / news)
- *http://www.britannica.com/bcom/eb/article/9/0,5716,71869+1,00.html* (sturgeon - Britannica.com / Acipenseridae / UK / English / encyclopaedia)
- *http://www.cabuzel.com/oleron/frames/esturgeon.html* (Esturgeon / *A. sturio* / France / French / general)
- *http://www.cco.asso.fr/ieo/ieo24/creac.html* (LO CREAC D'AQUITÀNIA/L'esturgeon d'Aquitaine. / *A. sturio* / France / French / general)
- *http://www.ccpclub.demon.co.uk/mas/quarry/sturgeon.htm* (MAS - The Maldon Angling Society / *A. sturio* / UK / English / general)
- *http://www.cemagref.fr/infos/com/ext/im19/sturio.html* (Pour la première fois sur un salon professionnel dédié à l'aquaculture... / *A. sturio* / France / French / general)
- *http://www.comptons.com/encyclopedia/ARTICLES/0175/01753600_A.html#P1A1* (Sturgeon / Acipenseridae / USA / English / encyclopaedia)
- *http://www.cplus.fr/html/ete97/poitou/st_seurin.htm* (Saint Seurin d'Uzet / *A. sturio* / France / French / news)
- *http://www.cucina.italynet.com/pesci/dati/88.htm* (Il pesci... / *A. sturio* / Italy / Italian / general)
- *http://www.daba.lu.lv/ldf/CORINE/Fish.html* (Appendix 1. Red Data Book: Fishes / *A. sturio* / Latvia / English / faunal lists)
- *http://www.dgf.min-agricultura.pt/leipesca/leipesca2_Anexo.html* (Lista actualizada dos peixes de água doce: anexo decreto 44623, 10 de outubro de 1962 / *A. sturio* / Portugal / Portuguese / faunal lists)
- *http://www.dierentuin.net/vissen/steur.html* (Dierentuin.Net Dieren Database Steur / *A. sturio* / Netherlands / Dutch / general)
- *http://www.ee/baltic21/publicat/R4.htm* (BALTIC 21: INTERNATIONAL BALTIC SEA FISHERY COMMISSION SECTOR REPORT ON FISHERIES / *A. sturio* / Estonia / English / faunal lists)
- *http://www.esvjahn.purespace.de/stoerbe.htm* (Stoer Beschreibung / *A. sturio* / Germany / German / general)
- *http://www.fetonte.polesineinnovazione.it/schede/s/storione.htm* ("Untitled" / *A. sturio* / Italy / Italian / general)
- *http://www.fishbase.org/Summary/SpeciesSummary.cfm?ID=2066&genusname=Acipenser&speciesname=sturio* (Species Summary / *A. sturio* / Philippines / English / general)
- *http://www.fishing.kiev.ua/riby/sab/ost01.htm* (Osetr / *A. sturio* / Ukraine / Ukrainian / general)
- *http://www.fribourg-peche.ch/peche/poissons/description/esturgeon_welcome.htm* (La pêche au pays de Fribourg - Description des espèces de poissons - L'esturge / *A. sturio* / Switzerland / French / general)
- *http://www.gamta.lt/Raimis/aamta/PADALIN/KBVD/BIS/sarasai.htm* (Istatymo blankas / *A. sturio* / Lithuania / Lithuanian / faunal lists)
- *http://www.gencat.es/darp/espesca/cespes19.htm* (Peixos: fitxa Esturió / *A. sturio* / Spain / Catalan / general)
- *http://www.geocities.com/CapeCanaveral/Hall/1345/sturgbibl.html* (Bibliography of Sturgeons / Acipenseridae / USA / English / references)
- *http://www.geocities.com/RainForest/3414/storione.htm* (Storione / *A. sturio* / Italy / Italian / general)
- *http://www.geocities.com/RainForest/8769/lista-roja/1069.htm* (Esturion, Acipenser sturio / *A. sturio* / Spain / Spanish / general)

- *http://www.gla.ac.uk/Acad/IBLS/DEEB/biomedia/pictures/acipe.htm* (Image Gallery - c / *A. sturio* / UK / English / images)
- *http://www.guldennet.nl/flevoland/urk/visserij/vissen/vis032.html* (Steur/Acipenser sturio / *A. sturio* / Netherlands / Dutch / general)
- *http://www.helcom.fi/news/news297.html#1* (HELCOM News 2/97 / *A. sturio* / Finland / English / news)
- *http://www.icn.pt/sipnat/especies/tcontpei.html* (Espécies - Peixes do Continente / *A. sturio* / Portugal / Portuguese / faunal lists)
- *http://www.iii.to.cnr.it/pesci/storione.htm* (STORIONE / *A. sturio* / Italy / Italian / general)
- *http://www.iksr.org/icpr/l2000/species-diversity-research.htm* (Salmon 2000 - The Rhine a salmon river again: Species diversity research / *A. sturio* / Germany / English / general)
- *http://www.italiaset.com/Ittiofauna/GallFish/Osteichtys/Acipenser%20sturio.htm* (Acipenser sturio / *A. sturio* / Italy / Italian / general)
- *http://www.izmiran.troitsk.ru/Boroda-t/Sabaneev/0s43-txt.htm* (Osetr nemetskií / *A. sturio* / Russia / Russian / general)
- *http://www.lib.washington.edu/fish/image_bank/i42a.html* (i42a / *A. sturio* / USA / English / images)
- *http://www.livingplanet.org/resources/publications/species/underthreat/page26.htm* (Common Sturgeon / *A. sturio* / Switzerland / English / general)
- *http://www.lusolink.net/especies/sturgeon.htm* (The Sturgeon / *A. sturio* / Portugal / English / general)
- *http://www.markuskappeler.ch/tex/texs/stoere.html* (Störe / Acipenseridae / Switzerland / German / general)
- *http://www.mir.gdynia.pl/akw/dydakt25.htm* (EDUCATIONAL HALL2 / *A. sturio* / Poland / English / images)
- *http://www.mma.es/docs/conservnat/naturalia/naturalia_hispanica/Peces/obrashidraulicas/CAP01.htm* (2 / *A. sturio* / Spain / Spanish / general)
- *http://www.mma.es/docs/conservnat/naturalia/naturalia_hispanica/Peces/pecescontinentales/cap3pecescontinentales/cap3_1_2.htm* (3 / *A. sturio* / Spain / Spanish / general)
- *http://www.mma.es/docs/conservnat/naturalia/naturalia_hispanica/vertebrados/LibroRojoVertebrados/ESTURION_SOLLO.htm* (Acipenser sturio Linnaeus, 1758 / *A. sturio* / Spain / Spanish / general)
- *http://www.multimania.com/estuairegironde/CEG-407.html* (Estuaire de la Gironde: milieu naturel / *A. sturio* / France / French / general)
- *http://www.museocivico.rovereto.tn.it/MuseoAperto/zoologia/zoologiaIttiologiaStorione.htm* (MCR - Zoologia - Ittiologia / *A. sturio* / Italy / Italian / images)
- *http://www.nic.funet.fi/pub/sci/bio/life/pisces/actinopterygii/acipenseriformes/acipenseridae/acipenserinae/acipenser/index.html* (Acipenser / Acipenseridae / Finland / English / faunal lists)
- *http://www.nrm.se/ve/pisces/swedfish.shtml.en* (Swedish Fishes / *A. sturio* / Sweden / English / faunal lists)
- *http://www.omne-vivum.com/v/12583.htm* (Foto-album: Acipenseridae / Acipenseridae / Netherlands / English / images)
- *http://www.peche.org/esturgeon/esturgeon_europeen.htm* (sauver l'esturgeon européen / *A. sturio* / France / French / general)
- *http://www.peche.org/poissons_eau_douce/esturgeon.htm* (esturgeon / *A. sturio* / France / French / general)
- *http://www.pescados-basilio.es/productos/esturi.htm* (La mar de recetes... / *A. sturio* / Spain / Spanish / general)
- *http://www.pfri.hr/fish/f063.html* (Ribe jadranskog mora / *A. sturio* / Croatia / Croatian / images)
- *http://www.proszynski.pl/Zwierzaki/1998/maj/czerwonaksiega/czerwonaksiega.htm* (Zwierzaki 4/98 - Polska czerwona ksiega czesc V / *A. sturio* / Poland / Polish / general)
- *http://www.provincia.milano.it/ambiente/pesca/fauna/storione.htm* (Storione / *A. sturio* / Italy / Italian / general)
- *http://www.provincia.venezia.it/cacciapesca/manifest/pescimare/storione.htm* (STORIONE COMUNE / *A. sturio* / Italy / Italian / general)
- *http://www.sns.dk/netpub/rodliste/715.htm* (8 kontor - rødliste 1997 / *A. sturio* / Denmark / Danish / faunal lists)
- *http://www.stabi.hs-bremerhaven.de/pesta2/privat/zoo/stoer.htm* (Stör / *A. sturio* / Germany / German / general)
- *http://www.terroir.sud.fr/site/produit/mer/esturgeon.htm* (Untitled Document / *A. sturio* / France / French / general)

- *http://www.uhc.lublin.pl/mazury/ryby/jesiotr.htm* (Ryby / *A. sturio* / Poland / Polish / general)
- *http://www.unife.it/geneweb/sturio.htm* (Acipenser sturio / *A. sturio* / Italy / English / general)
- *http://www.uni-rostock.de/fakult/manafak/biologie/abt/zoologie/stoer/arefev2.htm* (Perspectives of... / *A. sturio* / Germany / English / general)
- *http://www.uni-rostock.de/fakult/manafak/biologie/abt/zoologie/stoer/stoersw.JPG* (JPEG image 640×350 pixels / *A. sturio* / Germany / English / general)
- *http://www.urnerbarry.com/lithographs/acipense.htm* (Acipenser Sturio Sturgeon / *A. sturio* / USA / English / images)
- *http://www.waterland.net/ovb/kenniscentrum/visportret/steur.html* (De Steur/ *A. sturio* / Netherlands / Dutch / general)
- *http://www.waterland.net/visschenwinkel/sturgeon.htm#sturgeon* (Acipenseriformes / *A. sturio* / Netherlands / English / general)
- *http://www.wcmc.org.uk/species/data/species_sheets/sturgeon.htm* (World Conservation Monitoring Centre - WCMC/WWF Species Under Threat / *A. sturio* / UK / English / general)
- *http://www.welt.de/daten/1997/07/05/0705ws91106.htx* (DIE WELT online vom 05.07.1997 - Wissenschat / *A. sturio* / Germany / German / news)
- *http://www.wildernis.nl/helgoland/helgoland.htm* (Foto´s uit de Europese Wildernis / *A. sturio* / Netherlands / Dutch / images)
- *http://www.wkap.nl/issuetoc.htm/0378-1909+48+1/4+1997* (Environmental Biology of Fishes Table of Contents / Acipenseridae / Netherlands / English / references)
- *http://www.wms.com.pl/jesiotr0.htm* (jesiotr_03 / *A. sturio* / Poland / Polish / general)
- *http://www.worldstar.com/~dlarson/stofworld.htm* (Sturgeon of the World / Acipenseridae / USA / English / faunal lists)
- *http://www.y.lst.se/miljosidor/analys/Hotade%20arter/Appendix/ryggrads.html* (HOTADE ARTER / *A. sturio* / Sweden / Swedish / faunal lists)
- *http://www.zone.sk/animals/jesetery.html* (ANIMAL PLANET - VERTEBRATES / Acipenseridae / Slovakia / Slovak / general)
- *http://www-cal.univ-lille1.fr/~cv/ecologie/by_thema/biodiversite/frl96_compacte.htm* (World Conservation Monitoring Centre: French Animal Redlist / *A. sturio* / France / French / faunal lists)

Sturgeons conservation

- *http://www.euro-sturio.de/* (EURO-STURIO - Internationale Gesellschaft zur Rettung der Donau-Störe e.V. / Acipenseridae / Germany / German / general)
- *http://www.scientificamerican.com/explorations/1998/061598sturgeon/index.html* (Scientific American: Explorations: The Last Sturgeon: June 15, 1998 / Acipenseridae / USA / English / news)
- *http://www.traffic.org/cop11/briefingroom/sturgeon.html* (TRAFFIC: COP11 / Acipenseridae / Switzerland / English / news)
- *http://www.worldstar.com/~dlarson/html/welcome.html* (Sturgeon Page / Sturgeons / USA / English / general)

Additional information found on the WWW regarding *A. sturio* conservation is included in Appendix I (*Acipenser sturio* on international Red Lists and Conventions), together with relevant locations on related subjects (Conservation, Fishes, and European Union Legislation). The WWW also features information on national and regional regulations regarding *A. sturio* conservation, but these Web sites are not included in our lists.

Web pages selected for *A. sturio* conservation were created in 24 European countries, the USA and the Philippines. In fact, those European countries already involved in recovery programmes on Atlantic sturgeon conservation, led by France, have produced the highest number of locations (figure 1). Of the 20 different languages used in these pages, English is the most important, followed by French and Italian (figure 2).

DISCUSSION

Databases and the WWW have overwhelmed the information market. Organisations increasingly present their activities on the Web, which allows them to disseminate updated information about

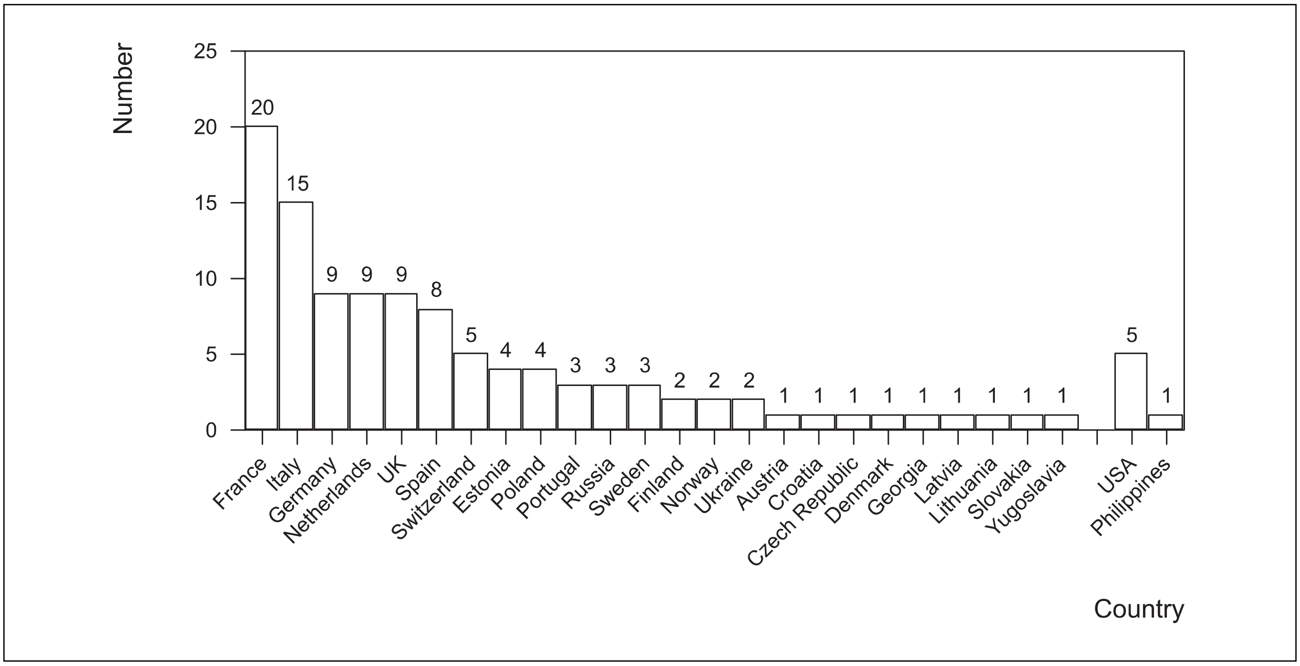

Figure 1. Number of Web sites with relevant information on *Acipenser sturio,* by countries

their experts, publications and on-going research projects, in a much more efficient way than was possible previously. Web technology has a major advantage over printed products since it allows end-users to search, browse, and print the information in different formats, according to their own specific needs. For this reason, the possibility of exchanging data via the Web has the potential to increase our knowledge concerning sturgeon conservation worldwide.

In summary, the Internet is a powerful tool for accessing information about nature conservation. We hope to contribute, with this paper, to the use of this wealth of valuable information.

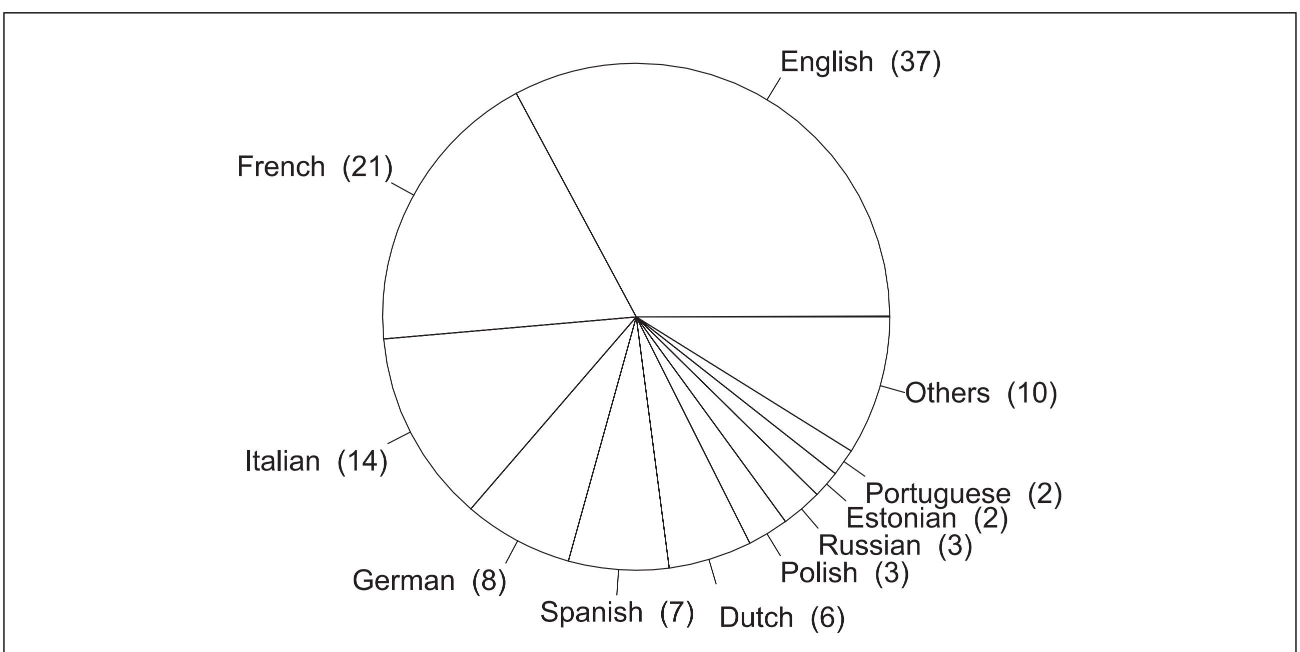

Figure 2. Number of Web sites with relevant information on *Acipenser sturio,* by languages

ACKNOWLEDGEMENTS

Several persons helped us to search for Web pages and provided us with URL addresses. Thanks also go to two anonymous referees for improving the manuscript. Finally, the WWW is a living creature, in continuous motion, and we would appreciate the submission of any further information on this subject, in order to include it on our Web page.

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APPENDIX I

***Acipenser sturio* on international Red Lists and Conventions**

- *Acipenser sturio* in Annex I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES - Washington Convention): *http://www.wcmc.org.uk:80/CITES/common/append/fauna12_correct.shtml*
- *Acipenser sturio* in Appendix II of the Convention on the Conservation of Migratory Species of Wild Animals (Bonn Convention): *http://www.wcmc.org.uk/cms/cms_app2_inter.htm*
- *Acipenser sturio* in Appendix II of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention): *http://www.coe.fr/fr/txtjur/a_ii(104).htm*
- *Acipenser sturio* in 2000 IUCN Red List of Threatened Species: *http://redlist.cymbiont.ca/species.asp?id=230*

Conservation

- Barcelona Convention, Mediterranean Action Plan (MAP): *http://www.unepmap.org/*
- Convention on Biological Diversity (CBD): *http://www.biodiv.org/*
- Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES): *http://www.wcmc.org.uk:80/CITES/*
- Convention on Migratory Species (Bonn Convention): *http://www.wcmc.org.uk/cms/*
- Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention): *http://www.nature.coe.int/english/cadres/berne.htm*
- European Environment Agency (EEA): *http://eea.eu.int/*
- European Topic Centre on Nature Conservation (EEA-ETC/NC): *http://www.mnhn.fr/ctn/*
- United Nations Environmental Programme (UNEP): *http://www.unep.ch/*
- Wildlife Trade Monitoring Programme of WWF and IUCN (TRAFFIC): *http://www.traffic.org/*
- World Conservation Monitoring Centre: *http://www.wcmc.org.uk/*
- World Conservation Union (IUCN): *http://iucn.org/*

Fishes

- Catalogue of Fishes: *http://www.calacademy.org/research/ichthyology/catalog/index.html*
- FishBase: *http://www.fishbase.org/*
- Ichthyology Web Resources: *http://www.biology.ualberta.ca/jackson.hp/iwr/iwr.html*

- Internet Resource Guide for Zoology - Fish: *http://www.york.biosis.org/zrdocs/zooinfo/#f*
- World Wide Web Virtual Library - Fish: *http://actwin.com/WWWVL-Fish.html*

European Union Legislation

Bonn Convention

- Convention on the conservation of migratory species of wild animals: *http://europa.eu.int/eur-lex/en/lif/dat/1979/en_279A0623_01.html*
- 82/461/EEC: Council Decision of 24 June 1982 on the conclusion of the Convention on the conservation of migratory species of wild animals: *http://europa.eu.int/eur-lex/en/lif/dat/1982/en_382D0461.html*
- 98/145/EC: Council Decision of 12 February 1998 on the approval, on behalf of the European Community, of the amendments to Appendices I and II to the Bonn Convention on the conservation of migratory species of wild animals as decided by the fifth meeting of the Conference of the parties to the Convention: *http://europa.eu.int/eur-lex/en/lif/dat/1998/en_398D0145.html*

Directive on the quality of fresh waters needing protection or improvement in order to support fish life

- Council Directive 78/659/EEC of 18 July 1978 on the quality of fresh waters needing protection or improvement in order to support fish life: *http://europa.eu.int/eur-lex/en/lif/dat/1978/en_378L0659.html*

Habitats Directive

- Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora: *http://europa.eu.int/eur-lex/en/lif/dat/1992/en_392L0043.html*
- 97/266/EC: Commission Decision of 18 December 1996 concerning a site information format for proposed Natura 2000 sites: *http://europa.eu.int/eur-lex/en/lif/dat/1997/en_397D0266.html*
- Council Directive 97/62/EC of 27 October 1997 adapting to technical and scientific progress Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora: *http://europa.eu.int/eur-lex/en/lif/dat/1997/en_397L0062.html*

Washington Convention (CITES)

- Council Regulation (EC) no. 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1997/en_397R0338.html*
- Commission Regulation (EC) no. 939/97 of 26 May 1997 laying down detailed rules concerning the implementation of Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1997/en_397R0939.html*
- Commission Regulation (EC) no. 2307/97 of 18 November 1997 amending Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1997/en_397R2307.html*
- Commission Regulation (EC) no. 2214/98 of 15 October 1998 amending Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1998/en_398R2214.html*
- Commission Regulation (EC) no. 1476/1999 of 6 July 1999 amending Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1999/en_399R1476.html*
- Commission Regulation (EC) no. 939/97 of 26 May 1997 laying down detailed rules concerning the implementation of Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1997/en_397R0939.html*
- Commission Regulation (EC) no. 767/98 of 7 April 1998 amending Regulation (EC) no. 939/97 laying down detailed rules concerning

the implementation of Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1998/en_398R0767.html*

- Commission Regulation (EC) no. 1006/98 of 14 May 1998 amending Regulation (EC) No 939/97 laying down detailed rules concerning the implementation of Council Regulation (EC) no. 338/97 on the protection of species of wild fauna and flora by regulating trade therein: *http://europa.eu.int/eur-lex/en/lif/dat/1998/en_398R1006.html*

Water protection

- Cooperation Agreement for the protection of the coasts and waters of the North-East Atlantic against pollution: *http://europa.eu.int/eur-lex/en/lif/dat/1993/en_293A1028_01.html*
- Convention for the protection of the marine environment of the North-East Atlantic: *http://europa.eu.int/eur-lex/en/lif/dat/1998/en_298A0403_01.html*
- Protocol concerning specially protected areas and biological diversity in the Mediterranean: *http://europa.eu.int/eur-lex/en/lif/dat/1999/en_299A1214_01.html*
- 99/800/EC: Council Decision of 22 October 1999 on concluding the Protocol concerning specially protected areas and biological diversity in the Mediterranean, and on accepting the annexes to that Protocol (Barcelona Convention): *http://europa.eu.int/eur-lex/en/lif/dat/1999/en_399D0800.html*
- 1999/802/EC: Council Decision of 22 October 1999 on the acceptance of amendments to the Convention for the Protection of the Mediterranean Sea against Pollution and to the Protocol for the Prevention of Pollution by Dumping from Ships and Aircraft (Barcelona Convention): *http://europa.eu.int/eur-lex/en/lif/dat/1999/en_399D0802.html*
- Annex V to the Convention for the Protection of the Marine Environment of the North-East Atlantic on the protection and conservation of the ecosystems and biological diversity of the maritime area: *http://europa.eu.int/eur-lex/en/lif/dat/2000/en_200A0519_01.html*

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Atlantic sturgeon *Acipenser sturio* L., 1758 in the Guadalquivir River, Spain: A further contribution to its recent population dynamics and present decline

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ABSTRACT

Using as its starting point a major expansion of the historical data from the caviar and smoking plant that formerly existed in Coria del Río (southern Iberian Peninsula), some characteristics of this population are reviewed that were previously studied by other authors. A continuous series is presented of the annual captures of males and females from 1932 to 1969, as well as the evolution of the proportion of sexes and the average yearly weight. Using the space distribution of the locality of capture along the estuary and the springtime water flows, a locality index of capture for every year has been drawn up which enables us to know the possibility of successful reproduction for each spawning season. The faulty environmental conditions of the estuary at the species's recovery time, due to the progressive shortage of flow in the spring, and the bad quality of the water during the 1960s, made reproductive failure more and more frequent. Together with this, the pressure of fishing to which potential reproducers were subjected every spring in the lower estuary led to the practical extirpation of this population.

Key words: Databases, locality index, reproductive failure.

RESUMEN

El esturión atlántico* Acipenser sturio *L., 1758 en el estuario del Guadalquivir: contribución al conocimiento de su reciente dinámica poblacional y actual declive

Partiendo de una importante ampliación del registro histórico de datos de la fábrica de caviar y ahumados que existió en Coria del Río (Sevilla), se analizan algunas de las características de esta población que ya estudiaron otros autores. Se muestran las capturas anuales de machos y hembras desde 1932 a 1969 en una serie continua, así como la evolución de la proporción de sexos y el peso medio anual. Utilizando la distribución espacial de las localidades de captura a lo largo del estuario y los flujos de agua primaverales se obtiene un índice de localidad de captura para cada año que permite conocer la posibilidad de éxito reproductor para cada estación de freza. Las deficientes condiciones ambientales del estuario en la época de remonte de los peces, debido a la progresiva escasez de caudal en primavera, y la mala calidad del agua durante los años sesenta tuvieron como resultado el fallo reproductor cada vez más frecuente. Esto, unido a la presión de pesca a que se sometió cada primavera a los potenciales reproductores en la parte baja del estuario, tuvo como resultado la práctica extinción de la población.

Palabras clave: *Bases de datos, índice de localidad, fallo reproductivo.*

INTRODUCTION

Acipenser sturio L., 1758 was a common species in the Guadalquivir River during its upstream migration, reaching Cordoba, 230 km far away from the mouth of the river (Steindachner, 1866). Until the 1930s, the species was occasionally caught in the sea by trawlers (Classen, 1944) and by fishing lines near Sanlúcar de Barrameda. In 1932, a dam was finished at Alcalá del Río, 100 km away from the sea. It represented a barrier for the migrating fish, which could not reach the spawning areas above the dam.

A caviar and smoking plant was opened the same year in Coria del Río, 70 km from the river mouth. Until 1933, all migrating fish were captured with trammel nets close to the dam. In march 1933, the Ybarra company brought skilled fishermen from Romania, who built and installed the special fishing device which made it possible to work all around the estuary, and specifically in deep areas (from 6 to 11 m deep) in the lower estuary. This meant that the entire estuary could be accessible to fishermen, and fish could be captured from the brackish water to the spawning areas downstream from the dam. From that time on, every year in January fishermen sounded the river bottom and installed the fishing lines in deeper areas. The fishing effort was relatively uniform through the years, and the factory contracted six to eight pairs of fishermen for the harvest season. Each pair used 1 575 hooks divided into three lines. Captures started at the end of January in the lower estuary and ended in the middle of May.

The factory remained open until 1972, when catches were vanishing. The last records were in 1974 and 1975 (Hernando, 1975) and 1992 (Elvira and Almodóvar, 1993), one fish each year.

For a few years after the dam was built, local fishermen still continued catching young sturgeons in the lower estuary's muddy beaches, using the same kind of spoon nets they used for mullets (Mugilidae) (Classen, 1936, 1944; España-Cantos, 1948a, b, c).

The effects on sturgeon reproduction stemming from the fact that the dam was isolating their spawning areas and also controlling spring water flows were partially studied by Fernández-Pasquier (1999). The aim of the present work is to use the expanded database to analyse the population characteristics again, and try to find new reasons for understanding the decline of this population.

MATERIALS AND METHODS

So far Classen (1936, 1944), Rada (1954) and Muñoz-Goyanes (1959) have obtained the database used in the studies on the sturgeon population in the Guadalquivir estuary. Data from Classen between 1932 and 1943 and from original notebooks between 1944 and 1954 were studied by Elvira, Almodóvar and Lobón-Cerviá (1991). Subsequently, new original notebooks (with new data to 1967) were used by Fernández-Pasquier (1999).

The present paper represents a major expansion of that database, including the original notebooks of daily records of all the fish entering the caviar factory. This expansion of the original database consists of:

- Entries on all the males captured between 1944 and 1949, including date and locality of capture, life weight, and total length.
- Entries on all the females captured between 1944 and 1949 including date, locality of capture, life weight, and ovary weight.
- Entries on females from 1955 to 1972, including date, locality, total length, life weight, and ovary weight.

This important new data has been compared with information from several other sources. Therefore, we now have data on 3 098 females and 1 074 males in a series of 38 years of fishing. Among these data the most outstanding features are live weight, total length, locality of capture, date, and weight of the ovary, for almost the entirety of females taken between 1932 and 1969. Because the data on females have continuity over time, whereas data is lacking on males for some years, we made most of our analyses for females only.

The estuary was divided into seven sections (figure 1) from the mouth to the dam, respectively. Sections 1 and 2 were the deepest, highly influenced by seawater, with sandy and muddy bottoms and strong tidal flows. Sections 3, 4, and 5 were in the middle of the estuary, with muddy bottoms and brackish water. Sections 6 and 7 were characterised by continental water, low influence of tidal rhythms, and deep holes with gravel used as spawning areas after the dam construction.

First of all, we examined whether the improvements in database of historical registration confirm the theses of other authors. On the other hand the

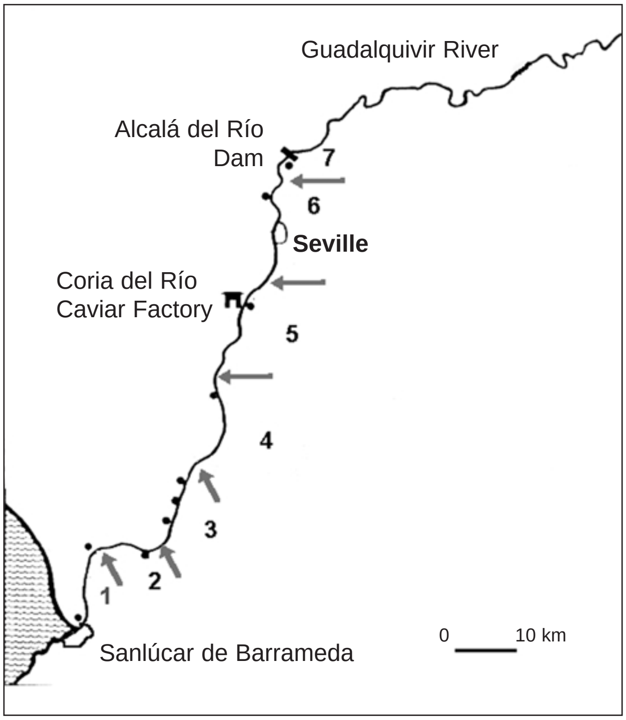

Figure 1. Map of the Guadalquivir estuary, localities and river sections. Modified from Elvira, Almodóvar and Lobón-Cerviá (1991)

important time sequence which we provide will enable us to study possible factors influencing the species's decline.

A χ^2 and a correlation rank test were used to study the sex ratio between the estuary sections. Spearman rank correlation coefficients were calculated to check relations between the adjusted average and water flows.

RESULTS

Evolution of captures and sex ratio

Between 1932 and 1972, the capture of 3 098 females and 1 074 males was recorded. Males caught in 1932, 1933, 1936, and 1941 were not recorded in the factory books.

The annual evolution of the captures is shown in figure 2, where a major annual increase in the first years can be seen, peaking in 1935 with the capture of 342 females, a figure that was never reached again. The captures in the late 1930s were very low, with the number of captured females staying below 100 until the early 1940s, a decade which saw the return of the previous stability in the captures. In the 1950s, a descending trend can be seen, reaching a critical point in 1962, with a drastic drop in captures below 20 females annually.

Figure 2 also shows the captures of males, highlighting that in several years they were not registered. Generally, the number of males was considerably lower than females, with important year-to-year differences. Although data on males is unavailable for the beginning of the fishery, and in the years of maximum captures of females (1934 and 1935) males were very scarce, later on there were years in which their number approached and even overtook that of the females. Although the evolution of the number of annual captures is different to that of the females, in both cases there are important year-to-

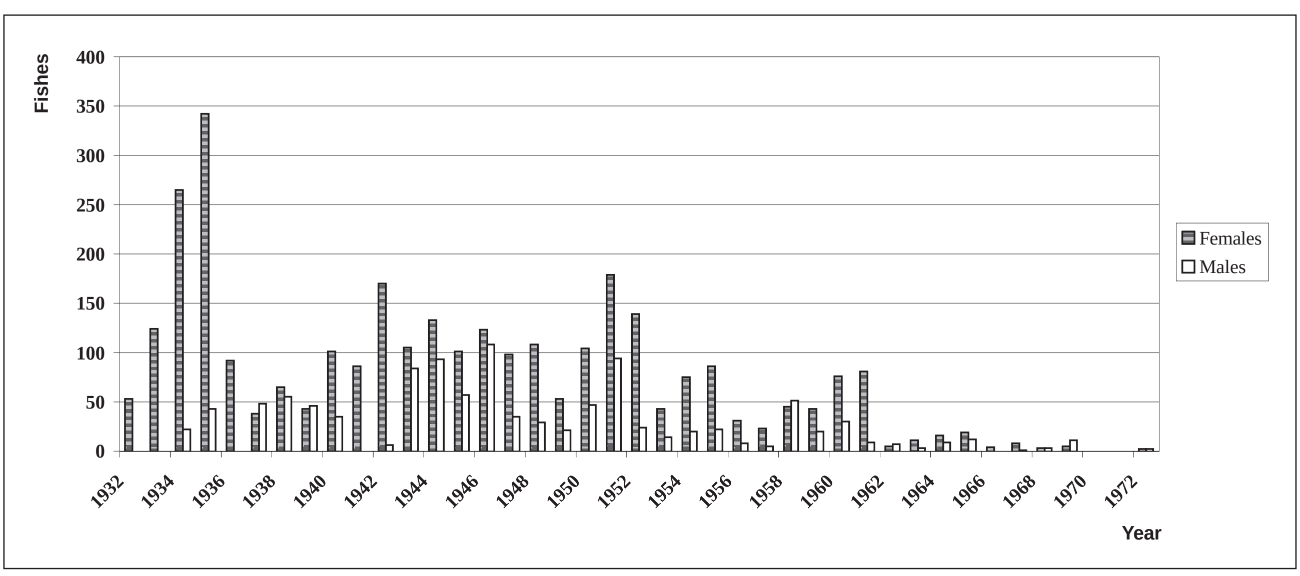

Figure 2. Annual variation of the number of captured fish

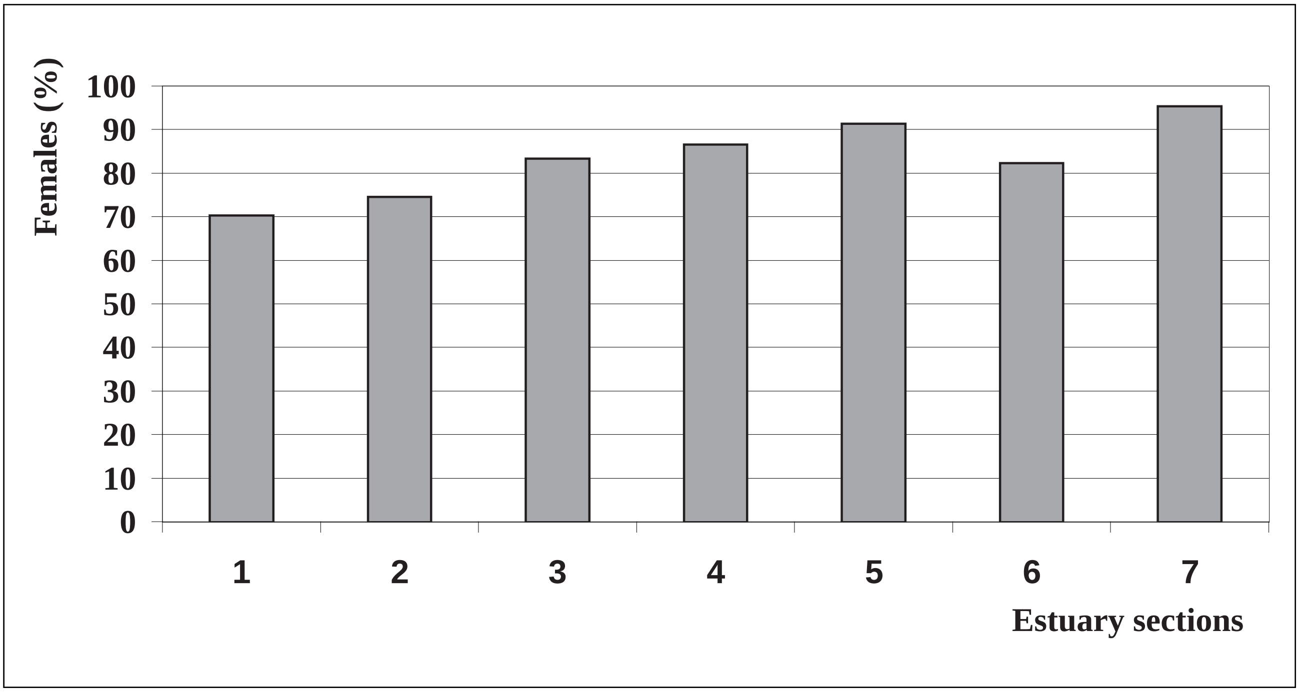

Figure 3. Percentage of females accounting for all the catches throughout 38 years in different sections of the estuary

year variations and a drastic decrease in captures starting from 1962.

In order to study space-time patterns, we divided the estuary into seven sections, from Elvira, Almodóvar and Lobón-Cerviá (1991), represented in figure 1. These seven sections, although they have several longitudes and their pressure of fishing varied, cover the entire available estuary for the species from the mouth to the spawning area, and they enable us to identify the zones of capture.

Figure 3 shows the percentage of females captured in each estuary section accounting for all the catches throughout 38 years. It stands out that the females always topped 70 % of total captures, with a tendency to increase this proportion closer to the dam, where they reached 96 % of the captures. These differences in sex ratios were statistically significant between sections ($\chi^2 = 116.6$; $p < 0.0001$), increasing progressively from sections 1 to 7 (correlation rank $r = 0.786$; $p = 0.036$).

Biometric characters

Table I shows biometric characters obtained from all fish recorded between 1932 and 1972. It is noteworthy that the average total length of the females was 36 cm higher than that of males, and that their average weight was exactly double.

A more detailed vision of the evolution of the average weight of the females over the years is shown in figure 4. The average weight of the captured females oscillated between 50 and 45 kg in the first 11 years of organised fishing. In the 12 following years, from 1942 to 1954, it had lower values, oscillating between 45 and 40 kg. Starting from 1954, there seems to be a recovery in the average weight, with annual values between 44 and 52 kg. From 1962 on, coinciding with the sharp drop-off in captures, larger inter-year weight differences can be seen, as well as a notable rise in the standard error.

Table I. Biometric characters of all fish recorded between 1932 and 1972

	Males			Females		
	Average	n	Standard error	Average	n	Standard error
Average weight	22.5	1 078	0.2	45.2	3 037	0.2
Average total length	150.5	621	0.3	186.6	2 551	0.3

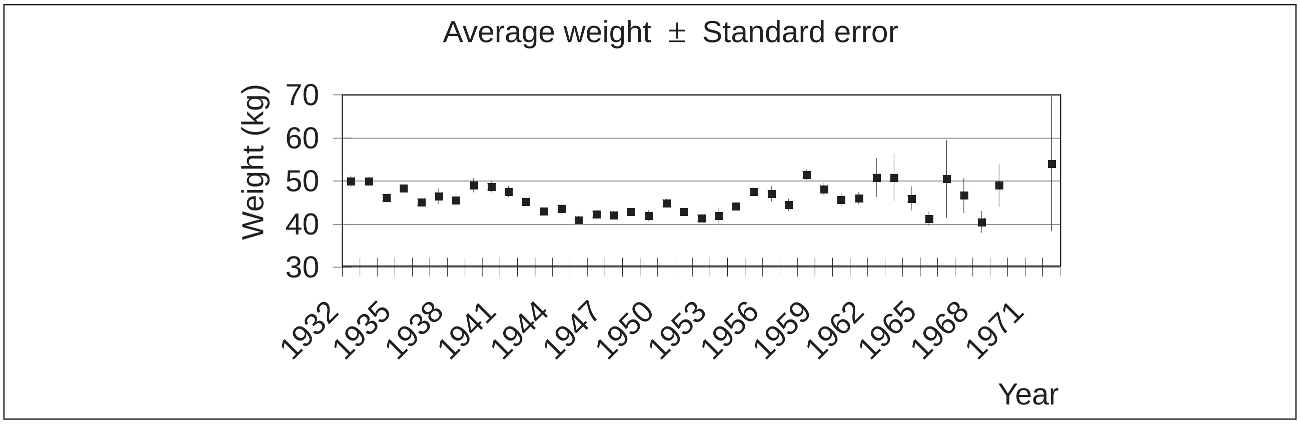

Figure 4. Yearly variation of the average weight of females between 1932 and 1972

Weight dispersion

In figure 5, the live weight of each female captured in the fishery is shown. We can see that in most years, individual weights vary widely, from 25 to 85 kg, with a lesser frequency of extreme values. The minimum weights take the shape of a well-defined curve, with two peaks corresponding to 1939 and 1958, which means scarcity of small-size breeders. The maximum weights were, in general, scat-

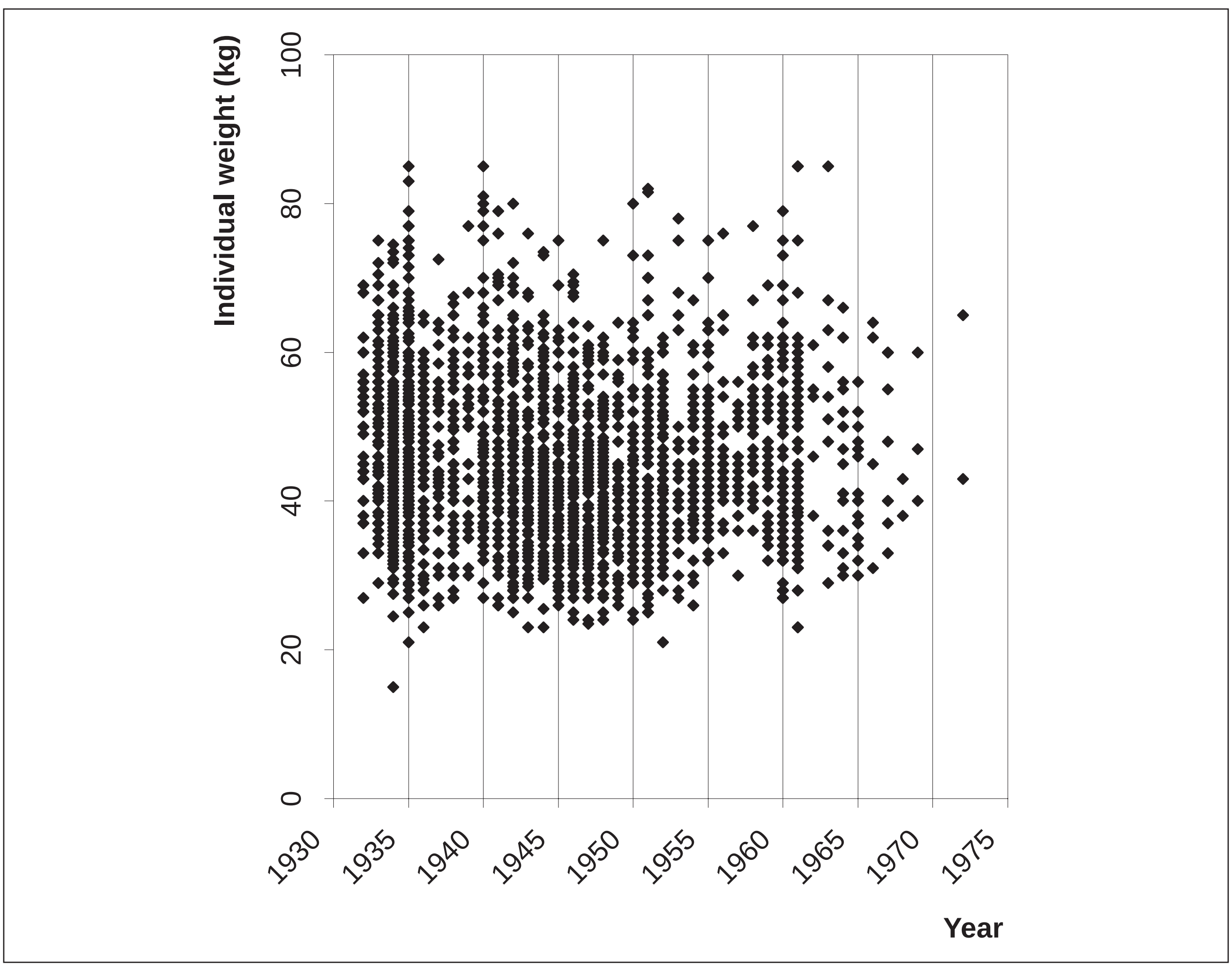

Figure 5. Weight of all the females captured for which data are available (n = 3 037)

tered more widely until 1962, with a of lack of fish larger than 65 kg in some isolated years. The biggest fish captured weighed 85 kg.

It is noteworthy that during the years in which the catches were more important, reaching more than 100 females, the lower weights were more frequent than in years with a scarcity of captures. It is also evident that in 1962 there was a drastic drop in captures. The following years show weights more and more concentrated around the median values, with scarcity of both large and small fish.

Relations between locality index and water flows

Yearly adjusted average values from 1934 to 1969 were obtained with the number of females captured in each section of the estuary versus the number of their corresponding capture section from 1 (mouth) to 7 (dam). This yearly value, hereafter termed the locality index (LI), gave us an instrument to compare the relative success of each fishing section of the estuary from one year to another. A value of 1.5 for this LI means that most of the fish were captured in the lower estuary, and a value of 4 means that a relevant number of fish were captured overcoming the estuary. We do not use data from 1932 and 1933, because in both years the fishery was settled only in the Alcalá dam, since it was still not possible to capture fish in the lower estuary.

Therefore the LI shows a first period, 1934 to 1948, with an alternation in values between 1.5 and 4. From 1948 onwards, the totality of values is around 2 (except in 1968, with only two fishes captured in the season), meaning that the lower estuary produced most of the captures. In figure 6, the values of LI versus average water flows during the breeding season (March, April and May) are shown, measured at Alcalá dam. The strong similarity of both values from 1934 to 1948 is remarkable.

A Spearman rank correlation coefficient was fitted in between LI and water flow (table II). The results confirm the strong relationship between locality index and water flows, at least until 1961, when there were still important captures. After 1962, the relationships lose their strength. The relationship shows that fishes were captured throughout the estuary only in years of a wet spring; in years with a dry spring, catches ocurred only in the lower estuary.

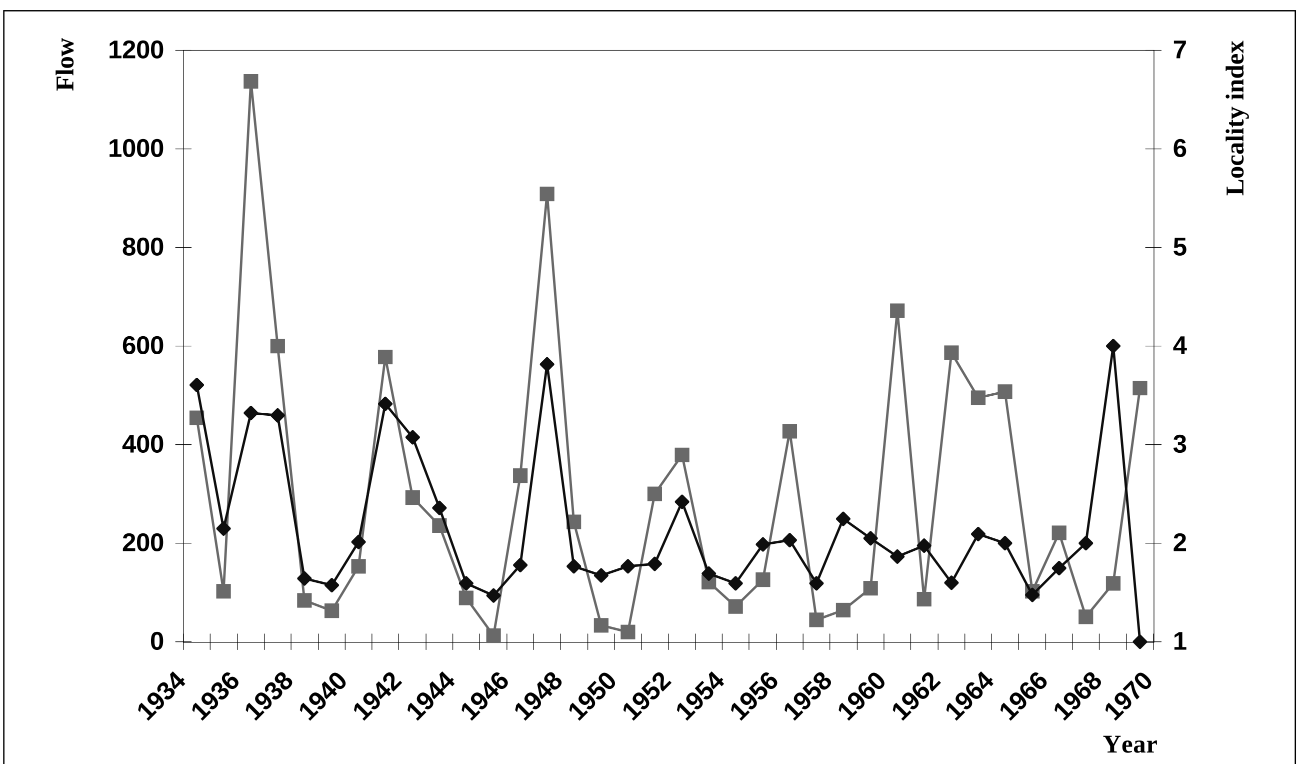

Figure 6. Locality index (◆) versus average (March, April, May) water flows (■) (m^3/s)

Table II. Correlation between locality index and water flow

Rank	Correlation coeficient	p
1934-1948	0.8762	< 0.001
1934-1961	0.7206	< 0.0001
1934-1969	0.4545	0.0056

DISCUSSION

Average weight and total length for 1932-1972 are similar to the average weight and total length that have recently been reported for the Gironde population by Williot *et al.* (1997).

Yearly average weight from 1932 to 1954 has the same tendency remarked by Elvira, Almodóvar and Lobón-Cerviá (1991) after incorporating data on the weight of females from 1944 to 1949 lacking in their study. After 1954, the average annual weight suffered important variations. These variations of average weight and its corresponding standard error are associated with scarcity of captures, being particularly notable from 1962 onwards, when the standard error is discharged coinciding with a definitive shortage of captures.

Sex ratio (74 % F, 26 % M; n = 4 172) is similar to those reported by Magnin (1962) for a five-year sampling period in the Gironde (n = 96), and by Elvira, Almodóvar and Lobón-Cerviá (1991) for the Guadalquivir captures from 1932 to 1943. The opposite ratio (74 % F, 24 % M) was reported by Williot *et al.* (1997) for catches of juveniles in the Gironde estuary. Juveniles have a similar size for males and females, and both have a similar chance to be captured by nets. In the case of adult fish, the ratios obtained were very probably an artefact of the selectivity of long lines, which capture females easier than males because they swim nearer to the bottom since the swimbladder is not functional when gonads are near maturity. Furthermore, in our database there are also four years in which no males were registered, which could have influenced the ratio.

The evolution of individual weight throughout the fishery indicates that there could have been two episodes in which new breeders failed to incorporate, before the definitive halt in captures: one at the end of the 1930s, and the other at the end of the 1950s.

The drastic scarcity of yearly catches from 1962 onwards must have been caused by pollution processes affecting both the entire estuary and breeding stock. There are reports from fishermen (pers. comm.) describing overflows of black waters polluted by subproducts of olive oil. This pollution produced an escape of sturgeons near asphyxia swimming at the surface of the water towards the sea, killing other fishes recently captured in the meantime waiting on a line to be transported at the factory.

The magnitude of these processes of organic contamination was so heavy that they caused the practical disappearance of one of the main fishing resources of the estuary at this time. The shrimp *Palaemon longirostris* Milne-Edwards, 1837 disappeared for a period of three years, so that the fishermen who were dedicated to this species during the summer had to leave and seek employment in the countryside until this fishery was recovered.

The existing relationship between freshwater flows in spring and the annual LI means that only in wet springs could the fish have massively entered the estuary, and they were captured everywhere, from the mouth to the dam. The immediate consequence of this is that only in wet springs could fish get into spawning grounds with the turbid, fresh water needed for spawning (Fernández-Pasquier, 1999).

