CONVENCIÓN SOBRE EL COMERCIO INTERNACIONAL DE ESPECIES AMENAZADAS DE FAUNA Y FLORA SILVESTRES

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Trigésima primera reunión del Comité de Fauna
En línea 31 de mayo, 1, 4 y 22 de junio de 2021

Cuestiones de interpretación y aplicación

Reglamentación del comercio

ADENDA AL DOCUMENTO IDENTIFICACIÓN Y TRAZABILIDAD DE ESTURIONES Y PECES ESPÁTULA (ACIPENSERIFORMES SPP.)

1. Este documento ha sido preparado por la Secretaría.

Progresos logrados desde mayo de 2020

2. El Fondo Mundial para la Naturaleza (WWF) Austria ha presentado un proyecto de estudio sobre "Identificación de especies e híbridos, procedencia y origen geográfico de los especímenes y productos de esturión y pez espátula (Acipenseriformes spp.) en el comercio". El mandato y las especificaciones del estudio fueron acordados por el Comité de Fauna, tal y como se solicitó en la Decisión 16.137 (Rev. CoP18). El proyecto de estudio se presenta en el Anexo a esta adenda para ser examinado por el Comité de Fauna.

3. Se recuerda al Comité de Fauna que el Comité Permanente estableció un grupo de trabajo entre períodos de sesiones sobre el "Sistema de etiquetado para el comercio de caviar" y el estudio puede ser importante para este grupo de trabajo que tiene el mandato siguiente:

   Teniendo en cuenta el trabajo realizado anteriormente por el Comité de Fauna y el Comité Permanente, el Grupo de Trabajo:

   a) *examinará los retos prácticos para la aplicación de las disposiciones de la Convención en lo concerniente a la aplicación de las "Directrices de la CITES para un sistema de etiquetado universal para el comercio y la identificación de caviar" que figuran en el Anexo 1 de la Resolución Conf. 12.7 (Rev. CoP17) sobre* Conservación y comercio de esturiones y peces espátula*, habida cuenta del cambio reconocido en muchos casos de especímenes capturados en el medio silvestre a especímenes no silvestres producidos en instalaciones de acuicultura;;*

   b) *según proceda, formulará recomendaciones a la CoP19 para abordar los retos identificados a fin de aportar un enfoque práctico respecto del comercio de caviar obtenido de la acuicultura, incluyendo las enmiendas a la Resolución Conf. 12.7 (Rev. CoP17) que se consideren necesarias; y*

   c) *presentará un informe sobre lo anterior al Comité Permanente.*

Recomendaciones revisadas

4. Se invita al Comité de Fauna a:

   a) examinar el estudio mencionado en la Decisión 16.136 (Rev. CoP18) y presentado en el Anexo de esta adenda; y

   b) formular recomendaciones, según proceda, para ser examinadas por el Comité Permanente, en cumplimiento de la Decisión 16.137 (Rev. CoP18).

IDENTIFICATION OF SPECIES AND HYBRIDS, SOURCE AND GEOGRAPHICAL ORIGIN OF STURGEON AND PADDLEFISH (Acipenseriformes spp.) SPECIMENS AND PRODUCTS IN TRADE (based on SC70 Doc. 44.2)

This study follows the recommendations 16.136 (Rev. CoP18)[1] and therefore aims 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;
ii) review relevant developments in this area, including the availability and reliability of uniform identification systems;
iii) evaluate the advantages and disadvantages of the different methods (including practicality, costs, time-efficiency, reliability, technical requirements, etc.); and
iv) formulate relevant guidance for CITES Parties, enforcement agencies, the private sector and other stakeholders.

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* Determination of age requires sophisticated equipment and expertise. All established methods utilize hard body structures (bones or fin rays). In all cases, the prerequisite for age determination is a marked seasonal difference in growth (hibernation). This implies that animals reared under farmed conditions and especially in aquaculture systems under constant temperatures and feed supply do not reveal any meaningful annual signal that would allow accurate age determination. For this reason the age determination in trade is only possible in those occasions where bony structures are present in the product, and the fish has been subject to annual changes in rearing conditions, thus rendering its application in trade an exception restricted to very few cases and for this reason has not been discussed in this study.

