Chapter

Action before the Extinction of Endangered Sturgeon Species: With Emphasis on Stock Enhancement and Conservation

Yusuf Bozkurt and Michael Chebanov

Abstract

Sturgeons are one of the most valuable fish species in the world due to their pleasant meat, famous black caviar, and isinglass. Sturgeons are believed to be among primitive groups of vertebrates in the world and have been described as "living fossils". It is assumed that they have been living in the natural waters of the northern hemisphere of the world for 200 million years. Their natural populations are now highly endangered because of overfishing for their black caviar and delicate flesh, destruction of spawning habitat, pollution of freshwater, and human-constructed barriers to access spawning migration. Nowadays, living populations have little chance of survival without stock enhancement and sustainable conservation programs. This review document presents general features of current restoration programs and describes how stock enhancement and conservation strategies can be used to recover Eurasian-based sturgeon resources in peril.

Keywords: sturgeon, stock enhancement, *ex situ* conservation, live gene banks, cryobanks

1. Introduction

Sturgeon is a common name for the 27 fish species belonging to the families of *Acipenseridae* and *Polyodontidae* which are some of the most important aquacultured freshwater fish. Their evolutionary history dates back to the Triassic period some 208–245 million years ago [1].

These fishes are highly appreciated not only because of their quality meat but also for their caviar and isinglass, a kind of gelatin used for various industrial aims derived from sturgeon's swim bladder. In terms of aquaculture, sturgeons are the most promising temperate freshwater species. Today, sturgeons are thought to be the perfect candidate species for aquaculture because of their high commercial value. Furthermore, the enormous financial gains of sturgeon farming are the main impulse for sturgeon aquaculture [2].

Today, sturgeons are described as 'living fossils' and are threatened with extinction due to some reasons such as habitat loss and overharvest according to the Red List compiled by the International Union for Conservation of Nature [3]. It has become clear that conventional measures such as decreasing pollution and protecting habitats to maintain and restore these populations did not reach their aim. Thus, biotechnological tools should be implemented as soon as possible in terms of *ex-situ* conservation programs. This review is aimed at describing the ways of stock enhancement and conservation strategies can aid the protection of endangered Eurasian-based sturgeon species.

2. Taxonomy

Sturgeons are classified within the *Acipenseridae* and *Polyodontidae* families include 27 species in four genera, which are *Acipenser* with 19 species, *Huso* with 2 species, *Scaphirhynchus* with 3 species, and *Pseudoscaphirhynchus* with three species [1, 4, 5].

3. Morphological characteristics

Sturgeons have been known as "primitive fishes" or "relict species" because their morphological characteristics have remained relatively unchanged since the earliest fossil record, which evolved some 200–250 million years ago [6].

Sturgeons are distinguished generally by their cartilaginous skeleton from other bony fish species. They are recognized by their flattened rostra, elongated bodies, heterocercal tail lobes, and distinctive barbels. Additionally, their body is covered by bony dermal plates. The notochord is surrounded by a perichord supporting the cartilaginous structure. The spinal cord is located above the notochord. The caudal fin is typically heterocercal, with the continuation of the spinal cord into the upper body. The sturgeons have an intestinal spiral valve that increases the surface availability for nutrient intake and the time required for food digestion. The swimbladder is simple and physostomous, connected with the gut [7].

4. Biological characteristics

The sturgeons are an anadromous and migratory fish species. Most sturgeon species return from marine systems to the rivers, where they were born when sexually mature. Some species spend their whole life in fresh waters while others migrate into the brackish or seawater after a certain size (e.g. Russian sturgeon (*Acipenser gueldenstaedtii*), Siberian sturgeon (*Acipenser baerii*), sterlet (*Acipenser ruthenus*), and beluga (*Huso huso*) [5]. River currents carry the larvae from the upstream areas to downstream towards backwater areas. During their first year of growth, they reach 18–20 cm in length and begin to migrate to the salt waters [8].

Sturgeons exhibit a very long life cycle their first spawn does not occur until they reach around 7–12 years old. A female sturgeon may release 100,000–3 million eggs. They can grow to very large sizes (up to 6–7 m long). Most of the sturgeon species are anadromous. There are also potamodromous (landlocked) species and forms that spend their entire life cycle in freshwater [9].