LI indicate that the last possibilities of breeding success could have ocurred in 1947 and 1952, when the fish entered the estuary, even up to the spawning areas located in the proximity of the dam. In accordance with the age estimates of Classen (1943), females in the Guadalquivir estuary were 13 years old at first reproduction. Therefore, some small-sized females captured in 1960-1961 must have been first-time spawners belonging to a cohort of females which were born during the successful breeding season of 1947 (figure 5). The shortage of captures from 1962 on made it a little more difficult to appreciate the phenomenon again during the years 1965-1966, but it appears that it is probably more exact to estimate that females need between 12-14 years to get into the reproductive stock.

Reproductive failure became more and more frequent, especially from 1952 onwards, preventing the maintenance of a group of reproducers large enough to sustain the population. Due to this, and to more and more frequent reproductive failures (Vélez-Soto, 1951; Fernández-Pasquier, 1999), together with overfishing (Vélez-Soto, 1951; Gutiérrez, 1962; Lelek, 1980; Elvira, 1996; Fernández-Pasquier, 1999), the population was heading for a definitive decline. Other factors, such as fishing of juveniles in feeding areas of the lower estuary (Classen, 1936; España-Cantos, 1948), destruction of spawning grounds because of gravel exploitation (Gutiérrez, 1962), and ovary parasites, all of them known although not quantifiable, had contributed, to a much lower extent, to the present decline of the species.

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Reasons for the decline of *Acipenser sturio* L., 1758 in central Europe, and attempts at its restoration

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ABSTRACT

Sturgeons are becoming increasingly threatened world-wide by commercial exploitation and environmental stress. A species that anticipated the development of the remaining sturgeon species is *Acipenser sturio* L., 1758. Since the end of the last century, diminishing stocks have caused severe concern with regard to the survival of the species. Several attempts at management measures have been suggested, but due to economic and political pressure, as well as to very limited knowledge on the biology of the species, rarely have any effective protection measures been taken. Conservationists and fisheries biologists in many countries had given up hope of saving the species from extinction after early attempts at artificial reproduction and restocking had failed. Additionally, protection and conservation of the species, until the 1990s, was at the national level only. Under these conditions, French researchers have set an example with a long-term recovery programme that also tries to close the gaps of knowledge in many fields of biology. Until the mid-1990s this was the only active approach to protect and save the species from extinction. Recently, the restoration and conservation of *A. sturio* have received increased attention in several countries. The impacting factors upon the decline, based on the data from Central European rivers, as well as their relevance for restoration, are discussed. The national and international attempts to protect the species are summarised. The evaluation of their efficiency and an outline of potential alternatives are also presented.

Key words: Atlantic sturgeon, management, protection, recovery.

RESUMEN

Razones del declive de* Acipenser sturio *L., 1758 en Europa central e intentos para su recuperación

Los esturiones están siendo crecientemente amenazados en todo el mundo por la explotación comercial y la presión ambiental. Una especie que anticipó lo ocurrido con las restantes especies de esturiones es Acipenser sturio *L., 1758. Desde finales del siglo XIX, los mermados stocks han causado grave preocupación respecto a la supervivencia de la especie. Se han intentado diversas medidas de gestión, pero debido a las presiones económicas y políticas, así como al muy limitado conocimiento de la biología de la especie, raramente las medidas tomadas han sido de protección efectiva. En muchos países, los conservacionistas y biólogos pesqueros han perdido la esperanza de salvar a la especie de la extinción después de haber fracasado los primeros intentos de reproducción artificial y repoblación. Adicionalmente, la protección y la conservación de la especie, hasta la década de los noventa, se acometían sólo a escala nacional. Bajo estas condiciones, los investigadores franceses han constituido un ejemplo, con un programa de recuperación a largo plazo que también trata de llenar los vacíos de conocimiento en muchos campos de la biología. Hasta la mitad de los noventa, ésta fue la única perspectiva para la protección de la especie y salvarla de la extinción. Recientemente, la restauración y la conservación de* A. sturio *han recibido creciente atención en varios países. Se discuten los factores de impacto para su declive, a partir de los datos de los ríos centroeuropeos, así como su relevancia para la restauración. Se resumen los intentos nacionales e internacionales para proteger a la especie y se presentan la evaluación de su eficacia y un bosquejo de las potenciales alternativas.*

Palabras clave: *Esturión atlántico, gestión, protección, recuperación.*

INTRODUCTION

Sturgeon conservation has been the object of growing interest during recent years worldwide (Rochard, Castelnaud and Lepage, 1990). Management of the sturgeon species is demanding, due to their long life-cycles and diversity of habitats. In addition, such programmes might prove their efficiency after 15-25 years only –a timeframe that is almost inapplicable for administrative policies. For the European Atlantic sturgeon *Acipenser sturio* L., 1758, management and conservation are even more difficult, because the major decline had already begun in the 19th century. For most sturgeon species, overfishing was considered to be one of the main threats to the stocks (Debus, 1997). Previously, the decline in *A. sturio* was also attributed solely to fishing pressure on the species. A closer look at the impact of environmental alterations caused during the Industrial Revolution has shown that this cause-effect relationship seems rather preliminary. Important reasons for the decline of the sturgeon should be identified more precisely in order to outline persistent critical factors, adversely affecting successful restoration.

WHAT IS RESTORATION?

Restoration is defined as the "Return to a healthy and vigorous state or to an unimpaired and vigorous position" (Fowler and Fowler, 1971), thus emphasising the quality of the alterations as the main criterion to separate restoration from other remediation options. According to the National Research Council (Anon., 1992): "The aim should be the restoration of the whole ecosystem, even if sometimes some particular components or attributes are emphasised". In contrast, Bradshaw (1996) points out that "The endpoint of full restoration, although it might seem ethically the most justifiable and therefore the most obvious to adopt, may in fact not always be the most sensible in practical or biological terms".

For *A. sturio*, any attempt to restore either stocks or habitat has to be preceded by an analysis of the reasons for the decline of the species, thus directly imposing demands upon planned restoration attempts.

CASE STUDY

An analysis of the reasons for the decline of sturgeon populations and the restoration requirements deriving therefrom is examplified for the Elbe River, outlining mechanisms observed in Central Europe in general (Kirschbaum and Gessner, 2000).

Figure 1 gives the catch data of the lower Elbe River in northern Germany from 1858 until 1920. Since 1840 until the end of the 1880s catches from the Elbe River averaged 7 000 mature sturgeons annually.

Initially, the fishery targeted migrating adults in the river from April to July with a knot to knot mesh size of 20 cm, allowing fish of less than 1.5 m to escape. Since the 1870s, the number of fishing-vessels increased drastically (Koos, 1924), thereby decreasing the CPUE. Beginning in 1888, the catch declined drastically to approx. 50 % of the average return of the previous decades (Mohr, 1952). The fishermen therefore were moving to the lower estuary and the Wadden Sea (Blankenburg, 1910), not waiting for the fish to enter the river. A decrease in mesh size to 15 cm in the river and 12.5 cm in the coastal waters resulted in the increased catch of juvenile fish (Anon., 1914). In the 1890s, the proportion of marine and coastal catches therefore increased to 66 % of the total catch (Blankenburg, 1910). Despite the increased effort, the landings were decreasing continuously. After the First World War, the sturgeon fishery in the Elbe River was insignificant, being eliminated within 25 years.

In addition to the described fisheries-related impact, other human activities altered the environment for the species significantly (figure 2). Since the 11th century deforestation along the rivers has led to increased sediment transport, re-forming the deltas of large rivers such as the Vistula (Hoffmann, 1996). The anthropogenic impact was intensified throughout mediaeval times (mill-weirs and deposition of wastes), and peaked during the Industrial Revolution of the 18th-20th centuries (Schirmer, 1994).

Taking into account a generation period for *A. sturio* of 20-25 years (Elvira, Almodóvar and Lobón-Cerviá, 1991; Fernández-Pasquier, 2000), the critical period for the development of the stocks must have occurred before 1870.

Two major factors unrelated to the fishery could have contributed to this development. Firstly, the

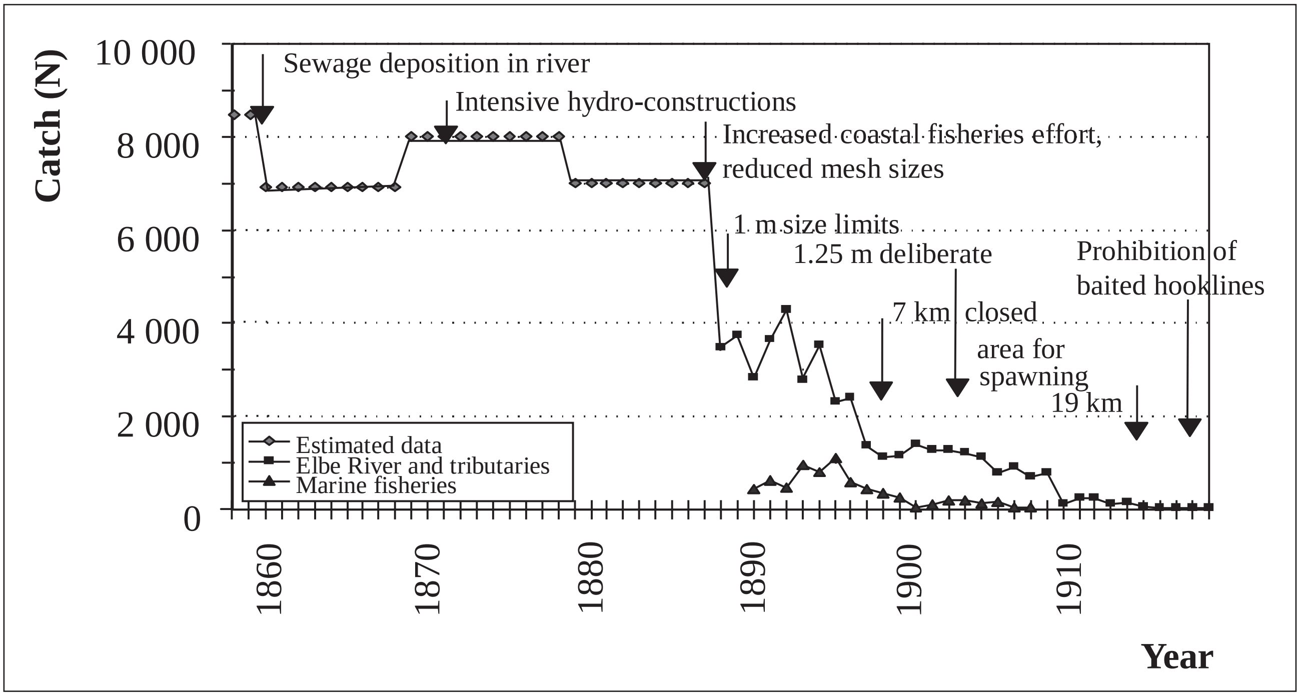

Figure 1. Sturgeon *A. sturio* catches in the lower Elbe River, its confluences and adjacent coastal and marine areas of northern Germany from 1858 to 1918; arrows indicate selected impact (after various authors)

cities of Hamburg and Altona began to dump their sewage into the Elbe River above the main spawning grounds at Koehlbrandt, in 1862 and 1868, respectively (Bonne, 1905). Additionally, intensively developing industrial settlements along the Elbe (Kisker, 1926; Bonne, 1905) also utilised the river

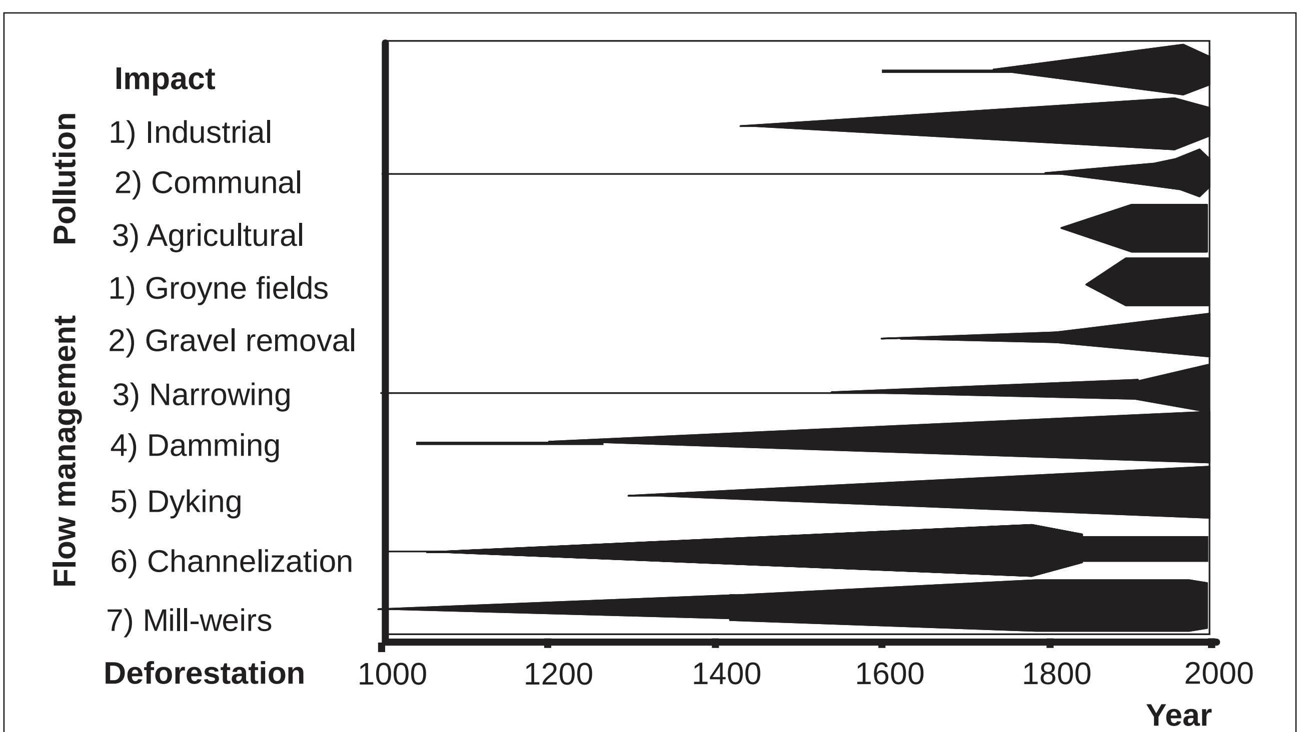

Figure 2. Development and relative intensity of alterations in river habitat in Central Europe over the previous 1 000 years (after various authors)

Table I. Impact assessment for major anthropogenic influences on different life stages of sturgeons; factors were assessed for direct impact on life-cycle stage; effects classified as T = leading to total loss; (–): adverse; (o): neutral; (+): beneficial

Life-cycle stage	Pollution			River management							Fisheries
Factors	Industry	Comm.	Agric.	Mill Weirs	Melior-ations	Dyking	Narrow	Groyne	Subst. removal	Dam	
Maximum cummulative effect	T	T	T	T	–	T	T	–	T	T	T
Adult mating	–	–	o	o	–	o	o	–	–	o	–
Adult migration	–	–	o	–	–	o	–	–	o	–	–
Pre-adult	–	–	o	o	o	o	o	o	o	o	–
Juvenile	–	–	–	–	o	–	–	+	o	–	–
Fingerling	–	–	–	–	–	–	–	+	o	–	o
First feeding	–	–	–	–	–	–	o	+	o	–	+
Yolk-sac larvae	T	T	T	T	–	T	T	o	–	–	+
Embryo	T	T	T	T	–	T	T	o	–	–	+

to remove their production wastes. Since the 1860s this led to oxygen depletion, as was described in detail by Bonne (1905).

Secondly, increasing demand for mass transportation of goods and raw materials resulted in increased hydro-constructions (Keweloh, 1985), thus increasing the uniformity of the riverbed and leading to losses of important habitats (Kausch, 1996a, b). The potential impact of different habitat alterations upon the subsequent life-stages of sturgeons are given in table I, mainly reflecting a worst-case scenario.

These factors, interacting with fisheries pressure upon larger juveniles and adult fish, can be considered the main reasons for the decline of *A. sturio*, although certain fishing activities might have a positive effect upon early life stages by decreasing predatory pressure, as well as decreasing the concurrence for habitat and food. Anadromous fish stocks, as well as rheophilic species, were largely affected by the habitat alterations (Bauch, 1958). As a consequence of the changing environment, ubiquists became the dominant species and the typical fauna extirpated (Lozan *et al.*, 1996; Wolter and Vilcinskas, 1997). Here again, *A. sturio* seems to have anticipated the development for other species.

EARLY MANAGEMENT EFFORTS AND EFFECTS

Various catch regulations were applied throughout Europe. From 1273, regulations excluding *A. sturio* from common fishery rights are documented from the Baltic fresh lagoon (Benecke, 1881). Management was mainly a question of the utilization of the resource, with the aim of increasing income from the fishery rights.

Management as a means to protect the resource was a reaction to the apparent decrease of the catches in the 19th century. The measures taken were comparable throughout the range of the species. Comparable restraints were anticipated when their application was attempted. Basically, three different management tools were used: catch regulations by size limits or mesh size limitations, closed areas, and the protection of the species by temporal or total inhibition of catches.

Figure 1 indicates the main measures taken in the Elbe River tributaries (after Blankenburg, 1910; Ehrenbaum, 1913, 1936):

1886 increase in size limit to 1.5-2.0 m neglected (too few juveniles in the fishery)

1890 size limit of 1.0 m

1904 deliberate size limit of 1.25 m by the fisheries co-operatives

1915 baited hook-lines prohibited in the Eider River (to avoid mass mortality of juveniles)

1918 baited hook-lines forbidden in the Oste River (see above)

1923 a size limit of 1.5 m after 9 years of ongoing debate was adopted

1924 size limit abolished

In other European regions, the struggle for the protection of the sturgeon was comparable. In the Gironde River basin (Trouvery, Williot and Castelnaud, 1984), for example:

1890 size limit imposed was 0.14 m

1923 increased size limit to 1.5 m

1924 reduction of size limit to 1.0 m (following protest from fishermen)
1939 size limit increased to 1.5 m
1950 size limit reduced to 1.3 m
1952 size limit of 1.45 m was agreed upon

Closed areas and closed seasons for sturgeon fisheries were applied in a variety of combinations for the Elbe River (after Quantz, 1903; Ehrenbaum, 1923):

1890 closed seasons from 26 July until 26 August
1898 Elbe River annually varying fishing leases to allow limitation of the fishing effort
1898 closed seasons from 15 July until 26 August
1898 Oste River, 7 km spawning sanctuaries where fertilised eggs were found
1904 fisheries ban after 1 August until the end of the year
1914 Oste River, 19 km of spawning sanctuaries

In the Gironde basin, the main measures, according to Trouvery, Williot and Castelnaud (1984) were:

1939 closed season during June/July (the fishery was still vital)
1950 closed season from July-December
1952 Gironde estuary transformed into a closed area

Summarising the fisheries regulations –especially the size limits applied– to protect *A. sturio*, they must be classified as ineffective. This was already known at the time when the size limits were discussed (Ehrenbaum, 1916). Sufficient size limits, which would have protected the spawners for at least one reproduction, were not applicable from a political point of view (*ibid*; Trouvery, Williot and Castelnaund, 1984). Closed areas, such as spawning sanctuaries, did not increase significantly the number of juveniles (Quantz, 1903; Koos, 1924).

CONSERVATION

A total ban of fisheries for sturgeons was first initiated by the Polish government in 1932 (Witkowski, 1992). In the former USSR, the necessity for increased protection of the species in the Newa River was recognised timely, as well (Berg, 1935). Since 1967, the species was protected at sea in the Georgian SSR (Ninua and Tsepkin, 1984). Attempts to artificially reproduce occasional catches of the species have been unsuccessful since the 1970s due to the unavailability of ripe fish of both sexes (Zarkua, pers. comm.).

In France, a total ban on fishing and marketing of the species was applied in 1982. Spain followed in 1983, with a total protection status for the species.

German attempts to conserve *A. sturio* had their onset in 1886 and 1891, when artificial reproduction and subsequent release of yolk sac larvae were carried out. Further trials failed due to the lack of simultaneous availability of ripe males and females. Comparable attempts were made in the Vistula area after 1906 (Seligo, 1907), and more recently in the Eider River, since 1953 (Spratte, 1994), without success.

Although various environmental alterations have been discussed as reasons for the decline of the anadromous fish species (Benecke, 1881; Volk, 1910; Schiemenz, 1913; Seligo, 1931), practical consequences for protection have not resulted.

CURRENT STATE OF STOCKS

Today, only one population with proven reproduction persists, in the Gironde and its tributaries in France. The situation of the population is described in great detail in the present volume (Elvira (ed.), 2000). One population, in extremely uncertain conditions, thrives in the Rioni River, Georgia. Furthermore, only single catches throughout the former range (Holčík *et al.*, 1989) have been confirmed for Lake Ladoga (Podushka, 1985), in Cadiz Bay (Elvira and Almodóvar, 1993), in the North Sea (Spratte and Rosenthal, 1996), and in the Baltic Sea (Paaver, 1997).

RECENT PROTECTION EFFORTS FOR *A. sturio*

General protection was considered only after the main phase of decline was witnessed and the stocks were reduced to an economically insignificant factor. The ban on catches throughout the range, their background and intentions, were not communicated to the fisheries effectively. Therefore, despite the protection status, sturgeons were caught and sold. Overall population sizes continued to decrease. Recent attempts in France, including public awareness campaigns, seem to have improved the interaction with fishermen significantly.

Active measures for the restoration of the population were begun in only one case while a stock was still present (Elie, 1997; Williot *et al.*, 1997). In other countries, fisheries biologists have long considered the extirpation of the species as an inevitable consequence of the alterations that followed the economic development of Central Europe and have only served as its witnesses (Nellen, 1992).

The disappearance of *A. sturio* was finally accompanied by its listing in the Red Books of the European Union. With the Washington Convention on Trade with Endangered Species, it was listed in Appendix I of the CITES Regulations in 1973. This attempt was aimed at prohibiting or strictly regulating international trade in the species.

Furthermore, the EU made an attempt to protect not only the species, but to provide measures to protect its habitat, when listing *A. sturio* as a species of special concern in Appendix II of regulation 92/43/EEC, thereby requiring measures for the restoration of habitat in order to protect the species.

Finally, the listing of the species in Appendix 2 of the Bern Convention in 1998 is aimed at the prohibition taking into possession of catches of sturgeon in international waters. This measure is extremely important, as outlined by Lepage and Rochard (1997), because the main losses from the population are attributed to the accidental captures in fisheries today. According to recent estimates, these catches alone would be sufficient to extirpate the population in a course of approximately 15 years.

OUTLINE OF CURRENT REMEDIATION ATTEMPTS

The attempts or considerations for the protection and re-establishment of *A. sturio* in Europe are summarised in table II. In general, *ex-situ* measures, including the catch of fish in the wild and subsequent rearing under controlled conditions as a means of protection, are the general objectives. Most of the measures are aimed at restocking natural water bodies with the F1 of these catches. The subsequent development of breeding plans based on sound genetical analysis and broodstock management is the next step necessary in this approach. Habitat assessments and recovery measures seem inevitable, given the reasons for the decline discussed above.

France

Since 1980, intensive activities to restore the Gironde stock of *A. sturio* have been carried out, including: stock assessment, habitat identification, artificial reproduction and stocking from artificially reproduced spawners, as well as social mediation

Table II. Current status of remediation efforts for *A. sturio* in Europe. (BSD): Broodstock Development (rearing of specimens to contribute to a broodstock in captivity, origin not defined); (ESM): *Ex-Situ* Measures (catch of animals from the wild and rearing under controlled conditions); (ER): Experimental Restocking (release of artificially propagated fish under current scientific contol and assessment); (HI): Habitat Identification; (HR): Habitat Remediation; (RS): Restocking (release of juveniles after artificial reproduction); (SA): Stock Assessment; (SE): Stock Enhancement (support of native stock by restocking)

Country	Onset	Status	Main aims
Albania	1995	Suspended	ESM, BSD, SE
France	1980	Ongoing	ESM, BSD, HI, HR, ER, SA, SE
Georgia		In preparation	ESM, HI, HR, SA, SE
Germany	1996	Ongoing	ESM, BSD, ER, HI, HR
Hungary		In preparation	BSD, HI, RS
Poland		In preparation	BSD, HI, RS
Russia	1997	Ongoing	ESM, BSD, RS
Romania		Planned	BSD, RS
Spain		Suspended	?
Sweden		Planned	BSD, RS
Baltic Sea (HELCOM)	1997	Ongoing	ESM, BSD, HI, co-ordination of national programmes, enhancement of collaboration

of the recovery plan. The techniques and results are described in a series of publications, also in the present volume (Elvira (ed.), 2000).

Germany

Since 1994, the Society to Save the Sturgeon (*A. sturio*) has tried to develop and co-ordinate projects for the restoration of *A. sturio* in Germany and to increase scientific collaboration. The programme is presented in some detail in the present volume (Elvira (ed.), 2000). The measures comprise *ex-situ* measures, genetical analysis, broodstock development, optimization of rearing techniques, habitat assessments, and planned experimental release of fish after tagging with transmitters to assess habitat utilization.

Georgia

The documentation on the Rioni stock dates back to Marti (1939) and Ninua (1976). Until 1990, annual monitoring activities were carried out on the shelf, estimating the stock size to comprise 200-400 individuals of the age classes 2+ (Zarkua, pers. comm.). Since 1990, no further surveys or research on *A. sturio* have been conducted. Recent activities in conservation and stock assessment are limited to a critical extent by the country's devastating economic situation. During 1997 and 1998, fishing trials for migrating adults and juveniles have been carried out in an attempt to check the status of reproduction of this stock. Neither adults nor migrating juveniles of *A. sturio* were caught during these trials.

Remediation activities in other European countries

In other European countries, the interest in restoration or remediation of *A. sturio* has increased recently. Here, the main target is to re-establish the species in its previous range, with the increasing tendency to use the available material rather than hypothetical remains of historical populations. Anyhow, analysis of the *A. sturio* complex seems required to precisely define the species that was historically dominant in the area in question (Holčík, 2000). In most cases, the absence of *A. sturio* from the waters can be documented for a number of years, if not decades. All remediation attempts or plans mainly suffer from the unavailability of the species in question. This is an obstacle that in future years is very unlikely to be overcome on more than a very limited scale. This obstacle also prevents administrators and researchers for expending more efforts on the subject.

The CITES listing banned transfer over international borders, but commercialization persisted (Germany 1992, Estonia 1996, UK 1998). Listing in EU directive 493/92 provided the legal status for future implementation which should lead towards the urgently-needed effective, active protection in European waters.

CONCLUSIONS

Information requirements

The anthropogenic impact on *A. sturio* was not assessed in quantitative or qualitative terms (Riedel-Lorje and Gaumert, 1982). Cause-effect relationships or rankings of effects on population dynamics are available neither for the impact of pollution nor the effects of hydro-construction. Today, the available resources are restricted to only one confirmed population with irregular natural reproduction –thus limiting the potential for experimental work on the species. Additionally, general transfer of information between different sturgeon species is of limited applicability, due to differences in behaviour (Jatteau, 1998). In any case, the potential for transfer of results from *Acipenser oxyrinchus* Mitchill, 1815 to *A. sturio* should be the subject of further research.

Broodstock development is essential in order to obtain stocking material, but requires the development of management plans (US Fish and Wildlife Service) (Anon., 1995; Elie, 1997). Furthermore, problems related to rearing *A. sturio* in captivity have been described recently (Williot *et al.*, 1997; Kirschbaum and Gessner, 2000), and are as yet unresolved.

Research targets

The main factors to be dealt with in the near future are the genetic characterization of remaining as well as missing/extinct populations (Holčík, 2000; Ludwig *et al.*, 2000). This is urgently required to outline the status of the species and to support the development of breeding programmes to increase genetic heterogeneity.

The physiological requirements of the species have to be determined for successful rearing. In order to improve the security of *ex-situ* measures, biotechnologies such as cryopreservation, androgenesis and gynogenesis should be focused upon in order to evaluate their potential for the conservation of genetic heterogeneity.

Furthermore, a variety of topics concerning the environmental interaction and ecology of the species requires in-depth research, e.g. an assessment of the reasons for the decreasing fertility of male sturgeons in the Gironde described by Williot *et al.* (1997).

Targeted management strategies

Ex-situ measures seem to be the only remaining tool to allow the species to survive, and to conserve remaining genetic heterogeneity. These measures should provide a sensible division of risk to protect available resources. Therefore, such research should be carried out under the joint responsibility of at least two countries working on restoration to allow effective management.

Improvement of rearing techniques aimed at achieving high survival rates and effective growth under *ex-situ* conditions should be stimulated to increase chances for success.

To improve future research and effective utilization of funds, increased international collaboration is considered highly necessary.

In order to improve public awareness, effective utilization of available communication channels to mediate the work to the public should be developed further.

Political targets

To date, international harmonization of activities and adoption of international agreements among the various countries involved are still lacking to protect the species effectively during its marine phase (Rochard, Lepage and Meauze, 1997).

To increase effective protection for the survival of migrants, to allow the long-term aims to be reached and effective re-establishment to be successfully carried out, the initiative of the German government to list the diadromous sturgeon species under the Bonn Convention should be supported.

It is strongly recommended that the managing authorities adopt viable *A. sturio* populations as one of the main criteria for successful habitat restoration in European rivers.

In general, the inclusion of all anadromous fish species into the protection management of river basins should lead to a significant increase in effectiveness when approaching the restoration of habitat or ecosystems.

The development of an international pressure group to affect and influence political decisions is considered vital for the fate of this species in the future (Pustelnik and Guerri, 2000).

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Sturgeon spawning grounds in the Odra River tributaries: A first assessment

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ABSTRACT

As part of a feasibility study for the re-establishment of *Acipenser sturio* L., 1758 in its previous range in German and Polish waters, a habitat assessment was initiated for the Odra River watershed. Spawning habitat for sturgeons is considered to be of major importance for the successful restoration and subsequent reproduction of the species, since it supports the most vulnerable life stages. The ongoing restoration project comprises three distinct phases; the first phase included the evaluation of readily available habitat. Habitat requirements were identified based on published information on sturgeon reproduction, historical catch data, and early life history. Potential spawning habitats were determined in a first step based on historic records. For the identified historic spawning sites, recent data on migration obstacles and water pollution were evaluated, thus excluding non-accessible or adversely affected sites. In the Drawa River –described as a sturgeon spawning habitat until 1939– potentially suitable habitats were determined to be readily available. Data were gathered on the dynamic of the discharge, water quality, longitudinal profiles and cross-sections of the river, as well as substrate composition. Five river stretches comprising approximately 15 000 m^2 were identified as being potentially suitable for sturgeon spawning. Assuming an average fertility of 1 million eggs per female and a maximum density of 3 500 eggs/m^2, the spawning-site surface required for an average female would comprise approximately 350 m^2. Thus, the Drawa River could provide a spawning habitat for approx. 50 females.

Key words: *Acipenser sturio*, Germany, Poland, North Sea, reproduction.

RESUMEN

Lugares de freza del esturión en los afluentes del río Oder: una valoración inicial

Como parte de un estudio de viabilidad para el restablecimiento de Acipenser sturio *L., 1758 en su distribución original en aguas alemanas y polacas, se inició una valoración del hábitat en la cuenca del río Oder. Se considera que el hábitat de freza para los esturiones es de mayor importancia para el éxito de la recuperación y subsecuente reproducción de las especies, ya que mantiene los estados vitales más vulnerables. El proyecto de recuperación en marcha comprende tres fases diferentes; la primera incluyó la evaluación de hábitat fácilmente disponible. Los requerimientos de hábitat fueron identificados a partir de información publicada sobre la reproducción del esturión, datos históricos de capturas, e historia natural temprana. Los hábitats potenciales de freza fueron determinados, en una primera fase, a partir de citas históricas. Para los lugares de freza históricos identificados, los datos recientes sobre obstáculos a la migración y contaminación del agua fueron evaluados, excluyendo los lugares no accesibles o adversamente afectados. En el río Drawa –descrito como un hábitat de freza para el esturión hasta 1939– los hábitats potencialmente apropiados fueron determinados para ser fácilmente disponibles. Se reunieron datos sobre dinámica de la descarga, calidad del agua, perfiles longitudinales y secciones transversales del río, así como de la composición del sustrato. Cinco* *secciones fluviales, comprendiendo aproximadamente 15 000 m², fueron identificadas como potencialmente apropiadas para la freza del esturión. Asumiendo una fecundidad media de un millón de huevos por hembra y una densidad máxima de 3 500 huevos/m², la superficie de lugar de freza requerida para una hembra media comprendería aproximadamente 350 m². Así, el río Drawa podría proporcionar un hábitat de freza para aproximadamente 50 hembras.*

Palabras clave: Acipenser sturio*, Alemania, Polonia, Mar del Norte, reproducción.*

INTRODUCTION

Historically, the European sturgeon *Acipenser sturio* L., 1758 inhabited all major Central European rivers, including the Njemunas, Vistula, Odra, Elbe, Eider, Weser, Ems and Rhine (Holčík *et al.*, 1989). Since the beginning of the 20th century, sturgeons have become rare due to habitat alterations and overexploitation (Gessner, 2000). Today the sturgeon is considered extirpated in the North Sea tributaries and extinct in the confluences to the Baltic Sea (Bless, Lelek and Waterstraat, 1994).

Water quality has improved recently in the Weser, Elbe and Odra Rivers. This has been a consequence of changes in industrial production and the increased efficiency of wastewater purification due to German unification and subsequent developments (Anon., 1998), –a major prerequisite for the return of diadromous fish species to the rivers (Sych *et al.*, 1996). In any case, historical reasons for the decline of *A. sturio*, *Salmo salar* L., 1758, *Alosa alosa* (L., 1758), *Petromyzon marinus* L., 1758 and others must be understood in detail in order to assess the requirements for future remediation measures (Kirschbaum and Gessner, 2000).

The Odra River was selected for the present work because its lower section is largely unaltered, thus providing flood plains, backwaters and side-arms. Large areas of the river are still freely accessible. The decreasing pollution following the effectuation of international agreements (HELCOM) (Anon., 1993) provides a sound basis for the future development of rheophilic fauna. Spawning runs of *Salmo trutta* L., 1758, *Vimba vimba* (L., 1758) and *Coregonus lavaretus* (L., 1758) are still observed in the river and *S. salar* was successfully re-established in the Odra River tributaries during the early 1990s (Bartel, 1997). Furthermore, the available infrastructure for joint research and planning in the Baltic Sea makes it possible to closely monitor tagged fish after stocking.

The Odra River system has a catchment area of 120 000 km², to which Poland contributes 89 %, the Czech Republic 6 % and Germany 5 % (figure 1). The river is 865 km long with an average slope of 0.07 ‰. The runoff during the year varies between 250-1 500 m³/s, with an annual mean of 17 km³ for the lower Odra River. The main tributary is the Warta River, which contributes 40 % of the runoff.

The main alterations to the Odra River system can be summarised as follows. In 1740, major dyking and regulation activities were begun to develop arable land. Between 1740 and 1850, further regulations to increase navigability and improve drainage of the pasture lands led to the transfer of the main stem to the eastern river arm. The river constructions resulted in a maximal reduction in length of up to 60 % in some mid-river sections (Meier, 1992). For the sturgeon, the historic spawning grounds in the upper reaches of the river near Raciborcz were blocked by dams and weirs since the early 18th century. In addition, these hydroconstructions reduced the accessible habitat for juveniles in the middle part of the river. The altered habitat structure resulted in drastic changes to the fish population's composition (Albrecht, 1964; Wolter and Vilcinskas, 1998). Since the middle of the 19th century, increasing loads of communal wastes and effluents from primary and secondary industries polluted the riverine ecosystems (Schiemenz, 1905). The last recorded *A. sturio* from the Odra tributaries was caught in 1952 in the Warta River (Przybyl, 1976).

In Germany, a programme for the re-establishment of *A. sturio* was begun in 1996. This remediation programme is divided into three phases. The first phase included the identification of readily available habitats for reproduction and early life stages in the Odra River tributaries. During the subsequent phase of the project, the actual status of historical spawning grounds are to be described and an assessment of necessary improvements will

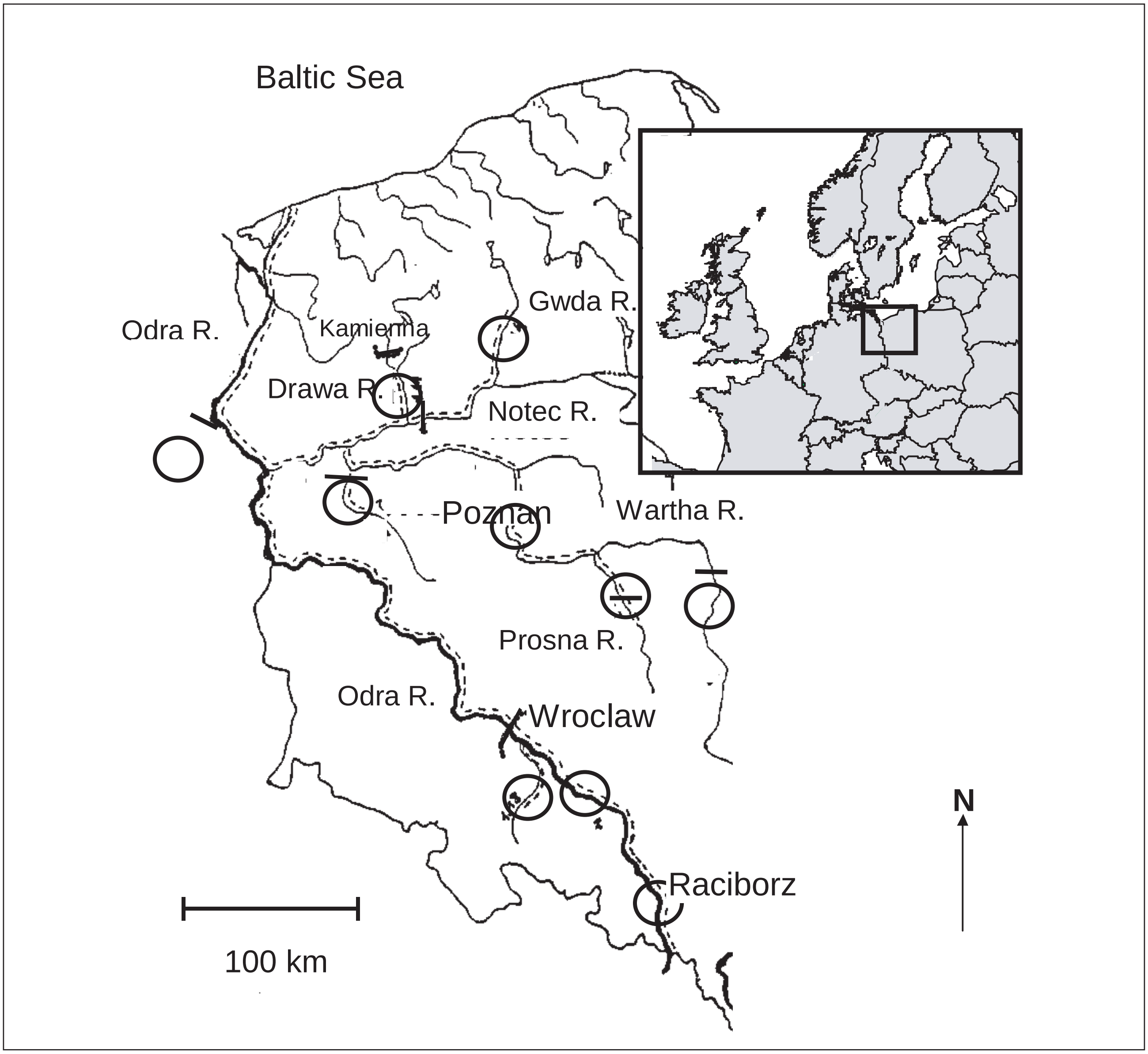

Figure 1. Odra River and tributaries, its localization, and the historic distribution of *A. sturio* (after Sych *et al.,* 1996); migration routes are indicated by dotted lines, migration obstacles are given by lines across the river, spawning sites are indicated by circles

be carried out. The third phase will deal with the experimental release and follow-up of *A. sturio* to determine habitat utilization and potential threats.

MATERIALS AND METHODS

A list of criteria for spawning habitats for *A. sturio* was compiled, based on published information (table I). This was used as a basis for a decision tree, to compare the suitability of potential sites with the given criteria (figure 2).

Identification of potential spawning grounds was based on data on the historic spawning grounds in the Odra River system from Wittmarck (1875), Grabda (1968), Przybyl (1976) and Sych *et al.* (1996). Migration obstacles and structural alterations were determined using maps. In cases where the degree of alterations were unclear, direct observation was carried out.

An impact assessment for habitat alterations and their potential effects upon the reproductive efficiency of *A. sturio* was performed, considering the degree of the alterations.

Table I. Criteria for spawning habitat of *A. sturio*, compiled after various authors. (1): determined for *A. oxyrinchus desotoi*; (2): determined for *A. brevirostrum*

Criterion	Quality	Reference
Substrate	Pebble > Ø3 cm or rock Ø10-Ø30 cm	Elie, 1997
Sedimentation	Absent	Sulak and Clugston, 1999(1)
Oxygenation	Interstitial	Richmond and Kynard, 1995(2)
Current velocity	0.4-2.0 m/s	Elie, 1997; Ninua, 1976
Temperature	17-20 °C	Ehrenbaum, 1936
Depth	1-12 m	Vlasenko, 1974
BOD	Low	

Firstly, the absence of migration obstacles (e.g. dams) was chosen as the primary criterion for potentially leading to blocking of migration routes. Modification of hydrology by effluent regulation disrupts the natural runoff in intensity and temporal scale. An alteration of temperature regime is likely under these circumstances.

Secondly, river-bed modification was evaluated. These impacts reach from gravel or sand removal to sealing of natural substrate with increasing structural uniformity. As a consequence, reduction of spawning habitat for rheophilic fish species occurrs, resulting in the alteration of community structure.

Thirdly, ameliorations (e.g. straightening of river) and disconnection of meanders results in the reduction of connected backwaters, the increase of flow velocity and the increase of runoff intensity.

Water quality criteria of particular importance for spawning habitat evaluation were biological oxygen demand (BOD), oxygen contents, sanitary standards, chlorinated carbohydrates, and temperature. Water analysis for the Polish river stretches was performed by the regional water authorities (WIOS 1993-1997) (Anon., 1997). For the German part of the Odra River, data were provided by the Landesumweltamt Brandenburg (Anon., 1998; Prein-Geitner, Sonnenburg and Drewes, 1993).

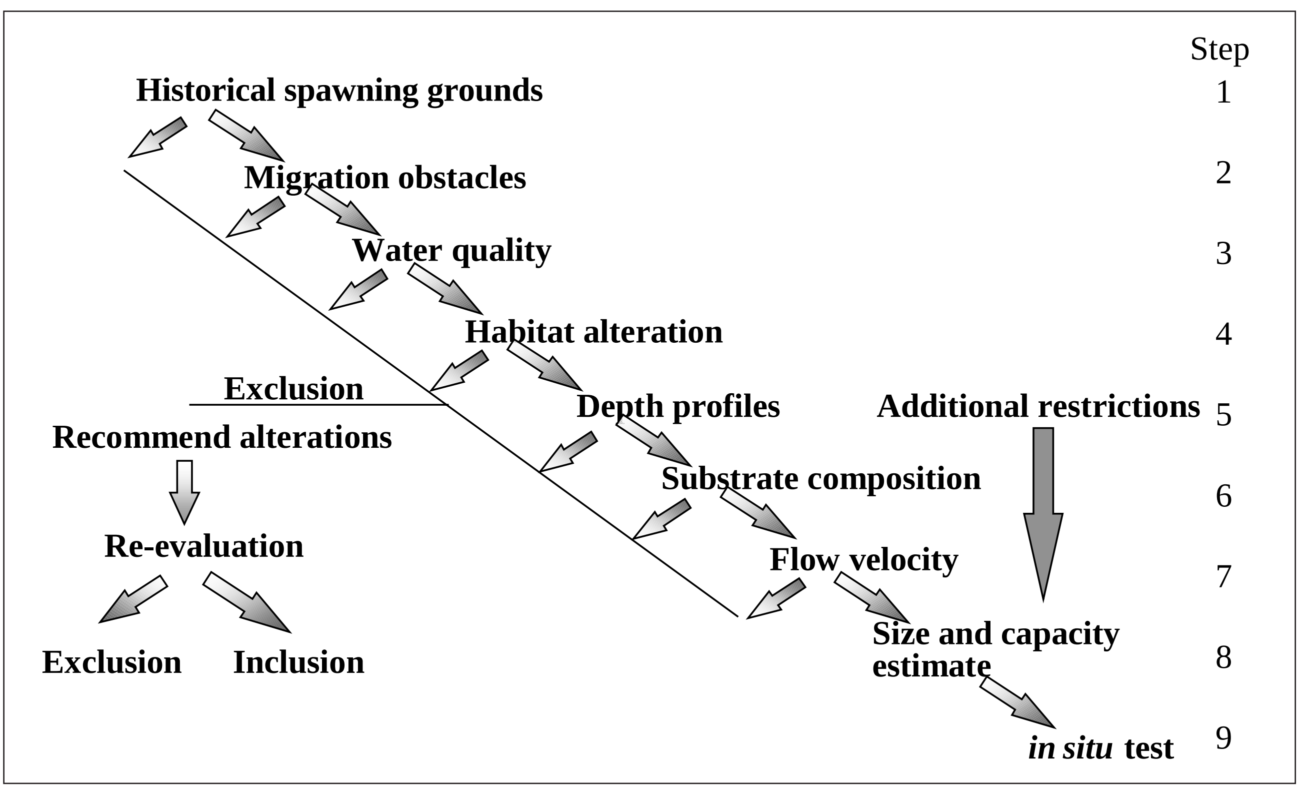

Figure 2. Basic decision tree for the determination of potential spawning grounds for *A. sturio*

The results of the impact assessment for hydro-constructions and their effects on the reproductive efficiency of *A. sturio*, based upon a ranking system using a scale from 0 to 10 for each of the given criteria, can be integrated into a model (after Wild and Kunz, 1992) for structural assessment of lotic waters, including additional indices for relevant aspects of structural properties, thus providing the potential to assess the structural quality of the river stretches in question.

After criteria 1-4 of the decision tree (figure 2) were met, a depth-profile of the river was determined in the river channel by echo-sounding. The profile was recorded during downstream passage on the lower 32 km of the river, below Kamienna dam. The data were collected using a Simrad® echosounder (Model EA300) with a Trimble NT200D GPS. Oxygen, temperature, pH and conductivity were determined every 100 m using Oxyguard® probes to expand data from Poland's Inspectorate for Environmental Protection, known as WIOS. Based on these data, potentially suitable river stretches were selected. Here, transects including flow velocity, depth, temperature, oxygen contents, Secchi depth, and substrate composition were taken. Flow velocity was determined using a Höntzsch ZS25GFE-mn40/60/p3 meter with a 1 m extension grip. Measurements were made at a water depth of 75 cm in order to allow comparison with previous publications (Ninua, 1976).

Substrate composition was determined using an Eckman dredge. However, when this was not applicable sediment was inspected by a diver and/or by sampling with plasticiline attached to a pole, to which < 1 cm particles could adhere, reproducing the image of larger substrate items.

Spawning grounds comprise river habitats with gravel substrates, sufficient water exchange in interstitial water, and absence of sedimentation (Sulak and Clugston, 1999; Elie, 1997; Richmond and Kynard, 1995). Direct observations of the spawning process have not been made for *A. sturio* (Trouvery, Williot and Castelnaud, 1984), although various attempts have been made to identify spawning grounds. They were restricted to the detection of fertilised eggs (Quantz, 1903), localization of potential spawning habitats based on hydrography, the catch of ripe females and early juveniles (Ehrenbaum, 1923) and the catch of ripe (running) females (Blankenburg, 1910), or the catch of predators with sturgeon eggs in their stomachs (Letaconnoux, 1961; cited after Guerri and Pustelnik, 1995). Only Sulak and Clugston (1999) were able to precisely identify and describe the spawning habitat for the closely related *A. oxyrinchus desotoi* Vladykov, 1955. Be that as it may, hydrography and hydrobiology of the river (Suwanee, Florida) limit a general application of the results. However, a distinct preference for certain habitat features (e.g. sedimentation, substrate, flow pattern) became obvious. Data gathered from other sturgeon species indicate that flow patterns as well as substrate quality have a major impact on the acceptance of spawning habitat. Limitations with regard to water depth have been suggested for various species, but the validation of these findings is still lacking.

Carrying capacity can be estimated based on incubation density (Derzhavin, 1947). Assuming an average fertility of 1 million eggs per female and an optimum density in the range of 1 000-3 500 eggs/m^2, the average spawning site for one female is calculated to cover 350 m^2.

RESULTS

Suitability of historic spawning sites

Historic sturgeon distribution in the Odra River has been reported up to Raciborz (figure 1). However, these sites have been inaccessible since the construction of a dam in Wroclaw (Breslau) in the 19th century. In the Warta River migration, obstacles are found only in the upper reaches of the river, as well as in some of its tributaries (Notec, Obra, Prosna).

In order to assess the potential suitability of the present habitat, the historic spawning sites of *A. sturio* in the Warta system, as given in figure 1, were evaluated according to the decision tree (figure 2) using the habitat criteria as outlined in table I.

Alterations to the river stretches of Odra, Warta and Drawa due to hydro-constructions were assessed using the structural index. The results for the Odra River, the Warta and the Drawa Rivers sum up to 61.5 %, 52.3 %, and 21.5 % of the maximal alteration for the given criteria, respectively.

Table II gives the assessment of habitat for sturgeon spawning in the Odra River and its tributaries. Especially, in the Odra River sediment transport can be observed throughout the river-bed in

Table II. Assessment of the present conditions of historic spawning sites for utilization by *A. sturio.* (+): suitable/favourable; (–): unsuitable; (?): status unknown

Criterion	Lower Odra (National-park)	Middle Odra (Frankfurt-Wroclaw)	Wartha (Poznan)	Prosna	Notec	Drawa
Migration obstacle	+	+	+	–	+	+
Substrate	– (small scale)	+	?	?	–	+
Sedimentation	–	–	–	–	–	+
Adjacent feeding habitat	+	+	+	+	+	+
Flow dynamics	+	+	+	+	+	+
Temperature	+	+	+	+	+	+
Depth	+	+	+	+	+	+
BOD	–	–	–	–	–	+
Other adverse criteria	Water quality	Water quality	Water quality	Water quality	Water quality	

the middle and lower part of the river. This sediment transport excludes the main river channel as potential spawning sites for sturgeons. Only very limited areas of suitable substrate could be found in groyne fields and river-bank structures where sedimentation was not detectable.

In the Warta River, at least two historic spawning sites are readily accessible; hydro-constructions for increased navigation capacity have altered the river's structure. As shown in table II, the high nutrient load is the main reason for the negative assessment.

Drawa River

The only tributary which already matches the postulated requirements for a sturgeon spawning habitat today is the Drawa River. In all other tributaries with historic spawning sites, substantial impact from pollution and hydro-construction reduces their suitability.

The Drawa River flows from north to south through Pomerania with its mouth being situated 270 km from Pomeranian Bay, in the southern Baltic Sea. It is accessible via the Odra, Warta, and Notec Rivers. The river has a length of 186 km, and its catchment area comprises 3 295 km^2. The flow rate varies from 13.8-65.9 m^3/s, with an annual mean of 37.9 m^3/s (Anon., 1997). The river is freely accessible for 32 km up to Kamienna dam, built in 1898. The catchment area is dominated by agriculture and forestry.

Historically, sturgeons utilised the spawning grounds in the lower 15 km of the Drawa River until 1939. The river section studied below Kamienna dam appears largely unaltered. It meanders through a glacially formed moraine refuge. Backwaters and side-arms are still present, but are temporarily disconnected. Identified salmon and sea trout spawning habitats comprise 12 400 m^2 throughout a 6 km stretch below Kamienna dam.

In its lower 20 km the river has an average width of 20 m and a mean depth of 2.2 m, increasing towards the river mouth. Depressions in the lower part of the river reach depths below 5 m.

The river below the dam can be divided into three sections. The upper part of the river (river km 32-24) is comparatively shallow, with an average depth of 1.6 m. Here, sandbanks and cobblestone riffles frequently form in the channel. Submerged macrophytes are abundant. The middle river segment (river km 24-17) is a transition area. Mean water depth increases to 1.9 m. The main channel shows sand banks on inner bends and in areas of reduced flow only. Macrophytes are limited to bank structures.

The lower part of the river (river km 17-0) becomes increasingly narrow, especially in the lower 3 km. The mean water depth increases to an average of 2.2 m, with deep pools exceeding 5 m depth. The flow velocity in the main river channel at a depth of 0.75 m varies between 1.1 and 1.4 m/s at mean discharge. The substrate in the main channel is dominated by compacted cobbles and stones (diameter < 30 cm). Gravel and cobble dominate the banks of the river channel. Sandbanks are present

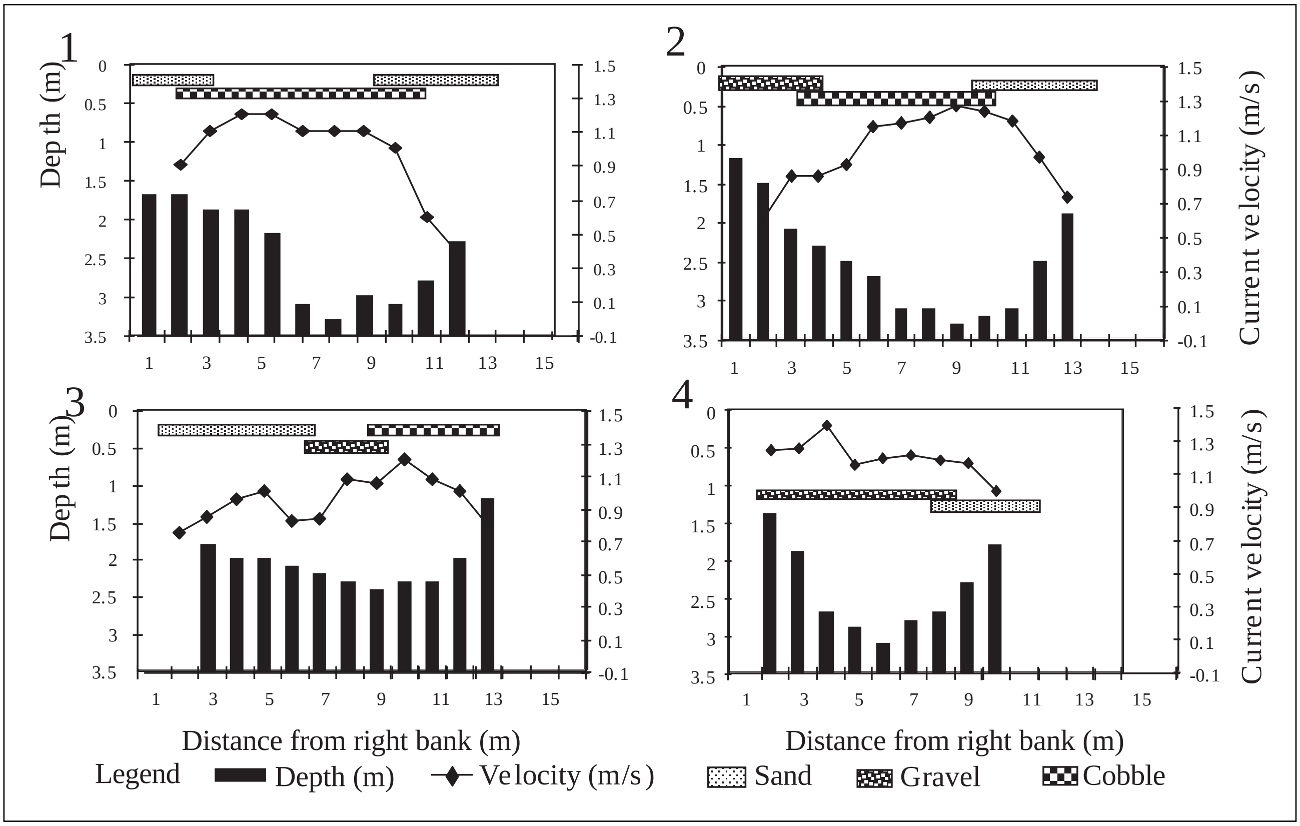

Figure 3. Depth profiles and current velocities of four transects in the lower 15 km of the Drawa River, substrate composition is indicated according to the legend

and sedimentation largely depends upon the flow pattern. Debris is found in the backwaters and in zones of low current. Examples for the transects displaying river morphology are given in figure 3. Here, the pronounced slope with gravel and stone substrate becomes clearly visible.