Please note: To ease reading, the term “sturgeon” in the study refers to both sturgeon and paddlefish species. When referring to individual species, the study utilizes the three letter CITES species codes for the Acipenseriformes as detailed in CITES Conf. 12.7. The full list of CITES codes and corresponding Latin names can be found in Annex 3 of this study.

[1] 16.136 (Rev. CoP18) - 16.138 (Rev. CoP18) Identification and traceability of sturgeons and paddlefish (Acipenseriformes spp.) https://cites.org/eng/taxonomy/term/42059.

0. Introduction

According to IUCN, sturgeons and paddlefish (Acipenseridae) are the world's most endangered group of species (IUCN 2010). A major factor contributing to this status is a long-lasting overutilization of the natural populations for the production of their processed eggs being sold as caviar, which is considered one of the most valuable wildlife products.

In response to the dramatic decline in sturgeon populations globally and due to concerns of overharvesting, **all of the 27 species of Acipenseriformes have been listed in Appendix II of the Convention on International Trade in Endangered Species (CITES)** in 1998[2] to ensure that trade in sturgeon products becomes more transparent and thus more sustainable. With the listing under Appendix II, international trade in specimens or products must be controlled and supervised in order to avoid utilization incompatible with the survival of the species in the wild. **Two species,** STU and BVI, were already **listed in Appendix I,** whereby all international commercial trade of these species deriving from the wild is prohibited.

In a second step, in 2000, a **universal labelling system for caviar** was introduced to assist the Parties in identifying legal caviar in trade. CITES Resolution Conf. 12.7 (Rev.CoP17)[3] recommends, that Parties implement the universal labelling system for all caviar (wild sourced and derived from aquaculture) for international as well as domestic trade. The labels must be non-reusable, i.e. they cannot be removed undamaged or transferred to another container. The label may seal the container, or if not, the packaging should permit visual evidence of any opening. Until today, the labelling requirements of CITES Resolution Conf 12.7 (Rev CoP17) have not been implemented for the domestic trade in key markets with the exception of the European Union. China, Japan, Russia and the US have not revised relevant national legislation and CITES labels are therefore not mandatory for domestic trade in these countries, undermining the original purpose of the CITES labelling system to ensure a traceable and thus legal trade.

Resolution Conf. 12.7 (Rev. CoP17) also recommends that Parties **register legal exporters, processing and repackaging facilities** of caviar or sturgeon and paddlefish species, maintain a register of these licensed facilities to which official registration codes should be assigned, and provide this information to the Secretariat.

CITES Resolution Conf. 12.7 (Rev.CoP17) further recommends that range States with shared stocks set catch and export quotas for caviar and meat of Acipenseriformes spp. by consensus on an annual basis. However, no export quotas for meat or caviar from wild caught sturgeon have been communicated to the CITES Secretariat by range States since March 2011; As a result, no international trade in wild sourced caviar or meat of Acipenseriformes spp. from shared stocks (such as the Caspian Sea, the Danube, the Black and Azov Sea, the Amur/Heilongjiang River, the Saint John River/Bay of Fundy) can be considered legal. It should also be noted that today, there are national fishing and trade bans for wild sturgeons in many relevant range States.

Global caviar production and trade dynamics have changed profoundly over the last decades with the rapid growth of aquaculture production. According to CITES trade data, global caviar imports declined from 229t in 2000 to 108t in 2015. During the same time the import ratio of farmed versus wild caviar in international trade switched from 5% aquaculture source in 2000 to 95% in 2015 (Bronzi et al. 2018).

Despite the introduction of CITES regulations and the rapid growth of aquaculture production, illegal fishing of sturgeon and illegal trade in wild meat and caviar is known to occur in many regions and remain a serious threat to drastically reduced wild populations (Cohen 1997, Zabyelina 2014, Van Uhm et al. 2016, Knight 2017, Savić et al. 2018, Hruby 2019, Daea 2019, Luca et al. 2020).

A recent report by TRAFFIC, submitted to the 30th meeting of the CITES Animals Committee (AC30 Inf. 33) in relation to agenda item 17, identified several **types of illegal trade** of caviar and sturgeon products[4]

- Caviar and sturgeon meat taken from allegedly poached wild stocks are on sale at open air markets, "under the counter", or through individual contacts or online offers;
- Caviar labelling is not in compliance with the relevant CITES Resolution (e.g. labelling not containing all required information);
- Wild sourced caviar from sturgeon species can be deliberately mislabelled as aquaculture derived species to allow laundering through the legal trade;
- Aquaculture derived products are deliberately declared as wild sourced or different species to sell at a higher price;
- Falsified or forged CITES documents, or genuine CITES documents issued corruptly, are used to permit exports.