Furthermore, hybrids between the beluga (Huso huso) and the sterlet (Acipenser ruthenus) obtained artificially are widely used in aquaculture in the Soviet Union [10], Germany [11], and Japan [12]. It is known that the intraspecific or intergenetic crosses (2n × 2n or 4n × 4n) are fertile, while the interploid hybrids (2n × 4n) are triploid and usually sterile (with a chromosome number of approximately 160–180) in sturgeons [6]. This situation is important in case the escaping of hybrids into the wild from sturgeon farms can lead to genetic contamination of sturgeon populations in the natural waters.

5. Distribution

Sturgeons are especially distributed throughout the Northern Hemisphere where living in lakes, large rivers, coastal waters, and interior seas in Russia, Turkey, Azerbaijan, Iran, Kazakhstan, Turkmenistan, Ukraine, Romania, Bulgaria, China, and Western Europe countries as well as North America (**Figure 1**) [13].

The sturgeons reaching sexual maturity, migrate to the larger rivers in the Northern Hemisphere such as Volga, Kuban, Don, Ural, Danube, Terek, Kura, Sefidroud, Tajan, Gorgan, Atrak, Kızılırmak and Yeşilırmak [14]. Furthermore, most of the natural sturgeon stocks inhabit the basins of the Sea of Azov, the Black Sea, and the Caspian Sea where 90% of the world's supply of caviar and flesh comes from these areas.

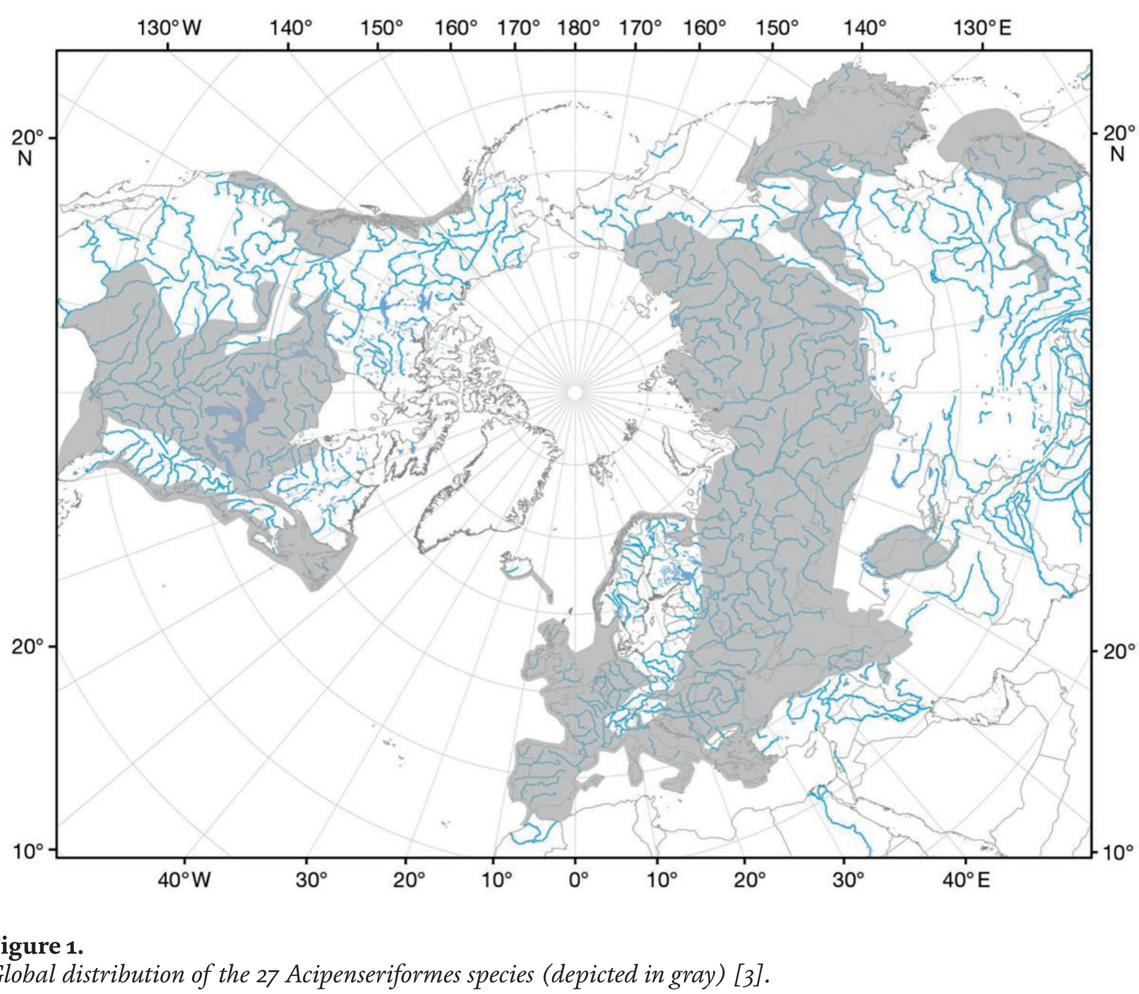

Figure 1.
Global distribution of the 27 Acipenseriformes species (depicted in gray) [3].