When comparing the criteria for spawning habitat with the structures in the Drawa River, five areas in the lower 15 km of the river were identified that should be suitable habitats for *A. sturio*.

According to the outlined technique to calculate carrying capacity, the Drawa River substrate would be sufficient for up to 50 females, under the assumption that simultaneous spawning would take place evenly distributed over the available substrate.

DISCUSSION

Outline of suitable habitat

Information about spawning habitats in *A. sturio* is very rare. No observed spawning has been recorded. Data on spawning habitat derive from catches of ripe females, of simultaneous catches of males and females in one spot, or from occasional detection of eggs, either in predators or on site. Additionally, the spawning sites have mainly been recorded under conditions already altered with regard to turbidity, flow regime, hydro-construction and damming.

The substrate requirements outlined by various authors have been summarised by Elie (1997). Bedrock or stones are frequently described as the preferred substrate for *Acipenser oxyrinchus* Mitchill, 1815. For *A. sturio*, *Acipenser stellatus* Pallas, 1771 and *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833, larger gravel to cobble seems to be utilised for depositing the eggs in lowland rivers (Holčík *et al.*, 1989). An adjacent habitat for yolk-sac larvae is essential for the acceptance of spawning habitat. The experimental data on tigmotaxis in larval sturgeons (Richmond and Kynard, 1995; Jatteau, 1998; Gessner, unpublished) indicate that gravel exceeding 1.7 cm in diameter is an effective substrate for the developing yolk-sac larvae.

The role of water depth and current velocity are not yet fully understood. According to Vlasenko

(1974), the role of water depth is mainly a function of turbidity and light exposition. He described sturgeon spawning on suitable substrate in turbid water at depths of 1-5 m compared to clear water, where spawning took place at depths of 2-24 m. Furthermore, the reported depth requirements might be related to the fact that the spawners utilised the depressions or pools as resting areas between subsequent spawns, where they were frequently caught. Additionally, in northern European lowland rivers, suitable gravel/cobble substrate is available only in exposed sites, which are subjected to erosion. In the Oste and Eider Rivers, spawning was described in deep areas on the outer bench of meanders where coarse gravel to rocky structure (of dyke foundations, sunken groynes or bedrock) was exposed which resemble the only areas where suitable substrate, from a hydrological viewpoint, can occur. This combination of high current velocity and coarse substrate leads to even distribution of eggs on the surface during spawning and good oxygenation for the developing embryo, as well as to avoidance of sedimentation on the eggs. The latter criteria were also met on alternative spawning substrates, such as branches and plants, which have been described in the Oste River (Ehrenbaum, 1923; Quantz, 1903).

The morphology of spawning sites is difficult to determine. The identified habitat for *A. oxyrinchus* in the Hudson River consists of irregularly shaped bedrock (Bain *et al.*, 2000). Substrate conditions that match the situation in European lowlands and marshes were experimentally determined below dams in the Kuban and Volga rivers for *A. stellatus* (Khoroshko and Vlasenko, 1970). According to Alyavdina (1953, cited after Khoroshko and Vlasenko, 1970), the slope of the gravel bed should preferably be convex and exposed to the current, with a 20-30 cm thick layer of 7-10 cm cobble, imitating a bank sunken in spring flood.

Apart from the status of maturation when the fish were reaching the spawning sites, the current pattern was determined to limit habitat acceptability. Khoroshko and Vlasenko (1970) described the effect of current pattern in two adjacent artificial spawning sites on the Volga River. The upper, bank-like structure was subjected to eddies along its length. The downstream site was exposed to laminar flow. Although, both sites revealed low spawning activity, which was attributed to the maturational status of the fish. In any case, the site exposed to laminar flow showed higher spawning activity.

The structural index provides an assessment for the alterations to river structure due to hydro-constructions. The results for the Drawa River indicate a deviation of approx. 22 %. From a practical viewpoint, 15 % deviation could indicate an absolute insufficiency for natural reproduction if reached with only one criterion. In this case, the alterations could be interpreted as a warning sign as long as they do not directly affect the spawning habitat. Since it is not known to what extent alterations to river habitat affect natural populations, assessments of the determination of potential habitat are, to some degree, speculative. Perhaps the only realistically feasible way to run a test involving introductions of *A. sturio* to determine the suitability of the habitat would be by close control (e.g. radio-tracking the fish).

Habitat identification

In the Odra River system, only the Drawa River currently meets the requirements for sturgeon spawning habitat. Substrate suitable for spawning can be found in at least 5 sections of the river, comprising approximately 15 000 m^2.

Limitations for the utilization of spawning habitat in the Drawa River might be imposed by the altered flow regime caused by the Kamienna dam. Therefore, it might become necessary to alter the spring runoff to a magnitude providing sufficient discharge to relocate gravel and to increase flow enough to attract the migrants. Modification of dam discharge to match the requirements of the species has to be established before the return of adults into the river.

Future studies

For the years 2000-2004, additional studies are planned to determine and effectively characterise the available habitat. These include video-documentation of the identified habitat, as well as a more precise size estimate for potential spawning grounds. Furthermore, current patterns are to be determined by Acoustic Doppler Profiles. Since ide *Leuciscus idus* (L., 1758) and asp *Aspius aspius* (L., 1758) utilise the same habitat for spawning as sturgeons do (Khoroshko and Vlasenko, 1970), these indicator species will be used to more precisely narrow the potential habitat.

In addition to refining our determination of potentially suitable habitats in the Drawa River, during these future studies historical spawning grounds in the Odra River catchment area, which are currently considered unsuitable, are to be subjected to in-depth analysis over the next four years. Analogous procedures are planned for the Elbe River. Potential habitats are to be identified, and the requirements for necessary alterations will be defined. Attempts to reconnect backwaters and side-arms of the river are in the planning stage for the lower Odra River in the international park along the border (Köhler and Chonjacki, 1996). If performed properly, these measures might be well suited to increase the habitat requirements of early life stages of the sturgeon in the upper reaches of the river, as well. Additionally, they will provide sufficient experience with the management of the reconnected waters to allow transfer of the results to other parts of the river, where comparable measures are planned for flood prevention.

Perspective

The removal of migration obstacles might be one option to alter the adverse situation for diadromous species in some parts of the river systems. The improvement of water quality standards via installation of effective purification plants is considered more urgent. These measures are currently being implemented by the Polish government in order to comply with international agreements to protect the Baltic (Anon., 1993).

A significant concern is the future development of the rivers for shipping, since the environmental alterations needed to accommodate the increasing size of ships will further decrease available spawning habitat for sturgeon. Of particular importance in this respect are increased water depth, steep bank profiles, and building of additional dams in order to provide navigable waters for bigger ships leading to canal-like structures (Hermel, 1995). Thus there is a conflict of interest between commercial and natural users of the river. This largely contrasts with the identified requirements for the connection of backwaters to the river systems in order to increase natural habitat and floodplains. From a conservational point of view, the reduction of navigable waterways would be the desired aim (Köhler and Chonjacki, 1996).

After the preparatory work has been finished and stocking material is available, the subsequent life stages of the sturgeon will find suitable habitat, as can be derived from the findings on exotic sturgeons in the area (Arndt *et al.*, 2000). Improved fisheries management will be required to adapt fishing pressure along the migration routes (Gessner and Nordheim, 1998).

In general, future objectives have to be dealt with including all partners involved (Holland, 1996). International collaboration is essential, not only on a bilateral basis but, considering the migration distances covered as given by Rochard, Lepage and Meauzé (1997), the planned activities must also count on the co-operation of the states adjacent to the Baltic Sea, thus aiming at the most effective and well co-ordinated approach possible to minimise the potential for failures.

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Major problems concerning the conservation and recovery of the Atlantic sturgeon *Acipenser sturio* L., 1758

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ABSTRACT

Analysis of existing data and information show that the present status of the Atlantic sturgeon *Acipenser sturio* L., 1758, is much more complex than that of any other sturgeon worldwide. The name *A. sturio* includes 9-12 geographically, and probably also reproductively, isolated populations with disrupted gene flow. If so, and if there are both morphological and genome differences among them, then this name is used to designate several distinct species. Restoration programmes for the particular geographic area in which the Atlantic sturgeon has been known must be based on the stocking of offspring of the same genome from ancestors coming from the same geographic area. The stocking of specimens from other geographic areas would be similar to introduction of an exotic species *sensu stricto*, resulting in wide-ranging, unexpected and irreversible consequences. Therefore, the ultimate decision on such stocking has to be preceded by careful and scientifically sound analyses of the intended action. Any final solution to this problem needs close international co-operation and financial support from the countries concerned.

Key words: Distribution, morphology, genome, taxonomy, ecology, recovery.

RESUMEN

Problemas importantes relacionados con la protección y recuperación del esturión atlántico* Acipenser sturio *L., 1758

El análisis de los datos e informaciones existentes muestra que el estado actual del esturión atlántico Acipenser sturio *L., 1758 es mucho más complejo que el de cualquier otra especie de esturión en todo el mundo. El nombre* A. sturio *comprende 9-12 poblaciones geográficamente, y probablemente también reproductivamente, aisladas con una corriente interrumpida de genes. Si es realmente así, y entre estas poblaciones existen diferencias tanto morfológicas como genómicas, este nombre denomina entonces varias especies distintas. Los programas de recuperación para un área geográfica particular en la que* A. sturio *ha sido conocido tienen que estar basados en la repoblación con descendientes con idéntico genoma de ancestros procedentes de la misma región geográfica. La repoblación con ejemplares de una región geográfica diferente significaría la introducción de una especie exótica* sensu stricto*, resultando una ampliación de su rango, con consecuencias imprevistas e irreversibles. Por eso, la decisión final sobre tal repoblación tiene que basarse en un análisis cuidadoso y científicamente sólido de la acción proyectada. Cualquier solución final de este problema exige una estrecha colaboración internacional y también el apoyo económico de los países involucrados.*

Palabras clave: *Distribución, morfología, genoma, taxonomía, ecología, recuperación.*

To my friend, former colleague and co-worker, frequent opponent and sometimes also rival and competitor, Dr. Eugene K. Balon on the occasion of his 70th birthday

INTRODUCTION

"Basic systematic data are important for conservation. Without detailed surveys and accurate taxonomy, it is impossible to identify the various species and evaluate their real conservation status, it is impossible to properly manage their fisheries, it is impossible to establish strategies and it is impossible to set priorities. Without accurate names, it is impossible to list a species as endangered or threatened and to take conservation action" (Kottelat, 1998).

The Atlantic sturgeon belongs on the list of critically endangered fish and is included in the Red Data Book of almost all countries where it was known to occur (e.g. Borodin, Bannikov and Sokolov, 1984; Botev and Peshev, 1985; Anon., 1986; Baruš, 1989; Keith, Allardi and Moutou, 1992; Glowaciński, 1992; Witkowski *et al.,* 1999; Karandinos and Paraschi, 1992), and also in the IUCN (Anon., 1996), CITES (Anon., 1992) and the Bern Convention (Anon., 1979). As reported elsewhere (Holčík *et al.*, 1989), the curve of its catches dramatic decline mirrors the decline of the species population density, which started at the beginning of the 20th century. The curve is both very similar in different countries and also almost perfectly synchronous in neighbouring regions. The main reasons for this rapid decline have been known for a long time, i.e. river regulations and the catch of juveniles (Roule, 1925). Attempts to save this species date back to the 1860s, when Kessler (1864) requested the total ban of its fishery in the Volkhov River "to ensure by this way, more or less the existence of the Atlantic sturgeon in the Gulf of Finland" (Kessler, op. cit., p. 212). Then Kulmatycki (1933) and Berg (1935) requested the conservation of this valuable fish, including a total ban on its fishery in Poland and Russia. However, their attempts were not successful in adjacent countries, and even in Poland and Russia the total ban on the fishery of this species was implemented only in 1952 and 1967, respectively (Dyduch-Falniowska, 1992; Ninua and Tsepkin, 1984). Ironically, the most abundant stocks of the Atlantic sturgeon, found in the North and Baltic Seas, were eradicated first, followed by those inhabiting the southern Mediterranean and Adriatic Seas. That it was the river regulations, along with the plundering fishery aimed at gaining its valuable flesh, and then mainly the caviar, is now generally admitted. It may be documented by the example of the Guadalquivir and Gironde Rivers' sturgeon, which were affected by fishery rather late in comparison with the northern stocks. Unfortunately, the catastrophic decline of the northern Atlantic sturgeon was not a sufficient warning, and the previous mistakes have been repeated. In both rivers, sturgeon was a common species, exhibiting migrations reaching far from the river mouth. In comparison with the northern countries, the demand for sturgeon started later. In the Guadalquivir River, its abundance and regular fishery initiated the establishment of a caviar and flesh factory in 1930 (Elvira, Almodóvar and Lobón-Cerviá, 1991b), and in the Gironde River the caviar industry started in 1920 (Williot *et al.*, 1997). Its existence lasted only a relatively short time: 30 and 50 years, respectively. In addition to fisheries, other factors including dam construction, river regulation, gravel and sand extraction, and pollution contributed to the dramatic decline and then the end of the sturgeon fishery.

Recent attempts to save this valuable species by means of restoration programmes (e.g. Elvira, Almodóvar and Lobón-Cerviá, 1991a, b; Debus, 1996; Sych, 1996; Williot *et* al., 1997) will be difficult, and involve some serious problems, as discussed below, and in addition to those mentioned by Birstein, Betts and DeSalle (1998).

WHAT IS *Acipenser sturio*?

This question forms the first, and also the most important, problem which has to be solved. The complex and situation for both Acipenseridae and the Atlantic sturgeon was tellingly characterised by Kottelat (1997, p. 30): "Due to their large size, museum samples of most species of Acipenseridae are rare. As a result, many species descriptions were based on few specimens only and their variability is not very well known… For various reasons (usually unsuitable concepts and methods), this has resulted in only few data usable in systematic analysis. One of the significant problems… is that authors very rarely compare specimens, but only compare data compiled from a variety of sources, whose consistency can always be doubted." In other words, the first problem is to determine which stock or population may be named as Atlantic sturgeon *Acipenser sturio* L., 1758. This task is rather difficult now, as there are only a few museum specimens and the data on the morphology of *A. sturio* found in various sources of literature are similar or identical, suggesting that they were simply copied from author to author (e.g. Brandt and Ratzeburg, 1833 → Heckel and Kner, 1858 → Siebold, 1863 → Kessler, 1864 → Berg, 1916 → Berg, 1948 → Staff, 1950 → Gąsowska, 1962 → Bryliňska, 1986; Marti, 1939 → Svetovidov, 1964 → Pavlov, 1980; Spillmann, 1961 → Tortonese, 1970 → Billard, 1997). Original data introduced by some authors are scarce, and taken from few specimens; therefore proper statistical analysis is impossible (e.g. Günther, 1870; Ivanović, 1973; Almaça, 1988; Oliva, 1995). According to Linnaeus (1758), the type locality for *A. sturio* are European seas ("... *in mari Europaeo*"). We know, however, that there existed at least 9-12 stocks of sturgeon recorded as *A. sturio,* which are geographically, and thus also reproductively, isolated (figure 1). This suggests the possibility of an allopatric speciation, and thus the existence of several species. This statement is supported by the following facts.

- First of all, it is necessary to know the geographical locations of syntypes. The text of Linnaeus is based on specimens from his own 1746 account and on the accounts of Gronovius and Artedi. One needs to know if he gives locality information in his 1746 account, and what Gronovius and Artedi say. The total locality assemblage is the type locality. If it is not possible to reach a conclusion, the syntypes of *sturio* in the British Museum (Natural History) may be used to identify the particular population, based on the tag description of this (these) specimen(s). If successful, one can designate a lectotype. If not, one should find a specimen suitable as neotype and ask the International Commission of the Zoological Nomeclature to designate this specimen as neotype.
- There are significant morphological differences between various stocks of the Atlantic sturgeon. As reported by Holčík *et al.* (1989) and recently also by Debus (1999), the number of dorsal and lateral scutes in the Baltic stock are significantly lower than in the Atlantic Ocean, the Mediterranean Sea and the Black Sea stocks (table I). On the other hand, the number of branchial spines in sturgeon from the Rioni River (Black Sea) and the Gironde River (Atlantic Ocean) are also different. In these two populations, a Student's *t*-test revealed statistically significant differences (table II). Although the values of the CD test are not statistically significant and show a wide overlapping of data, it need not be controversial in this respect. The European Atlantic sturgeon *A. sturio* and the North American Atlantic sturgeon *Acipenser oxyrinchus* Mitchill, 1815 were considered to be conspecific for 150 years, because of their seeming morphological closeness (Magnin, 1963, 1964). Only recently were they confirmed as a separate species by molecular analysis (Wirgin, Stabile and Waldman, 1997). Also, Almaça (1988) admitted the genetic and phenotypic differentiation between the populations ascending the Guadiana (Cadiz Bay) and the Douro (Bay of Biscay) Rivers.
- Fish frequently show clinal variation in their counts, which is both size and temperature dependent. Larger specimens from northern areas display a higher number of their meristic characters (Holčík and Jedlička, 1994). This has also been shown to be true for *Acipenser baerii* Brandt, 1869 (Ruban 1989, 1997, 1998). However, this association cannot be taken as *a priori,* meaning that such a rule applies for all sturgeon species, and has to be demonstrated (experimentally) case by case. Existing data on the Atlantic sturgeon, although very limited, show that the sturgeon stock from the Baltic Sea and the Atlantic Ocean, which are larger in size, have fewer dorsal and lateral scutes and branchial spines than the Black Sea sturgeon. In other words, the large sturgeon from the northern regions have a lower number of scutes and branchial spines than the small sturgeon from the southern regions. This clearly indicates that the clinal variation does not exist between the northern and southern populations of the Atlantic sturgeon, and that the observed data point to a specific differentiation between these populations.
- Data from other migratory and nonmigratory fish species show the homing or site fidelity of spawning populations. This ability was also postulated for *A. oxyrinchus desotoi* according to both genetic analysis and tagging (Wirgin, Stabile

and Waldman, 1997). Although there is some mixing of *A. sturio* stocks among particular populations inhabiting a region with several neighbouring and even extant tributaries (Castelnaud *et al.*, 1991), the sexually mature fishes spawn in their home river. In this respect *A. sturio* seems to differ from *A. oxyrinchus*, in which spatially restricted straying is limited to one region (Stabile *et al.*, 1996). In other words, stock mixing is possible within one region, but reproductive mixing is highly improbable among geographically isolated stocks. Nevertheless, this cannot be an *a priori* rule but has to be demonstrated on a case-by-case basis.

- It is known that Lake Ladoga (Baltic Sea watershed) was inhabited by a resident, non-anadromous population of sturgeon (Berg, 1948; Kozhin, 1964; Poduschka, 1985). In the southern regions similar populations of this species were not reported, despite the existence of at least one analogous situation in Lake Skadar (Adriatic Sea watershed). This lake was occasionally entered by sturgeon, prior to spawning, but the existence of a resident population was not recorded (Poljakov *et al.*, 1958; Ivanović, 1973).

Table I. Mean number of dorsal (SD) and lateral (SL) scutes in the Atlantic sturgeon from different watersheds (data from Magnin, 1963 and Ninua, 1976; according to Holčík *et al.*, 1989)

Watershed	SD	SL
Baltic Sea	9.6	27.7
Atlantic Ocean	12.7	35.1
Mediterranean Sea	13.0	35.1
Black Sea	13.5	32.0

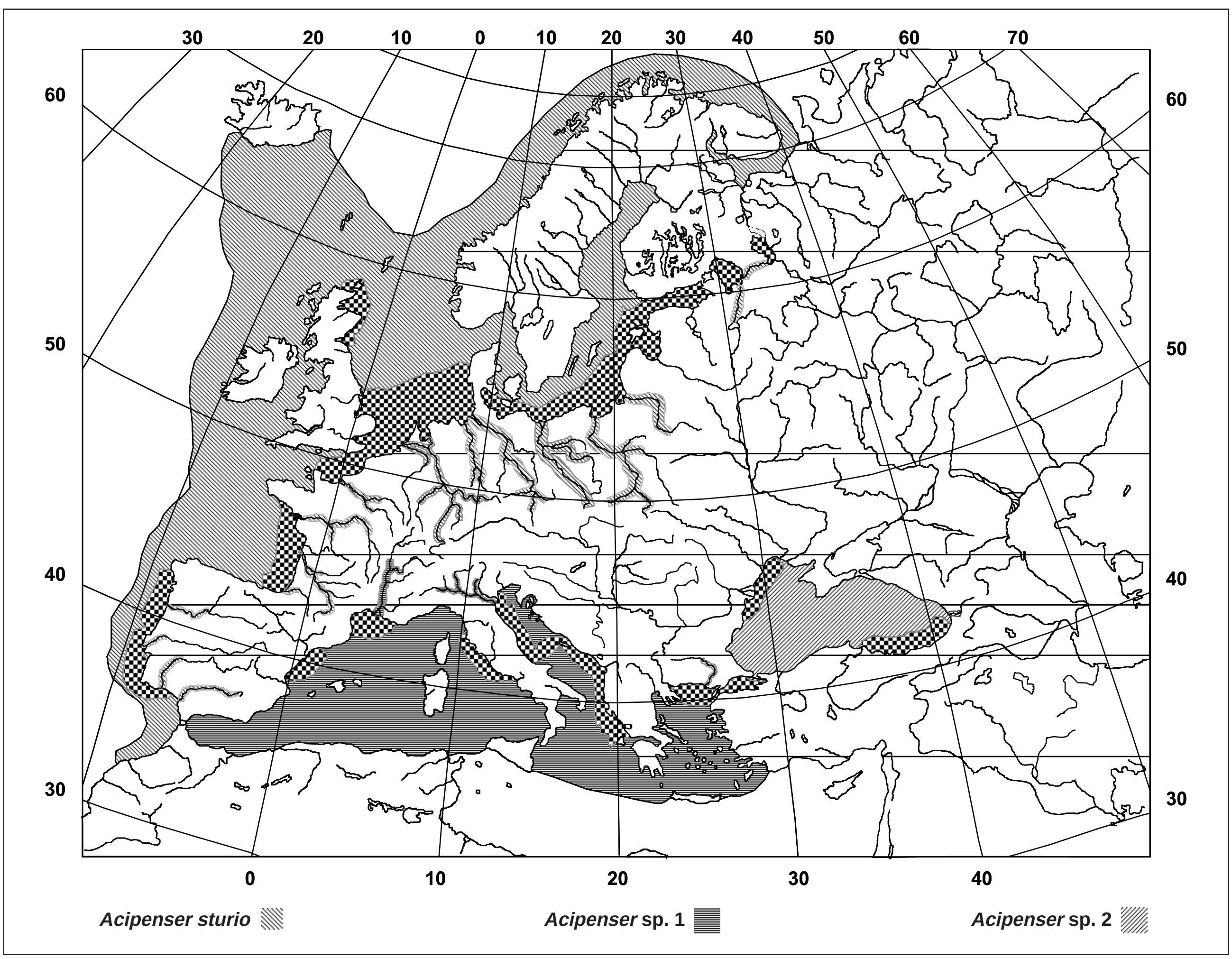

Figure 1. Distribution of *Acipenser sturio* and two supposed undescribed sturgeon species in Europe. Shaded regions denote areas with known local, geographically isolated populations. The draughtboarded drawing indicates the usual distribution of local populations. See text for explanation

Table II. Statistics on two populations of the Atlantic sturgeon. Data for the Rioni River from Ninua (1976) and those for the Gironde River from Magnin (1963). Student's *t*-test and coefficient of difference (CD) tests calculated by Holčík. (Du): number of rays in dorsal fin; (Au): number of rays in anal fin; (SD): number of dorsal scutes; (SL): number of lateral scutes; (SV): number of ventral scutes; (Sp.br.): number of gill rakers. (***): $p < 0.001$

Character	Rioni River (Black Sea)					Gironde River (Atlantic Ocean)					t	CD
	$\bar{x}$	s	$s_{\bar{x}}$	Ranges	n	$\bar{x}$	s	$s_{\bar{x}}$	Ranges	n		
Du	43.00	–	–	39-50	54	–	–	–	–	–	–	–
Au	25.00	–	–	23-28	7	–	–	–	–	–	–	–
SD	13.47	0.64	0.07	11-15	83	12.74	1.24	0.13	9-16	91	4.94***	0.39
SL	31.96	1.37	0.15	28-36	83	35.13	1.91	0.20	31-39	91	12.68***	0.97
SV	10.47	0.55	0.06	9-12	83	11.03	0.95	0.10	9-13	91	4.80***	0.37
Sp.br.	24.87	1.55	0.17	22-29	83	20.19	2.29	0.25	16-26	91	15.48***	1.22

Ironically, the details on the life history of this species of sturgeon are not properly known despite its wide geographical distribution. For instance, it is not known whether this species has spring and winter races, as is known for many sturgeon, especially in the Ponto-Caspian region (Berg 1934). Available data, however, suggest that both a spring and a winter race existed in the Rhine and Vistula Rivers, with the latter entering the river from August to October (Kulmatycki, 1932; Kinzelbach, 1987). The same is probably true for the Tagus and Guadalquivir, where catches of this species are known in late November (Almaça and Elvira, 2000) and in January (Classen, 1944; Holčík *et al.*, 1989). This does not imply that similar races exist(ed) in all populations. Also, the differences in the food quality between the northern and southern populations could affect their specific differences. While the main or preferred food of adult sturgeon from the northern seas are the bottom invertebrates (Mohr, 1952; Magnin, 1963), adult sturgeon from the Black Sea feed predominantly on the pelagic anchovy *Engraulis encrasicolus* (L., 1758) (Marti, 1939; Pavlov *et al.*, 1994). This also points to the different habitats of sturgeon in these regions: while the northern fish are of a benthic form, those from the Black Sea seem to be pelagic.

Generally, the morphological and ecological differences between the Atlantic sturgeon populations are more significant than the differences between *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 and *Acipenser persicus* Borodin, 1897, formerly considered conspecific (Vlasenko, Pavlov and Vasil'ev, 1989).

All of the above data strongly suggest that several species have been confused under the name *A. sturio*. Assuming larger geographical units, there are at least two and perhaps three closely related species isolated both geographically and reproductively (figure 1). The name *A. sturio* could be used for populations occurring in northern seas, and the *terra typica* of this fish could be the Baltic Sea or the North Sea (see note above). The distribution of *A. sturio sensu stricto* probably includes the entire Atlantic coast of Europe. The second presumptive species, for which no formal name is presently available, probably comprises populations from the Mediterranean, Adriatic and Aegean Seas. Eastern populations, especially those inhabiting the eastern part of the Black Sea, probably represent another species which also awaits formal description.

This hypothesis is now supported by the discovery of significant morphological differences between the North Sea sturgeon and the Iberian and Adriatic populations (Elvira and Almodóvar, 2000) and by Groeger and Debus (1999), who found significant differences in some morphometric characters between the sturgeon populations from the Baltic Sea and Atlantic Ocean. The generated polymerase chain reaction (PCR) data from sturgeon caught in the North Sea and the Bay of Biscay (Ludwig and Kirschbaum, 1998), sturgeon taken from the Gironde River and in the North and Baltic Seas (Birstein, Betts and DeSalle, 1998), and also sturgeon from the Baltic and Mediterranean Seas (Birstein and Doukakis, 1999) provide further support for this hypothesis.

The need for a precise revision of *A. sturio* also stems from the controversial paper by Artyukhin and Vecsei (1999). Although the specific distinction of *A. sturio* and *A. oxyrinchus* is now generally accepted (Birstein and Bemis, 1997; Birstein, Bemis and Waldman, 1997; Birstein, Hammer and

DeSalle, 1997), Artyukhin and Vecsei (1999) claim that both species are conspecific, and there are four subspecies within what they call the *A. sturio* complex. Unfortunately, their data lack depth and their discussion lacks critical statistical analysis. These authors also ignore not only the genetic data, but also the existing information on the morphology of non-Baltic populations of *A. sturio.* Moreover, their paper suffers from the crude absence of a clear concept of species and the gross ignorance of basic systematic principles and of the International Code of Zoological Nomenclature. Their "*Acipenser sturio occidentalis* subspecies nova" is therefore a *nomen nudum.* This paper illustrates the need to clarify the systematic status of populations occurring in particular regions, through a critical and competent systematic and taxonomic analysis, with clear principles and species concept (Kottelat, 1997). However, achieving this goal may be almost impossible, as many sturgeon populations are already extinct.

IS IT POSSIBLE TO USE FOREIGN POPULATIONS FOR THE RESTORATION OF THE EXTINCT ONES?

This question is the logical result of the conclusions from the previous section. If a species becomes extinct in some river or basin, it should be restored (the term reintroduction frequently used in conservation –e.g. Birstein, Bemis and Waldman, 1997– is correct only in cases where the first introduction of some species to its original home territory failed; the correct term for the translocation of a species to its native territory, in which it ceased to exist in the past, is restoration or restitution) by the introduction of the same species. However, it has to be proved that the foreign population belongs to the same species. As discussed in the preceding section, the similarities between sturgeon from different geographical areas are not clear. If the Baltic Sea watershed were stocked and then inhabited by sturgeon from the Black Sea, as suggested by the Polish recovery programme (Skóra, 1996; Sych, 1996) there would be a risk of unexpected results from such an introduction. In this case, the Black Sea sturgeon would be an exotic species in the Baltic Sea watershed. It is well known that the introduction of an exotic species is frequently harmful to native fishes and other taxa, through competition for food and space, predation, hybridization, introduction of exotic pathogens, and habitat and water quality alterations (Arthington, 1991; Holčík, 1991). Some of the effects of such introduction are irreversible, and may also have genetic consequences (Philipp, 1991). The question also is whether the introduced exotic species or foreign population survives. Moreover, the introduction of a foreign population may not result in the formation of a reproducing population; stocking of brown trout *Salmo trutta* L., 1758 from one river basin to another was not always successful (Arias, Sánchez and Martínez, 1995; Largiadèr and Scholl, 1995) because the foreign fish were not genetically adapted to the new environment. In Slovakia repeated stocking of both the broodfishes and juveniles from the Váh River into the Orava River did not result in the recovery of *Chondrostoma nasus* (L., 1758) (Bastl and Holčík, 1997). The same is true for the critically endangered huchen *Hucho hucho* (L., 1758), which did not establish self-reproducing populations in some rivers in which it was planted (Holčík, 1997). The juvenile huchen originated from a few broodfish collected in one river, which were used in a single hatchery for artificial reproduction.

AQUACULTURE AND HATCHERY IMPLICATIONS

Experiences with the planting of the aquaculture stocks of various salmonid species, including the attempts to restore the lake trout *Salvelinus namaycush* (Walbaum, 1792) in the Great Lakes, revealed that artificially bred stocks have reduced genetic diversity and their capability to reproduce in the new environment is decreased (Doyle *et al.*, 1991; Evans and Willox, 1991). It has also been found that the hatchery strain typically bears little genetic similarity to the wild population, and the genetic differences between hatchery and wild fish often translate into important differences in physiological, behavioural, and other traits related to fitness (Krueger and May, 1991). In the brown trout a very low number of stocked individuals have been observed, despite the long period of repopulation, and the hatchery-adapted genotypes may not survive or grow well when released into the wild (Arias, Sánchez and Martínez, 1995).

As is well known, the stocking of hatchery-reared juveniles has been used to maintain populations of the most important (from the commercial point of view) species of sturgeon, such as *Huso huso* (L., 1758), *A. gueldenstaedtii* and *Acipenser stellatus* Pallas, 1771 in the former Soviet Union. However, the initial success of the stocking has been replaced by a declining trend. In spite of a substantial increase in the number of the hatchery-produced juveniles during the last 30 years, the stocking of these three species did not affect either the catch or the number of spawners (Khodorevskaya *et al.*, 1997). Dam construction, the decreasing level of the Caspian Sea, poaching, and increasing water pollution are all blamed for this failure. It is likely, however, that the loss of genetic diversity within the decreasing number of spawners used for the artificial propagation is also a potential reason for this failure. The same can possibly explain the collapse of the rearing experiments with *A. sturio* in France (Williot *et al.*, 1997). One must agree, therefore, with Lelek (1996, p. 340) "that the stocking itself brings about no solution to repopulation of vanished species" and with Birstein, Bemis and Waldman (1997, p. 429) that "We must make the case throughout the world that even the very best stocking programs can only provide short-term solutions unless they are coupled to plans for protecting and increasing the levels of natural reproduction." In other words, it is vital to conserve the natural environment and, if disrupted, to help it to recover natural conditions. Shagaeva *et al.* (1993) show that in cases where natural reproduction is facilitated, poor environmental conditions can significantly reduce the survival of developing embryos and juveniles.

Birstein, Betts and DeSalle (1998, p. 97) offer the following warning: "A careful genetic evaluation of each captured putative *A. sturio* individual which potentially can be used for breeding in restoration projects is recommended"; i.e. each action ignoring these recommendations and warnings may result in quite the opposite effect. Instead of the conservation and rescue of the populations and/or species under consideration, their extinction may be sealed.

CONCLUSIONS

The situation of the European Atlantic sturgeon is much more complicated than in other species of sturgeons. It can be summarised as follows.

- The scientific name *Acipenser sturio* L., 1758 covers 9-12 geographically and apparently also reproductively isolated populations. If this is confirmed, then this name is currently used for several distinct species. The situation can only be clarified through critical research.
- The restoration programmes for particular geographic areas in which the Atlantic sturgeon has been known to thrive in the past must be based on the stocking of offspring of the same genetic make-up as the previous inhabitants.
- The stocking of specimens from other geographic areas would be comparable to introduction of an exotic species bringing with it wide-ranging, unexpected and irreversible consequences. This should absolutely be avoided, unless preceded by the careful and scientifically sound analyses of the intended action. Considering that the stocked species will potentially disperse into the waters of other countries, international co-operation is needed to avoid imperiling conservation or restoration efforts elsewhere.
- The final solution requires close international co-operation, with financial support from the countries involved.

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Re-establishment programme for *Acipenser sturio* L., 1758: The German approach

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ABSTRACT

Acipenser sturio L., 1758 was a prevalent species of the fish communities in all major German rivers' waters until the end of the 19th century. Since then, the population sizes decreased rapidly due to overfishing, pollution, and hydro-construction, and the last remaining population was observed in the Eider River until the 1960s. Recently, specimens have been observed in German waters only rarely. Until the end of the 1980s, neither politicians nor ecologists considered re-establishment measures feasible in Germany. In 1994, joint activities of scientists and aquaculturists led to the foundation of the Society to Save the Sturgeon (*A. sturio* L.), aimed at co-ordinating the *A. sturio* restoration measures in Germany. A federal programme was initiated, supporting in part the various subprojects dealing with this matter. Under a scientific co-operation agreement with France's Cemagref, the Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin received, in May 1996, juvenile *A. sturio* for the establishment of a broodstock, as an *ex-situ* measure to save the species. The IGB also carries out research on various aspects of *A. sturio* biology. Together with the Cemagref, genetic analyses of remaining populations and specimens are conducted as a basis for a long-term breeding programme. The broodstock development is carried out in co-operation with the Research Institute for Agriculture and Fisheries of Mecklenburg-Vorpommern, where experiments with model species are performed within the framework of the programme. Evaluation measures for the restoration of *A. sturio* in the Odra and Elbe Rivers are carried out by the Society in collaboration with the Institute for Inland Fisheries of Poland. Additionally morphometric and meristic studies are conducted at the University of Rostock to characterise the species. The evaluation of the remaining populations in other European regions, in particular Romania and Georgia, is considered an important element for its future development. International co-operation in general is an essential aspect of future attempts to save the species.

Key words: Restoration, decline, broodstock, habitat, water management.

RESUMEN

Programa de recuperación para* Acipenser sturio *L., 1758: la propuesta alemana

Acipenser sturio *L., 1758 fue una especie frecuente en las comunidades de peces en las aguas de todos los grandes ríos alemanes hasta finales del siglo* XIX*. Desde entonces, los tamaños de las poblaciones disminuyeron rápidamente debido a sobrepesca, contaminación y obras hidráulicas, y la última población superviviente fue observada en el río Eider hasta la década de los sesenta. Recientemente, sólo raramente han sido observados ejemplares en aguas alemanas. Hasta finales de los ochenta, ni políticos ni ecologistas consideraron posibles las medidas de recuperación en Alemania. En 1994 las actividades conjuntas de científicos y acuicultores llevaron a la fundación de la Sociedad para Salvar el Esturión (*A. sturio *L.), aspirante a coordinar las medidas de recuperación de* A. sturio *en ese país. Se inició un programa federal, financiando en parte distintos subproyectos. Bajo un acuerdo científico de cooperación con Cemagref de Francia, el Instituto de Ecología Dulceacuícola y Pescas Interiores (IGB) de Berlín recibió, en mayo de 1996, juveniles de* A. sturio *para el establecimiento de un stock de cría, como una medida* ex-situ *para salvar a la especie. El IGB también investiga sobre varios aspectos de la biología de* A. sturio*; colabora con Cemagref en la realización de análisis genéticos de las poblaciones y de ejemplares remanentes como base para un programa de cría a largo plazo. El desarrollo del stock de cría se lleva a cabo en cooperación con el Instituto de Investigación para Agricultura y Pesca de Mecklenburg-Vorpommern, donde se realizan estudios experimentales con especies modelo dentro del marco del programa. Las medidas de evaluación para la recuperación de* A. sturio *en los ríos Oder y Elba se establecen por la Sociedad para Salvar el Esturión en colaboración con el Instituto de Pescas Interiores de Polonia. Adicionalmente se llevan a cabo estudios morfométricos y merísticos en la Universidad de Rostock para caracterizar a la especie. La evaluación de las poblaciones remanentes en otras regiones europeas, particularmente en Rumania y Georgia, se considera un elemento importante para su futuro desarrollo. La cooperación internacional en general es un aspecto esencial de los intentos futuros para salvar a la especie.*

Palabras clave: *Recuperación, declive, stock de cría, hábitat, gestión del agua.*

INTRODUCTION

Sturgeons and paddlefish exhibit unusual combinations of size, behaviour, habits, and life history characteristics, which make them highly vulnerable to impacts from human activities: fisheries, hydroconstruction, and habitat destruction and degradation (Rochard, Castelnaud and Lepage, 1990; Beamesderfer and Farr, 1997; Boreman 1997). It is therefore not surprising that most of the 27 sturgeon species are currently endangered (Birstein, Bernis and Waldman, 1997). However, the combination of the various anthropogenic impacts is different for each species, and also particular to each river system. The historical distribution and the present situation of the stocks of the Atlantic sturgeon *Acipenser sturio* L., 1758 are a good example.

A. sturio was originally found from the Black Sea via the Mediterranean and the eastern North Atlantic to the North, Baltic and White Seas (Holčík *et al.*, 1989). During the 19th and particularly the 20th century, the stocks decreased drastically (the history of this decline in the European countries is described in detail in several papers in this volume), and today *A. sturio* occurs with certainty in fresh waters only in the Gironde-Garonne-Dordogne basin in France (Rochard, Castelnaud and Lepage, 1990; Lepage and Rochard, 1995; Williot *et al.*, 1997).

The decline of the species in Germany will be described in more detail: historically, *A. sturio* played a major role in fisheries in German waters (Benecke, 1881; Quantz, 1903; Blankenburg, 1910; Seligo, 1931; Ehrenbaum, 1936), especially in the lower parts of these rivers: the Eider, Elbe, Ems, Odra, Pregel, Rhine, Weichsel and Weser. For the Weichsel or Vistula, archaeological remains from the first millennium indicate that up to 70 % of the protein consumed derived from sturgeons (Hoffmann, 1996). In the 18th and 19th centuries sturgeon fisheries were economically important. British trade companies owned the leases in the Gdansk and Pregel area of the Baltic. In Germany (figure 1), the Eider, Elbe and Rhine were especially important for sturgeon

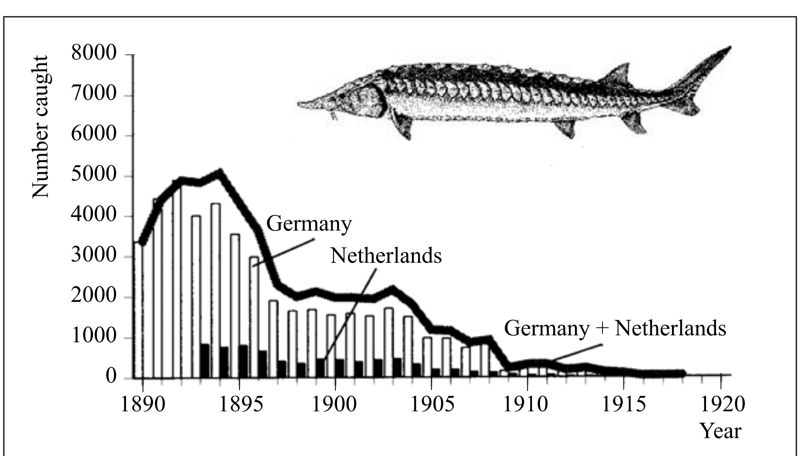

Figure 1. Total catch of *A. sturio* in Germany and Netherlands from 1879 to 1918. (After Lozan and Kausch, 1996 and Holčík *et al.*, 1989; modified)

fisheries (Mohr, 1952), trade (Kinzelbach, 1987, 1997), and caviar production. Until the end of the 19th century, the boom in sturgeon fishing continued, leading to a sharp decline in the catches from the Baltic Sea after 1894 (Debus, 1996). The level of landings was kept stable, despite the decline in riverine sturgeon catches, over 4-6 years by increased utilization of migrants in marine waters. The decline of *A. sturio* in Germany can be attributed to a combination of different impacts (Bonne, 1905; Bauch, 1958; Schirmer, 1994): overfishing, hydro-construction, degradation of water quality, and destruction of spawning habitat are the main factors contributing to the extirpation. The obvious increase in landings in the 1920s is a merely statistical problem. Since Hamburg had lost its sturgeon fleet due to the decrease of catches, the remaining fishing grounds were located in the Eider River. Therefore, sturgeon catches in the Wadden Sea were considered to be Eider River fish if not caught in the mouth of the Elbe River. This increased the proportion of the Eider catches. In any case, the sturgeon fishery continued despite the pressure imposed upon the fish. The management attempts (e.g. Ehrenbaum, 1916) presented in table I were all enforced too late, or were insufficient to effectively avoid the disappearance of the species.

Had fishing pressure been the major impact leading to the recruitment failure in all large German rivers, it should have been obvious in the Eider River, as well. The difference between the Eider and the other rivers is that only small settlements and little industry were located along the river in the 19th century. Apart from damming the tributaries Sorge and Treene in the 17th and 18th centuries and the upper reaches of the Eider in the 1860s, no major hydro-construction took place until 1890 (figure 2). Thereafter, the construction of the Kiel canal had a detrimental impact on the river's habitat, eliminating approximately 35 % of the catchment area from the main river (Fock and Ricklefs, 1996). The zone of tidal influence and sediment transport from the coastal areas into the river drastically increased (figure 3) due to flow reduction. This led to more frequent flooding, resulting in the second measure that finally broke

Table I. Measures taken to increase protection of *A. sturio* in northern Germany

1886		Proposed minimum size limit 1.5-2 m denied by authorities
1886 and 1891		Successful artificial reproduction (Quantz, 1903)
1890		Accepted minimum size limit 1.0 m, closed season July 26 - August 26
1898-1918:	Oste River	Closed spawning refuges comprising 7 km
	Elbe River	Closed season varying annually from July 15-August 26
1904	Elbe River	Closed post-spawning season (from August 1 on); deliberate minimum size limit 1.25 m
1914	Oste River	Closed areas increased comprising 19 river km
	Eider River	Prohibition of baited hooks; minimum size limits 1.25 m
1918	Oste River	Prohibition of eel longlines
1923		Minimum size limit 1.5 m
1924		Minimum size limit 1.2 m
1953	Eider River	Registration of catches for reproduction

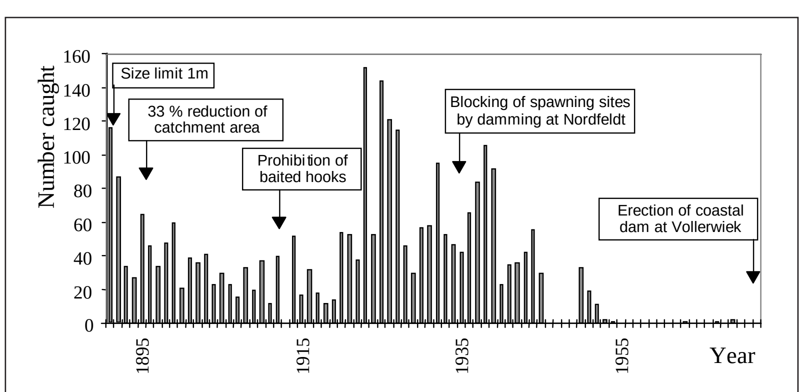

Figure 2. Sturgeon catches in the Eider River, northern Germany, between 1891 and 1973 (arrows indicate important events)

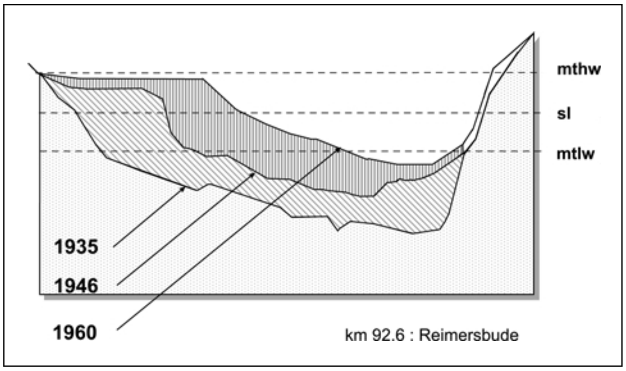

Figure 3. Reduction in cross section of the Eider River between 1935 and 1960 at river km 92.6 (mthw = mean tidal high water, sl = sea level, mtlw = mean tidal low water). (After Fock and Ricklefs, 1996; modified)

the back of the species in the Eider: erection of the dam at Nordfeld in 1934, which blocked the migration route to the spawning sites located upstream (Ehrenbaum, 1923). As a consequence of the recruitment failure, the sturgeon catches declined in the 1950s to incidental catches of single individuals. The idea in 1953 to artificially reproduce the few remaining fish arose too late (Spratte and Hartmann, 1992); no delivery of a ripe male or female was recorded in subsequent years. Additionally, the fishermen were not co-operative with this plan, slaughtering the catch before reporting it to the authorities (Spratte and Hartmann, 1992). In 1969, the last recorded sturgeon was caught from the Eider River (figure 4), just in time before the last migration obstacle, the dam at Vollerwiek in the river mouth, was constructed in 1972 to complete flood prevention.

The case of the Eider River demonstrates that each river system has to be analysed separately, and very carefully, to find the reasons for the decline of the population in question. The concentration of

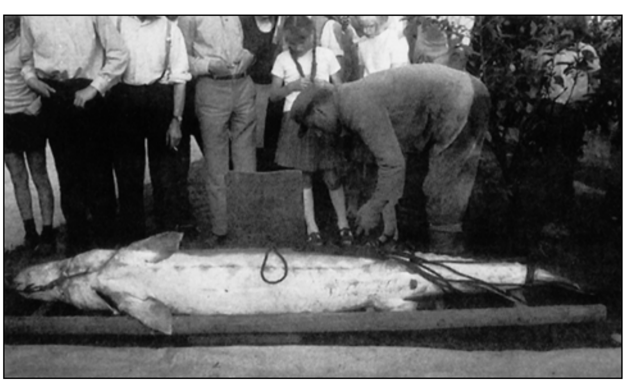

Figure 4. Female *A. sturio*, 2.6 m length and 105 kg weight, caught in the Eider River in 1969. (Photo: S. Spratte)

mature sturgeons in this river at a time when these fish had disappeared from all other German rivers indicates that homing in *A. sturio* might be a phenomenon more important than realised up to now.

HISTORY OF RE-ESTABLISHMENT FOR *A. sturio*

Until the 1990s the idea of conservation of a resource had not finally succeeded. Losing the species due to lack of prevention was widely accepted up to this time, as can be read between the lines of Nellen *et al.* (1994): "A restriction of the catches in the North Sea to protect the species (and to guarantee its survival) cannot be justified."

The international political readiness towards restoration measures and conservation of genetic diversity led to a series of international agreements on the conservation of endangered species (e.g. EU-Directive 92/43/EEC), leading to the protection and restoration of the required environment (Bern, Bonn conventions, RL 92/93/EEC). With increasing environmental consciousness, species that did not imply a commercial concern were integrated into active approaches. Finally, more complex structures were subjected to management measures, as expressed by the EUguideline demanding that management plans for entire water catchment areas of rivers are to be developed.

The dynamics and relationships between different sections of large river habitats have been identified in the past to a minor degree only. At the peak of the environmental impact of pollution, the idea to develop a more integrated understanding of the characteristics of river habitat was formed. River ecology became an increasingly dominant topic only recently (e.g. Baade and Fredrich, 1998; Buijse and Cazemier, 1998; Wolter and Vilcinskas, 1998; Thiel *et al.*, 1998; Neumann and Borcherding, 1998; Staas, 1998; Molls, 1998; Vricse *et al.*, 1998; Bischoff and Wolter, in press; Wolter, 1999).

The opinion concerning conservation measures for *A. sturio* in Germany in the 1990s also changed due to the side effects of German re-unification: the new political situation led to the foundation of the Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin in 1992, providing major resources for studies on large rivers, e.g. the Elbe and Odra, which after re-unification became available for fundamental research and restoration measures (table II). The new ideas concerning restoration of *A. sturio* were promoted by the success of the restoration of small streams (Bless, 1985) and the re-introduction of the salmon in tributaries of the Rhine (Schmidt, 1996) and recently the Elbe. Since the alteration of river management in the Rhine required symbolic parameters for the determination of environmental quality, the diadromous fishes seemed to fulfil the requirements quite precisely. They require accessibility to the rivers for reproduction and migration back into the sea; they need a highly oxygenated riverine habitat for the most vulnerable early life stages; they reach large sizes and are considered valuable and excellent game fish; and they are generally considered the typical victim of anthropogenic changes. During the marine phase, fisheries must play an important role for the survival of the species. All of this made the salmon the umbrella species for the restoration of the Rhine River (Neumann *et al.*, 1998). The reasons for not immediately considering *A. sturio* for restoration at that time, although the species is listed on the agenda of the International Commission for the Protection of the Rhine (IKSR) (Anon., 1987, 1991), were connected with its scarcity, deficits in the knowledge of its biology, and the comparatively longer time of its absence from the river (see above; Nellen *et al.*, 1994) because of its long generation span.

The increased interest in restoration of *A. sturio* in Germany in the early 1990s, the first positive results from the salmon restoration in the Rhine, as well as contacts with scientists working on restoration of the species, e.g. in Spain (Elvira, Almodóvar and Lobón-Cerviá, 1991), but particularly in France (Williot *et al.*, 1997), and the need to work on this subject by co-ordinating the various activities both nationally and internationally, led scientists, fish farmers and fishery administrators to the foundation of the Society to Save the Sturgeon (*A. sturio* L.) e.V. in Rostock in 1994. Subsequently, the Federal Agency for Nature Conservation began to support these activities providing the basic funding for the current programme in Germany.

CURRENT SITUATION OF RE-ESTABLISHMENT MEASURES AND FUTURE ACTIVITIES

The most important specific alternatives identified as potentially beneficial to the conservation of endangered sturgeon species are related to habitats, harvest, research, and culture stocking (Beamesderfer and Farr, 1997). The relative importance of these issues depends on the actual situation of the species in danger. *A. sturio* presently occurs with certainty only in the Gironde-Garonne-Dordogne basin in France, at a very low stock size level of a few thousand specimens exhibiting irregular natural spawnings (Rochard, Castelnaud and Lepage, 1990; Lepage and Rochard, 1995; Williot *et al.*, 1997). Therefore, research concerning broodstock development (Williot *et al.*, 1997; Williot *et al.*, 2000) and various life history stages is underway (Elvira (ed.), 2000) in France. The species has been protected in France since 1982. Habitat protection and restoration in the Gironde-Garonne-Dordogne basin is currently under discussion (Lepage, Rochard and Castelnaud, 2000).

The re-establishment measures in Germany, in order of importance, comprise the following issues: research, habitat protection and recovery, fisheries regulations, public awareness campaigns, and experimental restocking. Most of these activities are being carried out in close co-operation with many European institutions, in particular with Cemagref.

Table II. River systems in Germany potentially suitable for habitat restoration and sturgeon restocking measures

	Odra River	Elbe River	Rhine River
Length	865 km	1 091 km	1 320 km
Catchment area	120 000 km^2	148 268 km^2	185 000 km^2
Bordering countries	Germany, Poland, Czech Republic	Germany, Czech Republic	Switzerland, France, Germany, Netherlands
Research topics concerning *A. sturio*	Evaluation concerning restoration measures (Gessner and Bartel, 2000)		The potential of the lower Rhine River as habitat for reproduction and early life stages of *A. sturio* (Schmidt, pers. comm.)