Unfortunately, not many studies aiming to detect illegal sturgeon products in trade have been published, and very few include forensic methods to verify the origin of the products. Yet evidence for illegality in traded products (incl. missing labels as well as mislabelling) was provided for several European countries (such as Bulgaria, Romania, Germany and France) as well as in the US, Japan, Russia and China (Birstein et al. 1998, Doukakis et al. 2012, Fain et al. 2013, Jahrl 2013, Harris et al. 2018, Ludwig et al. 2015, Pappalardo et al. 2019, Van Uhm et al. 2016). A recent market survey in the Lower Danube Region (Jahrl et al. 2021) combined analysis of DNA and isotope composition to determine both species and source of a total of 145 samples

[2] A brief History of Sturgeon and CITES https://cites.org/eng/prog/sturgeon/history.shtml.
[3] CITES Resolution Conf. 12.7 (Rev. CoP17) https://www.cites.org/sites/default/files/document/E-Res-12-07-R17.pdf
[4] Harris, L., Shiraishi, H. (2018): Understanding the global caviar market: Results of a rapid assessment of trade in sturgeon caviar. Cambridge, United Kingdom: TRAFFIC and WWF joint report.

collected in Bulgaria, Romania, Serbia and Ukraine. Results show that products of poached wild sturgeon were available in all 4 countries and that 27 samples (19% of all samples) originated from wild-caught sturgeons, Furthermore, 17 caviar samples (12% of all samples) were sold in violation of CITES Regulations (without mandatory CITES labels, with incorrect CITES codes or imported without CITES permits)**.** Together with a minimum of 214 cases of illegality linked to sturgeons reported by enforcement authorities in the same region 2016-2020 (including the use of illegal fishing gear and seizures of poached sturgeon), this indicates ongoing illegal fishing, processing and trade and highlights the need to test and verify the legality of sturgeon products on the market with forensic methods in other geographic regions as well to combat such practices.

Resolution Conf. 12.7 (Rev.CoP17) specifically urges sturgeon range States to curtail the illegal fishing of and trade in sturgeon and paddlefish specimens by improving the provisions in and enforcement of existing laws regulating fisheries and export. However, current efforts of enforcement authorities to control the regulation of legal trade and prevent illegal trade are still limited to the control of the existence and placing of labels and completeness of the CITES code. To actually verify whether the content of a caviar container complies with the information provided on the label, forensic methods need to be applied. The same techniques are needed to verify the provenance of meat or whole fish offered for sale.

This study reviews the techniques currently available and their potential to be used in identifying Acipenseriformes species and their products in trade. The compilation represents an update of the CITES guideline for the species determination for sturgeons in international trade (Ludwig, 2008). In the meantime, new technologies have been developed and tested that allow a more in depth identification, not only of the species, but also of its geographic and production origin. The following chapters describe the different methods currently available, as well as their advantages and limitations. A summary table comparing the different methods is provided in chapter 6 (table 2).

The authors also provide an outlook for the improvement of the current labelling system and traceability in chapter 5.

1. The role of morphological species determination in trade control

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To allow easy, time efficient identification of sturgeon species encountered either on a vessel, on the bank of a river, in a farm or in trade, sturgeon morphology and morphometrics can be used for a first assessment. For this purpose, principal characteristics that are used to discriminate between the species have to be considered, making this method most suitable to identify intact specimens.

Commonly, an identification key is applied providing clear criteria that lead the user in a stepwise approach from option to option. Unfortunately, most of these keys focus on the typical regional species diversity and as such are of little use once transplants and international trade in sturgeons is concerned. Very few identification keys undergo the task to establish functional means to determine species globally. One of the very few examples of such a key that provides a sound basis for identification beyond regional limitations is the CITES Identification Guide – Sturgeons and Paddlefish 2001[5]. Information is provided in English, French and Spanish. A Ukrainian version of this guide was produced by WWF[6].