6. Current situation

In spite of their long history and abundance until 200–250 years ago, and also their adaptation ability to different habitats such as from the cold waters of Canada and Siberia to the warm waters of northern Italy and Spain, almost all sturgeon species are in danger of extinction (**Table 1**). Sturgeon species have experienced a significant decrease in abundance, as they are sought for their valuable flesh and caviar, by primarily human-constructed barriers to blocking spawning migrations, overfishing, and poaching, as well as due to impacts to their natural habitats such as reductions in river flow, and environmental pollution especially throughout the past century [15].

The decrease in sturgeon populations has prompted some international restrictions on fishing and trade of sturgeons and their valuable products. Currently, existing measures to protect sturgeon focus on holding live breeders in captivity through aquaculture. These attempts have demonstrated the possibility of maintaining a broodstock and showed potential for the conservation of endangered species through management under controlled conditions. For these reasons, those interested in sturgeon aquaculture have started to grow rapidly throughout the world and nowadays almost all sturgeon products such as caviar are the result of aquaculture production [15].

Species	Authority	Continent	Red List status	Year assessed
Acipenser baerii	Brandt, 1869	Eurasia	EN	2010
Acipenser brevirostrum	Lesueur, 1818	North America	VU	2004
Acipenser brevirostrum	Lesueur, 1818	North America	VU	2004
Acipenser dabryanus	Duméril, 1869	Eurasia	CR	2010
Acipenser fulvescens	Rafinesque, 1817	North America	LC	2004
Acipenser gueldenstaedtii	Brandt & Ratzeburg, 1833	Eurasia	CR	2010
Acipenser medirostris	Ayres, 1854	North America	NT	2006
Acipenser medirostris	Ayres, 1854	North America	NT	2006
Acipenser mikadoi	Hilgendorf, 1892	Eurasia	CR	2010
Acipenser naccarii	Bonaparte, 1836	Eurasia	CR	2013
Acipenser nadiventris	Lovetsky, 1828	Eurasia	CR	2010
Acipenser oxyrinchus	Mitchill, 1815	North America	NT	2006
Acipenser persicus	Borodin, 1897	Eurasia	CR	2010
Acipenser ruthenus	Linnaeus, 1758	Eurasia	VU	2010
Acipenser schrenckii	Brandt, 1869	Eurasia	CR	2010
Acipenser sinensis	Gray, 1835	Eurasia	CR	2010
Acipenser stellatus	Pallas, 1771	Eurasia	CR	2010

Species	Authority	Continent	Red List status	Year assessed
Acipenser sturio	Linnaeus, 1758	North America	CR	2010
Acipenser transmontanus	Richardson, 1836	North America	LC	2004
Huso dauricus	Georgi, 1775	Eurasia	CR	2010
Huso huso	Linnaeus, 1758	Eurasia	CR	2010
Pseudoscaphirhynchus fedtschenkoi	Kessler, 1872	Eurasia	CR	2010
Pseudoscaphirhynchus hermanni	Kessler, 1877	Eurasia	CR	2010
Pseudoscaphirhynchus kaufmanni	Kessler, 1877	Eurasia	CR	2010
Scaphirhynchus albus	Forbes & Richardson, 1905	North America	EN	2004
Scaphirhynchus platorynchus	Rafinesque, 1820	North America	VU	2004
Scaphirhynchus suttkusi	Williams & Clemmer, 1991	North America	CR	2004
Polyodon spathula	Walbaum, 1792	North America	VU	2004
Psephurus gladius	Martens, 1862	Eurasia	CR	2010

CR: critically endangered; EN: endangered; VU: vulnerable; NT: near threatened; and LC: least concern.
Source: [3].

Table 1.
IUCN red list status of sturgeon and paddlefish by continent in 2016.