Research

The establishment of a broodstock is the base of the restoration programme in Germany, as the species has practically disappeared from German waters. This *ex-situ* measure began in 1996, when on the basis of a co-operation programme the IGB in Berlin received 40 juveniles from the Cemagref in Bordeaux, originating from artificial reproduction in 1995 (Williot *et al.*, 2000).

The first experience with this species indicated (Williot *et al.*, 1997) that this fish is difficult to maintain, in contrast to many other sturgeon species. Cemagref and the IGB therefore decided to apply different strategies when raising these fish, in order to gain as much knowledge as possible: in France the fish are fed on different species of shrimps only (Williot *et al.*, 1997), whereas at the IGB a variety of natural food items are and will be tested (Kirschbaum, Gessner and Williot, 2000). In France, fresh, brackish and salt water is used; at the IGB, freshwater only. There is one specimen of *A. sturio* living in salt water in the biological station at Heligoland. This specimen will be used for feeding trials involving live marine invertebrates. The weighing intervals are several months in France, and only two weeks at the IGB.

In the context of early sexual maturation of *A. sturio*, research into the impact of environmental factors on the maturation of gonads using model species are being conducted at the IGB and the Research Institute for Agriculture and Fisheries of Mecklenburg-Vorpommern.

As *A. sturio* historically comprised many geographically distinct populations (Holčík *et al.*, 1989), the genetic characterization of these populations, using both recent and museum material (Ludwig et *al.*, 2000), is another key element of the project. In addition, related species (Ludwig and Kirschbaum, 1998), in particular *Acipenser oxyrinchus* Mitchill, 1815, will be included in the characterization of the species. These data will be important for decisions concerning stocking trials in the different German rivers.

In close co-operation between the Cemagref in Bordeaux and the IGB, studies on the genetic structure of the remaining population in the Gironde and the captive broodstocks are being conducted, aimed at developing a breeding protocol for future reproduction strategies, thus, making it possible to minimize inbreeding and to increase genetic heterogeneity in both the German and French breeding groups.

In addition to the genetic investigations, attempts at morphological characterization of *A. sturio* and related species are underway at the University of Rostock.

Research on the remaining populations outside Germany, for instance in Romania, Albania, Georgia, and Britain, is considered essential to increasing our knowledge about the current status of the species, as well as the collection of material for the European broodstock.

Furthermore, recent data on sturgeon catches (Gessner *et al.*, 1999) and the problem of alien species are being investigated (Arndt *et al.*, 2000) in order to keep track of the main alterations in the environment and to establish a basis for future activities.

At the IGB, fundamental studies, including temperature preference (Staaks, Kirschbaum and Williot, 1999) and acoustic and feeding behaviour (e.g. amino acids as possible attractants), are planned.

Habitats

Studies on habitat requirements of early life stages of model species will be performed in co-operation with several institutions.

Evaluation of potential spawning habitats has been carried out from 1996 to 1998 in the Odra River, as outlined in Gessner and Bartel (2000), indicating a general potential for successful restoration in this river. More detailed characterization of the Odra and Elbe Rivers as potential habitats (see also table II) for early life stages of *A. sturio* are currently underway.

Fisheries regulations

Development of adapted fisheries techniques to reduce probability of bycatch of sturgeons in the coastal waters of the Odra River will be carried out in co-operation with the IGB and the Research Institute for Agriculture and Fisheries of Mecklenburg-Vorpommern.

Public awareness campaigns

Enhanced public awareness of *A. sturio* restoration in Germany will be an important element of the project, aimed at the general public, but especially at fishermen and other users of the resources.

Experimental restocking

Last not least, after the first successful artificial reproduction (hopefully in a few years), future activities will include experimental release and tagging, tracking of fish for habitat analysis, and identification of potential threats.

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Restoration of *Acipenser sturio* L., 1758 in Germany: Growth characteristics of juvenile fish reared under experimental indoor conditions

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ABSTRACT

The survival of the highly endangered *Acipenser sturio* L., 1758 depends nearly exclusively upon the establishment of captive broodstocks. Such measures were initiated in Germany in 1996, due to a transfer of 40 artificially reproduced individuals from France under a co-operation agreement. We report the results of rearing these fish for a period of 3 years under freshwater conditions. During the first year the fish being exclusively fed frozen chironomid larvae (N = 40) grew from a median of 27 cm (with range of 23-31 cm), averaging 71 g (42-112 g), to 43 cm (38-51 cm) and 280 g (168-505 g) at a mean temperature of 20 °C (13-27 °C). Following their transfer to larger tanks (approx. 5 m³) at a mean temperature of 21 °C (17-24 °C), the fish (N = 27) reached a length of 68 cm (56-76 cm), averaging 1 281 g (512-2 097 g) at the end of the second year. Decreasing growth and increasing food conversion rates (FCR) during the first four months of the third year made us change the food composition (addition of large chironomids, krill and small marine fish). An increase in growth and a decrease in FCR was observed over the next four months resulting in a median length of 76 cm (58-89 cm), with a weight of 1 827 g (855-3 462 g) at the end of the third year (N = 27). Our fish showed rather large differences in growth, with some individuals reaching the maximum weight gain observed in wild fish, thus indicating the potential to optimise results by future testing involving additional natural food items in order to develop a formulated diet to stimulate early sexual maturation.

Key words: Growth curve, recovery, broodstock, behaviour, sexual maturation, semiclosed system.

RESUMEN

Recuperación de* Acipenser sturio *L., 1758 en Alemania: características del crecimiento de los peces juveniles mantenidos en condiciones experimentales de interior

La supervivencia del altamente amenazado Acipenser sturio *L., 1758 depende casi exclusivamente del establecimiento de stocks de cría en cautividad. Tales medidas fueron iniciadas en Alemania en 1996, gracias a la transferencia desde Francia bajo un convenio de cooperación de 40 individuos reproducidos artificialmente. Presentamos los resultados de la cría de estos peces por un periodo de tres años en condiciones dulceacuícolas. Durante el primer año los peces (N = 40) siendo alimentados exclusivamente con larvas congeladas de quironómidos crecieron desde una media de 27 cm (con rango de 23-31 cm), promediando 71 g (42-112 g), a 43 cm (38-51) y 280 g (168-505 g) a una temperatura media de 20 ºC (13-27 °C). A continuación de su traslado a tanques más grandes (de aproximadamente 5 m³) a una temperatura media de 21 ºC (17-24 ºC), los peces (N = 27) alcanzaron una longitud de 68 cm (56-76 cm), promediando 1 281 g (521-2 097 g) al final del segundo año. La disminución del crecimiento y el incremento de las tasas de con-*

versión de alimento (TCA) durante los primeros cuatro meses del tercer año nos hicieron cambiar la composición del alimento (adición de quironómidos de gran tamaño, krill y pequeños peces marinos). Un incremento en el crecimiento y una disminución de las TCA fueron observados durante los cuatro meses siguientes resultando una longitud media de 76 cm (58-89 cm), con un peso de 1 827 g (855-3 462 g) al final del tercer año (N = 27). Nuestros peces mostraron numerosas diferencias en crecimiento, con algunos individuos alcanzando la mayor ganancia en peso observada en peces silvestres, indicando así el potencial para optimizar resultados por futuras pruebas que incluyan alimentos naturales adicionales en orden a evaluar una dieta formulada para estimular la maduración sexual temprana.

Palabras clave: *Curva de crecimiento, recuperación, stock de cría, comportamiento, maduración sexual, sistema semicerrado.*

INTRODUCTION

Over the last decade, the protection of biodiversity has become a major subject of public awareness, and is now an aim of European policies (e.g. Agenda 21 - Convention of Biological Diversity, Rio de Janeiro, 1992). This applies in particular to most of the 27 species of sturgeons, all of which are endangered (Birstein, Bemis and Waldman, 1997).

Historically, three different sturgeon species were present in German waters (Lelek, 1987). *Huso huso* (L., 1758) (beluga) and *Acipenser ruthenus* L., 1758 (sterlet) occurred in the Danube River and its tributaries only, whereas *Acipenser sturio* L., 1758 (Atlantic sturgeon) was present in all major German rivers entering the North or Baltic Seas. In addition, this species was found along nearly all European coasts and previously was common in most of the large river systems of Western Europe, from the White Sea to the Black Sea. In the 1994 Red Book of Germany (Bless, Lelek and Waterstraat, 1994), *A. sturio* is considered threatened in the North Sea and extirpated in the Baltic Sea. Throughout Europe the species is considered threatened or extinct (Debus, 1993; Birstein, Bemis and Waldman, 1997).

A. sturio, meanwhile, occurs with certainty only in the Gironde-Garonne-Dordogne basin in France (Rochard, Castelnaud and Lepage, 1990; Lepage and Rochard, 1995; Williot *et al.*, 1997) and perhaps in the Rioni basin in Georgia (Ninua and Tsepkin, 1984). Its occurrence in the Adriatic Sea, especially in the Buna River in Albania, has yet to be proved (Gulyas, pers. comm.). The Gironde population in France has decreased dramatically since the 1970s. *A. sturio*, therefore, is a species of special concern to the European Union, being listed in Annex 2 of Directive 92/43/EEC. The recovery of the known remaining populations by natural reproduction does not seem probable. Therefore, a restoration programme should include artificial reproduction and supportive measures in order to stabilise the stocks. Additionally, habitat improvement and protection should accompany the management of the endangered stocks.

This strategy has been adopted in France by the National Agricultural and Environmental Engineering Research Centre (Cemagref) in the 1980s (Williot *et al.*, 1997). In Georgia, a similar attempt seems to be in preparation (Zarkua, pers. comm.). The first successful reproduction, including rearing of the larvae, was carried out in France in 1995 (Williot *et al.*, 2000). As part of a co-operation programme aimed at restoring *A. sturio* in German waters, 40 juveniles were obtained from the Cemagref in 1996 for behavioural studies (Staaks, Kirschbaum and Williot, 1999), and as a basis of a broodstock to be reared in captivity.

Little is known about the specific conditions for supporting optimal growth to provide a healthy stock in long-term rearing, and to achieve maturation of gonads in this species. On the contrary, the first results in France indicated (Williot *et al.*, 1997) that *A. sturio* is a species rather difficult to grow under controlled conditions, compared with most other sturgeon species, which are quite easy to grow, and can be weaned to dry food successfully (Williot *et al.*, 1988; Giovannini *et al.*, 1991; Charlton and Bergot, 1991; Ronyai *et al.*, 1991; Hung *et al.*, 1993; Jähnichen, Kohlmann and Rennert, 1999). The present paper reports on our first results of rearing *A. sturio* over a period of 3 years under indoor conditions.

MATERIALS AND METHODS

Specimens

The 40 *A. sturio* obtained from Cemagref as part of a cooperation agreement were 11 months old (Williot *et al.*, 2000) on their arrival at the Institute of Freshwater Ecology and Inland Fisheries in Berlin in May 1996. The fish were the smallest of those raised in the Cemagref facility at that time. The average length upon arrival was approx. 20 cm with a mean weight of 41 g. The 40 fish were separated for most of the time into 3 different groups of similar size.

Tanks

The fish were first stocked in three aquaria (1.2 m^2, 0.8 m^3; 0.9 m^2, 0.5 m^3; 0.8 m^2, 0.3 m^3), then transferred to small (1.4 m^2, 1.0 m^3) and later to two large rectangular tanks (6.8 m^2, 6.8 m^3) and one circular tank (3.3 m^2, 4.7 m^3), using recirculated tap water. The tanks were equipped with filters of spongy open-cell foam (10 cm thick), and filter material was cleaned every 3-5 months. Water circulation was mediated by Eheim pumps; their number was adapted to the size of the tanks to obtain effective filter systems (i.e. water content of the tank was recirculated approximately once per hour).

Physico-chemical parameters

Water quality was monitored once daily, including NH_4, NO_2, NO_3, using Aquamerck Test kits. The pH and O_2 levels were measured twice daily using an Oxyguard probe. Alkalinity of the water was tested once a week (Aquamerck). Partial water exchange was performed with chlorine-free tap water when water quality in the tanks was not within the following limits: pH 5.7-8.1 (mean 7.2); O_2 4.9-9.9 mg/l (mean 6.2); NH_4 0.8-0.025 mg/l (mean 0.05); NO_2 0.5-0.0 mg/l; NO_3 500-5 mg/l (mean 200 mg/l). Water temperature was regulated by aquaria heaters for each closed system, to obtain stability within the given limits of ± 2 °C per day at an average temperature of 20 °C (range 13-27 °C) during the first year and 21 °C (range 17-24 °C) thereafter. Photoperiod was regulated according to the continuous change of the natural photoperiod (light-dark) in Berlin (LD 16:8 in summer, 8:16 during winter), since it is well known that light has a large impact on the growth of temperate fishes (Trenkler and Semenkova, 1995; Boeuf and Le Bail, 1999).

Feeding and growth

From May 1996 to August 1998 feeding was undertaken *ad libitum* with small frozen chironomids. Each 100 g package of chironomids contained on average 44 % of water; the remaining wet biomass contained 12 % of dry weight. From August 1998 to January 1999 small frozen chironomids, large chironomids, krill and small marine fish (small elongated tropical fish, purchased from a wholesale dealer supplying aquarium shops) were given. Thereafter, the krill was left out of the feed.

Weight and length were first recorded at about 2-5 month intervals; later, at 4-week intervals, and finally every 2 weeks. Length was taken as total length, within 0.5 cm. Weight was measured using a Mettler scale with a d = 1 g for 1 below, after externally drying the fish with soft paper. Apart from sampling, the fish were handled only when graded and newly stocked. Individual growth was followed from April 1997, when the fish were tagged with a passive integrated transponder (Trovan®) for safe identification of the individuals. Tagging was undertaken under anaesthesia using a bath of 70 ppm MS222. The degree of intoxication was determined by the behavioural response of the fish following the criteria given by Conte *et al.* (1988).

Growth previous to this date was analysed for the three respective groups. The growth performance after tagging was calculated for each individual fish. Feeding rate was calculated as percentage of the body weight (weight of food administered) administered per day (% bw/d). Feed conversion ratio (FCR) was calculated as the ratio of feed administered compared to weight gain.

For the presentation of the growth characteristics, we used median and range because these variables represent more real values than mean (extreme values heavily influence the mean values) and standard deviation.

General management

Apart from handling during the procedure of weighing the fish no particular measures were taken to reduce any kind of disturbance. Our intention was to get the fish used to various kinds of manipulation, e.g. we put sufficient light over the tanks to enable continuous observation of the fish.

Statistical analysis

To locate significant differences ($p < 0.05$) between the growth of our fish and those of a wild population (Lepage, Lambert and Rochard, 1994) we used a *t*-test.

RESULTS

Growth characteristics

During the observation period of about 3 years, the fish grew from a median length of 27 cm to a median length of 76 cm (median of 43 cm after the first and of 68 cm after the second year, respectively). A length frequency distribution (figure 1) and a growth curve (figure 2) show more detailed information about these growth characteristics.

The individual growth values revealed that there was an individual who was, right from the beginning of our individual measurements, the smallest fish (36 cm), and continued to be so (58 cm) to the end of the observation period (20 May 1999). As far as the longest fish is concerned, there were 3 individuals (no. 1906, 81EF and D385) that changed position several times: for the first two weighing times after tagging fish no. 1906 was the longest (51 and 58 cm); then for the next three times it was fish no. 81EF (70, 75 and 77 cm); for nine times, fish no. D385 (80 to 88 cm), and the last time (20 May 1999), fish no. 81EF (89 cm) again.

As far as weight is concerned (figure 3), the fish grew from a median of 71 g, when they were all first

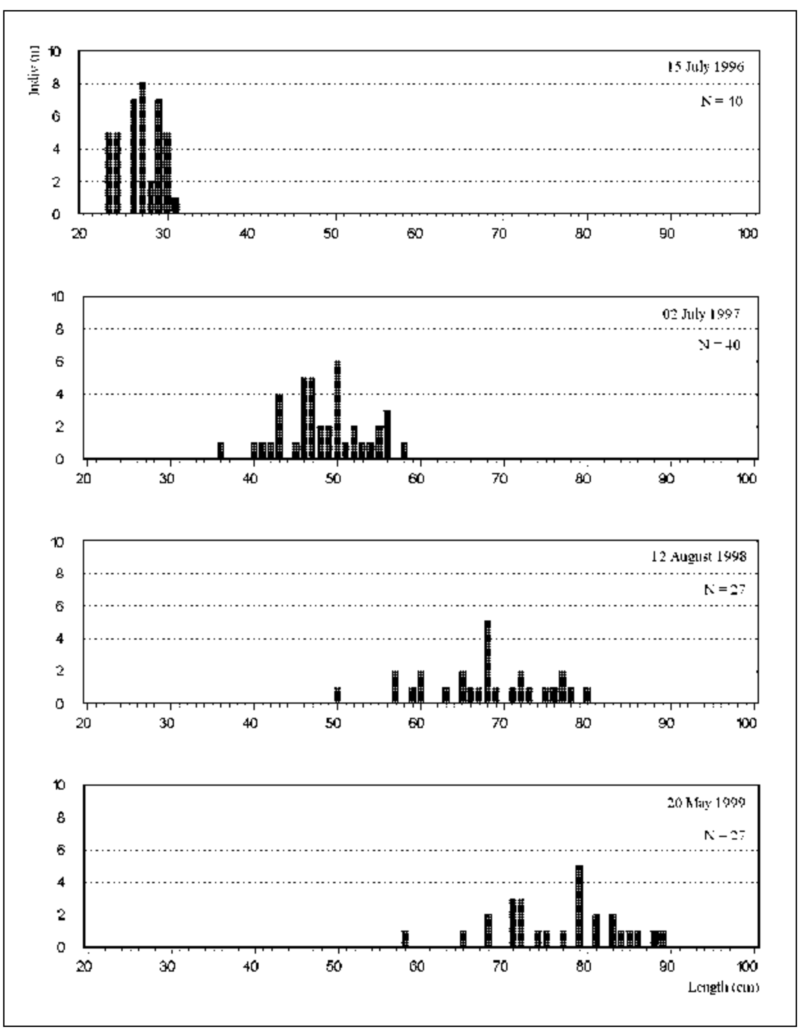

Figure 1. Length-frequency distribution of *A. sturio* representing the observation period of 3 years

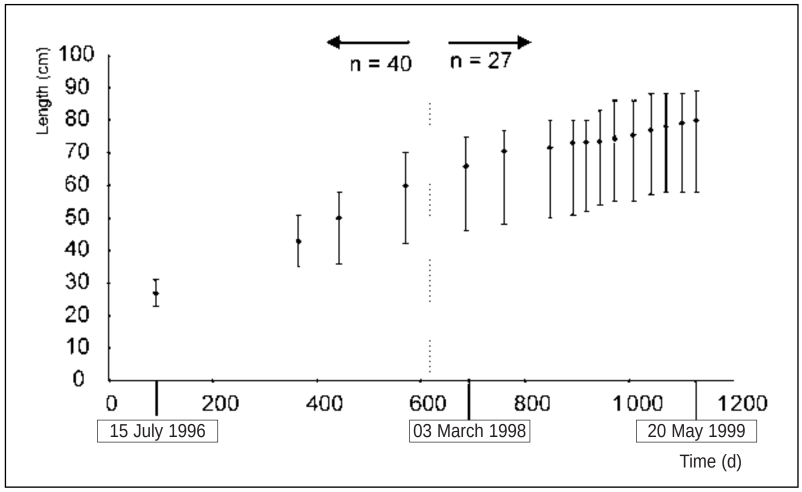

Figure 2. Growth characteristic of *A. sturio* concerning length (median and range) representing the observation period of 3 years. The dotted vertical lines represent the time of transfer of fish from one rearing unit into a larger one

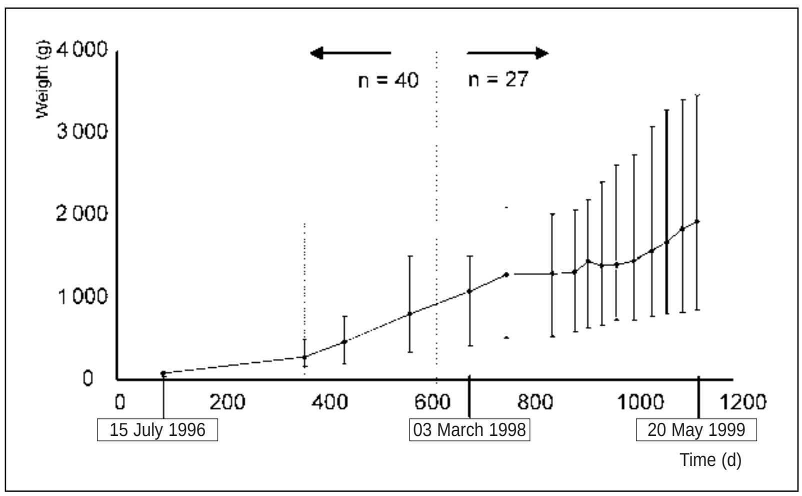

Figure 3. Growth performance of *A. sturio* concerning weight (median and range) representing the observation period of 3 years. The dotted vertical lines represent the time of transfer of fish from one rearing unit into a larger one

measured on 15 July 1996 (13 months old) up to a median of 1 827 g at the end of the third year (median of 280 g after the first and of 1 281 g after the second year, respectively).

The individual growth values concerning weight again showed the shortest fish (fish no. DB80) to be also the fish with the lowest weight (167 g), right from the beginning of the observation period up to the end (855 g). The heaviest fish after tagging was individual no. 1906 for a period of two weighing times (504 and 774 g), but afterwards fish no. 81EF (also the longest fish at the end of the observation period, see above) continuously gained more weight and remained the heaviest fish (1 498 g) from then on, reaching 3 462 g at the end of the observation period.

Figure 4 shows that the feeding rate varied between 2.5 and 4.8 % during the entire observation period. FCR, also shown in figure 4, reveals a drastic increase, from values of about 20 up to 100 at the beginning of the third year, and a decrease thereafter down to the values observed before.

Diseases and mortality

Two observation times (middle of December 1998 and of August 1999) showed the fish to be free of parasite infection. However, several times we observed haemorrhages of the ventral and lateral scales, skin, and ventral part of the pectoral girdle, in particular at the end of the observation period, when the fish, due to the transfer of the rearing tanks from one building to another, were insufficiently fed for several weeks.

No mortality was observed during the observation period due to regular rearing conditions. However, a combination of technical defects and insufficient backup devices caused the loss of 13 fish in January 1998.

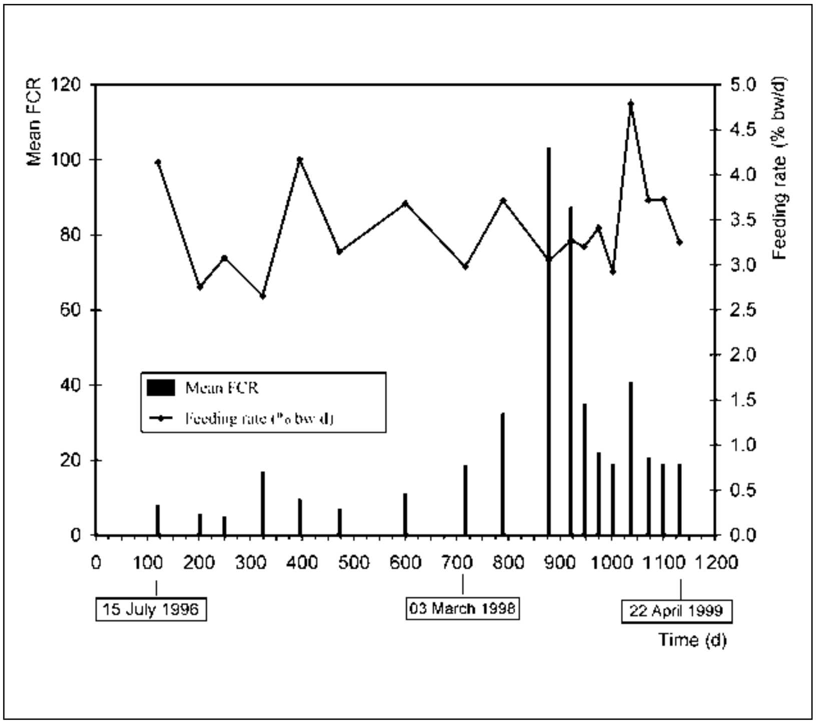

Figure 4. Feeding rate (in % body weight/day) and mean feed conversion ratio (FCR) observed in *A. sturio* during the rearing period of 3 years

Abnormal morphology and behaviour

Two fish showed a missing integumental nose cover (figure 5), and one fish had only a single eye. Two fish showed abnormal swimming movements, apparently due to insufficient buoyancy. One fish (which died accidentally in January 1998, see above) had a distorted backbone, apparently caused by an accidentally broken backbone, which resulted in abnormal swimming movements. One large fish performed regularly circular movements at the water surface. A few fish showed caudal and ventral fins bent at 90° angles (figures 5 and 6).

Figure 5. Example of a specimen of *A. sturio* showing a missing nose cover

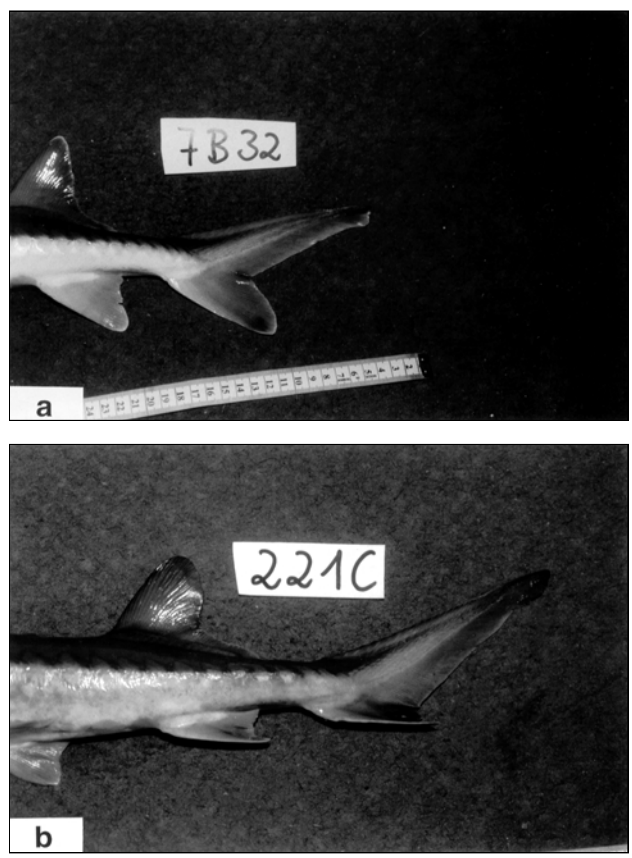

Figure 6. (a): Detail of normally shaped anal and caudal fins in a specimen of *A. sturio*. (b): Specimen of *A. sturio* with anal and ventral part of caudal fin abnormally bent at 90° angle

DISCUSSION AND CONCLUSIONS

Keeping conditions

Sturgeons seem to tolerate quite large variations in physico-chemical factors, and are more tolerant than teleosts (Salin and Williot, 1991), though exact values are scarce. Toxic levels of ammonia in *Acipenser baerii* Brandt, 1869 (Salin and Williot, 1991) are more than a magnitude higher than the maximum ammonia levels we tolerated in our tanks (0.8 mg/l). Intoxication in sturgeons is generally related to special behaviour (Salin and Williot, 1991); we never found any indication of intoxication based on this specific behaviour: our keeping conditions were apparently quite favourable for the fish.

The considerable increase in growth after the first transfer of the fish into larger tanks (figures 2 and 3, around day 360) could be interpreted as a reaction to more favourable keeping conditions in general, as the only major change seemed to be the increase in water volume and surface area per fish.

Abnormal morphology and behaviour

What was the cause of the malformations observed in some of our fish? The ventral and anal fins bent at 90° angles apparently resulted from frequent contacts with the tank bottom: those fish that showed very well-developed buoyancy had normally-shaped fins, whereas those that had slightly less well-filled gas bladders showed these malformations. These abnormalities were independent of size or condition of the fish. The one fish that had serious problems with buoyancy showed extreme malformations on all ventrally located fins. A few fish had very slightly bent pectoral fins: those fish that swam in the tanks in the clockwise direction showed these malformations on the left side only, and vice versa.

The missing integumental nose cover observed in a few large fish apparently did not alter the feeding behaviour of these specimens.

The malformations observed in general did not influence the growth of the fish, and were caused by various kinds of physical contacts with the wall of the tanks.

Growth and feeding

Comparing the overall growth of our captive reared fish with wild-caught fish (figure 7), we see that our fish showed similar growth characteristics to the wild fish (Lepage, Lambert and Rochard, 1994), taking into account that our fish (received from the Cemagref facility in 1996) were the smallest of all the 1995-born specimens available at that time.

During our experiments, however, constant, high water temperatures of approx. 20 °C were applied. Our *A. sturio*, therefore, should have shown better growth characteristics (assuming that *A. sturio* reacts in the same way as other sturgeons) than the wild fish, as has been shown for other species (Rosenthal and Gessner, 1992). Consequently, we

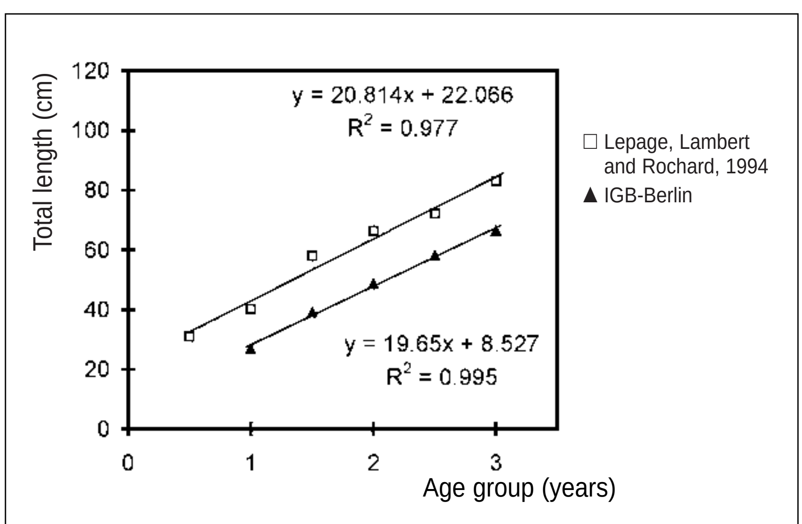

Figure 7. Growth characteristics of the *A. sturio* reared in Berlin (IGB) compared to Gironde fish (Lepage, Lambert and Rochard, 1994) based on mean values for age groups

have to conclude that our fish showed suboptimal growth. One explanation for this could be the suboptimal food supplied to our fish (see also Williot *et al.*, 1997). The natural food of *A. sturio*, however, is not yet well known. The food we supplied, chironomid larvae and marine tropical fish, cannot be considered a natural food, at least not for our larger fish, which would live, in nature, in a temperate marine environment. Future experiments, therefore, will include feeding trials with additional natural food items, experiments concerning the optimal temperature for growth, significance of tank size on growth characteristics, and the attempt to develop a formulated diet to maximise weight gain and thus stimulate early sexual maturation and gonadal development, as has been shown for other sturgeon species (Filippova, 1999; Doroshov, Van Eenennaam and Moberg, 1999).

The individual growth characteristics showed rather large individual differences. Some individuals, for short periods of time, had a growth rate comparable to the maximum length increase of about 20 cm per year, found in the wild (Lepage, Lambert and Rochard, 1994). Why this growth period in captivity is so short will be investigated in future experiments.

The decrease in growth performance at the beginning of the third year was probably due to the bad quality of the small frozen chironomids. These benthic insect larvae could have contained organic or inorganic pollutants, with adverse effects on growth; we have found a similar effect with live chironomids in feeding tropical fish (unpublished results).

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Cryopreservation of Atlantic sturgeon *Acipenser sturio* L., 1758 sperm: First results and associated problems

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ABSTRACT

We present our attempts to adapt the established cryopreservation techniques for sturgeon sperm to *Acipenser sturio* L., 1758, using the sperm of a wild male which matured *in vivo*. The sperm, from a ripe male caught in the Gironde estuary in 1996 and obtained 4 h after its catch and transport, was diluted 1:1 with media containing 56.0-76.0 % tris-HCl-buffer (49.5 mM tris(oxymethyl)aminomethane) (pH 8.0), 14.4-24.0 % dimethylsulfoxide and 9.6-20.0 % egg yolk. The suspension was poured into 1.5 ml tubes, sealed and frozen in -196 °C liquid nitrogen vapour in a three-stage programme. Thawing took place in a 40 °C water bath. The motility of thawed sperm was 10-15 %, whereas the motility in native sperm before cryopreservation was 50 %. After one month storage time in liquid nitrogen, sperm was thawed and used for fertilization of sterlet *Acipenser ruthenus* L., 1758 eggs with the help of two activator media. After fertilization in a medium containing 3.1 mM tris(oxymethyl)aminomethane, 5.3 mM NaCl and 58.3 mM sucrose, 38.3 % of the embryos were developing on the second day, compared with 23.2 % when activator without sucrose was used. In the control group, fertilization of sterlet eggs with fresh sterlet sperm resulted in 43 % developing embryos.

Key words: Fertilization, reproduction, conservation, recovery.

RESUMEN

Criopreservación del esperma de esturión atlántico* Acipenser sturio *L., 1758: primeros resultados y problemas asociados

Presentamos nuestros intentos de adaptar las técnicas establecidas de criopreservación al esperma de Acipenser sturio *L., 1758, usando el esperma de un macho silvestre madurado* in vivo. *El esperma, procedente de un macho maduro capturado en el estuario del Gironda en 1996 y obtenido cuatro horas después de su captura y transporte, fue diluido 1:1 en un medio que contenía 56.0-76.0 % tris-HCl-buffer (49.5 mM tris(oximetil)aminometano) (pH 8.0), 14.4-24.0 % dimetilsulfóxido y 9.6-20.0 % yema de huevo. La suspensión fue vertida en tubos de 1.5 ml, sellada y congelada en nitrógeno líquido a -196 °C en un programa de tres fases. La descongelación tuvo lugar en un baño de agua a 40 °C. La movilidad del esperma descongelado fue del 10-15 %, mientras que la movilidad del esperma nativo antes de la criopreservación fue del 50 %. Después de un mes de almacenamiento en nitrógeno líquido, el esperma fue descongelado y empleado para la fertilización de huevos de esterlete* Acipenser ruthenus *L., 1758 con la ayuda de dos medios activadores. Después de la fertilización en un medio conteniendo 3.1 mM tris(oximetil)aminometano, 5.3 mM NaCl y 58.3 mM sacarosa, el 38.3 % de los embriones se desarrollaron al segundo día, frente al 23.2 % obtenido cuando se usó un activador sin sacarosa. En el grupo control, la fertilización de huevos de esterlete con esperma fresco de esterlete produjo un 43 % de embriones desarrollados.*

Palabras clave: *Fertilización, reproducción, conservación, recuperación.*

INTRODUCTION

The Atlantic sturgeon *Acipenser sturio* L., 1758 was caught in many European rivers in large quantities until the onset of the 20th century (Kinzelbach, 1977). In recent years, only a few specimens have been reported (Elvira and Almodovar, 1993; Debus, 1995). For future work on the recovery of this species, it is important to create a storage stock of cryopreserved gametes, embryos and embryo cells, because only limited numbers of mature specimens are available from controlled conditions (Williot *et al.*, 1997). Availability of such a storage stock would enable us to restore the genome of Atlantic sturgeon at any time, with the help of androgenesis or substitution crossing, even in the absence of any males and females of this species. Only limited experience with sturgeon sperm cryopreservation is available to date. The first research was done by Burtsev and Serebryakova (1969), who used glycerol in concentrations of 5-14 % in combination with egg yolk and sucrose (or lactose) or salts as cryoprotectant media. The sperm of beluga *Huso huso* (L., 1758), kaluga *Huso dauricus* (Georgi, 1775), sterlet *Acipenser ruthenus* (L., 1758), and bastard *H. huso* × *A. ruthenus* had a motility from 10-100 % after freezing and thawing, but fertilised not more than 1 % of the eggs.

Kasimov *et al.* (1974) reported that stellate *Acipenser stellatus* Pallas, 1771 and Russian *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833 sturgeon sperm had 40 % of motile cells and fertilised 35 % of the eggs after freezing in 5 ml tubes in media with urea, chloralhydrat, sucrose, glycerol and egg yolk extract at -55 °C.

Kopeika (1982) demonstrated the possibility of cryopreserving sturgeon (beluga, sterlet, sturgeon, stellate) sperm with dimethylsulfoxide (DMSO), DMSO + polyethylenoxide-3000 (PEO-3000), DMSO + sucrose, and ethylenglycole. DMSO and DMSO + PEO-3000 were determined to be the best of these cryoprotectants (Kopeika, Belous and Pushkar, 1981). Cryopreserved *A. stellatus* sperm was stored for 4-22 days and had 40-60 % of motile cells, which fertilised 64.1 ± 8.8 % of the eggs and controls, reaching 77.5 ± 5.8 % using fresh sperm of identical species (Kopeika and Novikov, 1983). Frozen-thawed sperm of *A. ruthenus* fertilised 87.1 ± 2.9 % (control 97 %) of the eggs. The high sensitivity of sturgeon sperm to osmotic pressure changes was decreased by adding 20 % egg yolk and the gradual increase of osmotic pressure in suspension. After 6-year storage in liquid nitrogen, the sturgeon sperm, frozen according to this procedure in a medium with tris-HCl-buffer, egg yolk, and DMSO, revealed good motility (*A. gueldenstaedtii*, 20-40 %; *H. huso*, 25-40 %; *A. stellatus*, 55 % and 60 %; *A. ruthenus*, 35 % and 50 %; *Acipenser nudiventris* Lovetzky, 1828, 60 %) (Dzuba *et al.*, 1999).

Because of the importance of cell cryopreservation for conservation, we performed our experiments upon the incidental catch of a mature male Atlantic sturgeon in Gironde River in April 1996. This experiment was designed to transfer the method established for other sturgeon species, and to adapt the cryoprotectant medium to the sperm of this male.

MATERIALS AND METHODS

Immediately after catching the male *A. sturio* (23 April 1996) and its subsequent delivery to the farm, its weight and size were determined. The sperm was obtained at 23:00 h (4 h after its catch and arrival at the farm). In order to prevent interaction of water and faecal mass with sperm, a soft transparent polyethylene tube (5 mm diameter) was inserted into the genital hole. The effluent of the tube was placed in glass beakers for sperm storage. Sperm was flowing freely without previous massage of the abdomen. The procedure took place without anaesthesia. During the time of manipulation water was passed through fish gills. The sperm obtained was put into a refrigerator at 5 °C, where it was stored prior to cryopreservation for 1-4 h. Estimation of sperm quality was carried out on the basis of spermatozoa motility with the help of a ×800 microscope. For sperm motility activation, 0.2 ml of river water was put onto a glass slide, and then 0.005 ml of sperm was added. This suspension was thoroughly mixed with the tip of a pipette. We visually estimated the quantity of cells that were moving straight as well as all cells on the 3-5 segments of the slide. The ratio of these counts was expressed as a percentage. The duration of sperm moving straight was estimated in the first experiment. The concentration of spermatozoa was counted in a Nikon Chamber**.** Media for sperm cryopreservation were created *ex tempero* (table I). Cooled buffer medium (5 °C) was slowly added to the sperm via side of the rotating glass. Medium/

Table I. Cryoprotective media and freezing regimens used in different experiments

No. of experiment and cryo - preservation regimen	Medium	Compound of cryoprotective medium			Duration of first stage of cryopreservation (min)
		49.5 mM tris-(oxymethyl)-aminomethane-HCl (%)	DMSO (%)	Hen egg yolk (%)	
1	A	68	22	10	15
2	B	70	18	12	8
	C	56	24	20	
3	B	70	18	12	17
	C	56	24	20	
	D	76	14.4	9.6	
4	D	76	14.4	9.6	15

sperm ratio was in a 1:1 proportion. Sperm equilibration in media with cryoprotectants was for 30 min; then 1 ml portions of the cell suspension were poured into 1.5 ml cylindrical ampoules. These units were closed with plugs.

We used a scheme of multifactor experiments in our experiments 2 and 3 (media B and C) where we varied two factors (osmolarity of medium and duration of first stage of cryopreservation) on two levels. In the third experiment, the medium for sperm dilution had the lowest DMSO and yolk concentration tested (D).

Sperm cryopreservation

Ampoules with diluted sperm were placed above the surface of liquid nitrogen in the Dewar's liquid. One of the tubes containing sperm suspension with media A was equipped with the thermo-couple in all experiments (P510, Cole-Parmer Instrument Co., Dostman Electronic GmbH, Wertheim, Germany) and connected to the computer. Cryopreservation used a three-stage freezing regimen: (1) speed of 1-5 grad/min from 5 °C to -10 and -15 °C; (2) speed of 20-25 grad/min till -70 °C; (3) the tubes with suspension were plunged into liquid nitrogen. Temperature for transferring to the second stage of cryopreservation depended on the duration of stage 1 (table I). The speed of freezing could be changed by altering the distance of the tubes from the nitrogen surface. The temperature changes in the tubes were recorded on the display every 30 s.

Thawing

Sperm was thawed in a static water bath at 40 °C until liquid medium became visible. We checked the quality of thawed sperm after freezing in activator 1 for each cryoprotective medium utilised in order to estimate the suitability of the regimen for the sperm used (table II). In these cases, the sperm was in liquid nitrogen for 10 min.

Egg fertilization

The sperm destined for fertilization was stored in liquid nitrogen for one month. For fertilization, we used only the sperm (experiments 3, 4) which was cryopreserved in the cryoprotectant medium D containing 76 % 49.5 mM tris-oxymethyl-aminomethan-HCl-buffer(pH 8.0) + 14.4 % DMSO + 9.6 %

Table II. Sperm activators used for estimation of *A. sturio* sperm motility and fertilising ability. (*): this activator was used only once in experiment No. 1

Sperm activators used for	
Motility testing	Fertilization
1. 9.3 mM tris-HCl + 12 mM $NaHCO_3$	3. 3.1 mM tris-HCl+ 5.3 mM NaCl + + 58.3 mM sucrose
2. 9.3 mM tris-HCl + 12 mM $NaHCO_3$ + + 146 mM sucrose *	4. 3.1 mM tris-HCl + 5.3 mM NaCl

egg yolk. Fertilising ability of thawed *A. sturio* sperm was tested using eggs of 3 females of sterlet *A. ruthenus* due to the absence of *A. sturio* eggs. We then added 2 ml of thawed cell suspension and 2 ml of activator 3 or 4 to the batches (200-250 eggs/batch) of *A. ruthenus* eggs and mixed for 3-4 min, after which sperm was rinsed off, and the batch subsequently incubated in river water. In the control, 0.4 ml of fresh *A. ruthenus* sperm activated with 4 ml of water was added to the *A. ruthenus* eggs. The insemination results were estimated on the basis of developing embryos on the second day after fertilization.

RESULTS

A. sturio weight was 25.3 kg, and length was 170 cm. We obtained 35 ml of sperm. The cell concentration determined in sperm was 1.1×10^8 spermatozoids/ml. Initial motility was 50 % after activation with river water. Straight moving of spermatozoa was for 3 min. The results of cryopreservation experiments are presented in table III. After thawing the sperm in the first experiment, only 1 % of cells were activated in activator no. 1 and 10-15 % –in activator no. 2 with sucrose. But motility of sperm after cryoconservation was short, not exceeding 90 s.

Table III. Motility of spermatozoa of *A. sturio* after equilibration in cryoprotectant media and freezing-thawing

No. of experiment and cryopreservation regimen	Medium	Sperm motility % in activator 1 after	
		Equilibration in cryoprotectant medium	Thawing
1	A		1
2	B	40	10
	C	1	1
3	B	20	10
	C	10	1
	D	20-30	10-15
4	D		10-15

With the help of regression analysis of spermatozoa motility after cryopreservation in media B and C (experiments 2 and 3), we have calculated the regression equation:

$$Y = 22 - 18X_1$$

where Y = spermatozoa motility, and X_1 = first studied factor (osmolarity of cryoprotectant medium). It could be concluded from this equation that differences in the regimens of cryopreservation do not influence sperm motility. The negative coefficient of X_1 is evidence of the need to lower medium osmolarity in order to increase sperm survival. This was confirmed in experiments 3 and 4, by using medium D. Here, we observed the maximum survival of cells, although the increased storage time prior to cryopreservation led to a decline in sperm quality.

These results were confirmed in the fourth experiment, using medium D (table I) for cryopreservation of the sperm. One month after cryopreservation, the sperm was thawed in order to test its fertilising ability. Eggs of three *A. ruthenus* females were inseminated with this sperm (table IV). Using the eggs of two females (no. 1 and no. 3), neither the control nor the experiment set were successful.

Table IV. Fertilizing ability of frozen-thawed sperm of *A. sturio*

Eggs *A. ruthenus* 2nd female	Sperm	Activator	Quantity of developing embryos on the second day after fertilization (%)
Experiment	*A. sturio*	3	38.3
Experiment	*A. sturio*	4	23.2
Control	*A. ruthenus*	H_2O	43.0

DISCUSSION

Over the course of their reproductive life, males do not produce spermatozoa with identical genomes (Ayala, 1984). Therefore, the limited availability of reproductive material makes it important to store the maximum quantity of cells in order to decrease the possibility of inbred depression. The quality of cryopreserved sperm is dependant upon various factors. Their relevance could vary according to intrinsic (e.g. degree of gonadal maturation, initial quality of sperm) as well as extrinsic factors (critical factors in cryopreservation and cryoprotection). Initially, obtaining sperm with high quality and cryoresistance seems critical, due to the unpredictable availability of ripe males, hindering proper preparation for obtaining sperm. It could be supposed that in the present study the stress conditions imposed on the fish by catch and transportation, as well as the time when sperm was obtained, had an adverse impact on sperm quality. This is supported by the findings on Siberian sturgeon *Acipenser baerii* Brandt, 1869 (Williot, Kopeika and Goncharov, 2000), in which the degree of maturation, as well as the time of obtaining sperm, had significant influence on sperm quality**.** The sperm motility and cryoresistance changes show a bell-curve after hormonal stimulation (Kopeika, Williot and Goncharov, 1999a). The peak of motility has been identified at 5 a.m. 2-3 days after hormonal stimulation of males (Kopeika, Williot and Goncharov, 1999b). Therefore, half of the potentially available cells were lost during our first stage of obtaining sperm. Additionally, degradation of immobile cells could have imposed a negative influence on the remaining live cells, thereby affecting their cryoresistance. This interaction has been reported for mammal sperm (Bugrov and Sidashov, 1991). We obtained only 35 ml of sperm with cell concentration 1.1×10^8 spermatozoids/ml, i.e. close to the minimum. Ginzburg (1968) reported that sturgeons give from 25-500 ml of sperm with concentrations ranging from 0.14 to 7.55×10^9 spermatozoids/ml. Concentration of *A. baerii* sperm varied from 0.5×10^8 to 5×10^8 (Gallis *et al.*, 1991). With the increasing of delay after hormonal stimulation, there was quantitative growth of mature motile spermatozoa, as well as in general concentration (Williot, Kopeika and Goncharov, 2000). Therefore, we could suppose that we obtained sperm from males with low reproductive ability, or sperm that was immature. The latter supposition was confirmed, begining from our first experiment on cryopreservation, in which we obtained only 1 % of motile cells. Although we used cryoprotective media and universal three-stage freezing regimens that have provided good results for cryopreservation of sperm in other sturgeon species (Kopeika, Belous and Pushkar, 1981; Kopeika, 1982). This regimens was also used with good results for cryopreservation of sperm from carp *Cyprinus carpio* L., 1758 (Kopeika, 1986), salmon, and other fish (Kopeika, unpublished). In order to obtain reliable results, we investigated thawed sperm under critical conditions –in river water or activator no. 1, thus providing lower osmolarity than the usual activator for thawed sturgeon sperm (Kopeika, 1982; Kopeika and Novicov, 1983; Tsvetkova *et al.*, 1996; Jähnichen *et al.*, 1999). The increased sperm motility, 10-15 % upon activation with sucrose activator, compared to water activation, might be attributable to the higher osmolarity of the activator. The increased osmolarity led to a lower possibility of post-hypertonical shock of the thawed sperm membrane. Spermatozoa, like all other cells, become more sensitive to osmolarity deviations in the medium. This sperm immaturity could also be confirmed by the short time of sperm motility (90 s) after cryopreservation. Similar effects have been observed for carp sperm (Kopeika, unpublished). The duration of motility in immature carp sperm was 10-15 s after dilution with the cryoprotectant medium, compared to 60-90 s in mature sperm. Therefore, in the case of immature sperm, in which cryoresistance is lower, the need exists, as we assumed, to decrease the osmolarity of the cryoprotectant medium by lowering the concentration of cryoprotector (B). To exclude possible errors in our supposition, we also used media with higher osmolarity (C), and other methods, in each experiment. Crystallization in medium B, with a lower content of cryoprotectant, was expected to be at a higher temperature than that for medium C, with higher cryoprotectant concentration. Therefore, in medium B, we began increasing our freezing speed during the second stage earlier and at a higher temperature than in medium C. It is important to take into account here that increased freezing speed before the end of crystallization can cause increased cell destruction (Kopeika, unpublished). Therefore, we used two regimens of cryopreservation. However, the results of our multifactor experiment showed that these changes of regimens did not influence sperm motility. This could be evidence for the fact that crystallization was over before the suspension was transferred into the second stage of cooling. A negative coefficient of X_1 shows that for better results, it is necessary to decrease the cryoprotective medium's osmolarity. In order to confirm the significance of our obtained regression equation, it appeared necessary to repeat the experiment. But we did not do this, in the end, because we obtained additional confirmation regarding the relevance of our conclusion. The survival of sperm after cryopreservation was the highest in medium D, with lower osmolarity, than in the multifactor experiment media. The delay of sperm storage in the refrigerator could lead to the growth of sperm sensitivity to extreme factors.

The fertilization ability of thawed sperm was dependent on the activator used. Higher fertilization rates (expressed in the quantity of developed embryos) were observed in activator no. 3 with sucrose, compared to the activator without sucrose. Our results correlate with data that freshwater fish sperm (sturgeon, carp) becomes more sensitive to variation in osmotic pressure after cryopreservation (Kopeika, 1982; Kopeika and Novicov, 1983). Additionally, the fertilising ability of frozen-thawed sperm depends on the quality and quantity of the activating medium and insemination conditions. Comparing the average fertilization ability of cryopreserved sperm of *A. sturio* with that of control sperm from *A. ruthenus*, our results are still better than other published experiments (Tsvetkova *et al.*, 1996; Jähnichen *et al.*, 1999).

Our short and uncompleted (due to the restricted availability of sperm) study indicates that this material could successfully be stored, even with sperm of initially low quality, with the given method (Kopeika, 1982) of cryopreservation in tris-HCl-buffer with DMSO and egg yolk. The total survival of fish spermatozoa during cryopreservation could be increased if the causes of variability in sperm quality could be influenced. In the future, it will be necessary to create optimal conditions for obtaining high-quality sperm, even from reared species. In order to reach this objective, the males should be reared under optimal temperatures before and after injection. Only mature sperm should be obtained. The best time is early morning, but not earlier than 24-36 hours after hormonal stimulation, in order to increase the survival of cells during cryopreservation. The use of antifreeze fish proteins also provides possibilities of improving the results by increasing the sperm's initial cryoresistance. In any case, additional research efforts are urgently required in this field to eliminate the existing uncertainties.

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Atlantic sturgeon *Acipenser sturio* L., 1758 restoration and gravel extraction in the Gironde estuary

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ABSTRACT

The Gironde estuary, in the southwest of France, is the last Western European estuary where all diadromous fish still migrate. In addition, the Gironde-Garonne-Dordogne basin is the last system known where the reproduction of the Atlantic sturgeon takes place. The Gironde estuary is an essential habitat for the completion of the life history of this diadromous species. A European recovery plan launched in 1994 has shown the significance of the estuary for feeding and acclimatization of juveniles before their first journey to the sea. The Atlantic sturgeon has been threatened in the last 50 years, mostly by gravel extraction in spawning grounds and unadapted fishing regulations that have led to overfishing. Since the end of the 19th century, several measures connected to navigation (dykes, rockfill, groynes) have been carried out in the Gironde estuary. This estuary, although only industrialised to a slight extent, has suffered from dredging since the creation of the navigation channel in 1875. Although gravel extraction is commonly recognised as causing serious damage to fish habitats, new gravel extraction projects in the Gironde estuary are under consideration. A qualitative risk factor analysis, based on the latest knowledge on the biology and ecology of the Atlantic sturgeon, shows that these projects lead to maximum risk for the survival of this species. Taking into account the present fragility of the sturgeon population, protective measures must be taken as a precaution. These would rule out any further gravel extraction.

Key words: Conservation, France, recovery programme, river habitat.

RESUMEN

Recuperación del esturión atlántico* Acipenser sturio *L., 1758 y extracción de grava en el estuario del Gironda

El estuario del Gironda, en el sudoeste de Francia, es el último estuario de Europa occidental donde todavía migran todos los peces diadromos. Además, la cuenca Gironda-Garona-Dordoña es el último sistema conocido donde tiene lugar la reproducción del esturión atlántico. El estuario del Gironda es un hábitat esencial para la realización del ciclo vital de esta especie diadroma. Un plan de recuperación europeo iniciado en 1994 ha mostrado la importancia del estuario para la alimentación y aclimatación de los juveniles antes de su primer viaje al mar. El esturión atlántico ha sido amenazado en los últimos 50 años, principalmente por la extracción de grava en los frezaderos y por los obsoletos reglamentos que han provocado su sobrepesca. Desde finales del siglo XIX, diversas medidas relativas a la navegación (diques, relleno con piedras, espigón) se han llevado a cabo en el estuario del Gironda. Este estuario, industrializado, aunque sólo de forma ligera, ha sufrido dragados desde la creación de un canal de navegación en 1875. Si bien la extracción de grava se reconoce comúnmente como causante de graves daños en los hábitats de peces, se están considerando nuevos proyectos de extracción de grava en el estuario del Gironda. Un análisis de los factores de riesgo cualitativos, basado en los últimos conocimientos de la biología y ecología del esturión atlántico, muestra que estos proyectos conllevan el máximo riesgo para la supervivencia de esta especie. Teniendo en cuenta la fragilidad actual de la población de esturión, deben tomarse medidas protectoras como precaución. Éstas deberían excluir cualquier extracción adicional de grava.