1.1. Overall morphology of sturgeons

The gross morphology of sturgeons is characterized by a spindle-shaped elongated body with 5 rows of scutes - one dorsal, two lateral, and two ventral rows - that lend the cross section a pentagonal shape. Between the large bony scutes, smaller denticles/scutelets can be present. The size, shape, colour, and number of these bony scutes and scutelets vary between species. The dorsal fin is placed towards the caudal end of the body. Its shape helps to discriminate between different species. The tail (caudal fin) is heterocercal and has a long upper and a short lower lobe. In several species the upper lobe extends into a fibrillum. The head is conical, with a protruding snout, bearing 4 barbels on its ventral side, between the tip of the snout and the mouth. Their position in relation to the snout is species specific, as is the length of the snout in comparison to head length and body length. Additionally, several measurements like head width, the proportion of head length to head width, total length to head length etc. can support species determination.

1.2. Definition of the relevant characteristics for species determination

The characteristics and termini listed in Figure 1 are used to describe the morphological features. In general, the combination of the following characteristics is used to discriminate between species:

The shape and the proportions of the head provide valuable information for species discrimination. In addition, the coloration, especially the presence / absence of white margins on the snout, the presence / absence of dark patches underneath the tip of the rostrum and the presence of protrusions (wart like structures) underneath the rostrum can support the species assignment.

The following characteristics comprise the major criteria for species identification:

- Form of snout (round, conical, elongated, pointed) and length in relation to head length, rostrum bent up-/downward
- Shape and structure of the dermal cranial bones
- Shape (fan like, round, fringed) of barbels and their relative position between top of snout and mouth (closer to tip of snout, midway, closer to mouth)
- relative size (as wide as head, smaller than head width) and shape (crescent, round) of mouth
- Form of lower lip (split lip or continuous)
- Connection of head with first dorsal scute (free or connected)
- Attachment of the gill covers to the body on the ventral side (free at isthmus or attached to isthmus)

Body

The main information provided by the body and its appendices relate to

- Shape & Coloration

The shape is characteristic with regard to the location of the deepest point of the body in the longitudinal axis, whether this point lies immediately behind the head, in the first body section or in the middle of the body. Typical examples are NUD and SCH with the deepest body section being located immediately behind the head.

- Scute number, size and color

[5] © Environment and Climate Change Canada (ECCC) 2001, 181p. .ISBN 0-660-61641-6
[6] https://ua.danube-sturgeons.org/wp-content/uploads/2019/03/idguide_full.pdf

The number of bony scutes on the dorsal, lateral and ventral rows of scutes provides limited information since the overlap between species is high and only a few species are easily discriminated by this characteristic alone. The size and the contrast of the scutes in comparison to the skin color provide additional cues for identification. For instance, the largely reduced scutes in subadult and adult HUS and DAU discriminate the species from most of the other Acipenserids; the small lateral scutes of RUT occurring in high numbers exceeding those of other species form a white band along the lateral line;

- Additional plates in the anal and post anal fin as well as the post dorsal region present or absent

Around the anal fin, the bony scutes reveal a marked species-specific difference with regard to the position, number and size of the bony structures present. The structures to be considered include the pre-anal, post-anal as well as the scutes that run alongside of the anal fin (if any). Both STU and OXY are typical examples of a species-specific distribution of these scutes.

- Small denticles between the rows of scutes present or absent, form and size of denticles

The interdenticles or scutelets can differ markedly between species. In STU and OXY these are rhombic, in all other species (if present at all) they are star shaped. In GUE, STE and TRA the interdenticles sometimes form an additional row of scutes dorso-laterally, which often is incomplete.

A special shape of the denticles is revealed in the Scaphirhynchus and Pseudoscaphirhynchus species which both have hook-like structures on the top of the rostrum and on the skull.

Fins

The relative position of the fins and the number of fin rays as well as the shape of the fin can be used to discriminate a variety of species. Especially the length and shape of the dorsal fin varies considerably between species, with the extremes being represented by HUS (long and straight) and OXY (short and steep). Furthermore, the position of the anal fin in comparison to the dorsal fin also is species-specific in several species. In FED and HER as well as in the Scaphirhyncus species, the upper lobe of the caudal fin tapers off on a long fimbriate extension of the fin.

