CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

Twenty-eighth meeting of the Animals Committee
Tel Aviv (Israel), 30 August-3 September 2015

Interpretation and implementation of the Convention

Trade controls and marking

IDENTIFICATION OF STURGEONS AND PADDLEFISH SPECIMENS IN TRADE (DECISION 16.137)

The attached information document has been submitted by the Secretariat, and was prepared on the basis of information provided by Dr. Arne Ludwig, co-author of the study "*Identification of Acipenseriformes species in trade*", conducted for the CITES Secretariat in 2008. It is related to agenda item 11.

Dr. Arne Ludwig (ludwig@izw-berlin.de) of the Leibniz-Institute for Zoo- and Wildlife Research, Department Evolutionary Genetics, was invited by the Secretariat to provide his views on the study on sturgeons and paddlefish (Acipenseriformes spp.), called for in Decision 16.136. The information that Dr Ludwig provided is structured along the various elements that this study should address, as mentioned in Decision 16.136.

16.136 The Secretariat shall:

a) subject to external funding and in consultation with the Animals Committee, organize a study to:

i) provide an overview of molecular, DNA-based and other forensic methods that could assist in identifying the species and populations of Acipenseriformes specimens in trade, determining the origin or age of specimens, and differentiating wild from captive-bred or aquacultured specimens;

Species identification

There exist several DNA tests based on mitochondrial DNA for the identification of nearly each sturgeon and paddlefish species (reviewed in Ludwig 2008). Although non-direct methods (e.g. gel electrophoresis, restriction fragment length polymorphisms [RFLP]) are widely distributed and still commonly used, capillary sequencing has to be the method of choice because it is a direct method where the results can be compared with reference sequences in international databases. Costs are low and the equipment is available in most research institutes or universities worldwide. In addition, there are several nuclear markers (microsatellites or single nucleotide polymorphisms [SNP]) which are used in species identification. Whereas microsatellites are often used for detection of ploidy levels (Ludwig et al. 2001) which is useful for detection of hybrid among species of different ploidy, some microsatellites (Jenneckens 2001) and SNPs are species specific (Boscari et al 2014). These SNPs are powerful tools for the identification of hybrids too. Unfortunately, species-specific SNPs are missing for most sturgeon species. The Russian sturgeon complex (*Acipenser gueldenstaedtii, A. persicus, A. naccarii, A. baerii*) is the major problem in species identification because these species are closely related (especially *A. gueldenstaedtii* and *A. persicus*), and natural as well as artificial hybrids occurs in large numbers. Without nuclear markers – which have to be developed – a separation of these species is rather difficult because mitochondrial haplotypes are shared among these species (reviewed in Ludwig et al. 2008 and others). Moreover, the development of nuclear markers should possibly go in the direction of a multi-locus approach in order to provide independent diagnostic tools and a consequent higher detection power. In this sense the recent accessibility to genome wide approaches with limited costs has highly increased the potential of new markers discovery.

Population identification

Although catch quota were/are based on populations (e.g. river or water systems), this field received less interest from scientific research in recent years. Data are only available for a few species of interest (*A. naccarii; A. ruthenus; A. schrenckii; A. persicus*; and the North American species). These studies were mostly focusing on mitochondrial sequences and/or microsatellites. Also in this case, next generation sequencing techniques (e. g. Genotyping by sequencing; RADseq) will provide progress in the near future.

Sampling

For both issues mentioned above (species/hybrid identification and population characterization), the lack of reliable samples represents a major problem for several reasons:

i. The scarcity of natural populations makes the collection of samples of wild animals difficult.

ii. The genetic integrity of natural population might be compromised by previous restockings of individuals from different populations or hybrids.

iii. The delivering of samples for scientific purposes requires a time consuming bureaucratic to get the required permissions

Age

There are no genetic tests available to ascertain age.

Aquaculture vs. wild

This issue considers major concern because aquaculture production is increasing significantly. Considering the very recent origin of captive fish in hatcheries and the ongoing use of wild specimen for breeding, diagnostic genetic differences between wild and aquaculture fish cannot be expected. Genetic differentiating between aquaculture and wild fish is only possible based on pedigree analysis based on nuclear markers (microsatellites; SNPs). To do these pedigree analyses requires DNA from all possible parent breeders; if this material is available, the progeny of offspring can be proofed. Genetic tests for pedigree analysis are available or can be developed soon. These approaches would also allow a precise traceability of the hatchery of origin. Additionally, isotope analyses are powerful potential tools for the separation of aquaculture fish (caviar) from wild fish. In these analyses the isotope profiles of farm fish are compared with samples from their environment (e. g. water). However, these analyses were introduced very recently in sturgeon identification. Consequently, experiences are low and data for comparison are not available at the moment.

ii) review relevant developments in this area, including the availability and reliability of uniform identification systems;

As in many other species under CITES control, mitochondrial sequences are used for species identification (e. g. cytochrome b and d-loop sequences). There is a huge set of reference sequences available (reviewed in Ludwig et al. 2008). Normally, these sequences are reliable. But there are also wrongly declared sequences of doubtful origin archived in databases (recently there was a case for *A. sinensis* published in the JAI). We recommend the use of a set of reference sequences to avoid misinterpretations. Notably, mitochondrial sequences cannot be used to identify hybrids. Nuclear markers for the identification of species are only available for a few species, but progress can be expected in the next years.

iii) evaluate the advantages and disadvantages of the different methods (including practicality, costs, time-efficiency, reliability, technical requirements, etc.); and;

Mitochondrial sequences is easy to apply, has medium costs, takes about 2 days, is reliable, and requirements are knowledge in PCR techniques and a capillary sequencer. This test does not identify hybrids.

Nuclear marker are easy to apply, takes only a few hours, are cheap and reliable, but require advanced knowledge of PCR techniques or NGS techniques or a capillary sequencer or gel electrophoresis.

Isotope analyses are difficult to apply because they require reliable reference samples, are time consuming and have medium costs, and need specific equipped laboratories.

iv) formulate relevant guidance for CITES Parties, enforcement agencies, the private sector and other stakeholders;

The most crucial point is the differentiation between aquaculture fish and wild fish. There is no reliable test available at the moment. Genetic approaches are helpful if there is a breeding management (e. g. collection of samples from breeders for pedigree analysis). Isotope analyses are only at the beginning of development. Parties should provide reliable reference material (particularly from wild fish) for the development of isotope tests and pedigree analyses. A collection of reference samples has to be established. Also, the ongoing introduction of wild fish into aquaculture would need to be stopped. There are no signs of inbreeding in sturgeons until now and, especially in polyploid species, inbreeding is definitely not an issue (Ludwig 2011). Additionally, reference laboratories have to be evaluated regularly to verify their reliability.