The first sturgeon farming trials were performed almost simultaneously in the mid-nineteenth century in Russia, Germany, and North America to produce juveniles as stock material to prevent population declines in the wild. As a result of the investigations in artificial reproduction, the commercial production of a hybrid between *Huso huso* and *Acipenser ruthenus*, the 'bester', started in the 1960s in Russia [16]. Currently, sturgeon aquaculture has been developing in three directions: (a) to conserve and enhance natural populations via controlled propagation; (b) to generate farmed sturgeon broodstocks for artificial reproduction and commercial aquaculture (including meat and caviar production); and (c) to promote recreational farming such as for aquarium fish and garden ponds [17, 18].

7. Stock enhancement in the wild

In the past, a large number of sturgeon juveniles produced by hatcheries, located in the basins of the Azov, Black, and Caspian Seas (Russia, Turkey, Iran, etc.), were released into the natural water bodies to enhance sturgeon stocks. According to observations, it was seen that such traditional releasing of a large number of juveniles (weighing 2–3 g) from sturgeon hatcheries leads to strong food competition and inefficient use of food resources in natural waters. However, different age and size-graded juveniles that have different spawning ecotypes migrated into the river mouth at different seasons, which reduced competition among the juveniles and optimized the use of food resources in the wild [19].

Resultingly, it is well understood that expanded (from May to September) release of different size- and age-graded juveniles into different sections of rivers will contribute to an elevated survival rate of released juveniles and also to the conservation of biodiversity of established sturgeon populations and rational use of food organisms in different water bodies [19]. From this point of view, long-standing research on the seasonal dynamics of food organisms in the river and estuary, combined with monitoring of growth and survival of different sturgeon juveniles led to supporting of new strategies regarding stocking of sturgeon species to the water bodies. This novel approach implies the importance of optimization of the seasonality of reproduction, the releasing of different size- and age-graded juveniles, depending on the site, season of juvenile releasing, and climate variations (wet or dry years) [20–22].

Additionally, the implementation of controlled reproduction, breeding methods, and rearing systems made it possible to achieve significant success in restoring these species that have not been observed for several decades in the wild. As a result, the number of sexually mature adult beluga and stellate sturgeon in the Krasnodar Center's genetic collection significantly exceeds their current number in the Sea of Azov [6].

8. Stock enhancement in the hatcheries

A logical mitigation strategy to be carried out to reduce the fishing pressure on natural stocks facilitates the sustainable development of sturgeon aquaculture [6]. It is obvious that a genetically heterogeneous broodstock population, comprising from 40 to 200–300 individuals of the same species, should be ensured for the formation of a biologically valuable sturgeon population in the sturgeon hatcheries to back up for gene maintenance. On the other hand, it should be noted that another broodstock population should be provided at another hatchery within the same region as "safety precaution" [23].

By providing fertilized eggs for the growing of fish in aquafarms and also the formation of captive broodstock in wild breeders, the hatcheries contribute to the improvement of sturgeon farming effectively. From this point of view, the installation of a sturgeon broodstock based on hatcheries had to be made in view of the properly prepared breeding plans, considering the optimal species and age structure of the broodstocks. At present, technological feasibilities exist to apply these kinds of programs to provide control of the environmental conditions at the sturgeon hatcheries. Breeding technologies such as artificial spawning, egg incubation, and initial larvae rearing have been used over the last 50–70 years in sturgeon hatcheries [24].

Efforts have been made to broaden the reproduction period in order to provide more effective usage of the hatcheries [21]. It is known that expansion of the reproduction period also allows the insertion of the winter spawning stock into the stocking programs. In this regard, controlling the sexual cycle and spreading the spawning period over the year is possible by using captive broodstocks in the hatcheries. In this way, the combination of thermal and hormonal manipulations, ovulation, and spermiation can be possible earlier or later than the normal spawning period [25]. Additionally, hatchery technologies to collect ovulated eggs in place of performing cesarean surgery, have increased female survival following spawning [26].

9. Monitoring of the farmed broodstock

It is known that captive breeding is an important measure to conserve endangered sturgeon species and also serves as the prime strategy to overcome the challenges in the near future [27, 28]. Monitoring programs should record the biological characteristics of juveniles, including physiological-biochemical indices, teratology, melanophore adaptive response, and resistance to the extreme values of environmental factors (temperature, salt resistance, and oxygen deficiency).

Also, morphological, biological, genetical, and reproductive properties of the mature broodstock should be monitored frequently to ensure its quality and fitness in the hatcheries. Genetic monitoring should be used as a tool in each of the programs to assess whether genetic variation is being maintained and measure the efficiency of conservation programs for endangered sturgeon species [29].