Palabras clave: *Conservación, Francia, programa de recuperación, hábitat fluvial.*

INTRODUCTION

The Atlantic sturgeon *Acipenser sturio* L., 1758 is a diadromous fish species that spawns in fresh water, but matures in estuaries and at sea. In the past, it was present in almost every large river from the Black Sea to the North Sea, via the Mediterranean Sea and the Atlantic Ocean (Rochard, Castelnaud and Lepage, 1990). Today, its marine distribution is restricted to the French Atlantic coast, with only occasional captures around the United Kingdom and in the North Sea (Rochard, Lepage and Meauzé, 1997). The last known areas for reproduction are in the Garonne and Dordogne Rivers in southwest France. Since 1982, *A. sturio* has been fully protected in France. The species is listed on France's Endangered Species Red Book (Keith, Allardi and Moutou, 1992). On an international level, the species is protected by the Bern convention, the Washington convention (CITES) (Anon., 1997) and is listed in the International Union for the Conservation of Nature Red Book (IUCN) (Lepage and Rochard, 1995). Most European countries have signed these international treaties, and have put a ban on the fishing of this species.

THREATS

Dams, pollution and overfishing caused the disappearance of *A. sturio* from most of its historical distribution (Williot *et al.*, 1997). In the Gironde estuary, the decrease of the sturgeon population is mainly due to maladapted fishing regulations, which led to continuous overfishing and to the destruction of spawning grounds and nurseries by gravel extraction operations (Castelnaud *et al.*, 1991). The dams are located 210 km from the ocean on the Dordogne River, and 270 km on the Garonne River, and some sites suitable for spawning are still found downstream from both dams (Elie, 1997). Presently, these threats have been largely reduced by the ban on river gravel extraction and the protection measures on national and international levels. Although gravel extraction is commonly recognised as causing serious damage to fish habitats and was believed to be involved in the decline of several sturgeon stocks around the world (Rochard, Castelnaud and Lepage, 1990), new gravel extraction projects in the Gironde estuary are under consideration.

THE STURGEON AND THE ESTUARY

A European recovery plan (1994-1997) has shown the significance of the Gironde estuary for feeding and acclimatization of juvenile sturgeon before their first journey at sea. The estuary is a compulsory passage for the juvenile and represents an essential habitat for the completion of the life history in this diadromous fish (Elie, 1997). Sampling campaigns with trawl nets have shown that sturgeons were present in each of the determined zones (figure 1). Higher proportions of captures, exceeding up to 10 times the catches in other zones, were encountered in zones 1 and 7.

NAVIGATIONAL FEATURES AND UNDERWATER MINING ACTIVITIES

Since the end of the 19th century, several navigational features (embankments, rockfill, groynes) have been built in the Gironde estuary. Although it supports little industry, the estuary has been constantly disturbed by the dredging of the navigation channel since it was first created in 1875. Today, regular dredging is still carried out for its maintenance. Currently two areas of the estuary are authorised for gravel and sand extraction (figure 1) for construction, but exploitation remains relatively limited. In addition to agricultural practices that influence the availability of sediment, all these operations, from the past and present, have contributed to the enrichment of the maximum turbidity zone which moves with the tide between the river and the estuary. The dumping of dredging material, as well as the cleaning of the gravel and the "natural" maximum turbidity itself, can cause, especially during the summer, a sharp decline in dissolved oxygen or even localised total anoxia due to organic matter degradation and poor photosynthesis (Romaña and Thouvenin, 1990). Moreover, there can be activation and accumulation of bacteria and micro-pollutants at the dumping site (Pommepuy *et al.*, 1990).

EXTRACTION IMPACTS

Because of the complex balance between animal populations and their habitats, the impact of gravel and sand extractions on a fish population is of-

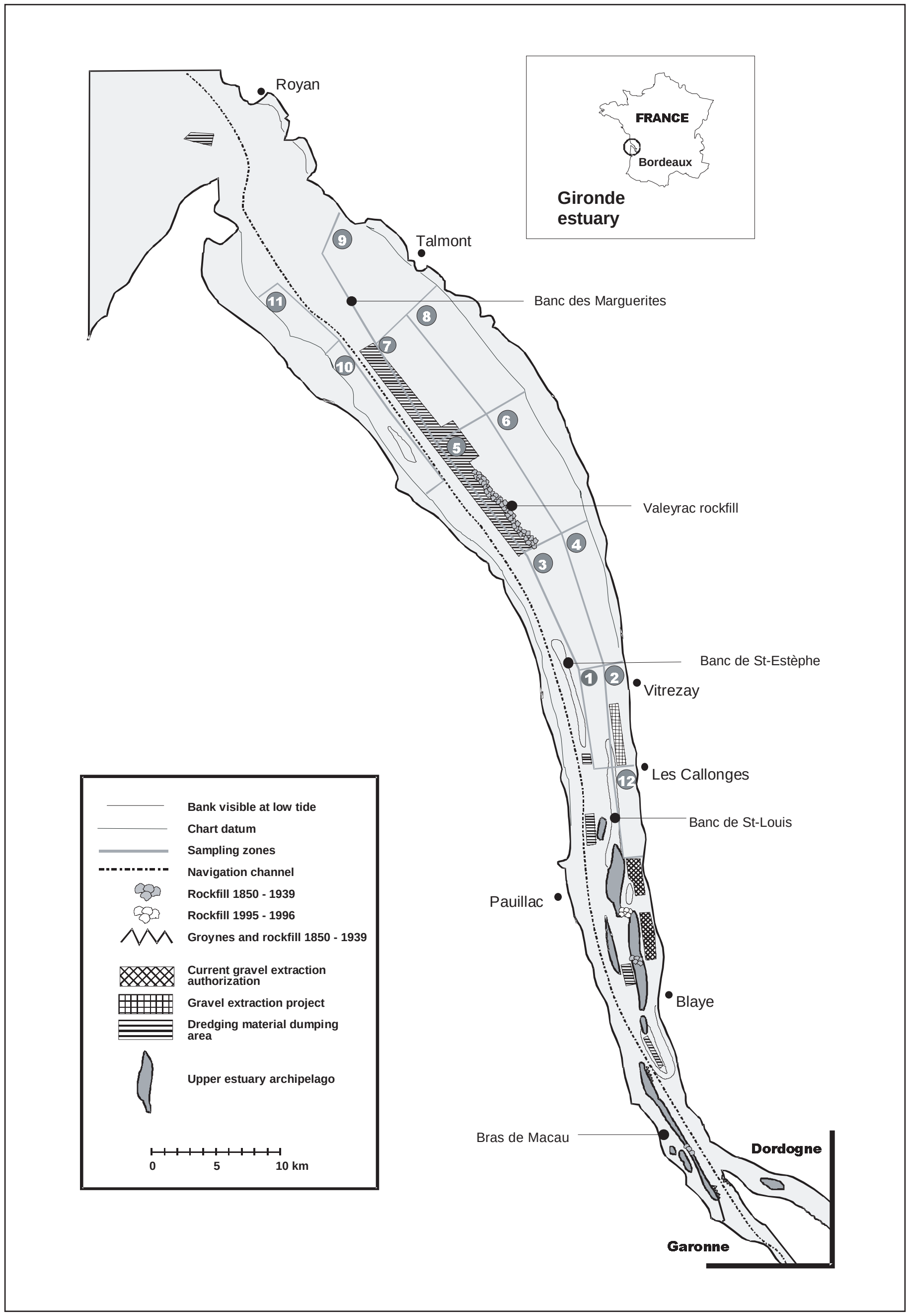

Figure 1. *A. sturio* sampling zone in the Gironde estuary and gravel extraction project

ten estimated *a priori*. There are direct impacts upon populations, but the indirect impacts imply much higher risks because of the impossibility of evaluating the effects of habitat destruction.

There is always a risk for an animal population when man decides to modify its habitat. Beyond the risk to a threatened population, extractions generate a major risk of disturbance to an ecosystem, by modifying the quality of the habitat, on the physical level as well as on a physico-chemical level (Bouchaud *et al.*, 1979; Malherbe, 1990; Mitchell, 1990; Pommepuy *et al.*, 1990).

Therefore, it seems more appropriate to apprehend the problem by a risk analysis based on current knowledge of the elements describing a population (Akcakaya and Ginzburg, 1991). Interference with the habitat of a threatened population, such as the sturgeon, will involve much higher risks than in a population without any particular problems.

Table I. Grid of risk analysis input for a fish population facing a deterioration of its habitat. The checkmarks indicate the conditions met by *A. sturio*

Condition of risk unknown	Condition involving moderate risk		Condition involving maximum risk
	Several wild populations	✓	Single wild population
	Wild population that does not exhibit drastic decline	✓	Wild population that is reduced to a crucial minimum threshold
	Balance structure of population	✓	Irregular population structure
	No particular problem with the status of the species in its country of origin	✓	Endangered status of the species in its country of origin
Information not available	Several river basins where reproduction takes place	✓	Single river basin where reproduction takes place
	Short life history	✓	Long life history
	No decline in the breeding stock	✓	Sharp decline in the breeding stock
	Little or no reduction observed, deduced or expected in the surface area and/or quality of the habitat	✓	Reduction observed, deduced or expected in the surface area and/or quality of the habitat
	Habitat not highly populated	✓	Essential habitat

In table I, the principal parameters considered are presented. They are inspired by the classification criteria from Appendix I of the Washington convention (CITES) (Anon., 1997), the Red Data Book of the International Union for the Conservation of Nature and Natural Resources (IUCN), and Akcakaya (1997). For each criterion, there are three possibilities to select, whether the information is available or not, and whether it matches one of the two situations described.

According to the criteria given in table I, concerning the risk resulting from damaging essential habitat, it is apparent that the risk is maximised for the sturgeon population.

The sturgeon displays all the aggravating: risk factors related to gravel extraction in the estuary. No factor of knowledge or even of current ignorance is able to balance this degree of risk. Establishing all necessary measures in order to make it possible to restore the population of sturgeon in the Gironde would imply extending the prohibition of gravel extraction for the entire Gironde estuary in order to minimise the risk involved.

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Genetic analyses of archival specimens of the Atlantic sturgeon *Acipenser sturio* L., 1758

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ABSTRACT

Genetic variability was analysed in *Acipenser sturio* L., 1758 and *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815 using variation in the D-loop region of mtDNA and a number of microsatellites (nuclear markers). The studied material included tissue samples from: (1) 38 *A. sturio* archival specimens collected in different German, Swedish, Danish, and French museums of natural history; (2) 27 live *A. sturio* representing a broodstock for restoration of this species in German waters (Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany); (3) 30 wild *A. o. oxyrinchus* caught in the Atlantic Ocean near the coast of New Jersey and in the Delaware River (USA); and (4) 60 individuals of *A. o. oxyrinchus* from an artificially reproduced stock originally obtained from several wild sturgeons captured in the St. John River (Canada). A 250-bp fragment of the D-loop region of mtDNA was cloned and sequenced. The length of a repeated unit was 80 bp in *A. sturio* and 79 bp in *A. o. oxyrinchus*. The repeated units of *A. sturio* and *A. o. oxyrinchus* differed by 11 substitutions and one deletion or insertion, respectively. No heteroplasmy was found. Three different haplotypes of mtDNA were observed in both species. Five microsatellites had polymorphic band patterns. In *A. sturio*, analyses of microsatellites showed a decrease in allelic numbers between the years 1823 and 1992. This decline resulted in a fixation of several alleles. For *A. sturio*, one mtDNA haplotype and seven alleles were observed only in archival samples. Genetic distance calculations showed a great genetic similarity between *A. sturio* populations in the Gironde River and the North Sea, and a basal position of the Mediterranean and Adriatic Sea *A. sturio* populations. In *A. o. oxyrinchus*, the number of microsatellite alleles ranged between 14 (Hudson and St. John rivers) and 22 (Delaware River). Genetic distance calculations showed a high genetic similarity between subpopulations of *A. o. oxyrinchus*.

Key words: *Acipenser oxyrinchus oxyrinchus,* breeding programme, genetic drift, restoration.

RESUMEN

Análisis genéticos de ejemplares almacenados de esturión atlántico **Acipenser sturio** ***L., 1758***

Se analizó la variabilidad genética en Acipenser sturio *L., 1758 y* Acipenser oxyrinchus oxyrinchus *Mitchill, 1815 usando la variación en la región D-loop del ADN mitocondrial y en un número de microsatélites (marcadores nucleares). El material estudiado incluyó muestras de tejidos de: (1) 38 ejemplares almacenados de* A. sturio *colectados en diferentes museos de historia natural alemanes, suecos, daneses y franceses; (2) 27* A. sturio *vivos correspondientes al stock de cría para recuperación de esta especie en aguas alemanas (Instituto de Ecología Dulceacuícola y Pescas Interiores, Berlín, Alemania); (3) 30* A. o. oxyrinchus *silvestres capturados en el océano Atlántico cerca de la costa de Nueva Jersey y en el río Delaware (USA); y (4) 60 individuos de* A. o. oxyrinchus *de un stock reproducido artificialmente obtenido originalmente de diversos esturiones silvestres capturados en el río San Juan (Canadá). Se clonó y secuenció un fragmento de 250 pares de bases de la región D-loop del ADN mitocondrial. La longitud de una unidad repetida fue de 80 pares de bases en* A. sturio *y de 79 en* A. o. oxyrinchus. *Las unidades repetidas de* A. sturio *y* A. o. oxyrinchus *difirieron por 11 sustituciones y una delección o inserción, respectivamente. No se encontró heteroplasmia. Se observaron tres diferentes haplotipos de ADN mitocondrial en ambas especies. Cinco microsatélites presentaron patrones polimórficos de bandas. En* A. sturio *los análisis de microsatélites mostraron una disminución en números alélicos entre los años 1823 y 1992. Este declive tuvo como consecuencia la fijación de varios alelos. Para* A. sturio, *se observaron un haplotipo de ADN mitocondrial y siete alelos sólo en los ejemplares almacenados. Los cálculos de distancia genética mostraron una gran similitud genética entre las poblaciones del Gironda y del Mar del Norte, y una posición basal de las poblaciones de* A. sturio *del Mediterráneo y del Adriático. En* A. o. oxyrinchus *el número de alelos de microsatélite varió entre 14 (ríos Hudson y San Juan) y 22 (río Delaware). Los cálculos de distancia genética mostraron una gran similitud genética entre las subpoblaciones de* A. o. oxyrinchus.

Palabras clave: Acipenser oxyrinchus oxyrinchus, *programa de cría, deriva genética, recuperación.*

INTRODUCTION

Knowledge of the dynamics of gene flow, genetic drift, and selection in natural sturgeon populations on a spatial and temporal scale plays an important role in recent and future conservation plans. A large literature exists on the genetic differentiation at the species level for extant sturgeons (Birstein, Betts and DeSalle, 1998; Birstein *et al.*, 1998; Birstein and DeSalle, 1998). To date, only limited data were available for archival samples taken from museum specimens. Ferguson and Duckworth (1997) included three specimens of *Acipenser fulvescens* Rafinesque, 1817 from the Natural History Museum in London in their study of this species's genetic structure. These specimens were collected between 1866 and 1873. Until now, the genetic analysis of sturgeons has focused on phylogenetic reconstructions and species identification (Birstein, Hanner and DeSalle, 1997; Birstein, Betts and DeSalle, 1998; Birstein *et al.*, 1998; Birstein and DeSalle, 1998). Only few data regarding the genetic structure of the European Atlantic sturgeon *Acipenser sturio* L., 1758 have been published (Wirgin, Stabile and Waldman, 1997; Ludwig and Kirschbaum, 1998; review in Birstein and Doukakis, 2000). However, recent developments of molecular methods in general and the PCR techniques in particular have opened up the possibility of studying DNA from museum specimens (Nielsen, Hansen and Loeschcke, 1997, 1999). Thus, information important for conservation programmes, such as the historic gene flow, migration routes, genetic distances, and subpopulation structure, can be obtained by using archival samples.

The objective of our study was to establish a method for the investigation of archival samples in order to analyse genetic variability and similarity among several extinct stocks of *A. sturio*, as well as to characterise the German broodstock that has been created at the Institute of Freshwater Ecology and Inland Fisheries (IFEIF), Berlin. Also, we wanted to assess the influence of population decline on the gene pool of *A. sturio*. Since sequence differences are low in several mitochondrial (mt) genes among sturgeon species (Birstein, Hanner and DeSalle, 1997), we studied microsatellites in addition to the partial sequence of the D-loop. In fishes, as in other animals, the D-loop is the most variable region of the maternally inherited mtDNA, whereas microsatellites are known as highly polymorphic nuclear markers (Meyer, 1993). Also, we performed a similar study on three populations of *Acipenser oxyrinchus* Mitchill, 1815, the American Atlantic sturgeon, which is a sister species to *A. sturio* (Birstein and Bemis, 1997), and compared data for these two species.

MATERIALS AND METHODS

Samples

We collected archival samples from 44 specimens of *A. sturio* kept in different German, Swedish, Danish, and French natural history museums (table I). Only limited data are available regarding the collection and storing conditions of these specimens. For DNA extraction, we took different tissue samples from each fish: fin clips, pieces of gill arches and/or of skin. Since historically the North Sea and its tributaries were the most important areas for sturgeon reproduction in German waters (Holčík *et al.*, 1989; Debus, 1995), we mainly used specimens collected in the North Sea or the Elbe River (table I). However, we also included two specimens from the Mediterranean and

Table I. Names of museum collections, and the location and year of the catch of archival specimens included in the present study. (*): Specimens from which the mtDNA sequences were successfully amplified. (1): BS = Baltic Sea, NS = North Sea, and MM = Mediterranean Sea. (2): The sign (<) before the date means that the sturgeon was caught before this date but included in the collection during this year. (3): This specimen came from the artificially reproduced *A. sturio* progeny of sturgeons caught in the Elbe River (Germany); a series of successful artificial reproduction of *A. sturio* was carried out in the town of Glückstadt, near Hamburg, between 1886 and 1891 (Mohr, 1952); no successful artificial reproduction of this species was reported after 1891; this specimen was about three days old, and it was included in the MfN collection in Berlin in 1903

Collection	Museum number	Location of the catch[1]	Year of the catch[2]
National Natural History Museum (MNHN), Paris	4634	Russia, unknown location	< 1870
	4636	Russia, unknown location	< 1870
	4637	Russia, unknown location	< 1870
	4638	Europe, unknown location	< 1870
	5158	NS, Elbe River	< 1870
	5165 *	MM, Tiber River	1823
Natural History Museum (MfN), Berlin	No number (a juvenile) 3*	NS, Elbe River	1903
Koenig Museum, Bonn	8 *	NS, Helgoland Island	1993
Natural History Museum (NRM), Stockholm	1709	BS, Soedermanland	1890
	1837	NS, Bohuslän	1837
	13336 *	NS, Kattegeatt	1991
	18853	NS, Elbe River	1885
	35437	BS, Tosteberga	1871
	36001	NS, Hanstholmen Island	1985
	21705	NS, Bohuslaen	1838
	21706	NS, Bohuslaen	1897
	21707	BS, Soedermanland	1932
	21708	NS, Stroemstad	1910
	21710	NS, Fjaellbacka	1895
Rostock University (Germany)	A2	NS, Elbe River	1903
	A4	NS, Dutch coast	1992
	A14	BS, Warnemünde	1885
	A15	BS, Rostock	1883
	A16	BS, Warnemünde	1887
	A17	BS, Rostock	1882
	A18	BS, Rostock	1887
Biological Institute Helgoland (Germany)	E 01 *	NS, Elbe River	1959
	E 02 *	NS, Helgoland Island	1991
	E 03 *	NS, Helgoland Island	1988
Senckenberg Institute (MF), Frankfurt/Main	MF 7647 *	Adriatic Sea	1827
Zoological Museum, Dresden	*	Elbe River, near Dresden	1871
Zoological Museum, Hamburg	10476	NS, Oste River	1903
	10477	NS, Oste River	1903
	88	NS, Oste River	1907
Copenhagen University Zoological Museum (ZMUC), Copenhagen	42	BS, Copenhagen	1873
	CN16	BS, Copenhagen	1940
	P10202 *	NS, Helgoland Island	1956
	P10218 *	NS, Hirtshals	1986

Adriatic seas (MF 7647 and MNHN 5165, respectively; see table I).

Additionally, 27 individuals representing the German broodstock kept at the IFEIF for restoration of *A. sturio* in German waters were investigated. These originated from artificial reproduction of several specimens from the French stock obtained at the National Agricultural and Environmental Engineering Research Centre (Cemagref) in Bordeaux (France) from two individuals, a female and a male, caught in the Gironde River system in France in 1995 (Anon. 1995; Williot *et al.*, 1997).

Also, 90 specimens of the North American *Acipenser oxyrinchus oxyrinchus* were included in our study for comparison with *A. sturio*. Specimens of *A. o. oxyrinchus* were obtained from three different populations on the North American Atlantic coast: (1) 14 individuals were caught in the Atlantic Ocean near the town of Belmar (New Jersey, USA), close to the mouth of the Shark River –most likely, these sturgeons belonged to the Hudson River population (Waldman and Wirgin, 1998) and we will consider them as such; (2) 16 sturgeons were collected in the Delaware River (New Jersey, USA; see Secor and Waldman (1999) on the history of this population); and (3) 60 sturgeons were obtained from an artificially reproduced stock at the Sturgeon Conservation Centre in New Brunswick (Canada). Several breeders caught in the St. John River (Canada) were used to create this stock. These three populations represented *A. o. oxyrinchus,* one of the two *A. oxyrinchus* subspecies, the other being *A. oxyrinchus desotoi* Vladykov, 1955 (Waldman and Wirgin, 1998).

DNA extraction

Twenty-five µg tissue from each sample was washed in 0.68 % NaCl solution for 24 h at 4 °C, then homogenised and transferred to 180 µl Lysis Buffer (QIAGEN, Germany). After the complete lysis, DNA was extracted and washed following the QIAamp Tissue Kit (QIAGEN, Germany) standard protocol. The DNA concentration was estimated using the DipStick Kit (Invitrogen, Netherlands).

MtDNA amplification and sequencing

Traditionally, PCR fragments between 1.6 and 2.2 kb are used for the detection of heteroplasmy in sturgeons (Brown, Beckenbach and Smith, 1992; Brown *et al.*, 1996; Miracle and Campton, 1995). Taking into consideration the difficulties of amplification of long PCR fragments when studying archival samples, we used two primers closely located to the repeated region, Hetero I (5´-ACCCTTAACTCCCAAAG-3´) and Hetero II (5´-CATTTRATGGTAGATGAAAC-3´) (Ludwig and Jenneckens, 2000).

PCR amplification was performed in a total reaction volume of 25 µl containing approximately 10 ng template DNA, 2.5 µl 10× reaction buffer, 10 pmol of each primer, Hetero I and Hetero II (Ludwig and Jenneckens, 2000), 100 mM dNTPs, 2.5 mM $MgCl_2$, and 0.5 units *Taq* DNA polymerase (Oncor-Applegene, Germany) under the following reaction conditions: 94 °C, 20 s; 50 °C, 10 s; and 72 °C, 1 min with a final elongation step at 72 °C for 4 min. PCR products were separated on a 2.0 % agarose gel at 150 V for 2 h.

Potentially, different factors can influence the results of PCR reaction in the case of archival samples. Numerous examples of artefacts and pseudogenes have been described which included several mitochondria genes (Van der Kuyl *et al.*, 1995; Zhang and Hewitt, 1996). Sampling methods and changes in storing conditions of the selected museum specimens resulted in contamination. Thus, eight different sequences were observed from a single PCR amplification using DNA extracted from museum samples (Van der Kuyl *et al.*, 1995). To minimise these factors, we repeated all analyses twice and cloned PCR products before sequencing. In addition, sequencing analyses were done in two different labs: at the IFEIF (Berlin) and at the Institute of Animal Breeding and Genetics, University of Göttingen (Germany).

For sequencing the amplified heteroplasmic fragments, PCR products were extracted from agarose gel, purified with QIAquick Gel Extraction Kit (QIAGEN, Germany), and cloned with TOPO TA Cloning Kit (Invitrogen, Netherlands) using standard conditions. Ten clones of each sample were amplified in 50 µl reaction volumes containing 2.5 units AmpliTaq DNA Polymerase (ABI, USA), 10 pmol primer (Hetero I and Hetero II), and 25 ng DNA of each sample. The reaction was amplified in a Perkin Elmer thermocycler 2400 programmed for 30 cycles each for 94 °C at 10 s; 50 °C at 10 s; and 72 °C for 2 min. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Germany). Sequencing was performed in 20 µl reaction volume containing 3.5 µl BigDye RR Terminator Cycle Sequencing Kit (ABI, USA), 10 pmol primer and 15 ng DNA, and amplified in a Perkin Elmer thermocycler 2400 programmed for 25 cycles each for 94 °C at 10 s; 50 °C at 10 s, and 60 °C for 4 min. PCR products were sequenced in an ABI 310 automated sequencer (ABI, USA).

Statistical analysis of mt sequences

The 250-bp region of the tRNA^{Pro} and the D-loop sequences were used for calculation of p-distance values (Kumar, Tamura and Nei, 1993). Using the computer package MEGA (Kumar, Tamura and Nei, 1993) and the p-distance outcomes, we constructed a neighbour-joining tree (Saitou and Nei, 1987). Bootstrap analyses (500 steps) were also performed in MEGA.

Microsatellites

Genetic variability was screened using 11 primer pairs named Afu-19, Afu-22, Afu-23, Afu-34, Afu-39, Afu-54, Afu-57, Afu-58, Afu-62, Afu-68, and Afu-69 (May, Krueguer and Kincaid, 1997). Primers were labelled with 6-FAM and TET (ABI, USA). Amplifications were performed in a total volume of 25 µl containing 50 ng genomic DNA, 0.25 U *Taq* polymerase, 5 pmol primers, 0.10 mM Tris-HCl (pH 8.8 at 25 °C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 µg/µl bovine serum albumin (BSA), 0.08 % (v/v) Nonidet P40, and 100 µM of each dNTP. Amplification was performed with 40 cycles of the following steps: 30 s at 94 °C, 30 s at 57 °C (Afu-34, 39, and 68) or 52 °C (Afu-19 and 54), 30 s at 72 °C, and a 5-min final extension at 72 °C. For Afu-22, 23, 57, 58, 62, and 69, the results were identical at both temperatures, 52 and 57 °C. The size of alleles was determined using a 310 DNA sequencing machine (ABI, USA) with internal standards. We observed the number of alleles at each locus and in each population, as well as the allele frequences.

RESULTS

The D-loop (mtDNA)

We succeeded in amplification of D-loop region using DNA extracted from 12 of 44 archival samples of *A. sturio*. Amplification with the sturgeon specific primers resulted in a 250-bp fragment (10 individuals) or a 330-bp fragment (1 individual), including two or three repeats, respectively. In *A. sturio,* the length of the repeated unit was 80 bp, and in *A. o. oxyrinchus*, it was 79 bp. The repeated units of *A. sturio* and *A. oxyrinchus* differed by 11 substitutions and one deletion or insertion, respectively. No heteroplasmy was found in either species.

We observed three different mitochondrial haplotypes in *A. sturio*, and another three haplotypes in *A. o. oxyrinchus*. In *A. sturio*, the most common haplotype was identical to the haplotype found in sturgeon from the Gironde River. The second haplotype was present in an *A. sturio* juvenile artificially reproduced in the 1890s (MfN Berlin, see table I). In this case, a length variation in one of the repeated units was observed. The third *A. sturio* haplotype was found in two individuals: a sturgeon caught in 1823 in the Tiber River, Italy (MNHN 5165; table I), and a sturgeon captured in the Adriatic Sea (MF 7647; table I). This haplotype was differentiated from all other *A. sturio* haplotypes by a single T → C substitution.

We found the same *A. o. oxyrinchus* haplotype in specimens from both the Delaware and Hudson Rivers. It differed from the haplotype found in the St. John River by a single A → G substitution. Also, we observed the third *A. o. oxyrinchus* haplotype in only one specimen from the Delaware River. This haplotype had the same A → G substitution and an additional substitution (C → T) in the 3'-flanking region of the repeated unit.

Genetic distance calculations within *A. sturio* based on the D-loop data showed a high similarity between the North Sea and the Gironde River populations and a more basal position of the Mediterranean and Adriatic Sea populations (table II and figure 1). The genetic distance was 0.0004 between sturgeon from the Mediterranean/Adriatic Sea and the North Sea/Gironde River populations. In *A. o. oxyrinchus,* genetic distances ranged between 0.008 and 0.139. The St. John River and the merged Hudson and Delaware River populations of *A. o. oxyrinchus* had a high genetic similarity. In summary, a high level of genetic similarity was found within both species (table II and figure I).

Table II. p-distance values used for tree calculation calculated in MEGA (Kumar, Tamura and Nei, 1993). Haplotypes are named in relation to their sampling location

	A. sturio		*A. o. oxyrinchus*		
	Gironde River North Sea	Mediterranean Sea Adriatic Sea	Delaware River	Hudson and Delaware Rivers	St. John River
Gironde River, North Sea		0.004	0.135	0.139	0.139
Mediterranean Sea, Adriatic Sea			0.131	0.136	0.136
Delaware River				0.021	0.008
Hudson and Delaware Rivers					0.021
St. John River					

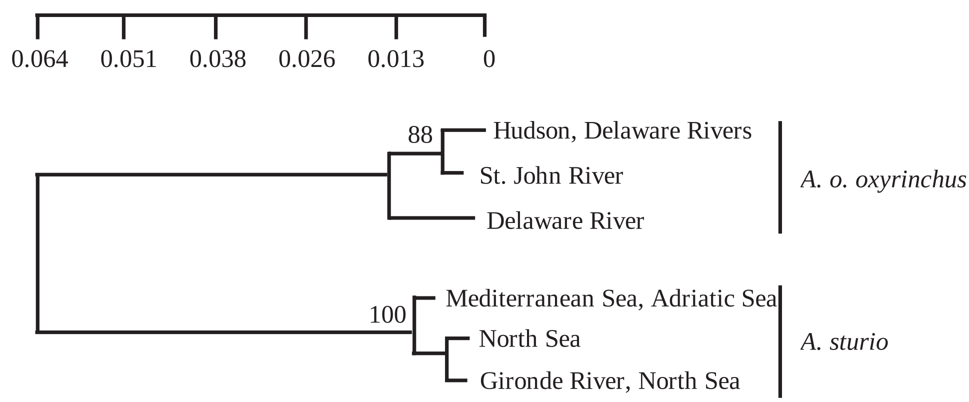

Figure 1. Genetic distance tree using a 250 bp of the mitochondrial D-loop and the tRNA^{Pro} gene basing on the different haplotypes found in this study. The tree was calculated in MEGA (Kumar, Tamura and Nei, 1993) based on p-distance values and neighbour-joining. Bootstrap values > 50 are shown on the branches

Microsatellites

We obtained microsatellites from 38 archival samples of *A. sturio*. The following microsatellites were used for statistical calculations: Afu-19, 34, 39, 54, and 68. The microsatellites Afu-22, 23, 57, 58, 62, and 69 were not included in the analyses since the PCR products were not synthesised or artefacts were formed. The microsatellites Afu-19, 34, 39, 54, and 68 showed polymorphic band patterns in both species, *A. sturio* and *A. o. oxyrinchus*.

In *A. sturio*, five alleles were observed for Afu-19, four for Afu-34, two for Afu-39, three for Afu-54, and four for Afu-68. Allele frequencies of the polymorphic loci in *A. sturio* and *A. o. oxyrinchus*, as well as the differences in the allele frequencies and distribution observed between the North and Baltic Sea specimens of *A. sturio*, are shown in table III. For example, the 121-bp allele of Afu-19 was observed two times: first, in a sturgeon caught in 1910 in the North Sea near the town of Stroemstad, Sweden (NRM 21708), and second, in a sturgeon captured in 1992 in the North Sea off the Dutch coast (Universität Rostock A4). This allele was missing in specimens from the Baltic Sea. On the whole, the following alleles were observed exclusively in the North Sea specimens: the 121 and 124 bp of Afu-19; the 147 bp of Afu-34; the 120 bp of Afu-39; and the 152 bp of Afu-68. On the contrary, the alleles 130 of Afu-19 and 180 of Afu-180 were present exclusively in the Baltic Sea specimens.

Combining the data for the microsatellites, we found 18 alleles in all archival specimens. In contrast, the German broodstock showed only eight alleles (table IV). The following alleles were missing: the 121, 127, and 130 bp of Afu-19; the 140 and 144 bp of Afu-34; the 120 bp of Afu-39; the 180 bp of Afu-54; the 128, 136, and 152 bp of Afu-68. The 136-bp allele of Afu-68, which was missing in the German broodstock, was the most common allele of this locus in archival samples. Possibly, if more specimens from the Gironde River population had

Table III. Allele frequencies of polymorphic loci observed in archival samples of *A. sturio* and in *A. o. oxyrinchus*

		A. sturio		*A. o. oxyrinchus*		
Locus Allele (base pair)		North Sea	Baltic Sea	Delaware R.	Hudson R.	St. John R.
Afu-19		n = 15	n = 9	n = 16	n = 12	n = 60
	118	0.067	0.111	0	0	0
	121	0.133	0	0.062	0.042	0
	124	0.133	0	0.062	0	0.016
	127	0.667	0.778	0.625	0.666	0.742
	130	0	0.111	0	0	0
	145	0	0	0.250	0.292	0.242
Afu-34		n = 15	n = 9	n = 16	n = 12	n = 10
	138	0	0	0.062	0.457	0
	141	0.034	0.111	0.062	0.500	0
	144	0.233	0.167	0.625	0	0.550
	147	0.200	0	0	0	0.050
	150	0.533	0.722	0.125	0	0.400
	153	0	0	0.125	0.042	0
Afu-39		n = 17	n = 7	n = 16	n = 14	n = 60
	120	0.176	0	0.094	0.036	0
	123	0.823	1.000	0.906	0.964	1.000
Afu-54		n = 17	n = 9	n = 16	n = 13	n = 60
	180	0	0.222	0	0	0
	184	0.500	0.611	0.812	0.810	1.000
	188	0.500	0.167	0.156	0.190	0
	192	0	0	0.031	0	0
Afu-68		n = 17	n = 9	n = 16	n = 12	n = 59
	132	0	0	0.031	0	0
	136	0.794	0.833	0.094	0.208	0
	140	0.148	0.111	0.125	0.458	0.364
	144	0.029	0.056	0.281	0.125	0.220
	148	0	0	0.031	0	0.102
	152	0.029	0	0.219	0.208	0.144
	156	0	0	0.156	0	0.017
	160	0	0	0.062	0	0.152

been included in our study, more alleles would have been found within this population.

The length of the microsatellite alleles in *A. o. oxyrinchus* was in the same range as in *A. sturio*. Frequencies of all alleles are shown in table IV. The number of alleles ranged from 14 (Hudson and St. John Rivers) to 22 (Delaware River) (table IV). Specifically, the highest number of alleles was observed for Afu-68 (n = 8), followed by Afu-34 (n = 5).

The following alleles were found exclusively in *A. sturio*: the 128 bp of Afu-68; the 180 bp of Afu-54; and the 118 and 130 bp of Afu-19. The alleles 138 and 153 of Afu-34; 192 of Afu-54; and 132, 148, 156, and 160 of Afu-68 were present in *A. o. oxyrinchus*, but not in *A. sturio*.

DISCUSSION

Analyses of archival samples

Until now, the only analyses of genetic variability at the population or subpopulation level among fishes using archival samples have been performed on the Atlantic salmon *Salmo salar* L., 1758 (Nielsen, Hansen and Loeschcke, 1997, 1999). Therefore, ours is the first detailed research on genetic variability of extinct sturgeon stocks. Previously, Ferguson and Duckworth (1997) studied only a few *A. fulvescens* specimens from a museum collection in London. However, our results on DNA extraction and amplification were similar to those of Nielsen, Hansen and Loeschcke (1997), who had an extraction and amplification rate in the case of archival samples of the Atlantic salmon of more than 90 %. The extraction and amplifica-

Table IV. Distribution of alleles observed in *A. sturio* and *A. o. oxyrinchus*. ([1]): The broodstock kept at the Institute of Freshwater Ecology and Inland Fisheries (Berlin) originated from artificial reproduction of two *A. sturio* individuals caught in the Gironde River basin in France (Williot *et al.*, 1997)

	A. sturio		*A. o. oxyrinchus*		
	Archival samples 1823-1996	Broodstock (Berlin)[1]	Delaware River	Hudson River	St. John River
Locus	n = 38	n = 27	n = 16	n = 14	n = 60
Afu-19	118, 121, 124, 127, 130, 145	118, 145	121, 124, 127, 145	121, 127, 145	124, 127, 145
Afu-34	141, 144, 147, 150	147, 150	138, 141, 144, 150, 153	138, 141, 153	144, 147, 150
Afu-39	120, 123	123	120, 123	120, 123	123
Afu-54	180, 184, 188	184, 188	184, 188, 192	184, 188	184
Afu-68	128, 136, 140, 152	140	132, 136, 140, 144, 148 152, 156, 160	140, 144, 148, 156	140, 144, 148, 152, 156, 160
Overall loci	19	8	22	14	14

tion rate for archival samples of *A. sturio* in our study was 25 % for mtDNA, and about 86 % for microsatellites.

Our data support the statement of Nielsen, Hansen and Loeschcke (1997) that archival samples are useful sources of information for tracking genetic changes in fish populations, and are therefore valuable for conservation and restoration programmes. The analysed archival samples provided us with a unique opportunity to review the historical structure of the gene pool of *A. sturio* and the dynamics of its change.

Genetic variability and conservation aspects

Sturgeon from the Delaware and Hudson River populations are part of a mixed-stock of *A. o. oxyrinchus* that is located along the North American Atlantic coast and reproduces mainly in the Hudson River (Waldman, Hart and Wirgin, 1996; Waldman and Wirgin, 1998; Secor and Waldman, 1999). The existence of native Delaware River individuals is questionable. However, the large number of alleles that we found in specimens from the Delaware River population points to possible crosses between different strains or to a hybrid origin of this stock. It is doubtful that the small number of alleles observed in specimens from the St. John River population reflects the entire genetic variability in this population: specimens analysed in this study resulted from artificial reproduction of a limited number of breeders. The inclusion of more specimens would probably result in a higher number of microsatellite alleles in sturgeon from the St. John River population. Our data on the analysis of mtDNA support this assumption. We detected only one mitochondrial haplotype in the St. John River sturgeon, and two haplotypes in individuals from the Delaware/Hudson population.

In contrast to Miracle and Campton (1995), but in agreement with Brown, Beckenbach and Smith (1992) and Brown *et al.* (1996), we observed no heteroplasmy in *A. o. oxyrinchus*. Brown *et al.* (1996) described one specimen of *A. o. oxyrinchus* showing three repeated units. In our study, we found a single *A. sturio* showing three repeated units. However, a very low rate of length variation and the absence of heteroplasmy in *A. sturio* and *A. o. oxyrinchus* indicate a genetically-based mechanism of restriction which prevents the inclusion or deletion of repeated units described in other American sturgeon species (Buroker *et al.*, 1990; Brown, Beckenbach and Smith, 1992; Brown *et al.*, 1996).

Our analyses of archival samples of *A. sturio* showed a decline in genetic variability between 1823 and 1992. These data illustrate the coincidence between the decline in genetic diversity decrease and periods of rapid decrease in population size or population crashes. Obviously, the drop in population size led to a fixation of particular alleles in the populations of surviving individuals. For example, the 120-bp allele of Afu-39 was observed in only four sturgeon caught between 1870 and 1890 in the Elbe River and the North Sea, as well as in two specimens of unclear origin. One specimen (MNHM 4634) was labelled with the sampling location “Russia before 1870”, and the sampling location of the second specimen caught between 1798 and 1806 was unknown (NRM 94). The aforesaid allele has been missing since 1890. The following seven microsatellite alleles were also missing after 1950: the 124, 127, and 130 bp of Afu-19; the 144 bp of Afu-34; the 120 bp of Afu-39; the 180 bp of Afu-54; and the 128 bp of Afu-68. Additionally, the mitochondrial haplotypes found in the Mediterranean and Adriatic Sea populations, as well as one from the North Sea individuals, were not found after 1950, either.

Overfishing and damming of rivers were the most significant factors that caused sturgeon population crashes (Birstein, 1999). Currently, *A. sturio* is on the verge extinction in German waters (Debus, 1995). In the 19th century it was the most economically valuable fish species in Germany (Debus, 1995; Spratte and Rosenthal, 1996). The last catches of *A. sturio* individuals –during the spawning migration of sturgeon into the Eider River, a tributary of the North Sea– were reported in 1969 (Spratte and Rosenthal, 1996).

At present, *A. sturio* inhabits only the Gironde-Garonne-Dordogne River basin in France (Holčík *et al.*, 1989; Birstein, Betts and DeSalle, 1998). Unfortunately, no data are available on the genetic structure of the Gironde River population. The 27 individuals representing the first generation of two sturgeons caught in French waters in the early 1990s had eight of 19 alleles found in the archival samples. These individuals represent the broodstock for potential restoration of *A. sturio* in German waters. Therefore, restoration of *A. sturio* in German waters using juveniles produced from these fish would be based on a maximum of about 42 % of the original genetic variability.

The microsatellite data are a valuable genetic characteristic of a sturgeon broodstock for establishing a breeding programme to safeguard the existing genetic variability. The genetic screening of specimens reared at the Cemagref facility in France and a joint German-French breeding programme are important to increase the chances of a successful restoration of *A. sturio* in German waters, as well as for successful stocking of this species in French waters. It is crucial for the restoration programmes to focus their future genetic analyses on the entire stock of the artificially produced *A. sturio* as well as on the wild individuals caught in the Gironde-Garonne-Dordogne River basin in France.

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Decline of the Atlantic sturgeon *Acipenser sturio* L., 1758 in Poland: An outline of problems and prospects

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ABSTRACT

Historical data on the occurrence and exploitation of Atlantic sturgeon and the causes behind its decline were reviewed, using publications from the 18th to the 20th centuries. The Atlantic sturgeon *Acipenser sturio* L., 1758 in Poland was originally found in the Vistula and Oder Rivers and their tributaries, to the upper parts of their watercourses. Over the following centuries, its range has been greatly reduced, and by the mid-1960s it had completely disappeared from Polish waters. The last sturgeon were caught in the lower Vistula River in 1965. Today, the species is considered extirpated. From the Middle Ages, the Atlantic sturgeon was intensively caught in rivers and lagoons, mainly by floating nets. The period of maximum fishery exploitation in the lower Vistula River and Gulf of Gdansk was about 1500, and the first symptoms of sturgeon overexploitation occurred around 1600. The main reasons for stock decline in Polish rivers are, chronologically: (1) long-term overexploitation; (2) water extraction; (3) hydraulic engineering (including stream regulations, damming and construction of harbours; and (4) industrial and agricultural pollution. At the end of the 19th century, the accumulated and prolonged influence of these factors reached a critical level. Legal regulations for sturgeon protection, introduced from the 19th century, did not stopped its decline. In the last decade, a new programme for the Atlantic sturgeon's restoration in Polish rivers has been proposed, based on the experience in production of other acipenserid species under controlled conditions. The main idea of the programme is to establish some parts of former spawning populations in the Oder and Vistula River systems by introducing fish reared artificially. This proposition has not been implemented due to the lack of spawners required to produce stocking material, a requisite for the establishment of reproductive populations.

Key words: Distribution, exploitation, conservation, North Sea.

RESUMEN

Declive del esturión atlántico* Acipenser sturio *L., 1758 en Polonia: un esbozo de problemas y perspectivas

Se revisaron los datos históricos de presencia y explotación del esturión atlántico Acipenser sturio *L., 1758 y las causas de su declive, utilizando publicaciones de los siglos* XVIII *a* XX. *El esturión atlántico en Polonia se encontraba originalmente en los ríos Vístula y Oder y sus afluentes hasta las partes superiores de sus cursos de agua. En los últimos siglos su distribución se ha reducido de forma importante, y a mediados de la década de los sesenta desapareció completamente de las aguas polacas. El último esturión se capturó en el tramo inferior del río Vístula en 1965 y hoy la especie se considera extirpada. Desde la Edad Media el esturión atlántico fue capturado intensamente en ríos y lagunas, principalmente con redes flotantes. El periodo de máxima explotación pesquera en el río Vístula inferior y en el golfo de Danzig tuvo lugar hacia 1500, y los primeros síntomas de sobreexplotación del esturión se manifestaron alrededor de 1600. Las principales razones para el declive del stock en los ríos polacos son, cronológicamente: (1) la sobreexplotación a largo pla-*

zo; (2) la extracción de agua; (3) la ingeniería hidráulica (incluidas las regulaciones fluviales, la construcción de presas y la de puertos), y (4) la contaminación industrial y agrícola. A finales del siglo XIX, la influencia acumulada y prolongada de estos factores alcanzó un nivel crítico. Las regulaciones legales para la protección del esturión, introducidas desde el siglo XIX, no detuvieron este declive. En la última década se ha propuesto un nuevo programa para la recuperación del esturión atlántico en los ríos polacos, basado en la experiencia en la producción de otras especies de acipenséridos en condiciones controladas. El planteamiento principal del programa es establecer algunas partes de las anteriores poblaciones reproductoras en las cuencas de los ríos Vístula y Oder introduciendo peces criados artificialmente. Esta propuesta no se ha podido llevar a cabo debido a la falta de los reproductores necesarios para producir el material a repoblar, un requisito para el establecimiento de las poblaciones reproductivas.

Palabras clave: *Distribución, explotación, conservación, Mar del Norte.*

INTRODUCTION

The Atlantic sturgeon *Acipenser sturio* L., 1758 inhabited the coastal waters of Europe from the Peczora River to the Rioni River in the Black Sea. Being an anadromous fish, with a wide range for feeding migrations, the species has always been subjected to intensive catches. Historic sources reveal mass and wasteful exploitation of this species throughout Europe (Mohr, 1952). Continously decreasing abundance of all European sturgeon populations were noted at the turn of the 19th and 20th centuries. The present paper describes the course of and identifies the main reasons for the disappearance of the sturgeon in Polish waters.

CHANGES IN STURGEON OCCURRENCE IN POLAND

Until to the end of the 19th century (figure 1)

Publications from this period recorded the occurrence of sturgeon from these waters, which might be considered as its maximal area of natural distribution. The sturgeon was a common fish in the Polish Baltic Sea until the second half of the late 19th century (Rzaczynski, 1721, 1736; Kluk, 1780; Bloch, 1784; Rathke, 1824; Zawadzki, 1840; Klinsmann, 1848; Heckel and Kner, 1858; Siebold, 1863; Holland, 1871; Benecke, 1881; Möbius and Heincke, 1883; Nowicki, 1889; Walecki, 1889). The species occurred massively in the Vistula River (Rzaczynski, 1721, 1736; Kluk, 1780; Walecki, 1863; Jachno, 1867; Taczanowski, 1877; Nowicki, 1879, 1880, 1889; Sobieszczanski, 1894; Fiszer, 1896; Anon., 1898b), the Oder River (Bloch, 1784; Gloger, 1833; Heinrich, 1856; Holland, 1871; Borne, 1882; Anon., 1898a), and in some rivers (e.g. the Pasleka) entering the Vistula lagoon (Anon., 1899). The Oder and Vistula Rivers, together with their tributaries, were major spawning grounds of the Baltic sturgeon populations. Their estuaries were major feeding grounds. According to Polish literature, the sturgeon was found throughout the Vistula River. The species has also been recorded in its tributaries: the San River (Ladowski, 1804; Heckel and Kner, 1858; Walecki, 1863, 1864; Nowicki, 1879, 1889; Wilkosz, 1896), the Dunajec and Wisloka Rivers (Nowicki, 1879, 1880), the Wislok River (Nowicki, 1880), the Bug, Narew and Drweca Rivers (Walecki, 1864; Anon., 1883) and the Bzura River (Walecki, 1863). Grabda (1968) supposed that the main spawning grounds of sturgeon were located in the San River, and right-bank tributaries of the upper Vistula River. In the Oder River, sturgeon occurred as far as Raciborz. The spawning grounds were probably located in the regions of Olawa and Brzeg (Przybyl, 1976). From the Oder River, sturgeon entered the Warta River (Rzaczynski, 1721; Walecki, 1864; Borne, 1882; Anon., 1898a) up to near Kolo. The main spawning grounds of this population were found in the middle Warta River, south of Poznan, and in the Gwda and Drawa Rivers (Schulz 1911).

The period 1900-1945 (figure 2)

Publications from this period presented some information on declining abundance in particular regions, changes in occurrence, and discussion of possible reasons. Information about occurrence of sturgeon in the Baltic Sea and its gulfs is cited in Seligo (1902), Demel (1924, 1925, 1927, 1933), Jakubski (1924), and Kulmatycki (1932a). Yet at

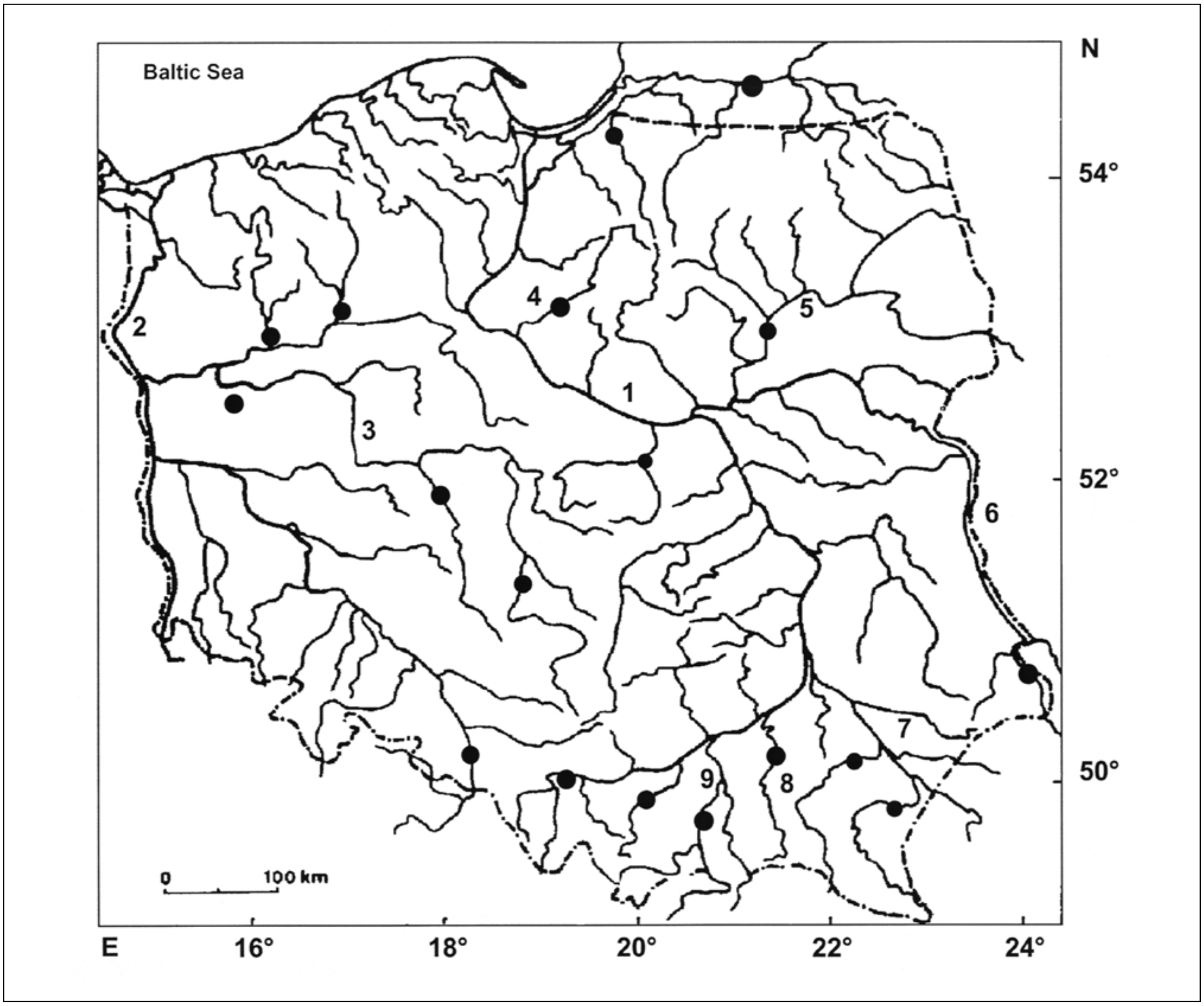

Figure 1. Occurrence of *A. sturio* in Poland before 1900. Black dots show the uppermost known locations in particular rivers recorded by different sources in the literature. Rivers: (1): Vistula; (2): Oder; (3): Warta; (4): Drweca; (5): Narew; (6): Bug; (7): San; (8): Wisloka; (9): Dunajec

the beginning of the 20th century, sturgeon was caught in the Vistula River in “considerable quantities” (Seligo, 1902, 1906, 1919, 1920; Wilkosz, 1904; Berg, 1911); however, later it declined rapidly (Kowalski *et al.*, 1910; Sasorski, 1922; Anon., 1923; W. J. S., 1929; Kulmatycki, 1926; Sakowicz, 1931; Blazejewski, 1934). The species presence was still confirmed in Vistula River tributaries, such as the Drweca (Seligo, 1902), Dunajec (Anon., 1925), and San Rivers (Kowalski *et al.*, 1910). After World War I, the main sturgeon spawning site was in the Drweca River (Grabda, 1968). In the Oder River, sturgeon occurrence was already considerably rarer (Anon., 1900, 1919, 1936; Szmyt, 1904; Pax, 1916, 1921, 1925a, 1925b; Wunder, 1933). A similar situation was noted in the Warta River (Kornaszewski, 1907; Kowalski *et al.*, 1910; Schulz, 1912; Kulmatycki, 1926, 1932b, 1936; and Sakowicz 1930).