Additionally, behavioral experiments including assessment of the background reactivity to the effect of low/high-frequency sounds and light of long/short duration, swimming capacity of larvae, and fry should be observed [6].

Resulting monitoring of the broodstock at the hatcheries can help avoid inbreeding depression (e.g. late maturation, reduction of reproductive properties, and disease resistance) and also will help to provide broodstock quality and proper health conditions.

10. Monitoring of sexual structure of the broodstock

One of the main problems of the management of sturgeons is their late sexual maturation and long reproduction period between spawnings. But in captivity, males reach maturity in from 3 to 4 years (Russian, Siberian, stellate sturgeon) to 8–15 years (beluga, kaluga). Moreover, in captivity, males can reproduce each year and females every 1–3 years [30].

The gonadal status as an important component of the sexual structure of the sturgeon broodstock requires exact determination and should be monitored throughout the lifetime of the broodstock. Taking into account the lack of external signs of sexual dimorphism in the sturgeon, the sex ratio of the stock should be monitored with the help of a specific ultrasound technique of sexing and staging at the early age of the sturgeon (1–2 years) [31]. For this aim, echograms are used to determine the stages of gonadal maturity in both male and female sturgeons.

The maturity stage is species-dependent and can be attained at different body weights and ages. When analyzing the sexual structure of the domestic broodstock several factors should be considered as follows:

- sexual differences in gametosomatic properties and age of maturity for each species,
- different ages of maturity for females and males,
- intervals between successive spawnings for females and males,
- maximum time of spawning observed for females.

When programs reach the optimum restocking targets, sufficient juveniles and non-mature fish at different age groups should be kept in the hatcheries to establish and maintain a continuously efficient reproductive stock. Thus, the sexual ratio of the broodstock should be under regular control. Consequently, all the broodstock data should be recorded according to their reproductive characteristics, and the variations observed during the monitoring should be recorded as well. In view of these data and results of ultrasound monitoring (gonads, liver, heart) the specimens that do not match the requirements for the brood fish destined for reproduction should be discarded.

11. Solution with the *ex-situ* conservation

When considering the future biodiversity status of sturgeon in nature, conservation of its gene pool is important to maintain for these species to keep from becoming threatened. It is therefore important to take immediate measures to safeguard the genetic diversity of this species. There are several ways to conserve endangered sturgeon species, yet long-term preservation of its genetic structure is an inevitable need. From this point of view, *ex-situ* conservation is one of the effective preservation ways with the aid of cryopreservation biotechnology.

Ex-situ conservation can be described as the "conservation of components of biological diversity outside of their natural habitats". This could be in the form of captive broodstock held in "live banks" like aquaria or aquafarm and also in the form of a frozen state in "gene banks". There is a growing concern for *ex-situ* conservation programs worldwide. *Ex-situ* conservation indicates an integral component of whole conservation programs in many countries [32]. The main purpose of *ex-situ* conservation is to maintain the same genetic structure as the source of the original population. It should be noted that cryopreservation deals with cryobiology which is related preservation of biological materials at ultra-low temperatures such as −196°C. Cellular viability can be preserved in a genetically stable form at this low temperature [33, 34].

In this regard, cryopreservation biotechnology provides the availability of genetic resources (sperm, egg, and embryo) for later use such as propagation material for the (re)stocking or base population for selective breeding programs [32]. Therefore, cryopreservation of genetic material, mostly sperm cells is preferred, and can be used for genetic management programs and also for genetic resource banking of those endangered species [35]. From this point of view, e*x-situ* conservation of genetic material via cryopreservation is an important strategy to preserve genetic diversity. Moreover, the original strain can be reconstructed of the population or variety after required environmental restoration with the help of this biotechnology. On the other hand, these efforts must be done simultaneously with *in-situ* conservation of habitats and the identification of the specific population structures, because only by recognizing the importance of distribution, environmental factors, and mechanisms that have conditioned the adaptive process can conservation of fish biological diversity be achieved.

Growing interest in cryopreservation biotechnology has enhanced the scientific studies in this research field [36, 37]. It is possible to use cryopreserved semen in routine reproduction implementations in aquaculture [38]. To date, more than 200 fish species reproducing with external fertilization, have been tested for sperm cryopreservation [39]. In terms of aquaculture, cryopreserved sperm can be used in breeding programs to produce developed lines and to form genetic resources for aquaculture. Additionally, cryopreserved sperm cells can be used to fertilize viable mature eggs during the off-season and even during the breeding season if the maturity of male and female fishes does not synchronize [40].