The period 1945-1965 (figure 3)

Publications from this period recorded a decreasing number of specimens in catches. Sturgeon occurrence was sporadically recorded in the Pomeranian Gulf (Kraczkiewicz, 1967; Grabda, 1968; Grabda and Waluga, 1968), in the Szczecin lagoon (Kozikowska, 1957; Gasowska, 1962), and in the Gulf of Gdansk (Staff, 1950; Gasowska, 1962; Rudnicki, 1963, 1966; Grabda, 1968; Turoboyski,

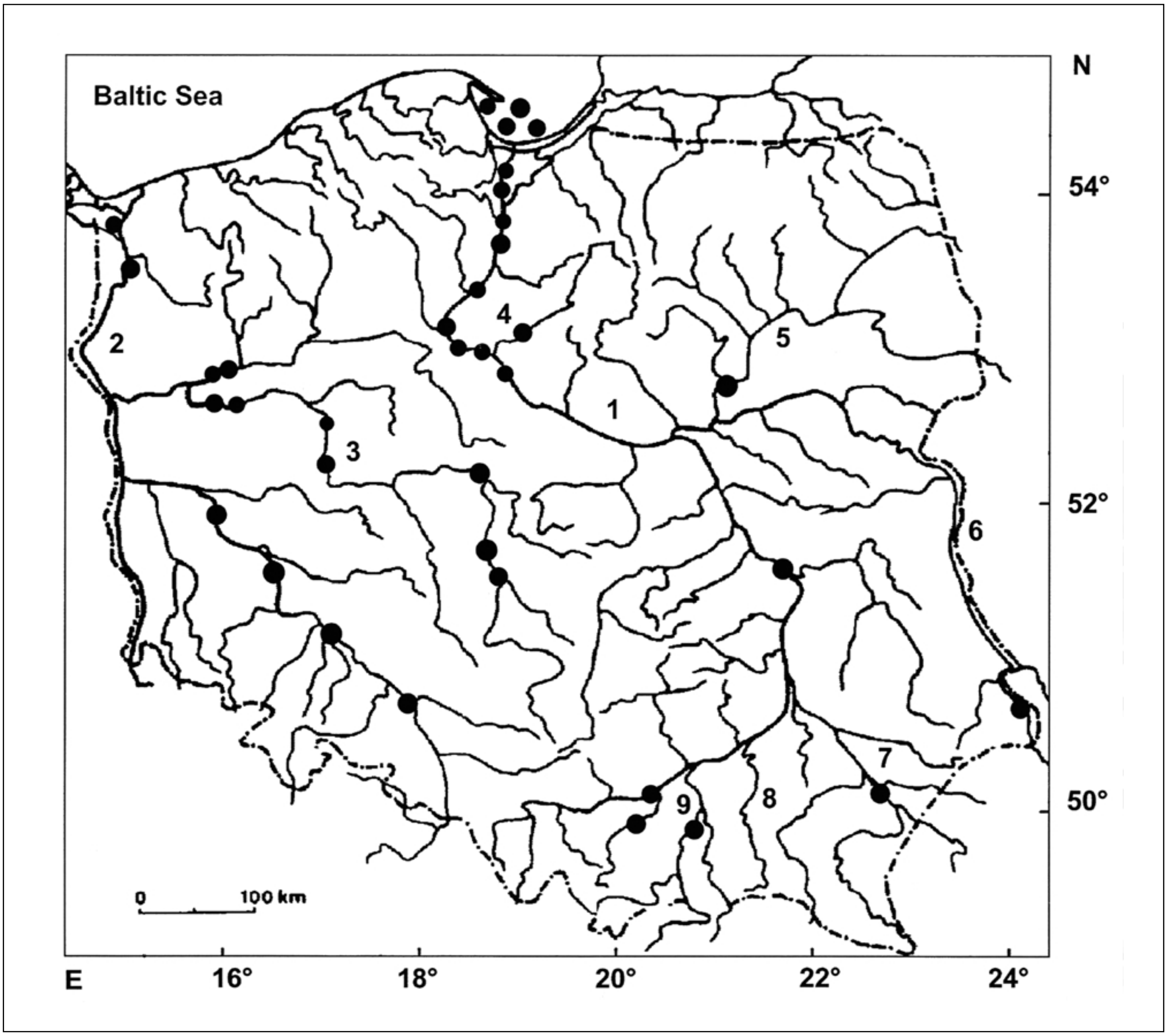

Figure 2. Occurrence of *A. sturio* in Poland between 1900 and 1945. Black dots show where groups or single sturgeon were caught, as recorded by different sources in the literature

1968). Isolated specimens were still caught in the lower and middle Vistula River (Chrzanowski, 1947; Poczopko, 1955; Kujawa, 1962; Rudnicki, 1963, 1966; Zelechowska, 1964; Kajzer, 1966; Grabda, 1968) and in the lower and middle Oder River (Kardaszewski, 1947; Kaj, 1948). They were also recorded in Oder River tributaries, such as the Gwda and Reda Rivers (Kardaszewski, 1947) and the Warta River (Kaj, 1948; Jaskowski, 1962; Rudnicki 1966), as well as Vistula River tributaries, such as the Drweca (Grabda, 1968), Narew (Staff, 1950; Rolik, 1959; Rudnicki, 1963, 1966; Anon., 1965) and San Rivers (Staff, 1950; Anon., 1956; Rolik, 1959; Rudnicki, 1963).

MAIN CAUSES OF STURGEON POPULATION DECLINE

Intensive exploitation

From the earliest times the Vistula lagoon and Gulf of Gdansk were an area of intensive sturgeon exploitation. During the 10th-12th centuries, sturgeon was one of the main fishes caught in Gdansk (Lega, 1949). Excavations of a former fishery settlements there, dating from the 10th-14th centuries, found gigantic quantities of sturgeon bony plates, in a good state of preservation (Urbanowicz, 1965). About 80 % of the fish remains, from layers

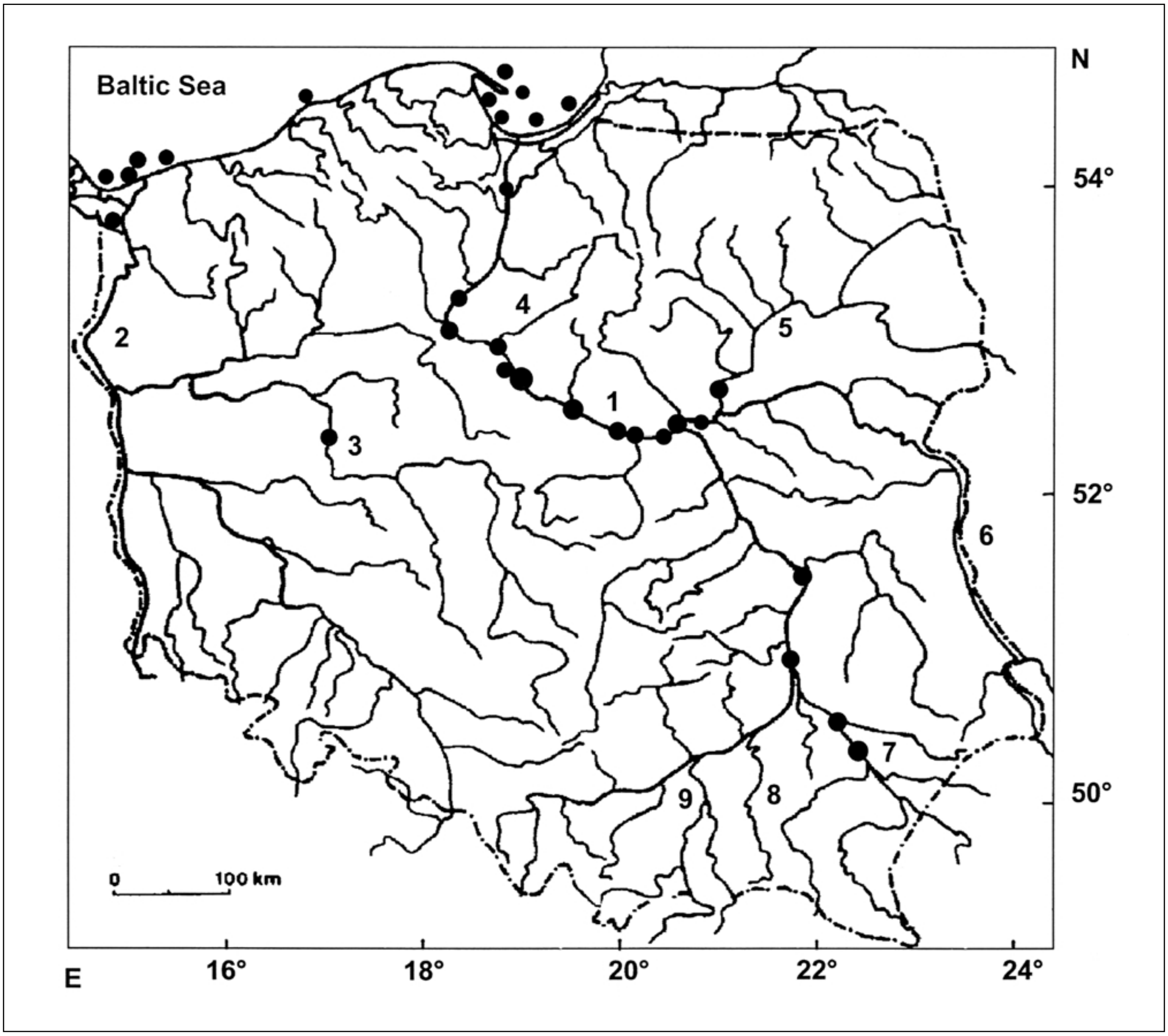

Figure 3. Occurrence of *A. sturio* in Poland between 1945 and 1965. Black dots show where single sturgeon specimens were caught, as recorded by different sources in the literature

of the 13th and 14th centuries, originated from sturgeon, mostly from specimens with a length of about 200-300 cm (Jazdzewski, 1954). The most important route in sturgeon migration between the lower Vistula River and Vistula lagoon must be considered the Nogat River. In 1395, after filling the Nogat River channel with sand from the Vistula River, the disappearance of sturgeon in catches from the lower course of the Vistula was noted (Olszewski, 1948). For many centuries, sturgeon was an object of intensive exploitation, reaching its maximum in the 16th-17th centuries. At that time, sturgeon catches and trade were monopolised by the city of Gdansk, and caviar was exported to England, France, and Lithuania (Ropelewski, 1963). Pilawa was the main centre of fishing and trade for the Vistula lagoon. In the early 18th century an increased quantity of small sturgeons was noted in the catches, a first sign of overexploitation. With the end of the 18th century, catches of sturgeon in Vistula lagoon and the Gulf of Gdansk lost all importance (Ropelewski, 1963). In the 19th century, the main sturgeon fishery became the outlet of the Vistula River.

In the Warta River (Oder River drainage) a period of increased sturgeon catches was reported in 1892-1894 (Grotrian, 1896). Fishes were most often caught in the region between Oborniki and Srem

(Kornaszewski, 1907). Grotrian (1896 after Przybyl, 1976) hypothesises that wasteful catching of spawned sturgeon from the Warta River caused the onset of the fishery's decline in that area.

In the mid Vistula River, the most intensive sturgeon fishery was between the cities of Pulawy and Zawichost, and between Nieszawa and Ciechocinek. In the 1850s and 1860s, sturgeon was the leading fish on the Warsaw market. At that time, Vistula sturgeon were the main source of caviar sold in St. Petersburg (Walecki, 1864). At the end of the 19th century, a stable sturgeon fishery still existed in the Gulf of Gdansk and Vistula lagoon. Fishes were caught using special nets, called *Störlanken* (Anon., 1886). The weight of individuals caught ranged from 25 to 35 kg, and length from 170 to 190 cm (Anon., 1887). The Vistula River sturgeon fishery rapidly declined at the turn of the 19th century. According to Grabda (1968), intensification of this decline was recorded in 1905-1908. The situation is well illustrated by catch data from the outlet of the Vistula River and Gulf of Gdansk, according to Zelechowska (1964) (figure 4). In later years, sporadic sturgeon catches were recorded in the lower Vistula River. Most often, there were individuals about 20-30 cm in average length. To catch sturgeons in this region, trap nets (sturgeon traps) have been used, whereas above the city of Torun, sturgeon were caught in floating nets, called *dryga*. According to official data, in 1919-1930 in the lower Vistula River, 39 sturgeon were caught, with a total weight of 3 774 kg (Grabda, 1968). According to Rudnicki (1963), in the middle Vistula River, in 1934-1935, about 1 t of sturgeon per year was caught, of which 45 % were caught illegally out of season. After World War II, catch records have been exceptionally rare. Grabda (1968) described 10 such records in the lower

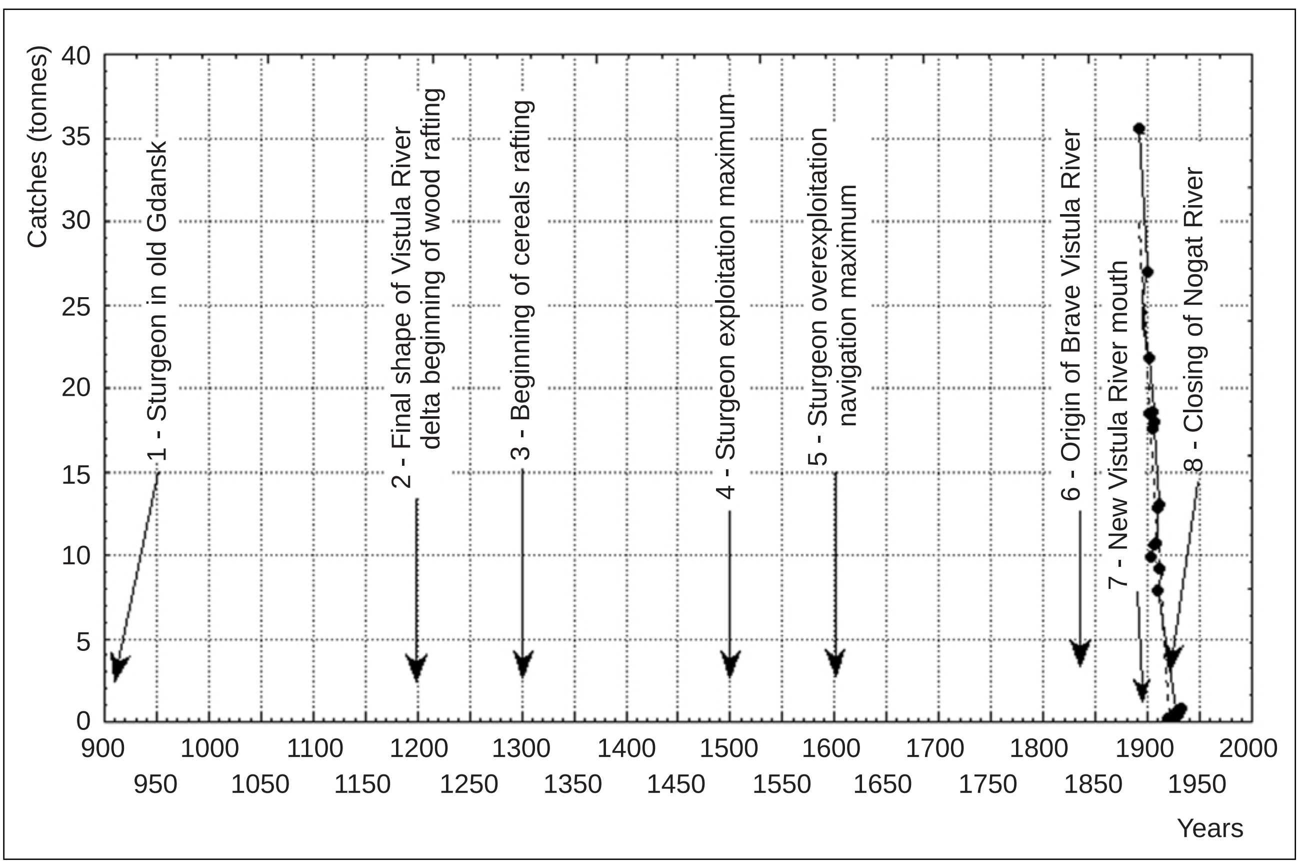

Figure 4. Timeline for *Acipenser sturio* occurrence in the Vistula River. The latest data about sturgeon catches taken from Kulmatycki (1932b) and Zelechowska (1964). Explanations of breaking points: (1): sturgeon comprised 54 % of catches in old Gdansk (Urbanowicz, 1965); (2): final shape of the Vistula River delta (Mikolajski and Wodziczko, 1929), sturgeons comprised 12 % of catches in old Gdansk (Urbanowicz, 1965), beginning of wood rafting (Rybczynski, 1935); (3): beginning of cereals rafting (Rybczynski, 1935); (4): maximum sturgeon exploitation (Ropelewski, 1963); (5): sturgeon overexploitation (Ropelewski, 1963), navigation maximum (Rybczynski, 1935); (6): origin of Brave Vistula River (Rybczynski, 1935); (7): new Vistula River mouth in 1895 (Rybczynski, 1935); (8): closing of the Nogat River (Rybczynski, 1934)

Vistula River. In the Polish zone of the Baltic Sea, approximately 10-15 sturgeons were caught in the postwar period (Grabda, 1968). After 1945 sturgeons were caught or observed 25-30 times (Dyduch, 1979). According to Kolman (1996), after introduction of full specific protection of sturgeon, during 1936-1965, 27 records of catches were officially confirmed in the Vistula River and its tributaries. Their recorded body lengths were over 150 cm, and weight ranged from 95-211 kg. In 1952-1955, 7 sturgeon were observed in the lower Vistula, and 2 in the San and Narew Rivers. Since the second half of the 1960s, no sturgeon catches have been recorded in Polish waters. Certain confusion regarding identification of later sturgeon specimens and where they were sighted was caused by the appearance of other acipenserids in Polish waters, originating from introduction or aquaculture. These specimens (mainly *Acipenser ruthenus* L., 1758; *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833; *Acipenser baerii* Brandt, 1869, or their hybrids) have been found in the Gulf of Gdansk (Bartel, 1968) and the Vistula River (Nabialek, 1974, 1976).

Reconstruction of river beds and navigation

At the beginning of the 13th century, wood exports from Gdansk began, and in the 14th century, cereal exports. This started a period of intensive development of navigation on the Vistula River, and river-bed regulation (Rybczynski, 1934, 1935). Maximum navigation activity was in the 17th century. Hydrotechnical works related with drainage of Vistula delta began in lower the Vistula River in 1856. At present the Vistula is modified partly in its upper course and completely in the lower. Closing of the Drweca River mouth with a dam near Lubicz blocked migration of spawning sturgeons in this river.

Regulation of the Oder River began in the 18th century

Between 1888 and 1895, the river was channelled from Kozle to the Nysa Klodzka River. In 1905-1917, regulation was extended below Wroclaw and 22 sluices were built there. At present, this river is regulated along its entire length. The Warta River was also channelled from the mouth of the Prosna River, as were the Obra and Notec Rivers (after 1869). According to Schulz (1911), steamship navigation was a major destructive influence on sturgeon spawning grounds in the Warta River. Kulmatycki quoted the opinion of fishermen from the Warta River, who said that the disappearance of sturgeon in the river was connected with its channelisation. This began in 1819. In 1896, the Warta River was dredged to the mouth of the Prosna River, which had an impact on sturgeon spawning grounds. As a result of the construction of the Bydgoski Channel (1774), the lower Notec River was transformed between 1891-1898 into a navigable channel with 11 sluices, cutting off sturgeon spawning grounds in the Gwda River (Przybyl, 1976). Blocking of passage to the spawning grounds resulted in a dramatic decline in the effectiveness of natural reproduction. Specimens living individually often died during the spawning period, as a result of negative changes caused by the resorption of unresorbed sexual products (Grabda and Waluga, 1968).

Pollution

Kulmatycki (1932b) highlighted the convergence of sturgeon disappearance with the development of industry. In waters of Greater Poland and Pomerania, this took place at the turn of the 19th and 20th centuries. Greater Poland concentrated over 55 % of all production of agriculture industry of Poland (Kulmatycki, 1929). In the Oder River basin, intensive development of industry (textile workshops, metal workshops, paper-mills, smelting works of iron and glasses) began in the 17th century (Maleczynski, 1963). Rapidly increasing quantities of sewage coal and chemical wastes had significant influence on spawning and feeding grounds of young sturgeons (Starmach, 1951).

Changes in the mouth of the Vistula River (figure 5)

The Vistula River delta gained its shape about 1200 (Mikolajski and Wodziczko, 1929). To the end of the 14th century, the Nogat and Vistula Elblaska Rivers were the main outlets of the Vistula (Rybczynski, 1935). From 1550, 87 % of Vistula waters passed through the Nogat, whereas by 1600 this had decreased to 75 %. Further decline took place around 1800, with only 60 %, and in the first half of the 19th century, only about 30 % of Vistula runoff was associated with the Nogat outlet (Mikulski, 1963). Sturgeon at that time could enter the Vistula through 15 branches. In 1840, the Vistula River broke a belt of dunes and formed a new outlet to sea (Brave Vistula). In 1895, the new mouth was constructed through the Schievenhorst dunes. The Gdansk and Elblag arms of the river were closed with sluices. In 1915, the Nogat River was closed with a sluice, as well (Rybczynski, 1934). For the sturgeon population, only one entry to the Vistula remained. Fishes feeding in Vistula lagoon lost previous connecting arms to the river. According to the opinion former fishermen, reconstruction of the Vistula River mouth in 1895 caused the disappearance of sturgeon in its lower course (Grabda, 1968).

According to Dyduch (1979), the following negative factors, in chronological order, influenced sturgeon populations in Poland: (1): long-term overexploitation (Michalski, 1967); (2): water extraction; (3): hydraulic engineering (Kulmatycki, 1932b); (4): change of hydrobiological conditions-pollution of water in rivers (Starmach 1951); and (5): changes of feeding conditions in the Baltic Sea.

At the end of the 19th century, the accumulated and prolonged influence of these factors reached a

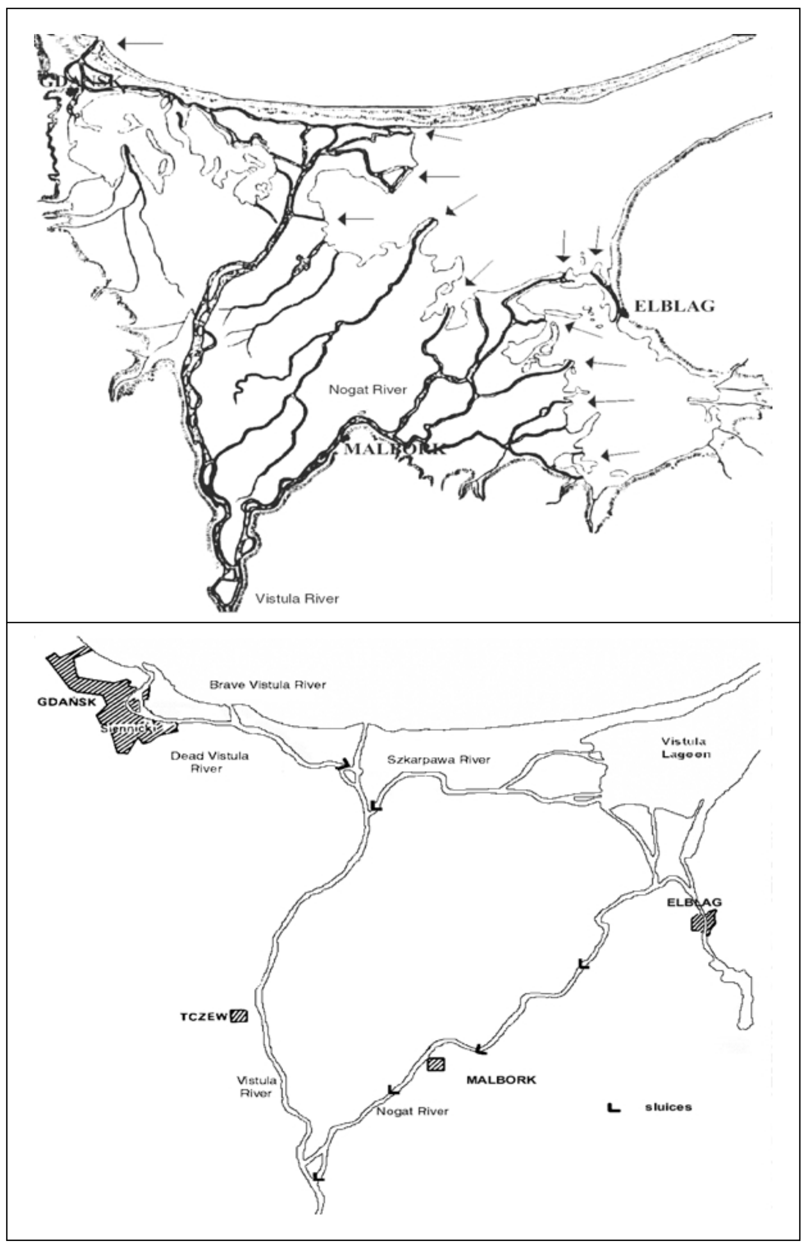

Figure 5. Changes in the Vistula River delta. Upper section-delta about 1300 (from Bertram, after Samsonowicz, 1967, modified). Lower section-delta in 1935 (after Rybczynski, 1935). Arrows indicate entries to the Vistula River for sturgeons

critical level for the last Polish sturgeon population (figure 5).

LEGAL PROTECTION AND PROJECT OF RESTORATION OF THE SPECIES

In the Middle Ages, a factor limiting common pressure on sturgeons was the *regale,* e.g. the royal monopoly on catching of fishes with large nets, and on special fish species (Lega, 1949). The rule of navigation's priority over fishery was very important. The ban on stable fishing devices (e.g. dams), blocking or partitioning the river indirectly favoured the protection of migrating sturgeons. In connection with intensive navigation development in the Oder River, Silesian princes forbade partitioning of rivers as early as 1337. This prohibition was renewed in 1349, 1355, and 1375 (Tobiasz, 1962). The Teutonic Order effected a limitation for Oliwa monastery, forbidding putting out sturgeon nets at a distance of about 840 m from the Vistula outlet (Grabda, 1971). Statutes of Piotrków from 1447 implemented the freedom of navigation and the prohibition of partitioning of the Dunajec, Wislok, San, Wieprz, Tyosemienica, Bug, Narew and Nida Rivers (Kutrzeba, 1918). According to 19th century Prussian legislation, sturgeon was protected from 15 July to 31 August. In 1932, the Minister of Agriculture instituted the first rules of sturgeon protection in Poland. At first, there was seasonal protection from 15 July to 15 August. This ban was then moved to an earlier period (1 June to 31 July). Additionally, individuals below 100 cm length were protected throughout the year. Simultaneously, authorities of the Free City of Gdansk introduced protection of sturgeon in the lower Vistula and the Gulf of Gdansk from 1 July to 31 August. However, this was partial protection only, because fishes migrated to spawning grounds as early as April, and from March to July the catches were greatest (Kulmatycki, 1932b). The law did not protect sturgeon during migrations to and from spawning grounds. Rudnicki (1963) estimated that in 1934-1935, only half of caught fish were registered in official data. From 1 October 1936, sturgeon in Poland became fully protected. This protection was confirmed in all following laws, which are still in force (from 1 April 1995).

Only one attempt at reproduction and artificial farming of sturgeon was undertaken, by Prof. A. Seligo in 1906-1907. He created a station for sturgeon reproduction at Tczew (Kulmatycki, 1932b). The undertaking did not have favourable results, because of problems with simultaneously acquiring males and females to reproduction.

In 1994, scientists from a few centres in Poland worked out a project for recovering migrating fishes, including sturgeon (Sych, 1996). On the basis of this project, sturgeon recovery in the Oder and Vistula River systems has been proposed, based in part on former migration routes. For the Oder River system, they propose restitution between the Oder-Warta-Notec to the Drawa and Gwda Rivers. The Vistula River system would have to be connected with the lower Vistula and Drweca Rivers. Recovery of sturgeon is still distant, because of the deficiency of stocking material (Kolman, 1996). A project for recovery of migrating fishes was submitted to the government of Poland in 1996.

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Early culture of the American Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815 and preliminary stocking trials

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ABSTRACT

We performed rearing studies with first-feeding fry and fingerling American Atlantic sturgeons *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815 of Hudson River parentage. Sturgeons were reared at initial densities of 3.7-22.2 fish/l and offered live *Artemia* sp., frozen *Artemia* sp., or a formulated diet (Biokyowa). After 26 days, mean specific growth rate was inversely proportional to fish density and ranged from 4.9-11.1 % per day. Fish fed frozen artemia were smaller but had the same survival rate as those fed live artemia. Sturgeons converted to a formulated diet with < 25 % mortality at mean length and weight (sd) of 34.5 mm (3.0) and 182 mg (50). Treatments of fingerlings established at initial densities of 0.37-2.22 g/l and fed a formulated diet (Zeigler) for 28 days exhibited mean percent survival (sd) of 87.0 (0.0) to 93.3 (2.3) and had feed conversion factors of 0.50 or less. Our study showed that first-feeding American Atlantic sturgeons require low initial rearing densities (7.4 fish/l or less) and 20-26 days of continuous live artemia to facilitate conversion to formulated feed. Fish reared similarly were released into the Hudson River in 1994 and into the Nanticoke River (a Chesapeake Bay tributary) in 1996 to evaluate survival and estimate wild recruitment. Sampling in the Hudson River from 1995 through 1997 showed that hatchery fish comprised 35-53 % of the total juvenile catch. Evaluation of fish released in the Nanticoke River from 1996 through 1998 showed that hatchery fish spread throughout Chesapeake Bay, made up 62 % of the total American Atlantic sturgeon catch, and had similar length-weight relationships as wild fish.

Key words: Density, specific growth rate, diet conversion, stocking evaluation.

RESUMEN

Primeras fases del cultivo de esturión atlántico americano* Acipenser oxyrinchus oxyrinchus *Mitchill, 1815 e intentos preliminares de repoblación

Hemos realizado estudios de cultivo con juveniles en estadios iniciales y más avanzados de esturión atlántico americano Acipenser oxyrinchus oxyrinchus *Mitchill, 1815 de linaje procedente del río Hudson. Los esturiones fueron cultivados a densidades iniciales de 3,7-22,2 peces por litro y alimentados con* Artemia *sp. viva,* Artemia *sp. congelada, o con una dieta formulada (Biokyowa). Después de 26 días, la tasa específica de crecimiento media fue inversamente proporcional a la densidad de peces y varió entre 4,9-11,1 % por día. Los peces alimentados con artemia congelada fueron más pequeños, pero tuvieron la misma tasa de supervivencia que los alimentados con artemia viva. Los esturiones transformaron una dieta formulada con menos de 25 % de mortalidad a una longitud y peso medios de 34,5 mm (desviación típica = 3,0) y 182 mg (50). Los tratamientos de estadios más avanzados, establecidos a densidades iniciales de 0,37-2,22 g/litro y alimentados con dieta formulada (Zeigler) durante 28 días, exhibieron un porcentaje de supervivencia medio entre 87,0 (d.t. = 0,0) y 93,3 (2,3) y fueron alimentados con factores de conversión de 0,50 o menores.*

Nuestro estudio mostró que los juveniles en estadios iniciales de esturión atlántico americano requieren densidades de cultivo iniciales bajas (7,4 peces/litro o menores) y 20-26 días seguidos de artemia viva para facilitar la conversión a dieta formulada. Peces cultivados de este modo fueron soltados en el río Hudson en 1994 y en el río Nanticoke (un afluente de la bahía Chesapeake) en 1996 para evaluar su supervivencia y estimar el reclutamiento silvestre. El muestreo en el río Hudson desde 1995 a 1997 mostró que los peces cultivados supusieron el 35-53 % de las capturas totales de juveniles. La evaluación de los peces soltados en el río Nanticoke desde 1996 a 1998 mostró que los peces cultivados se distribuyeron hacia la bahía Chesapeake, suponiendo el 62 % de las capturas totales de esturión atlántico americano, y tuvieron similares relaciones longitud-peso que los peces silvestres.

Palabras clave: *Densidad, tasa específica de crecimiento, conversión de dieta, evaluación de la repoblación.*

INTRODUCTION

The North American Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815 is considered the closest relative of the European Atlantic sturgeon *Acipenser sturio* L., 1758 through genetic analysis (Birstein and DeSalle, 1998) as well as morphological comparison (Artyukhin, 1995). Both species were distinguished by Magnin (1962) and both suffered severe declines over their respective ranges as a result of over-fishing and dam construction in the late 19th and early 20th centuries. In light of the highly endangered status of *A. sturio* (Williot *et al.*, 1997), experience gained in culture and experimental release of hatchery-reared *A. oxyrinchus* in the United States may serve as a valuable model for *A. sturio* when considering these activities as management tools for restoration. The Fishery Management Plan for Atlantic sturgeon (November, 1990) was adopted in the United States by the Atlantic States Marine Fisheries Commission to provide a framework for restoration of the species over its historical range. In anticipation of possible population management through use of hatchery-reared fish and potential interest in commercial aquaculture, the US Fish and Wildlife Service's Northeast Fishery Center - Lamar, Pennsylvania (NEFC) began developing culture techniques for the species in 1991. As a result of broodstock capture, transport, egg incubation, and rearing experiments at NEFC (McPeck, 1995; Mohler, Fynn-Aikins and Barrows, 1996; Mohler and Fletcher, 1999; Mohler, King and Farrel, 2000), five year-classes of hatchery-produced F1 generation Hudson River stock are maintained along with a number of captive wild fish.

As with many fish species, the success of a programme for restoration stocking or aquaculture depends largely on the development of reliable culture techniques for early life stages. However, development of culture techniques alone is no guarantee that hatchery-reared fish released into the wild will survive and eventually contribute towards rebuilding depleted populations. Many species are rare because of habitat loss and some may be readily propagated in the hatchery, but without suitable sites for their re-introduction into the wild, the role of the hatchery is reduced to that of providing refugia (Rinne *et al.*, 1986). Therefore, our studies were performed to: (1) refine culture parameters for first-feeding fry and fingerlings to optimise survival and growth in the hatchery; and (2) demonstrate the ability of hatchery-reared fish to survive and grow when released into two different drainages within the historical range of the species. Artificially-propagated progeny of wild American Atlantic sturgeons gill-netted from the Hudson River, New York were used in the studies.

REARING EXPERIMENTS

First-feeding fry

Six-day post-hatch sturgeons were reared at initial densities of 3.7-22.2 fish/l and offered: (1) live newly-hatched *Artemia* sp. delivered automatically via timer-controlled bellows pumps at a rate of 3 min feed delivery at 30-min intervals, 24 h/day; (2) newly-hatched frozen *Artemia* sp. hand-fed 3 times/daily (mean daily offering was 4.98×10^5 nauplii); or (3) a commercially-formulated diet offered at 10 % body weight per day (Biokyowa B-250, Biokyowa, Inc., Chesterfield, Missouri) via continuous-operation electronic feeders (model A-100, Double A Brand Co., Dallas, Texas). Feeding was observed at 10 days post-hatch and verified by the presence of artemia nauplii in the digestive tract. Random sampling showed that the number of artemia nauplii offered ranged from 3.82-5.72 $\times$ 10^5 per tank daily. Each treatment consisted of three 54-l circular, plastic tanks with a centre standpipe-drain assembly. Available grazing area on the bottom of each tank was approximately 1 631 cm^2. Water depth in each tank averaged 26.6 cm. Flows were set at 3 l/min, water temperature was 17 ± 1 °C, and dissolved oxygen ranged from 8.1-8.9 mg/l. Specific growth rate (SGR) expressed as percent gain per day was calculated using the following equation (Brown, 1957):

$$SGR = [\mathit{ln}\ (\text{final weight}) - \mathit{ln}\ (\text{beginning weight})] / \text{days} \times 100$$

After 26 days of feeding, mean SGR in live artemia treatments was inversely proportional to fish density and ranged from 4.9-11.1 % per day (figure 1). Fish fed frozen artemia were smaller but showed mean survival similar to those fed live artemia (> 93 %). Mean survival (sd) was lowest in tanks offered Biokyowa at 13.1 % (2.0) (table I). Since our supply of artemia cysts was depleted on day 26, sturgeons were offered only Biokyowa formulated diet (1:1 mix of size B-400 and C-700,

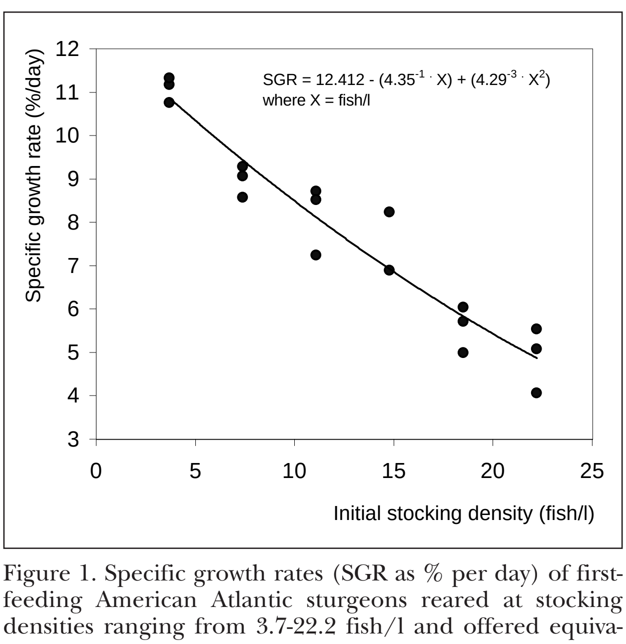

Figure 1. Specific growth rates (SGR as % per day) of first-feeding American Atlantic sturgeons reared at stocking densities ranging from 3.7-22.2 fish/l and offered equivalent amounts of live artemia nauplii for 26 days. The line of best fit is described by a second-order polynomial regression (SGR = 12.412 - (4.35^{-1} X) + (4.29^{-3} X^2) where X = density in fish/l. Dotted lines represent 95 % confidence limits for SGR. Only two replicates established for 14.8 fish/l treatment

Table I. Rearing densities, initial and final weights, feed type, and mortality in first-feeding American Atlantic sturgeon rearing study. Data with different letters are significantly different ($p < 0.05$)

Fry treatments				
Density (fish/l)	Mean weight (mg)		Feed type	% mean survival (sd)
	Initial	Final (sd)		
3.7	17.6	315 (55.7) a	Live artemia	93.8 (1.6) a
7.4	”	182 (49.6) b	Live artemia	96.3 (1.9) a
7.4	”	50 (20.6) e	Frozen artemia	94.7 (2.8) a
7.4	”	90 (25.3) e	BioKyowa B-250	13.1 (2.0) b
11.1	”	149 (45.3) c	Live artemia	96.6 (1.7) a
14.8	”	129 (43.5) d	Live artemia	96.2 (1.4) a
18.5	”	76 (27.5) e	Live artemia	97.7 (0.8) a
22.2	”	64 (22.2) e	Live artemia	96.9 (1.2) a

Biokyowa, Inc., Chesterfield, Missouri, USA) for a subsequent 11-day period at 3 % body weight per day. During this 11-day period, fish in the two lowest density treatments were most successful at diet conversion, showing mortality estimated at < 25 %. Random sampling of 30 fish per tank showed minimum mean length and weight (sd) for the two lowest densities at the time of diet change was 34.5 mm (3.0) and 182 mg (50). This size was achieved in 20-26 days when fry were reared at initial densities of 7.4 fish/l or less (figure 2). All higher density treatments exhibited high mortality during this 11-day period of diet conversion as fish became emaciated and had difficulty maintaining normal swimming posture. Subsequent analysis of a number of these moribund fish showed starvation as the probable cause of mortality (J. Coll, pers. comm.)

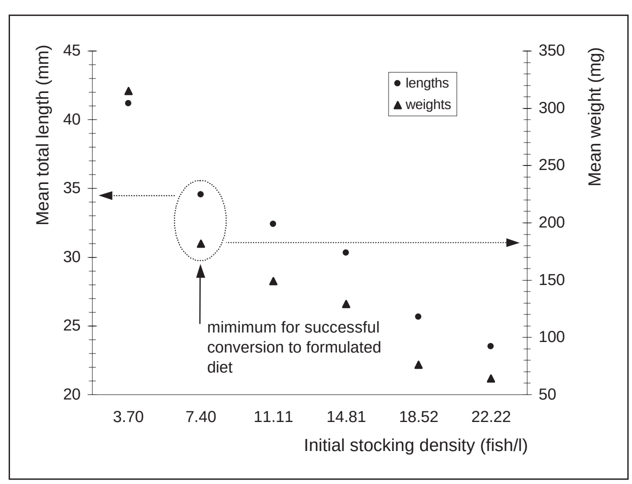

Figure 2. Mean total length (mm) and weight (mg) of first-feeding American Atlantic sturgeons offered live artemia nauplii for 26 days and observed minimum size for successful conversion to formulated diet

Fingerlings

Survivors of the fry study converted to formulated feed were pooled and redistributed at densities of 0.37-2.22 g/l using the same tanks and water quality parameters as above. Sturgeons were fed Zeigler sturgeon diet (Zeigler Brothers, Inc., Gardners, Pennsylvania) at 3 % of body weight/day via continuous-operation feeders. Afer 28 days, survival was 87 % or greater and not significantly different ($p > 0.05$) between density treatments. Feed conversion factor (FC), calculated as weight of feed offered/weight gain of fish, showed that fish reared at the low density were slightly more efficient at converting feed to flesh than those reared at the high density (FC = 0.44 vs. 0.50, respectively) (table II). Random sampling of 22-30 fish per tank showed mean weights (sd) ranged from 3.04 g (2.01) to 3.35 g (1.58) and were not significantly different between treatments ($p > 0.05$) (table II).

Table II. Rearing densities, initial and final weights, mortality, and feed conversion factors in fingerling American Atlantic sturgeon rearing study. Data with different letters are significantly different ($p < 0.05$)

Fingerling treatments				
Density (g/l)	Mean weight (g)		% survival	Feed conversion factor
	Initial	Final (sd)	Mean (sd)	Mean (sd)
0.37	0.48	3.22 (1.49) a	93.3 (2.3) a	0.44 (0.02) a
0.63	"	3.23 (1.69) a	91.3 (4.1) a	0.45 (0.02) ab
1.43	"	3.35 (1.63) a	90.0 (1.0) a	0.49 (0.03) ab
1.72	"	3.04 (2.01) a	87.0 (0.0) a	0.49 (0.03) ab
2.22	"	3.35 (1.58) a	89.3 (1.5) a	0.50 (0.03) b

EXPERIMENTAL STOCKING

Hudson River

In October 1994, 4 927 fish reared on live artemia followed by conversion to formulated feed as described above were marked with a pelvic fin amputation and a coded-wire tag under the first dorsal scute. Fish were then released into the Hudson River by the New York Department of Environmental Conservation to evaluate survival, growth, and estimate wild recruitment (mean length at release was 10.3 cm and mean weight was 4.1 g). Fish were released approximately 40 km downstream of the spawning area from which parental broodstock were captured. Gill-net sampling using stratified random sampling on the Hudson River in 1995 resulted in capture of 15 hatchery-reared fish and 14 wild fish, which were estimated to be the same age as hatchery-reared fish (Petersen, Bain and Haley, 2000). Age estimates of wild fish were based on length and pectoral spine analysis by Dovel and Berggren (1983) as cited in Petersen, Bain and Haley (2000). Assumed age-1 wild fish had greater total length than hatchery-reared fish (mean: 51.3 vs. 38.9 cm). Both marks (pelvic fin amputation + coded wire tags) were found on all hatchery fish, suggesting minimal tag loss. Petersen (1998) also reported results of targeted gill-net capture for juveniles in 1996 and 1997, where nearly 50 % of all juveniles captured were of hatchery origin (12 of 25) and (83 of 182), respectively. Hatchery-reared fish (age-3) captured in 1997 had mean fork length = 60.3 cm compared to assumed age-3 wild fish at 68.5 cm. Likewise, mean weight of captured hatchery-reared fish, at 1 554.7 g, was lower than that of assumed age-3 wild fish, at 2 403.5 g. Out-migration of hatchery-reared fish was documented since three marked fish (pelvic fin amputation + coded wire tag) were captured in the Delaware River estuary in 1997 and re-tagged with visible floy tags. One month later, one of these re-tagged indivuduals was recaptured in the Chesapeake Bay estuary (Bain, 1998). Hatchery-reared fish captured in the Delaware River three years after release clearly showed complete pelvic fin amputations (C. Shirey, pers. comm.)

Nanticoke River

In July 1996, a second experimental stocking was performed with 3 275 age-1 hatchery-reared fish. Fish were reared on live artemia followed by conversion to formulated feed in a manner similar to that described above. In October 1995, about one-half of the fish were transferred to the Maryland Department of Natural Resources, where they were held over the winter, and in June 1996 the remainder were transferred. Due to a heater malfunction, some sturgeons were kept in cold water over the winter of 1995 and ranged from 6-15 cm total length at the time of release. Others held in warm water (in Maryland) during the same time period ranged from 22-36 cm total length at release

(Welch, Skjeveland and Mangold, 1999). Weights of stocked fish were not obtained prior to release. All were marked with either a coded-wire tag or a floy T-bar tag and were stocked at two sites on the Nanticoke River, a tributary of Chesapeake Bay. Evaluation of hatchery-reared sturgeon was achieved via a monetary reward programme administered by the US Fish and Wildlife Service - Maryland Fisheries Resources Office from 1996 through 1998. Commercial fishermen were compensated for holding any live sturgeon obtained as by-catch until verified by programme administrators as either wild or of hatchery origin. Welch, Skjeveland and Mangold (1999) reported that hatchery-reared fish comprised 62 % of the total sturgeon catch (447 of 788). In addition, length-weight relationships for sturgeon ranging from 44.5 and 99.5 cm were similar between wild and hatchery-reared fish, but all sturgeon longer than 100 cm were wild fish. Age verification of wild sturgeon has not been completed to date. Even though stocked at only two sites on the Nanticoke River, hatchery fish showed wide dispersal, being captured throughout Chesapeake Bay (Welch, Skjeveland and Mangold, 1999).

DISCUSSION AND CONCLUSIONS

Rearing experiments

We found the mean minimum size (sd) for successful conversion to formulated feeds in *A. o. oxyrinchus* to be 34.5 mm (3.0) and 182 mg (50). This minimum size can be achieved in 20-26 days using initial rearing densities of 7.4 fish/l or less and a nearly continuous supply of live food (artemia nauplii). Since first-feeding fry were offered equivalent amounts of artemia nauplii regardless of density, the inverse relationship between SGR and stocking density (figure 1) suggests food was a limiting factor as density increased. However, Mohler, King and Farrel (2000) reported that after 26 days, first-feeding fry reared at 18.5 fish/l and offered supplemental artemia nauplii were similar in size to those reared at the same density which received a normal ration. This suggests that another factor limited growth regardless of ration. Observations of American Atlantic sturgeon fry from 1993 to 1998 at NEFC showed that first-feeding fry are substrate feeders, consistent with observations of Bardi, Chapman and Barrows (1998) with fry of the sub-species *A. oxyrinchus desotoi* Vladykov, 1955. There is some evidence that increasing substrate area of rearing tanks may affect sturgeon growth as reported by Sbikin and Budayev (1991) where young *Acipenser gueldenstaedtii* Brandt, 1833 reared in tanks with horizontal shelving had higher growth than those reared in a similar-sized normal tank. An attempt was made to demonstrate a density/grazing area relationship with first-feeding *A. oxyrinchus*, but excessive parasite-induced mortality precluded definitive results (Mohler, King and Farrel, 2000).

In the fingerling rearing experiment, mortality and growth after 28 days was similar in all density treatments (table II) implying that the threshold density which limits growth or mortality was not attained. Therefore, once switched to formulated feed, fingerling sturgeon can be reared at initial densities of at least 2.22 g/l and achieve feed conversion factors of 0.50 or less over a 28-day period. Hatchery-produced *A. o. oxyrinchus* fingerlings reared on live artemia as first-feeding fry then switched over to formulated food with no weaning period demonstrated adaptability in feeding behavior by subsequent exploitation of natural food items during their first three years of liberation, exhibiting growth, survival, and out-migration.

Experimental stocking

In the Hudson River stocking evaluation, hatchery-reared fish were reported as being smaller than their wild counterparts. However, ages of wild fish were not verified, but based on data obtained between 1975-1978 by Dovel and Berggren (1983). In evaluation of the Nanticoke River stocking, weight/length relationships were reported as similar between wild and hatchery-reared fish, but ages of wild fish have not been verified. Regardless of precision concerning size/age comparisons between hatchery-reared and wild fish, both experimental stockings demonstrated that American Atlantic sturgeons reared initially on live artemia with subsequent conversion to formulated diets can survive in the wild for at least 2-3 years and increase in size. Additionally, some individuals stocked into the Hudson River began out-migration at about age 3, as demonstrated by their capture in the Delaware River and Chesapeake Bay.

Long-term evaluation is needed to determine whether stocked fish have imprinted to the watershed of release and will eventually help to rebuild depleted populations through successful reproduction.

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Analysis of partnership and conservation requirements for a threatened species, *Acipenser sturio* L., 1758: Towards the implementation of a recovery plan

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ABSTRACT

The Atlantic sturgeon *Acipenser sturio* L., 1758 is a threatened species whose last sanctuary is the Gironde, Garonne and Dordogne ecosystem. It has been strictly protected throughout France since 1981. However, no significant increase of its population has occurred. Until recently, as with the programme developed for salmon, migratory species restoration plans have mainly been centred on knowledge acquisition and the development of strictly technical programmes. These approaches are insufficient to integrate species survival requirements in environmental management.

The analysis of needs necessary for the recovery of Atlantic sturgeon suggests easily manageable technical aspects, providing proper means are available. On the other hand, far more complex social aspects of recovery can often interfere with the implementation of technical actions. As a matter of fact, aquatic ecosystem management deals with multiple actors, with different concerns and various competencies, which can directly or indirectly influence the achievement of a restoration programme. Motivating each group of actors and including them in a collective project is one of the ways to achieve the goals of a restoration programme.

Key words: Atlantic sturgeon, protection, recovery programme, France.

RESUMEN

Análisis de asociación y requisitos de conservación para una especie amenazada,* Acipenser sturio *L., 1758: hacia la puesta en práctica de un plan de recuperación

El esturión atlántico Acipenser sturio *L., 1758 es una especie amenazada cuyo último santuario es el ecosistema Gironda, Garona y Dordoña. Se encuentra estrictamente protegido en Francia desde 1981. Sin embargo, no se ha producido un significativo incremento de esta población. Hasta recientemente, como con el programa desarrollado para el salmón, los planes de recuperación de las especies migratorias se han centrado principalmente en la adquisición de conocimiento y en el desarrollo de programas estrictamente técnicos. Estos enfoques son insuficientes para integrar los requerimientos de supervivencia de las especies en la gestión ambiental. El análisis de las necesidades requeridas para la recuperación del esturión atlántico sugiere aspectos técnicos fácilmente manejables, y las medidas apropiadas están disponibles. Por otro lado, aspectos sociales de recuperación más complejos pueden frecuentemente interferir con la puesta en práctica de acciones técnicas. Como un hecho natural, la gestión de los ecosistemas acuáticos trata con múltiples actores, con diferentes intereses y varias competencias, que pueden, directa o indirectamente, influir en la consecución del programa de recuperación. La motivación de todos los grupos de actores y su inclusión en un proyecto colectivo es una de las maneras de realizar los objetivos de un programa de recuperación.*

Palabras clave: *Esturión atlántico, protección, programa de recuperación, Francia.*

INTRODUCTION

The Etablissement Public Interdépartemental Dordogne, or Epidor, is a stated-funded organization responsible for developing integrated procedures for managing the 475-km Dordogne River, one of France's largest waterways, and all of its tributaries throughout the extensive Dordogne basin. It was set up in 1991 by the local governments of the six *départements* –France's large counties– through which the Dordogne flows.

By co-ordinating each *département*'s policies or projects for specific actions, Epidor develops overall management schemes for water, aquatic and adjacent habitats which have four main objectives: to restore water quality; to protect habitats and restore species; to achieve integrated management; to ensure respect for the environment.

In 1994, at the request of the French Ministry for the Environment, Epidor agreed to become involved in efforts to save the Atlantic sturgeon *Acipenser sturio* L., 1758. By approving this decision, the elected representatives on the *Conseils Généraux,* the six local governments to which Epidor answers, assumed their responsibilities as regards the threatened disappearance of a species which is of interest to all of Europe. Through their collective financial, technical and organisational commitment, they showed that the threat is not only a matter for the French State, scientists and local residents, but also an issue of general public concern. Epidor is now in charge of a European LIFE-Nature programme designed to this end, carried out jointly with scientists from the Cemagref institute in Bordeaux. A first phase of this programme was carried out during the period 1994-1997 (Anon., 1997). A second phase has been underway since 1998, and is scheduled to continue until the end of 2001 (Anon., 1998).

PRESENT STATUS OF STURGEON MANAGEMENT

As early as 1950, the French authorities had information which could have avoided the disappearance of sturgeon from the Garonne and Dordogne Rivers (Guerri and Pustelnik, 1995). Several documents blame over-fishing as the main cause for the increasing scarcity of the fish, along with large-scale poaching and lax legislation. A further cause was dredging in the river beds, which led to the disturbance and even destruction of the spawning grounds.

It was not until 1982 that a measure for total protection of the species was implemented, based on scientific proposals, whereby its catch, transport and marketing are now forbidden throughout French territory. This protection was reinforced at the European level in 1992 by the European Community Habitat Directive, and in 1997 by classification of the species in Appendix II of the Bern Convention. But these measures still did not solve the problem of accidental catches in the course of other fishing activities (for shad, lamprey, etc.), or due to poaching.

Despite repeated demands made to the administrative authorities by the nature protection associations and fishermen (Guerri and Pustelnik, 1995), no protective measures have been made applicable to the lower reaches of the Garonne (80 km) or those of the Dordogne (135 km) or the Gironde estuary (680 km^2) (figure 1). The argument advanced by the authorities for delaying the imple-

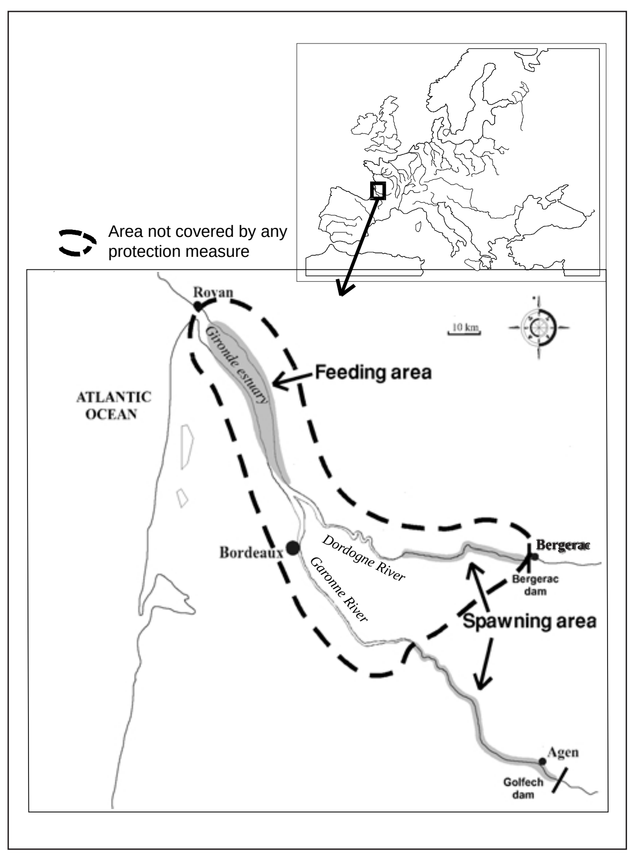


Figure 1. Habitat protection measures for *A. sturio* in the Gironde, Garonne and Dordogne basin

mentation of such measures rests on the alleged lack of accurate scientific information about the use of the various habitats by the different life stages of the sturgeon. This highly equivocal interpretation of the principle of precaution will result in the gradual elimination of many habitats. Indeed, approval and permits are still granted for the extraction of gravel and sand, whether for protected sectors or not, as well as for work or land use which will have serious consequences on the sturgeon's habitats.

Since 1990 the competent authorities have been provided with results containing all the elements required for the preparation of adequate measures to protect the sturgeon. Tables I and II summarise the scope of actions engaged, comparing the knowledge acquired, the management measures obtained and those still needed. It shows that, even if incomplete, scientific data and technical capabilities are fundamental elements. But success in terms of concrete results for the sturgeon depends on what happens to these technical aspects and the way they are used in the management process.

Until 1994, fishing activities were managed by several local services of the French State: the *département*'s offices of Agriculture and Forestry, Maritime Affairs, Maritime and Navigation Services, etc. French fishing law established a basis for

Table I. Habitat protection

Knowledge already acquired	Positive actions	Negative actions
Continued existence of areas of functional breeding (27 potential sites)	Upstream Garonne: 120 km upper reaches are protected	Permit delivered for channelling work 80 km lower reaches of Garonne unprotected
These areas are precarious and at risk from changes in the river bed		Dordogne not protected at all
Continued existence of feeding areas in the estuary specific areas frequented by 2-to-8-year-old sturgeon		No specific arrangements for protection of the estuary Authorization for gravel extraction currently valid
These areas are precarious and at risk from changes caused by dredging		
The Garonne and Dordogne are the last areas of functional breeding	International contacts made	

Table II. Population protection

Knowledge already acquired	Positive actions	Negative actions
The small size of the population	Protected species Population management chart	Lack of means for policing throughout French territorial waters
Rarity of natural breeding	Creation of conservation stock in captivity Development of methods and implementation of first re-stocking operation	
Strong impact on the population of mortality due to catching but low direct mortality due to accidental catches Survival depends on behaviour of the fishermen	Implementation of a monitoring and information plan for river, estuary and maritime fishing centres (underway at present)	
Stocks overlap the territory of at least 7 European countries	Classification of the sturgeon in Appendix I of the Habitat Directive and Appendix II of the Bern Convention	Lack of protection for the sturgeon in certain countries which signed the Habitats Directive and the Bern Convention

co-ordination and broader consultation which the defenders of the sturgeon could have expected to be a promising source of help -in the shape of the COGEPOMI, a committee for the management of migratory fish.

This committee brings together all the various kinds of fishermen, environmental associations, elected representatives (i.e. the county and municipal councillors), scientists and the river and maritime authorities. Its goal is to set up a management plan for migratory fish for each hydrographical river basin. Unfortunately, though, the sturgeon is not on the list of species covered by such plans. This follows a decision by the State Council (France's supreme legislative watchdog) that since the sturgeon is a protected species, it has no need of the management measures.

As a result, there is no operational and strategic body in which collective discussions can take place on the future of the sturgeon, and, above all, with the people principally involved, the fishermen, who are held responsible for the catching of specimens (whether accidentally or not). Paradoxically, then, the sturgeon is penalised by its very status as a protected species.

Furthermore, the moves to save the sturgeon have revealed serious limitations in the French authorities' ability to act in the field of environmental conservation. There are, for instance, considerable difficulties over collaboration between the Ministry for the Environment (highly motivated to save the sturgeon), whose powers are confined to rivers, and the Ministry for Agriculture and Fisheries, in charge of the maritime areas but far less concerned about the protection of species.

This has led to actions which are sometimes contradictory, resulting in considerable pressure on the fishermen to reduce activities in the river and estuary areas, while leaving relative freedom for activities in the maritime areas. In addition, there is a tremendous disparity between the policing resources allotted to control of activities and respect for legislation. They are present (albeit weak) in the river areas, but are practically non-existent in the estuary and maritime areas. This situation, added to a general lack of resources allocated to protection of the environment, explains why it is impossible for State authorities to play a significant role in promoting actions to save the sturgeon.

At present, therefore, it is paradoxically the non-governmental partners who, though having no powers nor legal obligations, are making the most energetic efforts to save the sturgeon. These actions are initiated by researchers, local authorities and certain associations or social-economic partners who are committing themselves to a project without any long-term guarantees.

A STRATEGY TO SAVE THE STURGEON

Fifteen years after publication of the ministerial decree banning fishing of this species, the sturgeon population remains seriously depressed. The framework, principles and means of managing *A. sturio*'s requirements have not worked. The sturgeon is the victim of its biological inability to survive easily in the same environment as man (e.g. such factors as its late maturity, that it inhabits estuaries and low valleys which are sensitive habitats, and that it is easily caught by fishing tackle).

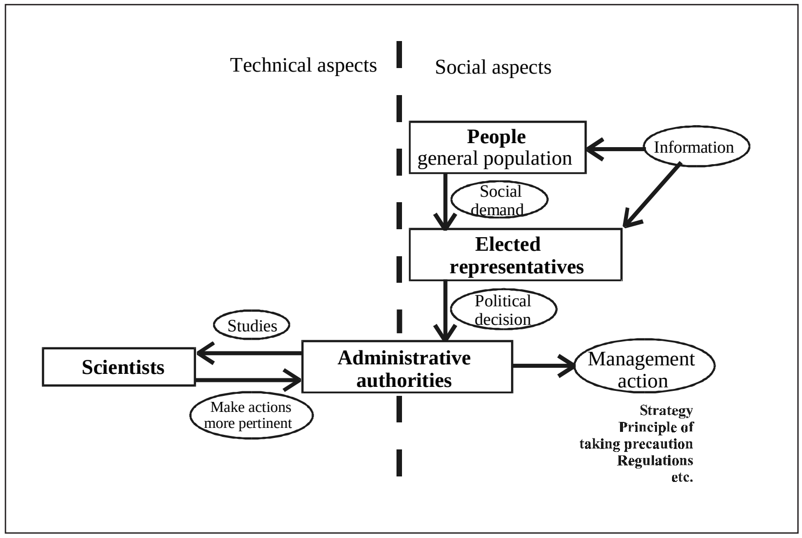
Setting up methods and operational technical action takes time and resources. The sturgeon is in a situation where the only way to guarantee it acceptable conditions for survival is to take certain of its needs into account immediately.

During the first phase of the LIFE programme (Anon., 1997), all-encompassing investigations were undertaken to design a strategy to enhance the involvement of those concerned in the efforts to save the sturgeon. This analysis resulted in three main lines for action:

(1) The sturgeon has been disappearing for the past 40 years; those rightly responsible for management processes affecting the species must now become truly involved and set up a suitable organization to put research efforts to use, turning them into operational management tools. To this end, stable, long-term funding must be secured, so as to support the indispensable scientific and technical operations and, above all, set up the essential organizational resources. In any democratic process (figure 2), it is up to the elected representatives and then the relevant authorities to decide on these steps. Pressure from the public will increase their commitment.

(2) Management of time is necessary in order to mobilise the people involved and to ensure

Figure 2. The mechanism of democratic management



the sturgeon does not disappear during that same period. Having seen how far the situation of the sturgeon species has deteriorated, how sluggish the management system is, and how the biology of the species permits only a very slow reinstatement of the population, it is essential to combine urgent action with others over the long term.

(3) The sturgeon is a major migrating species, ranging over distant territories where its survival can likewise be threatened. The problem of saving it must therefore be tackled at several levels, to ensure its conservation not only in local management situations, but also on the national and international plane.

The strategy proposed thus defines three priority target publics: the general public; their elected representatives, whose job it is to orient, decide and finance management policies; and the fishermen, because they are directly involved in day-to-day contact with the sturgeon. It can be assumed that members of the public, provided they are properly informed and made aware of the needs, will be anxious for their elected representatives to do something about saving the sturgeon. They will ask them to allocate all the resources needed for the project to succeed –that is, funding for programmes and indications of the guidelines for those who manage the situation.

Common sense and government responsibility have not been enough to ensure the sturgeon's survival. To start the debate all over again and decide what arguments should be put forward, it is necessary to establish the present-day social context and to identify the areas to which the people involved are sensitive when an ecological problem is being dealt with. Discussions during the first phase of the LIFE programme enabled several messages to be identified, which it has been possible to develop in the context of the sturgeon situation.

Several new and somewhat theoretical concepts have been adopted as starting-points for drawing up different schemes to inform people about the sturgeon. These relate to economics, the culture of fishing, maintenance of biological diversity, and sustainable development.

(1) Sturgeon used to make a significant contribution to the economy along the banks of the Gironde. Rebuilding a sustainable population is such a long-term and hypothetical question that it is totally impossible to consider realistic economic benefits from the fish.

(2) The culture of sturgeon fishing is reserved for certain knowledgeable practitioners and is tending to disappear at the same speed as the sturgeon themselves.

(3) The concept of biological diversity is highly scientific, and one which many people sometimes find difficult to understand.

(4) Because the sturgeon is rare and somewhat a creature of mystery, it has come to be an emblem and indicator of quality, its survival demonstrating the health of the habitat. It is this emblematic image of sustainable development, in a territory (river and estuary) known to be subjected to very considerable developmental pressure,

which has emerged as the most appropriate for gaining the attention of everyone who needs to be involved with the problem.

Based on all these elements, the LIFE programme has selected various distinct but complementary actions which address public awareness, communications, training, and advisory services. Some of these are detailed below.

A "Save the sturgeon" exhibit

This is a modular exhibit which presents the biology of the sturgeon, its life history, the main reasons for its disappearance (and those to blame for them). It involves the visitors by asking them to sign a petition, after first showing them what work is already in hand. A more mobile version of this exhibit has been designed in order to reach less centralised areas, schools and other small centres.

A documentary press file

Designed in collaboration with a magazine, the file contains interviews with fishermen, elected representatives and scientists, compiles key points of the information currently available, and present an overview of the whole problem of the disappearance and reinstatement of the sturgeon.

An educational pack

With the long term in mind, a pack for schools has been drawn up with professionals from France's national education system. It presents both teachers and pupils with an appropriate file on the sturgeon and the problem of the fish's threatened disappearance.

A competition

Under the catchy title of "The *A. sturio* affair", a competition for schools is being prepared on the Internet, with prizes for entrants who carry out the best investigations. The idea will be for children to scan the Web sites of bodies participating in the restoration project and seek out information concerning the causes and responsibilities for the sturgeon's disappearance.

A poster

Designed for display in public places frequented by fishermen (ports, fish auctions, co-operatives, maritime banks, etc.), the poster has already been distributed on the entire French coast. It gives the basic information needed to tell fishermen how to recognise the species, its status as a protected species, and what to do if they catch one. Several other documents (stickers, desk pads) are destined to accompany a second information campaign, which will be run by a person involved with organising activities in the field.

A database

It will be available on the Internet and will cover all the areas of information (historical, official and other texts, images, etc.) collected during the programme. The aim is to have a ready-made network of information available for future partners in the field of sturgeon protection.

A network of Web sites

This will be devote devoted to the sturgeon, permitting quicker exchanges of information between all the specialists involved.

Even taken together, all of these tools are obviously inadequate and cover only part of the needs identified in the strategy. They do, nevertheless, bring together the data required to inform the general public and schools in a simple and attractive form. They also make it possible for the media to access the essential information.

A tremendous effort is still required in this field and there is a need to investigate other means for motivating the general public, elected representatives, administrations and fishermen. Included among these other means are: setting up a permanent information site on the Gironde estuary; making media reports or setting up events at national level; and developing associations and information teams in the field.

DISCUSSION

One of the strategic aspects which should help progress for preserving the future of the sturgeon is based on the development of national and international mobilization. Until now, the question of the Gironde sturgeon has remained a very local concern. A first step has been taken in the launch of a European programme involving the European Commission, the national authorities (Ministry for the Environment), local authorities in the Garonne/Dordogne basin, the scientific community, and local associations. The establishment of an international scientific committee under the LIFE programme has made it possible to kick off the process of broadening the outlook for discussions about the sturgeon. The second phase of the LIFE programme takes this one step further by preparing restoration strategies for implementation at the international level, by means of enquiries in the major European drainage basins.

Care must be taken to involve all of the population segments concerned in these procedures in addition to the specialists and official bodies. Decision makers at all levels must be mobilised, in order to provoke positive interactions which would arise from the collective involvement of elected representatives in the various countries concerned. An example would be the creation of an international commission for the conservation of the European Atlantic sturgeon, along the same lines as that which is already in operation for the North Atlantic salmon (Anon., 1999). Its mandate would make it possible to initiate all of the appropriate debates at the right pan-European level and to propose to the member countries –with all the strength of an international body behind it– the lines along which organisation or action could be taken to enable significant progress.

As pointed out earlier, because the sturgeon is a protected species, it is excluded in France from discussions concerning the management of migratory fish. But its problems are not basically any different from those of other migratory species, such as the salmon, eel or river lamprey, which are also suffering difficulties. These species are most often victims of the same phenomena and suffer from the same disturbances: over-fishing, deteriorating habitat, etc. Thus, it would be advantageous to avoid dealing with the management of migratory species separately.

Comprehensive management of all migratory species would obtain savings in terms of economy of scale and ensure that species which receive less attention, because they are less well-known or less interesting economically, could benefit from the efforts invested for the more profitable species. This would at least bring the sturgeon back into the debates in those bodies which manage the habitats and practices, notably fishing, but at present have no real mandate for discussing this particular fish. It would put an end to the paradoxical situation whereby, of the eight species of migratory fish in the Dordogne basin, the sturgeon is the one least taken into account in management of the waterways –yet it is precisely the one for which the study programmes and technical follow-up are the best designed and best co-ordinated. This proposed global effort does not preclude more specific work but encourages co-ordination.

In the Dordogne region, work is progressing to establish such an overall management strategy, named *Objectif Retour aux Sources,* for migratory fish at a river-basin scale, and a similar process is being developed for the Garonne. This integrates eight fresh- and salt-water migratory species into the same collective programme, which is the subject of considerable consultation work and a strong desire to mobilise the general population. It is hoped by these means to construct a coherent project including the sturgeon, not just for the sturgeon.

CONCLUSIONS

Whereas the question of specific knowledge is for the scientists, the question of overall conservation is one for society as a whole. It has been suggested that governments and the different bodies involved in political, management and financial decisions, should develop a social charter for migrating fish which could be based on recognition that the management of migratory fish is one of the elements in integrated management procedures for rivers, and agreement on the fact that coherent strategies must be developed at local, national and international levels. The value of active and direct participation in the management of migratory fish would be enhanced by: initiating the establishment of management strategies at hydrographical river basin levels; checking that all the species are taken into account in the strategies developed; assuring that these strategies are supported by international bodies for the conservation of species; identifying the various local contacts involved and associating them with the project right from the beginning; ensuring that procedures for consultation, agreement and negotiation are implemented with the people and their representatives; and increasing financial investments in order to make all the above possible.

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Putative morphometric evidence of the presence of *Acipenser naccarii* Bonaparte, 1836 in Iberian rivers, or why ontogenetic allometry needs adequate treatment

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ABSTRACT

I have extended my initial analyses (Rincón, 2000) of a published morphometric data set of sturgeons caught in Iberian rivers and from a fish farm, claimed to prove the native status of both *Acipenser naccarii* Bonaparte, 1836 and *Acipenser sturio* L., 1758 in the Guadalquivir basin (Garrido-Ramos *et al.*, 1997), by applying the same uni- and multivariate techniques for allometric adjustment to a database expanded with further *A. naccarii* specimens. As previously, neither *log-log* scatterplots of head measurement vs. total length, nor graphical representation of the scores of individual specimens on the principal components (PC) extracted by PCAs performed on the covariance and correlation matrix, respectively, offered any suggestion of the existence of two morphologically dissimilar groups within wild Iberian sturgeons. In addition, the relatively weak (in terms of total variance accounted for) gradient in snout width that separated the one farmed Adriatic sturgeon from the wild fish found in my previous work (Rincón, 2000) was again detected, now clear and distinct, by all those techniques. Furthermore, a DFA showed that such dissimilarity produced almost perfect, highly statistically significant discrimination between farmed and wild fish, while there was no significant gradient separating the two supposed groups of wild Iberian sturgeons. Examination of the two composite variables that Garrido-Ramos *et al.* (1997) used to separate those groups in the extended database confirmed that they were negatively affected by ontogenetic allometry, therefore leading to the ascription of large and small specimens to different groups. I conclude that there is no morphological evidence to support the claimed autocthonous status of *A. naccarii* in the Iberian Peninsula.

Key words: Morphometrics, size correction, PCA, sturgeons.

RESUMEN

Supuesta evidencia morfométrica de la presencia de* Acipenser naccarii *Bonaparte, 1836 en ríos ibéricos, o por qué la alometría ontogénica necesita un tratamiento adecuado

He ampliado mi análisis inicial (Rincón, 2000) de los datos publicados sobre la morfometría de esturiones procedentes de aguas de la península Ibérica y de una piscifactoría, presentados como prueba del carácter nativo tanto de Acipenser naccarii *Bonaparte, 1836 como de* Acipenser sturio *L., 1758 en la cuenca del Guadalquivir (Garrido-Ramos* et al.*, 1997), aplicando las mismas técnicas uni- y multivariantes de ajuste de alometrías a una base de datos ampliada con un mayor número de ejemplares de* A. naccarii. *Como ya ocurrió, la representación gráfica de las diversas medidas cefálicas frente a la longitud total (todas transformadas a logaritmos) y de los componentes principales extraídos por ACP, realizados usando tanto la matriz de covarianza como la de correlación, no ofrecieron indicación alguna de la existencia de dos grupos morfológicamente distintos dentro de los esturiones capturados en aguas de la Península. Además, el relativamente débil (en cuanto a proporción de la varianza total que asumía) gradiente en la anchura del* *morro que separaba al único ejemplar de piscifactoría de los esturiones salvajes que hallé (Rincón, 2000), fue identificado de nuevo, ahora evidente y nítido, por todas las técnicas anteriores. Finalmente, un AFD mostró que tal diferencia permitía una casi perfecta, altamente significativa, distinción entre los especímenes de piscifactoría y los salvajes, mientras que no hubo gradiente morfométrico estadísticamente significativo que separase los supuestos dos grupos de esturiones salvajes ibéricos. El examen en la base de datos ampliada de las dos variables compuestas que Garrido-Ramos* et al. *(1997) usaron para distinguir tales dos grupos, confirmó que ambas se hallan gravemente influidas por la alometría ontogenética, llevando, por tanto, a la asignación de los ejemplares grandes o pequeños a grupos distintos. Concluyo, pues, que no hay evidencia morfológica alguna que apoye el supuesto carácter de especie autóctona de* A. naccarii *en la península Ibérica.*

Palabras clave: *Morphometría, corrección del tamaño, ACP, esturiones.*

INTRODUCTION

The Adriatic sturgeon, *Acipenser naccarii* Bonaparte, 1836, has been generally considered restricted to the Adriatic Sea, particularly its northern area, and the river basins draining into it (Holčík, 1989). Recently, however, Garrido-Ramos *et al.* (1997) have stated that individuals of the species comprise a substantial part of the sturgeons collected in the Iberian Peninsula (and in the Guadalquivir River in particular) from the end of the last century until the early 1980s; this is in direct conflict with the widely-held view that the Atlantic sturgeon *Acipenser sturio* L., 1758 is the only native sturgeon species in the Iberian Peninsula (Classen, 1944; Almaça, 1988; Doadrio, Elvira and Bernat, 1991; Elvira, Almodóvar and Lobón-Cerviá, 1991; Elvira and Almodóvar, 1993; Pereira, 1995). From this, Garrido-Ramos *et al.* (1997) moved on to claim the status of autochthonous, endangered species for *A. naccarii* in the Guadalquivir and, consequently, to demand the implementation of recovery plans for the species which entail its stocking in that area (Garrido-Ramos *et al.*, 1997; Ruiz-Rejón, Hernando and Domezain, 1998).

However, these assertions have been negated by a number of authors who have strongly criticised the evidence that allegedly substantiates them (Doukakis *et al.*, 2000; Elvira and Almodóvar, 2000; Rincón, 2000; Almodóvar, Machordom and Suárez, 2000). Such putative evidence is both morphometric and genetic. The genetic findings of Garrido-Ramos *et al.* (1997) could not be replicated by Doukakis *et al.* (2000) and have been directly contradicted by Almodóvar, Machordom and Suárez (2000). I have shown elsewhere that their morphological results are an artifact caused by their application of an inadequate methodology (Rincón, 2000) unable to separate ontogenetic and evolutionary allometry (Cock, 1966; Klingenberg, 1996).

The failure of Garrido-Ramos *et al.* (1997) to distinguish between allometry caused by growth processes within a taxon from that originated by phylogenetic variation between taxa, and to efficiently account for their effects, led them to misidentify large specimens of *A. sturio* as *A. naccarii.* The application of routine univariate and multivariate morphometric techniques, which adequately deal with allometry to the same data set, found that, indeed, there was only one group of morphometrically similar sturgeons among the specimens caught in Iberian rivers and traditionally identified as *A. sturio.* Furthermore, they detected the morphological dissimilarity of the only specimen *a priori* known to be *A. naccarii* (a sturgeon from a fish farm) present in the sample (Rincón, 2000).

However, this very scarcity of true *A. naccarii* in the Garrido-Ramos *et al.* (1997) database determined that gradients reflecting interspecific differences accounted for relatively little of the total variance of the sample in multivariate morphometric space. Thus, size-related gradients were magnified by default. Therefore, further assessment of the relative performance of the techniques used by Garrido-Ramos *et al.* (1997) and my previous work (Rincón, 2000), respectively, under different conditions seems warranted. To do this, I have added data from eight new *A. naccarii* to the original 25-specimen database that both articles examined, and then I have applied to this enlarged database the same common multivariate and univariate techniques for allometric correction I had previously used (Humphries *et al.*, 1981; Bookstein *et al.*, 1985; Reist, 1985, 1986; Klingenberg, 1996).

Specifically, the current paper seeks to answer the following questions: (1) how are my conclusions (Rincón, 2000) affected by the application of the same methodology to a sample where *A. naccarii* abundance is increased?; and (2) do the criteria in Garrido-Ramos *et al.* (1997) correctly distinguish between the two species over their entire size range, as claimed by those authors?

MATERIALS AND METHODS

Data

Table 1 in Garrido-Ramos *et al.* (1997) is one source of the morphometric measurements used in my analyses. It reports the values of the following six variables: (1) total length (Tl, cm); (2) distance from the tip of the snout to the base of the barbels (A, cm); (3) distance from the base of the barbels to the cartilaginous arch of the mouth (C, cm); (4) distance from the tip of the snout to the cartilaginous arch of the mouth (F, cm); (5) width of the snout at the point of barbel insertion (B, cm); and (6) distance from the tip of the snout to the frenulum (LFR, cm). From those measurements they also derive the subtraction C - A and the ratio F:B (Garrido-Ramos *et al.*, 1997, table 1, figure 2A,B). The values of these eight variables are given for 25 individuals (LFR is missing in specimen EBD-8174, number 2 in table 1 of Garrido-Ramos *et al.*, 1997) of which, one (PSN-1) comes from a farm-raised *A. naccarii* from an originally Italian stock. Therefore, the specific identity of PSN-1 seems certain. All the other are wild fish and could, hypothetically, comprise both *A. naccarii* and *A. sturio*.

To ensure comparability, morphometric data have been entered in subsequent calculations as they appear in table 1 of Garrido-Ramos *et al.* (1997) and have not been altered, save for the correction of obvious transcription or typographical error: e.g., the published measurements for specimen EBD-8174 are A = 5.20; B = 11.40; C = 12.60; F = 12.60; C - A = 2.20; and F:B = 1.14. I have inferred that the correct value for C should be 7.40, which I have used in further analyses. Garrido-Ramos *et al.* (1997) provide no information on their measuring protocols, but from their figure 2A,B it is clear that F should be equal to A + C. However, in some specimens (MUC1, PSN1), F is slightly smaller than A + C (e.g. MUC1; F = 10.60 cm; A + C = 11.00 cm) while in others F is larger (SE-1, SE-2, SE-3, F = 4.60, 4.30, 3.20 cm; A + C = 3.90, 3.45, 2.56 cm, respectively). Again, to maintain comparability, and given that the reported values of F are not always a linear combination of C and A, I have retained F in further analyses.

The cases of the subtraction C - A and the ratio F:B, the main basis of the claims made in Garrido-Ramos *et al.* (1997), deserve more detailed comment. Elsewhere (Rincón, 2000), I have shown that (1) they are both significantly influenced by overall size (represented by Tl in our case) and, therefore, cannot be used as substitutes of the standard statistical techniques employed to deal with the effects of size and allometric growth; and (2) as C - A and F:B do not represent distances between two identifiable morphological features, uni- or multivariate procedures for allometric correction involving them are no longer rooted in the model of animal ontogenetic development and growth first proposed by Huxley (1932), and which has received abundant further empirical and theoretical support since then.

For all of the above, I consider that the use of C - A and F:B, even with some form of size-correction, must be avoided. Moreover, preliminary multivariate analyses carried out including or excluding C - A (correcting for size effects) produced similar results and identical conclusions. Consequently, I here present analyses performed using the original measurements exclusively, save for further exploration of the properties and behaviour of C - A and F:B across a wider *A. naccarii* size range.

The remaining morphometric data were obtained from eight *A. naccarii*, 21.7 to 131.7 cm in Tl (table I), and coming from the same fish farm as

Table I. Morphometric data from the specimens of *A. naccarii* from the Sierra Nevada fish farm housed in the scientific collections of the Príncipe Alberto I de Mónaco Aquatic Ecology Station (PSN-2 to 5) and of the Doñana Biological Station (PSN-6 to 9), Seville

	Tl	A	B	C	F	LFR	C - A	F:B
PSN-2	21.70	1.32	1.86	1.18	2.50	4.73	-0.14	1.34
PSN-3	23.00	1.54	2.03	1.05	2.59	5.08	-0.49	1.28
PSN-4	75.50	2.60	5.90	3.40	6.00	13.20	0.80	1.02
PSN-5	78.00	2.75	6.30	3.45	6.20	14.00	0.70	0.98
PSN-6	124.00	3.50	8.00	3.95	7.45	21.50	0.45	0.93
PSN-7	131.70	3.70	8.90	4.70	8.40	20.50	1.00	0.94
PSN-8	123.00	3.20	7.50	3.30	6.50	16.00	0.10	0.87
PSN-9	49.50	2.15	3.31	2.18	4.33	8.80	0.03	1.31

PSN-1. I will henceforth refer to them as PSN-2 to PSN-9. Those specimens are housed in the scientific collections of the Príncipe Alberto I de Mónaco Acuatic Ecology Station (PSN-2 to 5) and of the Doñana Biological Station (PSN-6 to 9), both in Seville, but they have not been assigned catalogue numbers yet. Their six original morphometric variables (Tl, A, B, C, F, LFR) were measured and C - A and F:B were also calculated. See Elvira and Almodóvar (2000) for details on measurement procedures.

Data analysis

As in Rincón (2000), I have approached the data as a "problem sample" in which one or more groups of morphologically similar individuals may be present. Therefore, I have relied on exploratory techniques not requiring *a priori* group ascription to identify the patterns of morphometric variation within the sample. Then, these patterns can be contrasted against explanatory hypotheses, such as allometric growth or presence of several taxa in the sample, which, obviously, need not be mutually exclusive. Morphometric dissimilarity of the magnitude claimed by Garrido-Ramos *et al.* (1997) should be reflected in the results of one or more of the different techniques (ideally, all of them), if it is not merely a procedural artifact. See Smith (1973), Neff and Smith (1979), Humphries *et al.* (1981), or Humphries (1984) for applications of such an approach.

As recommended by Marcus (1990), as an initial step in my analyses I produced *log-log* bivariate scatterplots of A, B, C, F and LFR against Tl (not shown) and inspected those representations in search of any spatial arrangement of specimens that could be interpreted as evidence suggestive of the presence of two or more groups of sturgeons in the sample (e.g. spatially disjunct clouds of points in the bivariate plane). The plots did indeed show a spatial disposition indicating that the sample may contain more than one group of morphologically similar fish, although they all exhibited a common pattern of ontogenetic allometric covariation: all *log*-transformed head measurements appeared to increase linearly with *log* length, longitudinal measurements doing so more slowly than transversal ones, in all groups, but the details of the relationship seemed group-specific.

In such a situation, the common model (Huxley, 1932) of allometric growth is applicable. According to it, the formula for simple allometry between two morphological trait measurements *x* and *y* (see also Klingenberg, 1996) is

$$y = b\,x^{\alpha} \qquad [1]$$

very often linearised through *log*-transformation as:

$$\log y = \log b + \alpha \log x \qquad [2]$$

where α and b are constants. The constant b is frequently called an allometric coefficient.

I next applied a commonly used method for univariate size correction to the values of A, B, C, F and LFR. I employed the equation

$$\log y_{adj} = \log y - \alpha\,(\log Tl - \log Tl_{M}) \qquad [3]$$

where y_{adj} is the adjusted value of variable y, α is as in equations [1] and [2] and was estimated as the pooled slope of the *log-log* regression of variable y against Tl, and Tl_M is the grand sample's mean total length. This technique has been employed often (Ihssen *et al.*, 1981; Baumgartner, 1995; Hawkins and Quinn, 1996) and it has been discussed and preferred by Reist (1985, 1986) to other univariate methods of allometric adjustment. The use of a univariate adjustment is further justified because Tl is strongly correlated with generalised multivariate size in the present data set (correlation between *log* Tl and PC1 extracted by a Principal Components Analysis using the covariance matrix = 0.97; N = 32; $p < 0.00001$; see below for details), and the number of variables involved is small (Humphries *et al.,* 1981). Total length was excluded from subsequent analyses carried out on the adjusted variables (henceforth, $\log A_{adj}$, $\log B_{adj}$, $\log C_{adj}$, $\log F_{adj}$, $\log LFR_{adj}$).

I used PCA to explore the distribution of specimens in multivariate morphometric space. PCA is a valuable technique in situations such as the present one because it is not biased by information about group membership (Humphries *et al.,* 1981; Humphries, 1984). I used the covariance matrix of the *log*-transformed values of Tl, A, B, C, F, and LFR to extract the Principal Components (PCs) that ideally represent the major axes of multivariate morphometric variation (Humphries *et al.,* 1981; Bookstein *et al.*, 1985; Rohlf and Bookstein, 1987). Through use of *log* transformation and the covariance matrix allometries are preserved, the geometric space is not distorted, and the original variables influence the analysis according to their variance (Jolicoeur, 1963; Bookstein *et al.*, 1985).

These properties are desirable for two reasons. The first is that size is often the variable with the highest variance in morphometric data sets and, therefore, the first PC extracted (PC1) is often a general size factor. Subsequent PCs account more for shape differences, but will include size information not accounted for by PC1. The inspection of PC structure through examination of loadings and scoring coefficients of the original variables on them will indicate the extent of this. Secondly, when the length of the eigenvector is scaled to unity (as done here), the scoring coefficients convey direct information on the bivariate allometric relationships between the original variables (Humphries *et al.*, 1981; Bookstein *et al.*, 1985; Rohlf and Bookstein, 1987; Klingenberg, 1996).

Such a utilization of PCA is common in the ichthyological literature (Smith, 1973; Neff and Smith, 1979; Wood and Bain, 1995), including sturgeon systematics (Mayden and Kuhajda, 1996). Those references show how, when several groups are present in a sample, they are arranged as clouds of points spatially separated along the morphometric gradients that distinguish them. Such a disposition is what we would expect to see replicated if the current sturgeon sample contained more than one species. On the other hand, we would expect continuous distribution in multivariate morphospace if the sample comprises only one taxa.

When, as in our case, the ordination of specimens in morphospace is as interesting as the patterns of allometry, the use of the correlation matrix for PC extraction is justified (Klingenberg, 1996). However, unlike the situation with the covariance matrix, it would no longer be possible to derive information about allometries from the PC coefficients (Pimentel, 1979; Klingenberg, 1996). Consequently, I have carried out a PCA using the correlation matrix of the five size-adjusted variables to provide additional perspective and complementary insight and contrast to the results of the previous PCA from the covariance matrix.

Finally, I have assessed multivariate morphometric dissimilarity among groups of fish through Multivariate Analysis of Variance (MANOVA) of the five size-adjusted variables (Rice, 1990; Scheiner, 1993; see Wood and Bain, 1995 for an ichthyological example). Those variables that exhibited significant differences and, therefore, potential for among-group discrimination, were then subjected to a Discriminant Factor Analysis (DFA). Both techniques require *a priori* ascription of specimens to groups. Thus, I have distinguished between sturgeons from the Sierra Nevada fish farm, wild fish identified as *A. naccarii* by Garrido-Ramos *et al.* (1997), and wild fish identified as *A. sturio* by those same authors. The farm vs. wild origin distinction is not a morphometric criterium and, consequently, introduces no circularity into the procedure. However, Garrido-Ramos *et al.* (1997) separated *A. sturio* and *A. naccarii* according primarily to the value of C - A and, secondarily, F:B. While neither parameter has been used in my analyses, I have employed the size-adjusted measurements from which they derive. Hence, the information whose between-groups differences are explored may not be totally independent from that used for group assignment in the case of wild fish. However, I believe the analysis is still valid and useful because (1) I have used the size-adjusted variables and C - A and F:B where obtained from the original measurements and uncorrected themselves; and (2) the hypothetical bias the procedure may introduce would be towards magnification of differences among wild fish and minimization of those between farmed fish and the supposed wild *A. naccarii*. Therefore, results running against these trends are reliable, at least in this regard.

RESULTS AND DISCUSSION

Head allometry

Log-log scatterplots of A, B, C, F and LFR against Tl showed some separation between the *A. naccarii* from the fish farm and the *A. sturio* and putative *A. naccarii* caught in Iberian rivers, but provided no evidence of the existence of several groups within the latter (figure 1). They showed larger snout-barbel (A), barbel-mouth (C) and snout-mouth (F) distances and smaller snout width (B) than farmed fish of comparable size. LFR was similar for both groups (figure 1).

The spatial distribution of wild-caught fish in the bivariate planes appeared to be continuous, and adequately explained by allometric growth and normal interindividual variation (figure 1). Specimen AVG, identified as *A. sturio* (Garrido-Ramos *et al.*, 1997), seemed, however, an apparent

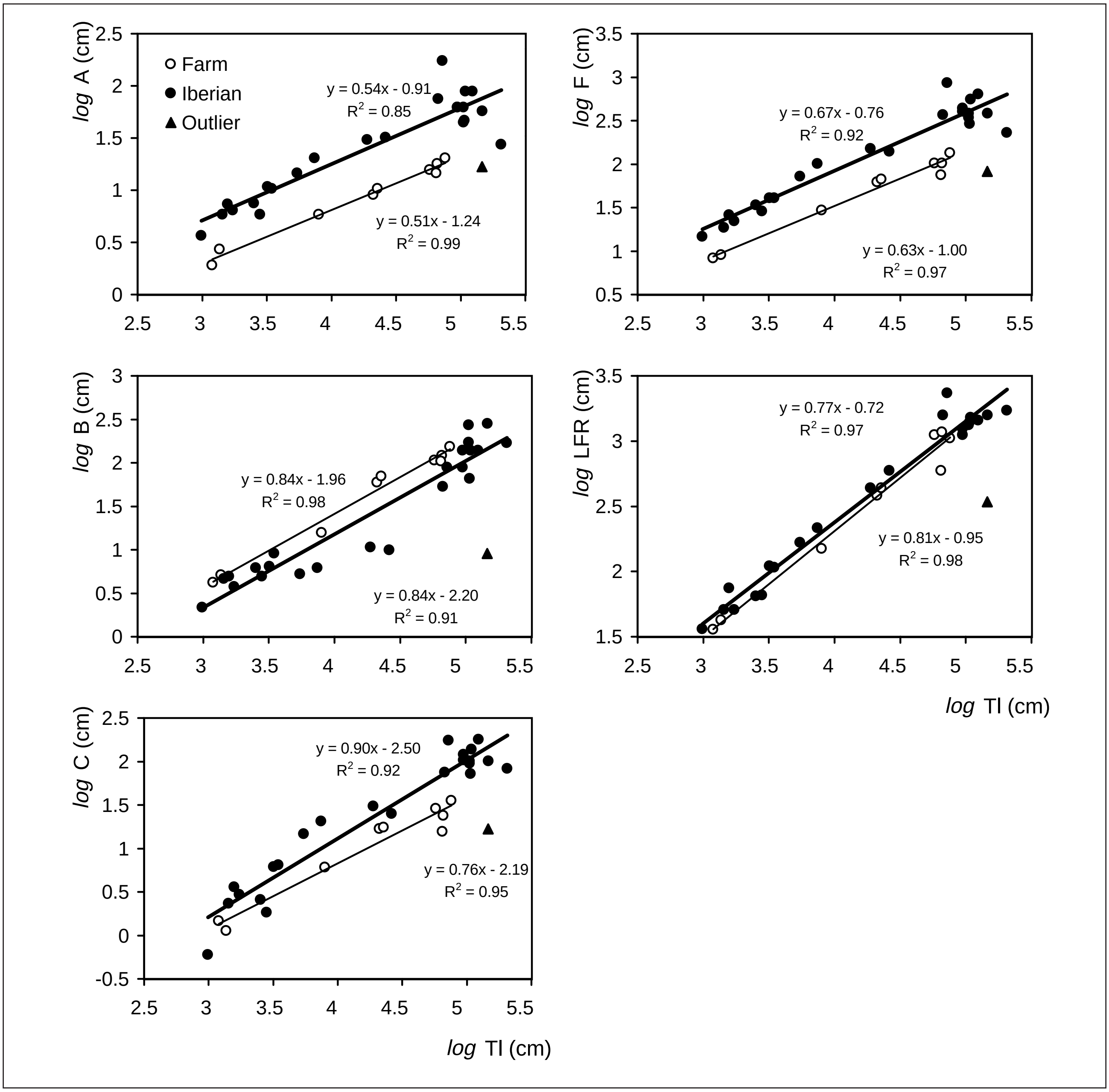

Figure 1. Bivariate *log-log* scatterplots and regressions of sturgeon cephalic morphometric traits (A, B, C, F, LFR, cm) against total length (Tl, cm). Open circles: *A. naccarii* from the fish farm; solid circles: wild-caught Iberian sturgeons; solid triangle: outlier (excluded from calculations)

exception. Its values for all cephalic morphometric traits were much smaller than would be expected in a fish of its size and it appeared as a clear outlier (figure 1). Therefore, it was excluded from the characterization of head allometry in the sample and those further procedures using the size-adjusted variables (i.e. fitting equation 2 to the data, PCA from the correlation matrix), but it was retained in exploratory analyses such as the PCA utilising the covariance matrix.

The allometric model (equation 2) showed a very good fit to the data (table II). Fish size, as measured by Tl, explained a high proportion of the variation in morphometric traits (85-98 %), the allometric coefficients (a) were all highly significant (i.e. different from zero), their estimates accurate (as shown by low standard errors), and the residuals sums of squares were small, as indicated by the high F values of the regressions (table II). Inspection of residual plots showed no apparent

Table II. Allometric coefficients (α) and parameters of the corresponding regression of *log*-transformed cephalic morphometric measurements against *log* Tl for both farmed *A. naccarii* and wild Iberian sturgeons

Variables	Group	R^2	F (df)	α	t (df)	p
log A	wild	0.85	117.65 (1,21)	0.5404	10.85 (21)	< 0.0001
	farm	0.98	487.35 (1,7)	0.5124	22.08 (7)	< 0.0001
log B	wild	0.91	202.93 (1,21)	0.8438	14.25 (21)	< 0.0001
	farm	0.98	390.35 (1,7)	0.8434	14.25 (7)	< 0.0001
log C	wild	0.92	257.73 (1,21)	0.9030	16.05 (21)	< 0.0001
	farm	0.94	121.53 (1,7)	0.7551	11.02 (7)	< 0.0001
log F	wild	0.92	247.73 (1,21)	0.6700	15.74 (21)	< 0.0001
	farm	0.97	271.50 (1,7)	0.6288	16.48 (7)	< 0.0001
log LFR	wild	0.97	663.88 (1,20)	0.7749	25.77 (20)	< 0.0001
	farm	0.98	286.93 (1,7)	0.8147	16.94 (7)	< 0.0001

systematic bias. These results further depict wild-caught Iberian sturgeons as a single morphologically homogeneous group.

All cephalic measurements were negatively allometric relative to total length for both *A. naccarii* from the fish farm and wild sturgeons (all as < 1, table II). This was particularly marked for A and, to a lower degree, F, while B exhibited faster rates of increase with size than either (table II). The allometric trajectories of both groups of fish appeared parallel (figure 1) and an Analysis of Covariance (ANCOVA) with Tl as covariate could find no significant differences between their respective allometric coefficients for either A, B, F, LFR ($F_{1,28}$ = 0.01-0.44; p = 0.51-0.94), or, although closer to significance in this case, C ($F_{1,28}$ = 2.84; p = 0.09). Nonetheless, once the effect of Tl was removed, the two groups were significantly different for A, B, C, and F (ANCOVA, $F_{1,28}$ = 8.85-52.49; p < 0.006 in all cases) due to the different elevations (parameter b in equation 2) of their ontogenetic trajectories (figure 1). LFR showed no significant difference (ANCOVA, $F_{1,28}$ = 1.82; p = 0.19). A, C and F, all longitudinal measurements, were significantly greater in wild specimens, whereas B, a transversal measurement, was smaller (figure 1). This means that farmed *A. naccarii* showed significantly shorter and wider snouts than wild sturgeons of the same size, this being one of the features that distinguishes *A. naccarii* from *A. sturio* (Holčík, 1989), and further indicating the absence of *A. naccarii* in the sample of sturgeons from Iberian rivers.

The net effects of the allometric trends common to both groups were a general reduction of head size relative to overall size with age, and, more significantly in our context, a reduction of pre-oral head dimensions in the longitudinal axis (F), particularly of their pre-barbel component (A), relative to transversal measurements (B). As a result, the snouts of sturgeons in both groups become proportionally shorter and wider relative to younger conspecifics. According to my results, these processes appear parallel in *A. naccarii* and A. sturio (the slopes of the respective regression lines vs. total length are not significantly different). I found no convergence or divergence, and thus the difference in snout width/length between the two species seems to be of similar magnitude throughout their ontogenetic development, at least while there is overlap in size, as *A. sturio* seems to grow to larger sizes than *A. naccarii*.

These findings, however, should be viewed with a measure of caution, given the relatively low number of specimens examined (particularly of *A. naccarii*), and the differences in their preservation methods and history. Nonetheless, they agree well with previous information on allometric variation for *A. sturio* and other sturgeon species. Magnin and Beaulieau (1963) also found a relative shortening of the snout, specifically of the pre-barbel area (i.e. distance A), relative to both total length and head length for both *A. sturio* and the American *Acipenser oxyrinchus* Mitchill, 1815. Similar results have been reported for *Acipenser baerii* Brandt, 1869; *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833; *Acipenser ruthenus* L., 1758; *Acipenser nudiventris* Lovetzky, 1828; *Acipenser stellatus* Pallas, 1771; and *Huso huso* (L., 1758) (see chapters on each species in Holčík (1989) and references therein). Therefore, this relative shortening and widening of the head as age and size increase seems to be a general allometric trend in sturgeons. In such a situation, specific ascription that depends solely on head morphometry requires a clear understanding of the allometric trajectories and degree of interindiviudal variation for each species. However, I have shown elsewhere (Rincón, 2000) that, even in the near absence of true *A. naccarii* of adequate size for comparison, the standard techniques for treatment of allometry correctly identify size-caused gradients and do not separate large and small *A. sturio* as morphometrically distinct. On the other hand, the choice of a less-than-apt methodology for size-adjustment while disregarding other potentially diagnostic anatomical traits (Holčík, 1989; Elvira and Almodóvar, 2000; can, of course, lead to confusion, as in Garrido-Ramos *et al.,* 1997).

The latter authors claimed that the value C - A discriminated between *A. sturio* (C - A ≤ 0) and *A. naccarii* (C - A > 0), and that this trait was size-independent (Garrido-Ramos *et al.,* 1997). However, simple algebra shows that the substraction of two size-dependent variables cannot be at the same time discriminating and size-independent (Rincón, 2000) and, unsurprisingly, C - A is in fact significantly correlated with Tl in both farmed *A. naccarii* and wild Iberian sturgeons (figure 2; $p < 0.001$). Moreover, C - A can be negative in small *A. naccarii* (table I, figure 2) and very similar in *A. sturio* and farmed *A. naccarii* of similar size (figure 2), being clearly inferior than measurements such as A, B or F .

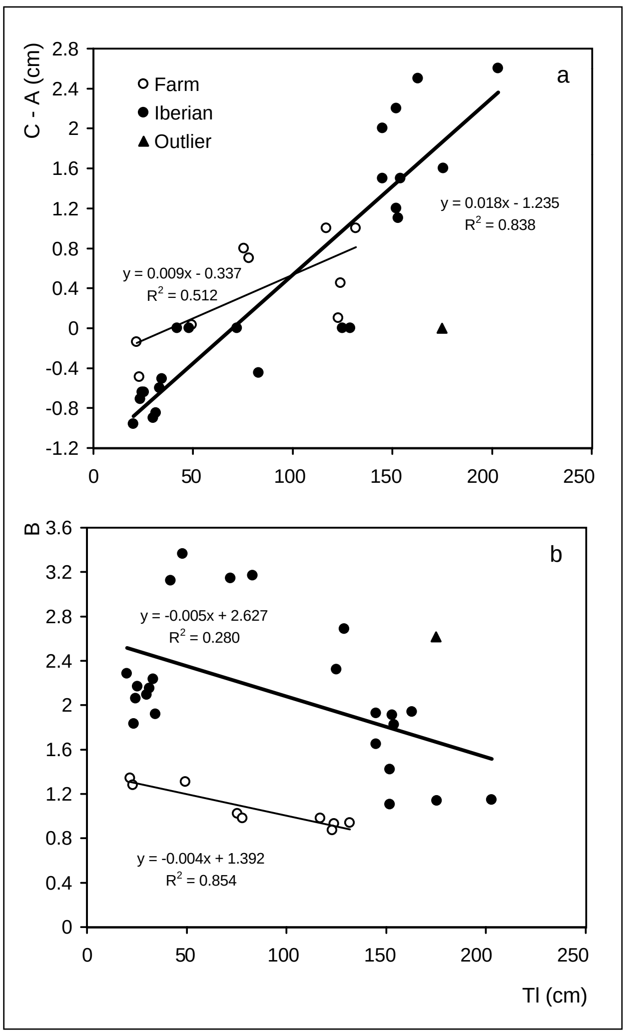

Figure 2. Scatterplots and regressions of (a) C - A (cm), and (b) F:B (dimensionless) against Tl (cm). Symbols as in figure 1

F:B, the other parameter Garrido-Ramos *et al.* used to separate *A. sturio* from *A. naccarii*, is, despite their claim to the contrary, also influenced by specimen size (figure 2; $p < 0.01$). As such, it offers no advantage over measurements such as A, B or F (figure 1), while being plagued by the problems derived from its being a ratio (Atchley, Gaskins and Anderson, 1976; Atchley and Anderson, 1978; Philips, 1983; Packard and Boardman, 1988; Prairie and Bird, 1989; Jackson and Somers, 1989) and not representing a distance between two identifiable features of sturgeon anatomy (Rincón, 2000).

Distribution in multivariate morphospace

The farmed *A. naccarii* specimens were clearly segregated from wild sturgeons taken in Iberian waters on the bivariate plane defined by the first two PCs extracted from the covariance matrix of *log*-transformed morphometric variables (figure 3).

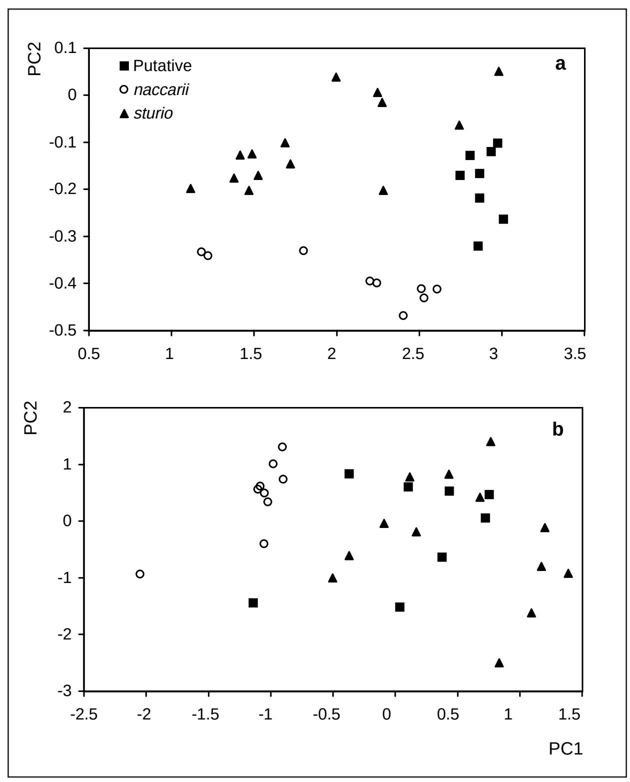

Figure 3. Sturgeon ordination on multivariate morphometric axes from PCAs using (a) the covariance matrix of *log*-transformed measurements, and (b) the correlation matrix of size-adjusted cephalic morphometric traits. Symbols denote specific ascription in Garrido-Ramos *et al.* (1997) and fish origin. Solid triangles: wild-caught *A. sturio*; solid squares, putative wild-caught *A. naccarii*; open circles: *A. naccarii* from the fish farm

This separation occurred along PC2 (5.41 % of variance; eigenvalue: 0.13) which reflected a gradient from longer, more slender snouts (positive end of the axis) towards those shorter and wider (negative end, figure 3a): B had the highest negative loading on it while those of A, C and F were all positive (table III). In contrast, putative wild *A. naccarii*, as recognised by Garrido-Ramos *et al.* (1997), and the other wild sturgeons overlapped extensively along PC2, and only displayed some separation along PC1 (figure 3a). As often happens (Humphries *et al.,* 1981; Bookstein *et al.,* 1985; Rohlf and Bookstein, 1987; Klingenberg, 1996), PC1 was both a general size factor (the loadings and coefficients of the original variables on it were all positive and of a similar magnitude; table III) and the major gradient in the morphospace defined by the current sample (92 % of total variance explained; eigenvalue: 2.28). Component 3 explained little variance (1.87 %) and had a small eigenvalue (0.05), and is not discussed further. Subsequent components were even less relevant.

Table III. Eigenvalues and proportions of variance explained of the first three principal components (PC1-PC3) extracted from the covariance matrix and loadings of the original variables on them

	PC1	PC2	PC3
log Tl	0.97	–0.13	–0.20
log A	0.92	0.37	0.03
log B	0.92	–0.35	0.26
log C	0.98	0.16	< 0.001
log F	0.96	0.27	0.03
log LFR	0.99	–0.04	–0.02
Eigenvalue	2.28	0.13	0.05
% Variance	91.9	5.41	1.87

Therefore, PC2 corresponds to the morphometric traits of the head acknowledged to differentiate *A. sturio* and *A. naccarii* (Holčík, 1989; Garrido-Ramos *et al.,* 1997), and it clearly separated those confirmed *A. naccarii* from the homogenous group formed by all wild Iberian fish, without any gap between the *A. sturio* and the supposed *A. naccarii* of Garrido-Ramos *et al.* (1997), whose only detected differentiation was in size (PC1). These findings replicate almost exactly, being, if anything, clearer than, my previous ones for a sample including only one confirmed *A. naccarii* (Rincón, 2000), and thus further validate them and the conclusions I derived therefrom. Besides, they are also completely consistent with the results of the exploration of head allometry presented immediately above. It is noteworthy that group assignment in that analysis was not based on morphometric traits (e.g. farm vs wild fish) and that the PCA entailed no *a priori* group ascription.

Wild Iberian sturgeons and farmed *A. naccarii* were again segregated on the plane defined by PC1 and PC2 obtained from the correlation matrix of the size-adjusted variables. In contrast, putative wild *A. naccarii* and *A. sturio* overlapped extensively, and showed no spatial separation (figure 3b). Most of the segregation was now along PC1 (figure 3b), which explained 70 % of the variance and had an eigenvalue of 3.49. PC1 reflected the same narrower vs wider gradient in head morphology as PC2 from the covariance analysis, and shared with the latter a substantially similar structure as indicated by the variable loadings on them (tables III and IV). PC2 arranged fish within each group (farmed and wild fish) along it according to their snout width (high loading of B), but explained less variance (16 %) than any of the original variables *per se*, as indicated by its eigenvalue < 1 (table IV). Further components had even lower eigenvalues, and proportions of explained variance and are not presented.

Table IV. Eigenvalues and proportions of variance explained of the first two principal components (PC1-PC2) extracted from the correlation matrix of size-adjusted variables matrix and loadings of the original variables on them

	PC1	PC2
log A_{adj}	0.96	0.04
log B_{adj}	–0.54	0.82
log C_{adj}	0.90	0.16
log F_{adj}	0.96	0.04
log LFR_{adj}	0.73	0.30
Eigenvalue	3.49	0.80
% Variance	69.8	15.9

Therefore, the morphological gradient that PC2 from the covariance matrix identified appears regardless of the method of PC extraction and with both *log*-transformed or size-adjusted variables. It also becomes the major one, capturing most of the remaining variance once the effect of size is removed, and, again, such a gradient neatly separates farmed *A. naccarii* from wild Iberian sturgeons, while it does not distinguish groups within the sample of wild fish. This confirms the results of the previous analyses in present paper. So far, univariate and multivariate examination of the patterns of allometry among morphometric measurements and multivariate ordination of specimens in relatively size-free morphospace has provided no evidence of the presence of two morphologically dissimilar groups among sturgeons caught in Iberian waters during the last century. On the other hand, the aforesaid methods have clearly identified known *A. naccarii* coming from a fish farm as distinct from the remaining fish captured in the wild in waters of the Iberian Peninsula.

These results agree completely with my findings (Rincón, 2000), but run counter to those of Garrido-Ramos *et al.* (1997). I have attributed this discrepancy to the fact that Garrido-Ramos *et al.* (1997) based their results on C - A and F:B without any correction of those parameters for size effects (Rincón, 2000), and the findings presented here provide additional support for this contention, as well as by presenting further instances of C - A and F:B having statistically significant correlation with size, and showing how supposedly diagnostic, species-specific values of C - A are, in fact, size-dependent.

A MANOVA performed on the five original, size-adjusted variables found significant differences between groups (Pillai's trace: 1.023; Wilk's $\lambda = 0.155$; df = 10, 50; $p < 0.0001$) for $log\,A_{adj}$, $log\,B_{adj}$, $log\,C_{adj}$, $log\,F_{adj}$, ($F_{2,29} = 3.71\text{-}14.83$; p = 0.03-0.00004 for each individual variable), but not $log\,LFR_{adj}$ ($F_{2,29} = 0.40$; p = 0.68). Hence, only the first four variables were then submitted to the DFA. The DFA elicited statistically significant between-group discrimination ($F_{8,52} = 7.17$; $p < 0.0001$) and extracted two canonical variates (CV). Only the first, CV1, was statistically significant and had a high eigenvalue (table V). Along it, farmed *A. naccarii* were clearly separated from wild Iberian sturgeons, whereas wild *A. sturio* and putative wild *A. naccarii* overlapped extensively (figure 4). CV2, in contrast, was not significant: its eigenvalue was very low, and provided no group separation (figure 4, table V).