At the same time, it is necessary to find ways to protect endangered species. Cryopreserved sperm makes possible the establishment of gene banks and also allows genetic combinations for the endangered species. In spite of the successful application of cryopreservation of sperm in many fish species, it is not possible for the eggs and embryos to exclude oysters. The main reasons are the large size of eggs, penetration of cryoprotectants, and uniform cooling and freezing during the cryopreservation process [40]. Investigations regarding the cryopreservation of teleost eggs and embryos are going on because of difficulties regarding storing and reanimating the following freezing process [41].

Thus, more research is needed to increase membrane permeability in terms of cryosolutions, microinjections of cryoprotectants, and antifreeze proteins In order to overcome this problem. Recent studies have focussed on the cryopreservation of primordial germ cells and tissues for the cryopreservation of both paternal and maternal genomes [40].

12. Establishment of cryobanks

One of the largest areas of interest in the field of cryopreservation biotechnology is in the creation of biodiversity sperm cryobanks [42]. With this biotechnology, it is possible to preserve semen samples for many species and cryopreserved sperm banks can serve as insurance against unforeseen events or outcomes [43]. The creation of sperm banks for the selected stock is necessary to establish genetic selection programs in commercial aquaculture. It is also necessary for the conservation of aquatic species in danger of extinction until the environmental conditions recovered [40].

Furthermore, sperm cryobanking has several significant advantages in terms of cost, security, and labor [44]. Additionally, the management and transportation of frozen samples are relatively simple which allows flexibility to design recovery programs. Also, cryogenic gene banks are less costly than the establishment of live gene banks. Consequently, the use of cryopreserved semen could support conservation efforts for the endangered species through stock enhancement [43].

There are some examples of cryogenic sperm banks in Russia [24], Europe, and North and South America established for aquaculture and conservation purposes [45]. To the best of our knowledge, the oldest fish cryobank in Europe was established in the former USSR. The second was at the Ukrainian Institute in Kharkiv. Other cryobanks were established in the Research Institute of Fish Culture and Hydrobiology (RIFCH-Vodnany) in Czechia and all Russian Research Institute of Freshwater Fisheries [46].

13. Establishment of live gene banks

It is well known that key elements of a biodiversity conservation strategy are not only the creation of controlled conditions but also the reserving of the gene pool of species that are on the verge of extinction. For this reason, the establishment of live gene banks is important to restore the diversity of target species and to increase the efficiency of their control in reproduction. The first live gene bank center of the Azov was established in 1994 in Krasnodar to overcome the challenges as follows [47]:

- collection of all sturgeon species (and seasonal biological forms) presented in the basin,
- capture of suitable brood fish as a source of the necessary material, using available technology and storing technological information in a data bank,
- rehabilitation of endangered sturgeon species,
- domestication of wild specimens of different age groups,
- construction of pilot facilities with a variety of conditions for holding individuals (in cold and warm water),
- monitoring of genetic and physiological peculiarities in the course of selection and building of the broodstock,
- revealing proper conditions and requirements for different seasonal forms.

This Center possesses a heterogeneous broodstock of five sturgeon species (beluga, stellate, Russian, sterlet, and ship sturgeons), all listed in the Red Book of the Krasnodar Region, with a total number of over 11,000 individuals. It is one of the largest living genetic banks of sturgeons in the World [47].

14. Conclusion

Since most sturgeon stocks in Eurasia are considered highly endangered, conservation and restoration of this valuable species are needed urgently. From this point of view, in order to conserve and replenish sturgeons stocks, it is necessary to:

- Conduct more research to improve cryopreservation techniques and embryo banking in conservation programs.
- Implement urgent measures to prevent sea and river pollution from any sources such as the petroleum industry, agricultural pesticide runoff, and domestic sewage outfalls.
- Establish natural refuges and restrict human activities within refuge boundaries.
- Implement urgent measures to maintain natural reproduction by rebuilding adequate spawning stock sizes
- Increase hatchery production in the stocking of juvenile fish per year for restoration and management purposes.
- Implement urgent strict measures against illegal fishing of sturgeon species.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Author details

Yusuf Bozkurt[1]* and Michael Chebanov[2]

1 Department of Aquaculture, Faculty of Marine Sciences and Technology, İskenderun Technical University, İskenderun, Hatay, Turkey

2 Department of Aquatic Bioresources and Aquaculture, Kuban State University, Kuban, Russia

*Address all correspondence to: yusuf.bozkurt@iste.edu.tr

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