This distinction between farm sturgeons and both groups of wild fish was highly statistically significant according to the F statistics associated with the squared Mahalanobis distances (SMD) between

Table V. Eigenvalues, statistical significance (Wilk's λ associated probability) and variable loadings for the two canonical variates (CV) extracted by a DFA of size-adjusted morphometric variables that exhibited significant between group differences in a previous MANOVA

	CV1	CV2
$log\,A_{adj}$	0.80	0.02
$log\,B_{adj}$	–0.38	0.61
$log\,C_{adj}$	0.43	0.26
$log\,F_{adj}$	0.82	0.34
Eigenvalue	3.04	0.09
Wilk's λ	69.8	15.9
p	< 0.00001	0.484

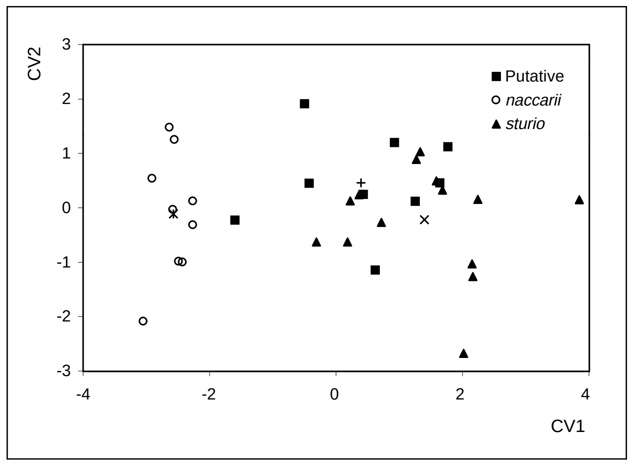

Figure 4. Sturgeon ordination on the plane defined by the two canonical variates (CV1, CV2) extracted by a DFA of size-adjusted, morphometric measurements of the snout. Symbols denote specific ascription in Garrido-Ramos *et al.* (1997) and fish origin. (*), (+) and (×) mark group centroid position for *A. naccarii* from the fish farm, putative wild-caught *A. naccarii* and wild-caught *A. sturio*, respectively. Remaining symbols as in figure 2

the respective group centroids on the plane CV1-CV2 (farmed-putative *A. naccarii*: SMD = 10.07, $F_{4,26} = 9.02$; p = 0.00011; farmed *A. sturio*: SMD = 17.42; $F_{4,26} = 19.34$; $p < 0.00001$). On the other hand, the two putative groups of wild fish were not significantly separated (putative *A. naccarii*-*A. sturio*: SMD = 1.63; $F_{4,26} = 1.81$; p = 0.16). Reflecting this lack of differences, the DFA classified 44 % (4 specimens) of the putative *A. naccarii* as *A. sturio* and 21 % (3 fish) of the *A. sturio* as putative *A. naccarii*. However, it correctly assigned the remaining 11 *A. sturio* (79 %) and all farmed fish to their respective *a priori* groups. Only one claimed wild *A. naccarii* was mistakenly grouped with the farmed sturgeon. This was the largest fish in the sample (Tl = 203 cm), specimen MUC1 in Garrido-Ramos *et al.* (1997), a stuffed specimen captured in the Tagus River (i.e., not from the Guadalquivir basin) in 1890, and which other authors have identified as *A. sturio* according to all other anatomical traits (Almaça, 1988; Elvira, pers. comm.).

CV1 reflected, once more, the difference between the narrower snouts of the wild Iberian sturgeons and the wider ones of the farmed *A. naccarii.* All size-adjusted longitudinal measurements of the snout had positive loadings on it (particularly $log\ A_{adj}$ and $log\ F_{adj}$), while the snout width's ($log\ B_{adj}$) loading was negative (table V). Therefore, the results of the DFA mirror and reinforce those of all previous analyses. Even DFA, a technique designed to maximise between-group discrimination, fails to uncover any significant dissimilarity in head morphology between the two supposed groups of sturgeons from Iberian rivers, once the effect of size is removed. At the same time, DFA clearly detects a highly significant difference in snout shape between all wild fish and the captive *A. naccarii.*

Concluding remarks

Extensive multivariate and univariate exploration that adequately accounts for ontogenetic allometry's effects on the morphometric data in Garrido-Ramos *et al.* (1997) fails to provide any evidence suggestive of the presence of two morphologically dissimilar groups in those sturgeons captured in rivers of the Iberian Peninsula throughout the last century and housed in different scientific collections. These same techniques, however, do adequately identify and separate the farmed *A. naccarii* specimens (acquired at a fish farm that raises sturgeons from Italian stock) from their wild-caught Iberian counterparts. Size is the major axis of morphometric variation within the sample, but, once its effect is statistically removed, a gradient in head shape that provides no discrimination between the two putative groups of wild-caught sturgeons, but that neatly separates farmed fishes from the rest, becomes readily apparent. I had previously identified that gradient (Rincón, 2000), but in my previous paper, due to the insufficient representation of *A. naccarii* in the database (both in terms of number and size range), it represented a smaller proportion of the total morphometric variance, and was weaker and somewhat less clear than in the present paper.

Therefore, the findings and conclusions presented above confirm and support those of my previous work (Rincón, 2000). Both, however, directly contradict those of Garrido-Ramos *et al.* (1997). This discrepancy is largely resolved by the examination of the methodology that produced the evidence that allegedly supported the claims of morphometric evidence proving the past presence of wild *A. naccarii* in the Guadalquivir. As has been shown both here (e.g. figure 2) and elsewhere (Rincón, 2000), Garrido-Ramos *et al.* (1997) employed procedures unable to deal with ontogenetic allometry and, as a consequence, they incorrectly identified larger specimens of *A. sturio* as *A. naccarii.* Again, I must note that according to these authors there would be no *A. naccarii* smaller than 145.00 cm in a sample where putative conspecifics comprise 75% of all adults; an unusual pattern in the light of what is known of the biology and migratory behavior of sturgeons (Holčík, 1989). At face value, this would mean that *A. sturio* is the only species that ever reproduced in Iberian rivers, while *A. naccarii* never did, despite their adults having been apparently quite common.

However, according to my results, the real situation is rather less intriguing: the results of Garrido-Ramos *et al.* (1997) are a methodological artifact, and there is no morphometric evidence to consider *A. naccarii* native to the Iberian Peninsula. Therefore, decisions concerning the management of Adriatic sturgeon stocks in Spain should always bear present Birstein *et al.* (1998) recommendations for farmed sturgeon species in Western Europe: "[farmed sturgeons]... should not be allowed to escape into the wild and should never ever be released."

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Genetic characterization of *Acipenser sturio* L., 1758 in relation to other sturgeon species using satellite DNA

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ABSTRACT

We obtained and characterized a satellite (st) DNA family named HindIII from the genomes of the Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836, Siberian sturgeon *Acipenser baerii* Brandt, 1869, and beluga sturgeon *Huso huso* (L., 1758). We did not find this stDNA in the genome of the Atlantic sturgeon *Acipenser sturio* L., 1758. The comparison of sturgeon species using the HindIII stDNA revealed the following: (1) *A. naccarii* and *A. baerii* are closely related; (2) *H. huso* appears to belong to the genus *Acipenser* and, probably, *Huso* is not a separate genus within the Acipenserinae; (3) *A. sturio* differs from the other three studied species by the absence of the HindIII stDNA and, most likely, it represents a separate evolutionary lineage within the Acipenseridae. The data on the HindIII stDNA can be successfully used for species identification of sturgeon specimens captured in different European regions.

Key words: Sturgeons, nuclear DNA, phylogenetic relationships, genetic identification.

RESUMEN

Caracterización genética de* Acipenser sturio *L., 1758 con relación a otras especies de esturión por medio de ADN satélite

En este trabajo presentamos la caracterización del genoma de Acipenser sturio *L., 1758 en relación con el genoma de* Acipenser naccarii *Bonaparte, 1836,* Acipenser baerii *Brandt, 1869 y* Huso huso *(L., 1758) utilizando una familia de ADN satélite (la familia HindIII). Nuestro análisis revela que: (1)* A. naccarii *y* A. baerii *son especies muy emparentadas; (2)* H. huso *aparece muy relacionada con las especies del género* Acipenser *y, probablemente, podría ser considerada como una especie perteneciente a dicho género, y (3)* A. sturio *difiere del resto de las especies analizadas, lo que sugiere que esta especie ha debido seguir una evolución independiente respecto a las otras especies. Estos datos pueden ser muy útiles, no sólo para establecer las relaciones filogenéticas entre* A. sturio *y las otras especies de Acipenseridae, sino también para la identificación de ejemplares de esturiones capturados en diferentes regiones europeas.*

Palabras clave*: Esturiones, ADN nuclear, relaciones filogenéticas, identificación genética.*

INTRODUCTION

Acipenser sturio L., 1758 is a sturgeon species of interest from conservation and evolutionary perspectives. It is considered an endangered species, for which recovery plans are needed (Birstein, 1993). However, recovery plans can be complicated by the fact that in some parts of the *A. sturio* area it is possible to find some other natural or introduced congeneric species (Rochard *et al.*, 1991).

Thus, historically *A. sturio* was sympatric with the Adriatic sturgeon *Acipenser naccarii* Bonaparte, 1836 and the beluga sturgeon *Huso huso* (L., 1758) in the Adriatic Sea basin (Rossi *et al.*, 1991). Of these species, *A. sturio* has been traditionally included in the genus *Acipenser,* while the beluga sturgeon *H. huso* and its close relative the kaluga sturgeon *Huso dauricus* (Georgi, 1775) were considered as representatives of a separate genus *Huso* within the subfamily Acipenserinae of the family Acipenseridae (Berg, 1904; for details, see Bemis, Findeis and Grande, 1997). However, recent molecular data (studies of mitochondrial DNA) indicate that *Huso* is not a separate genus within the Acipenserinae (Birstein, Hanner and DeSalle, 1997; Birstein and DeSalle, 1998). As for the introduced species, individuals of the non-indigenous Siberian sturgeon *Acipenser baerii* Brandt, 1869 caught in the rivers of Spain (e.g. Birstein, Betts and DeSalle, 1998) were escapes from aquacultured stocks.

In the present study we used a satellite (st) DNA family named HindIII in order to clarify the identification of and to explore the genetic relationships between *A. sturio* and *A. naccarii, A. baerii* and *H. huso.* stDNA consists of families of short, tandemly repeated sequences which are located in certain regions of chromosomes, mainly centromeres and/or telomeres. Because of their rapid evolution, the repeated sequences, including stDNA, are frequently used for taxonomic and phylogenetic purposes (Arnason, 1990; Mikhailova *et al.*, 1995; Garrido-Ramos *et al.*, 1999) and for species identification (e.g. Meredith *et al.*, 1991). The comparison of the HindIII stDNA from four sturgeon species showed the close relatedness of *A. baerii, A. naccarii,* and *H. huso,* and their distant relatedness to *A. sturio.*

MATERIALS AND METHODS

Sturgeon specimens were obtained from the Sierra Nevada S. L. Fish Farm at Riofrío, Granada, Spain (*A. naccarii* and *A. baerii*), from Azienda Agricola VIP, Brescia, Italy (*H. huso*), and from France's National Agricultural and Environmental Engineering Research Centre (Cemagref) in Bordeaux (*A. sturio*).

DNA was isolated from the liver and muscles of three individuals for each species following the method of Sambrook, Fritsch and Maniatis (1989). In summary, 1 g of liver from each specimen was ground with liquid nitrogen in a mortar into a powder. After the evaporation of the nitrogen, 10 volumes of the extraction buffer (10 mM Tris-HCl plus 0.1 M EDTA, pH = 8), 20 μg/ml pancreatic RNAase and 0.5 % SDS were added. The solution was transferred to a 30 ml centrifuge tube and incubated for 1 h at 37 °C. Thereafter, Proteinase K was added to a final concentration of 100 μg/ml and incubation was performed for 3 hours at 50 °C. The DNA obtained was purified using the common phenol/chloroform procedure and, finally, precipitated with ethanol. The pellet of DNA was then dissolved in the TE buffer (10 mM Tris-HCl plus 1 mM EDTA, pH = 8).

The HindIII sequences in the genomes of different sturgeon species were detected by Southern-blot hybridization. The hybridization and the detection of the hybridization sites were performed using the non-radioactive chemiluminescence method, ECL (Amersham), following the manufacturer's instructions. Filters were hybridized for 12-16 h at 42 °C with a peroxidase-labeled probe at 10 ng/ml of the ECL hybridization buffer containing 6 M urea, 0.5 M NaCl, and 5 % blocking agent. After hybridization, the membranes were washed twice, for 20 min each, in 6 M urea, 0.1 X SSC and 0.4 % SDS at 42 °C (high-stringency conditions) or 1 X SSC and 0.4 % SDS at 55 °C for 10 min (low-stringency conditions). The membranes were then washed twice in 2 X SSC at room temperature for 5 min. The 6 M urea in the hybridization and wash buffer is equivalent to 50 % formamide (Amersham). Hybridization sites were detected using the ECL detection reagents, which contained hydrogen peroxide and luminol, and the light emitted in the detection reaction was recorded radiographically.

To clone the HindIII stDNA from different species that have the HindIII sequences, samples of DNA obtained from these species were digested with HindIII restriction enzymes, electrophoresed on a 3 % NuSieve agarose gel, and stained with ethidium bromide. Intense DNA bands corresponding to the monomeric HindIII units were excised from the gel and the DNA was purified with phenol extraction. The HindIII units were cloned in the pUC19 plasmid vector. The ligation was used to transform *Escherichia coli* DH5-α competent cells, and bacteria clones containing recombinant plasmids with the repetitive fragment of interest were selected after screening with an aliquot of labelled DNA from the same band used for cloning. Eleven clones from *A. naccarii*, nine from *A. baerii*, and seven from *H. huso* were selected for sequencing.

Recombinant plasmids containing cloned stDNA sequences were used as templates for sequencing by the dideoxynucleotide chain terminator method of Sanger, Nicklen and Coulson (1977). Sequencing products were analysed using an automated laser fluorescent DNA sequencer (Pharmacia).

Sequence analyses were performed using the computer program GENEPRO v. 6.1 (Riverside Scientific Ent., 1993). For the HindIII sequences, interspecific analysis was studied using method II of Strachan, Webb, and Dover (1985). This method measures the mean variability per nucleotide site using all possible combinations between the different clones of the two species being compared. Basically, this calculation was made in a similar way to the calculation of nucleotide diversity (Nei, 1987) –i.e., adding together all the nucleotide differences between each pair of sequences being compared, and dividing this sum by the number of comparisons made.

RESULTS AND DISCUSSION

First, we analysed a stDNA family named HindIII present in the genome of *A. naccarii*. Our previous study had shown that this stDNA is absent from the genome of *A. sturio* (Garrido-Ramos *et al.*, 1997). Our new results confirm this conclusion: Southern-blot hybridization, using both low and high stringency conditions, did not detect the Hind III stDNA in *A. sturio*. However, we showed that the HindIII stDNA sequences are present in the genomes of *A. baerii* and *H. huso*.

Secondly, we cloned and sequenced several monomeric units of the HindIII stDNA family isolated from the genomes of *A. baerii* and *H. huso* (figure 1). Previously, the cytogenetic experiments (*in situ* hybridization) showed that this stDNA is located in the centromeres of several chromosomes of *A. naccarii* (Garrido-Ramos *et al.*, 1995). Later, Fontana *et al.* (pers. comm.) showed that the HindIII stDNA is also present in the centromeres of several chromosomes of *A. baerii,* the Russian sturgeon *Acipenser gueldenstaedtii* Brandt, 1833, the American white sturgeon *Acipenser transmontanus* Richardson, 1836, and *H. huso*. In contrast, the *A. naccarii* HindIII stDNA did not hybridize with chromosomes of *A. sturio*.

```
                        AGC C
A. naccarii CTTTTTCAAA  CTTTTGGGGC  ATTGAAATTA  TGAAAAAATA  AAATTGGCCA  AAATTATTAT  TTTTT*TGAC

                        CT  TA
A. baerii   ----------  AG--CG----  ----------  ----------  ----------  ----------  ----------

                             A
H. huso      ----C----  AG--CG-TA-  ----------  ----------  ----------  ----------  -----TC---


A. naccarii AGGACCGGAC  CAGACCACTT  TTTCAAAAAA  GGGGGATGTC  TAAATTTTGG TAGTTCTGAA  GATCAAAAAA

A. baerii   ----------  ---T------  ----------  -----*----  ---------- ----------  ----------
                                                       *
H. huso     ---T------  ---T------  -----G----  -----T-G--  -------G-- -----T----  ----------


A. naccarii TTGTGTTTTC  TTGACAGGAA  CGAACCTGTA  AG

A. baerii   ----------  ----------  ----------  --

H. huso     ----------  ------T-TC  T---------  --
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Figure 1. Nucleotide consensus sequences of the HindIII stDNA family in *A. naccarii*, *A. baerii*, and *H. huso*. A consensus sequence for each species was determined by comparative analysis of monomeric sequences isolated from each of the species: 11 monomers for *A. naccarii*, 9 monomers for *A. baerii*, and 7 monomers for *H. huso* were sequenced and analysed. The complete consensus sequence of *A. naccarii* is presented and, in each species, only the differences with respect to the first species are indicated. In variable positions the most frequently occurrent nucleotides are shown. If two nucleotides were equally frequent, the second is given above the consensus sequence. Asterisks indicate deletions

These data can be discussed in phylogenetic terms. The presence of the HindIII stDNA in four *Acipenser* species (*A. naccarii, A. baerii, A. gueldenstaedtii,* and *A. transmontanus*) and in *H. huso* supports the view of Birstein and DeSalle (Birstein, Hanner and DeSalle, 1997; Birstein and DeSalle, 1998) that the genus *Huso* is in fact not separated from *Acipenser*, and the doubts of Artyukhin (1995) concerning the systematic position of *Huso*. Therefore, *H. huso* appears to belong to the genus *Acipenser*. Additionally, the absence of the *A. naccarii* HindIII sequences from the genome of *A. sturio* supports the hypothesis that *A. sturio* (together with the American Atlantic sturgeon *Acipenser oxyrinchus* Mitchill, 1815) represents a separate evolutionary lineage within the family Acipenseridae (Birstein and Bemis, 1997; Birstein and DeSalle, 1998).

Our analysis revealed that the HindIII monomers in *A. naccarii, A. baerii* and *H. huso* are very similar. However, the slight differences in sequences that we found may be valuable evidence for determining the relationships among these species. In *A. naccarii* and *A. baerii,* the mean interspecific sequence divergence between the HindIII repeats was only 2.3 %. However, we found that the mean interspecific sequence divergence between *H. huso* and *A. baerii* HindIII monomers is 8.7 %, and between *H. huso* and *A. naccarii* it is even higher, 11.6 %. Therefore, in *A. naccarii*, and *A. baerii* the HindIII sequences clearly diverge from those in *H. huso*

These HindIII sequences can be used for the genetic identification of specimens captured in the rivers of western Europe. Previously, when we could find the HindIII stDNA in the DNA extracted from a museum specimen (EBD 8173, the museum collection of the Doñana Biological Station, Spain), we concluded that the specimen was not *A. sturio* (Garrido-Ramos *et al.*, 1997); this specimen had been captured in the Guadalquivir River (Spain) in 1974 and was initially identified as such. Notwithstanding, the presence of HindIII stDNA in a specimen should indicate that it belongs to one of the species having the HindIII stDNA (i.e., *A. naccarii, A. baerii,* or *H. huso*). For species identification, it would be necessary to obtain the stDNA from each specimen, sequence it, and compare it with the diagnostic nucleotide sites given in table I. Some sites (nos. 11, 12, 15, 67, and especially 106) provide an opportunity to differentiate *H. huso* from *A. naccarii/A.baerii*, and there are a few differences between the latter two species (sites nos. 10, 67, 87, and 106). Although some of the sites vary intraspecifically (table I), they can be used for species identification.

For example, for the identification of the EBD 8173 specimen, Garrido-Ramos *et al.* (1997) cloned and sequenced a HindIII monomeric unit named pAl1 (EMBL accession number Z50744). Although all three species (*A. naccarii, A. baerii,* and *H. huso*) have A in position 10, there are some monomer variants of *A. naccarii* in which the nucleotide T replaces A, and the only clone sequenced from the specimen EBD 8173 had T at this site, showing that it corresponds to the *A. naccarii* sequence. Similar reasoning can be used for the rest of sites shown in table I. Based on the sequence analysis of the HindIII monomeric units, we concluded that the EBD 8173 is an *A. naccarii* specimen (table I).

In a recently published paper (Doukakis *et al.*, 2000), three independent laboratories could not confirm our previous molecular identification of the two museum specimens, EBD 8173 and EBD 8174 from the collection of the Doñana Biological Station. The authors could not extract authentic DNA from these samples, and concluded that the DNA extracted and then cloned from the specimen EBD 8173 might have been a contaminant. Later

Table I. Diagnostic nucleotides in the HindIII stDNA sequences of *H. huso, A. baerii, A. naccarii,* and in the sequence from the specimen EBD 8173. Asterisks indicate a deletion at the site. At variable sites in which two possible nucleotides were found, the more frequent nucleotide is presented in upper case and the less frequent in lower case

Species	Nucleotide position						
	10	11	12	15	67	87	106
Huso huso	A	A	G	C	C	G	T/*
A. baerii	A	A/c	G/t	C/t	C/t	G	*/A
A. naccarii	A/t	C/a	T/g	T/c	T/*	G/t	A/c
Specimen EBD 8173	T	C	T	T	*	T	C

some of the co-authors of this paper (Almodóvar, Machordom and Suárez, 2000) managed to obtain DNA from the specimen EBD 8174 and identified the specimen as *A. sturio*. Be that as it may, we believe that we escaped some technical problems discussed in Doukakis *et al.* (2000): these authors used the PCR technique, and they could have amplified contaminants. This was the reason why, in our study, we used direct cloning of DNA extracts, which obviates the problems of minute contamination with the DNA from other sturgeon species.[1]

Finally, it is important to mention that, following an independent technique, comparative erythrocytic cytometry, it has been possible to show (Hernando *et al.*, 1999a, b) that specimen EBD-8173 is a multichromosomal sturgeon with approx. 240 chromosomes (as is well known, *A. sturio* is a species with approx. 120 chromosomes, while *A. naccarii* have approx. 240 chromosomes)[2].

CONCLUSIONS

Using the HindIII stDNA as molecular markers for studying the relationships among sturgeon species and for genetic species identification, we have shown the following:

1) *A. naccarii* and *A. baerii* seem to be closely related; the sequence divergence for the HindIII sequences between these species is only 2.3 %. Previously, the close relatedness of these species had been reported by Birstein (1999), Birstein, Doukakis and DeSalle (2000), and Tagliavini *et al.* (1999) using mitochondrial DNA. It was also described by Artyukhin (1995), on the basis of morphological traits.
2) *H. huso* is one of the *Acipenser* species which might be closely related to *A. naccarii* and *A. baerii*. As mentioned above, the fact that two *Huso* species are embedded within *Acipenser* was previously shown by Birstein and DeSalle (Birstein, Hanner and DeSalle, 1997; Birstein and DeSalle, 1998).
3) *Acipenser sturio* differs from the other three sturgeon species studied and, most likely, it belongs to a separate evolutionary lineage within the Acipenseridae. These data agree with the results of Birstein and DeSalle (1998) obtained using mitochondrial DNA.
4) HindIII stDNA can be a useful tool for molecular species identification of unknown specimens of sturgeons captured in different European regions.

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[1] Unfortunately, the authors of the paper have still failed to present serious proof that the DNA they extracted from specimen EBD 8173 (Garrido-Ramos *et al.*, 1997) was authentic, and not a contaminant. Also, they did not take into consideration the new morphology re-examination of specimens EBD 8173 and EBD 8174 discussed by Elvira and Almodóvar (1999; 2000) and Rincón (2000) (these papers are cited in Birstein and Doukakis, 2000). But it seems that the authors no longer insist that specimen EBD 8174 is not *A. sturio*, but *A. naccarii*, as they did in Garrido-Ramos *et al.* (1997) (co-editor's note; July 2000).

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[2] Both cited papers by Hernando *et al.* (1999a, b) do not contain any details on measurements of erythrocytes and any result of the measurements. The size comparison of erythrocytes from fixed tissues kept in different museum collections in Spain (EBD 8173) and Russia (specimens of *A. sturio, Acipenser nudiventris* Lovetzky, 1828, and *A. gueldenstaedtii*) should be taken with extreme caution and reservation: there is no guarantee that the sturgeon samples were fixed and then kept the same way in alcohol. For example, in Russian collections the alcohol-fixed specimens were usually transferred and kept for years in formalin, and then returned to alcohol. Since the Hernando *et al.* (1999a, b) publications cited do not contain any data on measurements and statistics, it is impossible to trust the authors' conclusion that "the average erythrocyte size in this fish (i.e., EBD-8173) did not differ significantly from that in Russian sturgeon *A. gueldenstaedtii* with a similar number of chromosomes (247-250) and was approximately 1.5-1.6 times larger than average erythrocytic size in the Baltic ($2n = 116 \pm 4$) and bastard sturgeon *A. nudiventris* ($2n = 118 \pm 2$)" (Hernando *et al.*, 1999b, p. 805) (co-editor's note; July 2000).

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Restoring *Acipenser sturio* L., 1758 in Europe: Lessons from the *Acipenser oxyrinchus* Mitchill, 1815 experience in North America

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ABSTRACT

Acipenser sturio L., 1758 was once wide-ranging and common in Europe, but it now persists precariously in the wild only in relict populations. Its sister species, the morphologically and ecologically similar *Acipenser oxyrinchus* Mitchill, 1815, exists in the western North Atlantic. Although both forms have suffered from the same three liabilities –overharvest, habitat modification, and pollution– their net effects have been far greater for *A. sturio*. Historically, there were at least 35 populations of *A. oxyrinchus* in North America; their present statuses range from moderately abundant to possibly extirpated. However, interest in conservation of *A. oxyrinchus* has increased greatly over the past two decades and great strides have been made toward their restoration. Techniques used have included surveys of anecdotal information, directed fisheries surveys, comprehensive genetic analysis, hatchery culture improvements, experimental stockings of hatchery-produced young, and fundamental life history research. Moreover, the seriousness of sharp declines in the face of suddenly enlarged fisheries mustered the political will to protect *A. oxyrinchus* from directed fisheries in U.S. waters for a period of up to 40 years (although by-catch remains a concern). Because their intrinsic rate of increase is very protracted, it will be decades before particular populations are once again abundant. Nonetheless, the future for *A. oxyrinchus* appears promising. *A. sturio* is so scarce that each specimen is precious; thus, it is risky to experiment with them. But given the similarities between the two species, those seeking to restore *A. sturio* should be able to adapt much of the information learned from managers of *A. oxyrinchus*.

Key words: Sturgeons, genetic analysis, hatchery culture, relict population, restoration.

RESUMEN

Recuperación de* Acipenser sturio *L., 1758 en Europa: lecciones de la experiencia con* Acipenser oxyrinchus *Mitchill, 1815 en América del Norte

Acipenser sturio *L., 1758 estuvo ampliamente distribuido y fue común en Europa, pero ahora persiste precariamente en estado silvestre sólo en poblaciones relictas. Su especie hermana, el morfológica y ecológicamente similar* Acipenser oxyrinchus *Mitchill, 1815, existe en el Atlántico norte occidental. Aunque ambas formas han padecido los mismos tres riesgos —sobrepesca, modificación del hábitat y contaminación— sus efectos netos han sido mucho mayores en* A. sturio. *Históricamente hubo, al menos, 35 poblaciones de* A. oxyrinchus *en América del Norte; su estado actual varía entre moderadamente abundante a posiblemente extirpado. Sin embargo, el interés por la conservación de* A. oxyrinchus *ha crecido importantemente durante las dos últimas décadas y se han dado grandes pasos hacia su recuperación. Las técnicas utilizadas han incluido inspecciones de información anecdótica, revisiones directas de las pescas, análisis genético detallado, mejora del cultivo en criadero, sueltas experimentales de juveniles producidos en criadero e investigación básica de la historia natural. Además, la gravedad de los bruscos declives frente a los repentinos aumentos de las pescas reunió la voluntad política para proteger a* A. oxyrinchus *de la pesca directa en las aguas esta-*

dounidenses por un periodo de hasta 40 años (aunque la captura ocasional continúa siendo un problema). Debido a que su tasa intrínseca de crecimiento es muy lenta, tendrán que pasar décadas antes de que poblaciones particulares sean de nuevo abundantes. No obstante, el futuro para A. oxyrinchus *parece prometedor.* A. sturio *es tan escaso que cada ejemplar es precioso; así, es arriesgado experimentar con ellos. Pero dadas las similitudes entre las dos especies, los que pretenden recuperar* A. sturio *serían capaces de adaptar mucha de la información aprendida de los gestores de* A. oxyrinchus.

Palabras clave: *Esturiones, análisis genético, cultivo en criadero, población relicta, recuperación.*

INTRODUCTION

Although western Europe has been hypothesised to be the centre of origin for Acipenseriformes (Bemis and Kynard, 1997), this region, and particularly its Atlantic coast, is depauperate in sturgeons. Unfortunately, its single native anadromous form, the Atlantic sturgeon *Acipenser sturio* L., 1758, only exists as relicts in the wild. Once found from northern Europe to the Black Sea, the single indisputably extant population exists in the Gironde River, France (Williot *et al.*, 1997). The precariousness of *A. sturio* is recognised officially (Birstein, Bemis and Waldman, 1997); they are classified as endangered by the IUCN (as of 1994) and critically endangered by CITES (as of 1996).

A. sturio is now the focus of restoration efforts across much of its former range. However, such efforts are complicated by the long absence of the species from many of its native waters, so that there is little localised species-specific scientific knowledge or experience available. Moreover, given the scarcity of *A. sturio*, there are few specimens with which to conduct experiments useful toward its restoration.

One potential source of information that could be helpful in developing effective programmes to restore *A. sturio* is the American experience with *Acipenser oxyrinchus* Mitchill, 1815. *A. oxyrinchus* is the sister species to *A. sturio* (Wirgin, Stabile and Waldman, 1997; Birstein and DeSalle, 1998); one which is so similar morphologically and ecologically that they were treated as a single species prior to the study by Magnin (1964), or as subspecies (Scott and Scott, 1988; Birstein, 1993). *A. oxyrinchus* is found in large rivers from the St. Lawrence, Canada, to the Mississippi, USA. Two subspecies are recognised, *A. oxyrinchus oxyrinchus*, which occurs along the Atlantic coast of North America and *A. oxyrinchus desotoi* Vladykov, 1955, which is restricted to the Gulf of Mexico region (Ong *et al.*, 1996).

The broad distribution of *A. oxyrinchus* is comparable to the native range of *A. sturio* in Europe. Today, however, the conservation status of *A. oxyrinchus*, although strongly compromised, is far better than that of *A. sturio*. The chief difference is that despite severe reductions in overall abundance, *A. oxyrinchus* has lost only a few populations of the approximately three dozen that were known to exist (Waldman and Wirgin, 1998). But concern about *A. oxyrinchus* increased in the 1980s and 1990s, as indications accumulated that many of its stocks were declining. In response, there has been a flurry of research, monitoring, and regulatory actions designed to restore *A. oxyrinchus* populations. In the present paper, I briefly review the life history of *A. oxyrinchus* and its decline and management response, and suggest ways in which this experience can aid European efforts to restore *A. sturio*.

LIFE HISTORY

A. oxyrinchus spawns in deep reaches of large rivers. Spawning times range from February in southern rivers to July in Canadian waters (Smith and Clugston, 1997). Spawning sites typically include flowing water over hard substrate. After several years in fresh or brackish water, most *A. o. oxyrinchus* leave their natal rivers and they may spend a decade or more in the sea before their first spawnings, whereas *A. o. desotoi* (because of temperature constraints) only uses marine waters during winter. Thus, the marine movements of *A. o. oxyrinchus* may be extensive, although there appears to be a tendency to remain within coastal zoogeographic regions (Waldman, Hart and Wirgin, 1996). Despite these extensive movements, estimated gene flow rates and strong stock structuring suggest that the species homes with high fidelity (Stabile *et al.*, 1996; Wirgin *et al.*, 2000).

A. oxyrinchus can reach approximately 300 kg and live as long as 60 years. They mature late (10-25 years), with a positive relationship between reproductive age and latitude (Smith and Clugston, 1997). Females do not spawn every year; in the Hudson River the average spawning interval was estimated at four years (Secor, Stevenson and Houde, 1997). Fecundity ranges between 0.4 and 2.0 million eggs (Van Eenennaam *et al.*, 1996). Males also may not spawn annually (Bain, 1997).

There is little detailed information on the food habits of *A. oxyrinchus.* Riverine juveniles feed on plants, aquatic insects, and small crustaceans and mollusks (Scott and Scott, 1988). Johnson *et al.* (1997) examined stomach contents of 275 specimens caught along the coast of New Jersey and found that they consumed mainly polycheates and isopods.

Secor and Waldman (1999) believe that *A. oxyrinchus* shows a mixture of life history traits selected for both stable environments (large fraction of population biomass composed of adults, extremely delayed maturity, infrequent spawning, large adult size, rapid juvenile growth rates, precocial young) and periodically salubrious environments (high individual fecundity, long reproductive life span, complex age structure). Because of these characteristics, population growth rates are slow (Boreman, 1997).

THE DECLINE OF *A. oxyrinchus*

Declines of sturgeons are commonly attributed to overharvest, habitat modifications (particularly damming), and pollution (Waldman, 1995), but for *A. oxyrinchus*, it is clear that overharvest has been the primary agent. Exploitation by Native Americans occurred as long ago as 2190 B.C. These sturgeon were caught by European colonists with a large variety of gear, including pound nets, weirs, stake row nets, trammel nets, trawls, harpoons, and snares. However, floating and anchored gill nets eventually became the primary means of capture. Total US landings of *A. oxyrinchus* (including low but unknown proportions of *Acipenser brevirostrum* LeSueur, 1818) were first estimated in 1880, and they peaked in 1890 at about 3 350 t (Smith and Clugston, 1997) due to pressure from the international caviar craze of that period (Secor and Waldman, 1999). Delaware Bay supported the largest fishery, constituting 75 % of harvests between 1890 and 1899 (Secor and Waldman, 1999). But after the mid-1890s catches declined rapidly and by 1901 the Delaware Bay fishery had crashed.

Catches in other rivers declined also, but not always as dramatically. For much of the twentieth century, both the abundance of *A. oxyrinchus* and their fisheries remained at relatively stable but low levels. The stated goal of the Atlantic States Marine Fisheries Commission (ASMFC) was to restore *A. oxyrinchus* to fishable abundance throughout its US range, with fishable abundance defined as ~317 t (10 % of 1890 landings). But localised increases in catches in the late 1980s raised concern about the species. For example, the Hudson River stock persisted throughout the 20th century at a moderate abundance while being fished at low levels, primarily for meat. But landings in the state of New Jersey from near the mouth of the river (mostly subadults for meat) rose from 5 900 kg in 1988 to a high of 100 000 kg in 1990 (Waldman, Hart and Wirgin, 1996). Over the same period, a caviar-based fishery developed in the Hudson River which focused on spawning female sturgeon. Most ominously, recruitment in the Hudson continued to decline (Anon., 1998b).

Largely in response to these fisheries, but also due to concerns about *A. oxyrinchus* across its US range, ASMFC pressured states to close their sturgeon fisheries. During 1986, total landings were 42 t, or only 1 % of those reported about 100 years earlier. Closure of the directed fisheries affected about 65 fishermen (Anon., 1998a).

In 1998, a petition to place *A. oxyrinchus* on the federal endangered species list was denied by the U.S. Fish and Wildlife Service and the U.S. National Marine Fisheries Service. Nonetheless, Anon., (1998b) did amend its 1990 Interstate Management Plan for Atlantic Sturgeon in response to deteriorating stocks, most notably the Hudson River stock. All US fisheries were closed by April 1998. Furthermore, an indefinite moratorium was enacted, with the potential to continue for up to 40 years. This potentially extraordinarily lengthy period was defined in order to establish 20 protected year-classes of females in each spawning stock. The amendment also seeks to reduce or eliminate by-catch mortality, protect spawning sites, and where feasible, reestablish access to historical spawning habitats.

MANAGEMENT AND RESEARCH EXPERIENCE WITH *A. oxyrinchus* POTENTIALLY RELATED TO RESTORATION OF *A. sturio*

Define clear and realistic goals

Between 1990 and 1998, the stated goal of the ASMFC (Taub, 1990) was to restore *A. oxyrinchus* populations sufficiently to support harvests that were 10 % of 1890 levels. But this objective was arbitrary, and not based on any biological rationale. Moreover, it did not examine the levels of restoration that could be achieved on a population-by-population basis. History proved this plan to be insufficient and unrealistic, as populations continued to decline.

Possible objectives for mode of restoration of *A. sturio* include development of (1) self-sustaining populations, (2) hatchery-reared-only populations, and (3) hatchery-supplemented populations. Ultimately, these populations could be (a) fished or (b) not fished as “hands-off” populations for “biodiversity” purposes. The initial choice in the pairing of mode and fate in relation to the opportunity presented in a given river system is critical in shaping subsequent efforts.

Determine present and historical status of populations

Anadromous sturgeons are large, long-lived fishes that usually occur in deep waters. Also, they mature late and females spawn only in intermittent years. These characteristics make these large fishes, ironically, rather cryptic. That is, low numbers of sturgeon may persist undetected by man. For example, *A. oxyrinchus* was thought to be extinct in Chesapeake Bay, but increased research and media attention to their possible restoration there stimulated commercial fishermen to notify authorities of incidental recaptures. And researchers discovered not inconsequential numbers of young specimens in Virginia tributaries to Chesapeake Bay. These findings shifted the focus from reintroduction of the species using fish from elsewhere to restoration of the native genetic stock.

The use of publicly-provided information to help steer restoration attempts worked particularly well for *A. o. desotoi.* A pamphlet and poster were developed soliciting anecdotal reports from citizens regarding sturgeon in Gulf of Mexico drainages (Waldman and Wirgin, 1998). This approach yielded a surprisingly large amount of knowledge which subsequently helped steer more rigorous investigations.

The possibility of additional, undetected populations of *A. sturio* should be taken seriously. The British Marine Life Society has catalogued a surprisingly large number of sightings and catches in UK waters (A. Horton, pers. comm.) in recent decades in a part of Europe where it is not even certain the species ever reproduced. And Williot *et al.* (1997) provided a figure of almost 100 captures of *A. sturio* by commercial fishermen in coastal waters from France to Denmark between 1980 and 1994, which must be an underestimate of total incidental catches. However, Rochard, Lepage and Meauzé (1997) interpreted the patterns of distribution of tagged and untagged *A. sturio* in marine waters as indicating a single source, from the Gironde River.

Because relict populations of sturgeons are cryptic, it may be useful to test the likelihood of their persistence before making the critical decision to introduce fish from other stocks. Statistical methods have been developed to accomplish this using incidental captures over time (e.g. Reed, 1996; Grogan and Boreman 1998).

Assess limiting factors

When a long-established sturgeon stock becomes extinct, some factor or combination of factors must have been responsible for its demise. For *A. oxyrinchus*, overfishing appears to be the major cause of population loss (together with habitat modification, to varying degrees). It seems more likely that in Europe, habitat modification has been the primary agent (e.g. Elvira, Almodóvar and Lobón-Cerviá, 1991; Nicola, Elvira and Almodóvar, 1996). But continued directed fishing on declining stocks and by-catch mortality also may have contributed to declines of *A. sturio* (Williot *et al.*, 1997). However, such generalities are insufficient in assessing the limiting factors particular to the loss of each population of *A. sturio.*

The circumstances that led to the loss of each *A. sturio* population deserve a detailed and forthright analysis. For example, it is obvious that if construction of a dam blocked the spawning run in a river and if that dam still stands, then restoration to a self-sustaining population is impossible. But if in another river intense overfishing collapsed the stock but newly stocked fish can now be protected, then that river may be a good candidate for restoration. Each restoration effort is a gamble involving scarce financial and specimen resources. Clear-headed initiatives in well-designed and likely-to-succeed situations will lead to successes that instigate additional programmes; misguided efforts with low probabilities of success will be counterproductive to the overall European effort.

Habitat restoration

Because sturgeons in fresh water occur mainly in large, deep rivers, opportunities for habitat restoration on their behalf are scarce. Beamesderfer and Farr (1997) summarised the few examples of habitat restoration for sturgeons in North America, including manipulations of water levels and addition of artificial substrate to assist spawning. None of these examples included *A. oxyrinchus*. But there was one recent project that should benefit the *A. oxyrinchus* population of the Kennebec River in Maine –the July 1999 breaching of the Edwards Dam– which may be instructive toward European efforts for *A. sturio*.

The Edwards Dam was constructed in 1837, blocking movement of spawning anadromous fishes past Augusta, Maine. Despite its continuing usage as a hydroelectric facility, in a precedent-setting decision, the Federal Energy Regulatory Commission determined that the value of its electric generation was outweighed by its environmental costs, particularly to anadromous fishes. *A. oxyrinchus* was one of several fish species that will benefit from the reopening of 11 km of additional river.

Breaching of the Edwards Dam is the greatest success to date in a new national movement in the US to remove unused or marginally beneficial dams to defragment rivers and restore spawning runs of fishes. Although such actions are politically very difficult, it is worth reviewing the possibilities for similar programmes in European rivers to assist *A. sturio* and other anadromous fishes, it is likely that no other action would have as positive an effect.

Improve culturing capabilities

A. oxyrinchus was first spawned artificially in 1875, but little culture was performed beyond 1912, until recently (Smith and Clugston, 1997). Most notably, a research hatchery of the US Fish and Wildlife Service at Lamar, Pennsylvania, has been improving culture techniques for *A. oxyrinchus*. Tens of thousands of young have been produced from only a few parents. For the most part, these fish have not been stocked in the wild because of concerns about effective population size, non-native stock transfer, and uncertainty about the extinction of certain stocks. Nonetheless, the protocols exist to reliably generate large numbers of young for stocking if required.

Thus, in the US, opportunities for stocking *A. oxyrinchus* are very limited despite the capabilities. The reverse is true in Europe, where almost every river system that hosted *A. sturio* is now apparently devoid of them, but where hatchery production, due to more limited experience with sturgeon, is not as highly reliable. Moreover, reproductively mature *A. sturio* are so scarce that (1) there is a more than trivial chance that individuals may die in captivity, and (2) the chances of successful hatchery production must be weighed against the probable loss of natural production. This raises the important question as to whether European hatcheries are already prepared to culture *A. sturio* in an environment where the loss of broodstock and their potential spawn is serious.

An alternative would be to first practice and refine culture techniques using *A. oxyrinchus* (available from US and Canadian research facilities). But this should only be tried where multiple safeguards exist against their escapement into the wild.

Genetic concerns

Introduction of limited numbers of hatchery-cultured *A. oxyrinchus* into the Hudson River and Chesapeake Bay and the prospects of larger stockings in those systems and elsewhere led to concerns about their potential genetic effects. Genetic issues were two-fold, but not mutually exclusive. One was that of maintaining effective population size to guard against inbreeding. General guidelines for fish culture suggest minimum effective population sizes of 100 to 200 should be maintained (Kincaid, 1995). But because spawning females were so scarce and because the hatchery culture that took place was for research purposes, only one or two females were used in each spawning of the offspring that were later stocked in the wild. The second issue was interstock transfer, i.e. concerns about introducing genes from a non-native stock to a river that might still support a relict stock.

These concerns led ASMFC to develop a protocol for breeding and stocking *A. oxyrinchus* (St. Pierre, 1996). Among its recommendations was to adopt a generation effective population size scheme with 100 as the minimum effective population size of brood fish (with an inbreeding rate of 0.5 %). Year-class effective population sizes should be at least six. Clearly, adherence to this protocol would involve a long-term commitment, e.g. 10 years at an average year-class effective population size of 10.

A second important recommendation was to use, whenever possible, broodfish from the same river in which stocking will occur. When this is not possible, the source of broodfish should be from the same regional genetic grouping as the river being stocked (e.g. Waldman, Hart and Wirgin, 1996; Wirgin *et al.*, 2000).

These protocols developed for *A. oxyrinchus* bear on any parallel attempts to restore *A. sturio*, but with a different emphasis, i.e. the single extant *A. sturio* population of the Gironde River could be degraded by inbreeding depression. This argues all the more for their protection from this stress by either allowing them to increase in abundance naturally or to develop a hatchery-based programme that assures generation effective population size.

However, because almost all European rivers that once hosted *A. sturio* appear to be devoid of them, and because the sources of hatchery-cultured fish to be stocked will be so limited, intrastock transfer is not a primary concern. But it would still be advantageous to the prospects for success of any stockings if the fish planted were outbred.

Protect stocked fish

The population viability of newly stocked *A. sturio* in a river system may be precarious because of limits on the numbers of fish available to be stocked and their subsequent survival. Even if directed fisheries are banned, sturgeon life history characteristics render them particularly susceptible to the forces of by-catch mortality, in that low levels of unnatural mortality forestall recovery (Boreman, 1997). Also, because of their broad dispersion in coastal waters, by-catch mortality may make up a considerable proportion of total fishing mortality; for *A. oxyrinchus* in 1987, nearly 77 % of total landings at a time when directed fisheries still operated (Smith and Clugston, 1997). To minimise all forms of non-natural mortality, sufficient regulations, enforcement, and penalties will need to be in place and be well publicised.

Appropriate monitoring of each restoration effort

Restoration of self-sustaining populations of *A. sturio* will involve documentation of reproduction.

In the US, aspects of the biology of *A. oxyrinchus* stocked in the Hudson River and Chesapeake Bay have been studied and information about their behaviour in comparison with wild juveniles has been learned (e.g. Secor, 1996). But although such monitoring has value, the true measure of success will be whether the stocked fish reproduce. This means holding the major thrust of monitoring in abeyance for more than a decade as the fish mature.

Develop long-term commitments

With a lengthy maturation schedule and a low intrinsic rate of increase, the emergence of results of restoration programmes for *A. sturio* will be extremely protracted in comparison with most other fishes. It is unusual for government agencies to make funding commitments in fisheries on the time-scale necessary for these programmes, that of decades. Management of *A. oxyrinchus* is now being steered by compacts among states, with committee representation by scientists, fishermen, regulators, and academicians. It may be most advantageous if restoration efforts for *A. sturio* coalesce around consortia that involve public, private, and university participation, in addition to government involvement.

CONCLUSIONS

Because of their origins, reaching back one-quarter billion years, their unique morphologies, sometimes great size, and their provision of one of the world's great delicacies, few fish generate as much public interest as sturgeons (Bemis and Findeis, 1994). However, although interest is presently very high, it is not apparent that conditions in many waters of western Europe will be readily conducive to the restocking of sturgeon. Thus, restoration of *A. sturio* to its native European waters is a noble but difficult endeavour.

I have argued for using some information from the North American experiences with management of *A. oxyrinchus*, together with honest assessments of the potential of conditions of individual European rivers to help steer restoration efforts of *A. sturio*. Nonetheless, given the vagaries of present knowledge of the biology of *A. sturio*, the plasticity of its environmental requirements, and imprecise knowledge of the actual environments, it may not always be clear that the present conditions in a river system are suitable for the survival and reproduction of *A. sturio*. In such cases, if sufficient specimens exist, it may be best to conduct an empirical experiment, i.e. stock some sturgeon and let them "test" the waters, and then, patiently, to await the outcome.

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A continuación figurarán un resumen en español y otro en inglés (*abstract*), con el título del trabajo en inglés.

El trabajo, cuando su naturaleza lo permita, se articulará en introducción, material y métodos, resultados, discusión, agradecimientos y bibliografía.

Los símbolos y signos químicos, físicos o matemáticos se escribirán siempre ateniéndose a las normas internacionales vigentes: SI (Sistema Internacional de Unidades), ISO (*International Standard Organization*) y UNE (Una Norma Española). Dichos símbolos, por tanto, se escribirán siempre sin punto y permanecerán invariables en plural. Las normas ISO y UNE servirán siempre de referencia en la elaboración de originales.

En español las mayúsculas también se acentuarán siguiendo las normas correctas de ortografía.

Para facilitar la lectura de números de muchas cifras, éstas pueden separarse en grupos apropiados, preferentemente de tres cifras, a contar desde el signo decimal en uno y otro sentidos; los grupos deben ir separados por un pequeño espacio, pero nunca por un punto u otro signo.

El signo decimal es una coma en la parte baja de la línea. En los textos escritos en inglés puede utilizarse también un punto, siempre en la parte baja de la línea.

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El nombre vulgar de las especies, cuando se citen por primera vez (en los títulos en español y en inglés, en el resumen, en el *abstract* y en el resto del texto), debe ir seguido de su nombre científico y éste, a ser posible, del nombre del autor que la describió y del año. En las veces posteriores en que aparezca el nombre de la especie no se volverán a citar ni autor ni año.

Irán en cursiva los nombres de géneros y especies, así como los nombres de revistas y simposios y los títulos de los libros.

No se aceptarán llamadas a pie de página.

Resumen y abstract

Ambos apartados no excederán de 125 palabras cada uno y darán a conocer los objetivos del trabajo así como los procedimientos seguidos y los resultados y datos más significativos obtenidos.

Al principio del *abstract* se incluirá el título del trabajo en inglés y al final de cada apartado figurarán hasta un máximo de ocho palabras clave, no incluidas en el título y por orden de importancia, representativas del trabajo.

Introducción

La introducción no excederá de 500 palabras, indicará brevemente los objetivos del estudio y proporcionará suficiente cantidad de información como para aclarar el planteamiento del trabajo y la hipótesis que se pretende comprobar.

Material y métodos

Este apartado será lo más conciso posible pero deberá proporcionar toda la información necesaria para permitir a cualquier investigador especializado evaluar la metodología empleada.

Resultados

El apartado de resultados será lo más claro posible y se ceñirá a los resultados de la investigación esenciales para establecer los principales puntos del trabajo.

Discusión

Se incluirá una breve discusión sobre la validez de los resultados observados relacionándolos con los de otros trabajos publicados sobre el mismo asunto así como un informe sobre el significado del trabajo. Se desaconseja discusiones extensas sobre la literatura existente.

Bibliografía

La bibliografía se limitará a los trabajos citados en el texto y sólo figurarán en ella los trabajos publicados o "en prensa". Esta última información deberá indicarse, en lugar del año, entre paréntesis. Las referencias en el texto a los autores se harán citando el apellido del autor (en minúsculas) y a continuación, entre paréntesis, el año de la publicación, o bien poniendo entre paréntesis el(los) autor(es) y el año, separados por una coma. Las observaciones no publicadas, las comunicaciones personales o los trabajos en preparación o en evaluación se citarán exclusivamente en el texto, sustituyendo el año de publicación por "observación no publicada", "manuscrito" ("MS") o "inédito"; "comunicación personal" ("com. pers."); "en preparación" o por "en evaluación", respectivamente. Cuando la publicación sea de más de tres autores sólo se citará el primero de ellos y a continuación la abreviatura *et al.* En la bibliografía, sin embargo, aparecerán los nombres de todos los autores, separados por comas. Las referencias bibliográficas figurarán por orden alfabético y, para un mismo autor, por orden cronológico. Los nombres de las revistas se escribirán preferentemente sin abreviar. Si se prefiere utilizar las abreviaturas, éstas se ajustarán siempre a lo indicado en el *Periodical Title Abbreviations*. 8.ª edición. Gale Research Inc. Detroit; Londres. 1992. Si esto no es posible se escribirán sin abreviar.

Ejemplos de citas bibliográficas:

– De una revista:
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– De un libro:
  Sinderman, C. J. 1970. *Principal diseases of marine fish and shellfish*. Academic Press. Londres; Nueva York: 870 pp.
– De un artículo de un libro que forma parte de una serie:
  Fraga, F. y R. Prego. 1989. Condiciones hidrográficas previas a la purga de mar. En: *Las purgas de mar como fenómeno natural. Las mareas rojas* (Cuadernos da Área de Ciencias Mariñas). F. Fraga y F. G. Figueiras (eds.) 4: 21-44. Ediciós do Castro. Seminario de Estudios Galegos. Sada (A Coruña), España.
– De un artículo de un simposio:
  Figueiras, F. G. y F. Fraga. 1990. Vertical nutrient transport during proliferation of *Gymnodinium catenatum* Graham in Ría de Vigo, Nortwest of Spain. En: *Toxic Marine Phytoplankton*. *Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton* (26-30 de junio, 1989. Lund, Suecia). E. Graneli *et al*. (eds.): 144-148. Elsevier. Nueva York.

Los autores serán responsables de que todas las citas bibliográficas estén completas y de la exactitud de las mismas.

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Todas las ilustraciones (figuras, láminas, mapas y fotografías o diapositivas) deben ser originales y se prepararán en papel de alta calidad de reproducción fotográfica, o en archivos de disquete independientes del texto (junto con copias de impresora laser). Sólo se incluirán aquéllas que muestren datos esenciales; nunca deberá producirse duplicidad de datos por la presentación de los mismos en texto, tablas e ilustraciones.

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Whenever possible, divide the paper into: Introduction, Material and Methods, Results, Discussion, Acknowledgements and References.

Chemical, physical or mathematical signs and symbols should follow standard international usage: SI (*Système International d'Unités*), ISO (International Standard Organization) and UNE (*Una Norma Española*). Therefore, these symbols should always be written without periods, and will remain unmodified when plural. Always refer to the ISO and UNE norms when preparing texts for publication.

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Numbers indicating years should follow this format: 1999 (for nineteen ninety-nine).

The first citation of the vernacular name of a species (in the Spanish and English titles, the abstract, the *resumen*, and the body of the text) should be followed by its scientific name, and then, whenever possible, by the name of the author who described it, and the year. Omit the author and the year in subsequent citations.

Italicize genus and species names, as well as the titles of journals, symposia, and books.

Footnotes will not be accepted.

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Include English and Spanish versions of the abstract (*resumen*, in Spanish), no more than 125 words each, setting out the paper's objectives, as well as the procedures followed and the most relevant findings and data obtained.

Include the title of the paper in Spanish at the beginning of the Spanish abstract. At the end of this section, list a maximum of eight key words, not included in the title and in order of importance, indicative of the paper's contents.

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The introduction should not exceed 500 words, briefly indicating the study's objectives and providing sufficient information to clarify the paper's basic focus and the hypothesis being tested.

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Make this section as concise as possible, while giving all the information necessary to enable any specialist to evaluate the methodology used.

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This section should be as clear as possible, and limited to findings essential for establishing the paper's main points.

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Include a brief discussion regarding the validity of the results observed in relation to those of other published papers on the same topic, as well as a report on the paper's significance. Extensive discussion of the literature is discouraged.

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Examples of bibliographic references:

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– Of a book:

Sinderman, C. J. 1970. *Principal diseases of marine fish and shellfish*. Academic Press. London; New York: 870 pp.

– Of an article from a book which forms part of a series:

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– Of an article from a symposium:

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