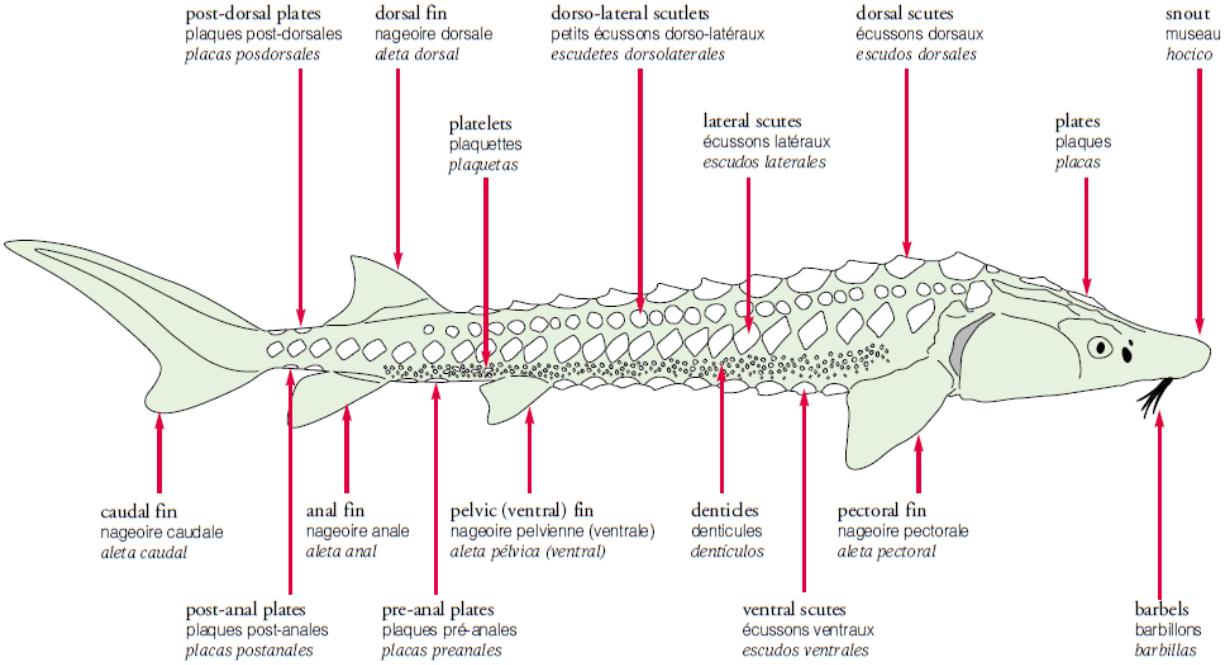

Figure 1: Gross morphology and typical traits used for sturgeon identification (modified after Environment and Climate Change Canada 2001)

How to apply the characteristics in species identification?

For identification purposes, principal characteristics that are used to discriminate between the fish have to be considered, provided that they are meaningful for the discrimination of the species in question. Table 1 provides an example of the overlap between different characteristics in different species.

Table 1: Overview of the main morphological features of different sturgeon species potentially co-occurring due to commercial transplants.

Species	DF	AF	DS	LS	VS	GR	poD	poA	prA
BAE	30-56	17-33	10-20	32-62	7-16	20-49	0	0	1-3
BVI	30-42	19-22	7-13	21-35	6-11	22-29	1-3	1-2	2-3
GUE	27-51	16-35	5-19	21-50	6-14	15-36	0-2	1-2	1-3
MIK	36-40	25-29	8-10	26-36	6-8	18-20	1-2	1-2	2-3
NAC	36-48	24-31	9-21	29-46	8-13	30-35	3-9	1-9	1-3
NUD	39-57	17-37	11-26	33-74	11-17	24-45	0	0	0-3
OXY	38-46	23-30	7-16	24-35	6-14	15-27	3-9	3-9	2-6
PER	27-51	16-35	7-19	23-50	7-13	15-31	0-2	1-2	1-2
RUT	32-49	16-34	11-18	56-71	10-20	11-27	0	0	1-4
SCH	38-53	20-32	11-17	32-47	7-9	36-45	6-8	6-8	3-6
STE	40-54	22-35	9-16	26-43	9-14	24-29	0	0	1-5
STU	30-50	22-33	9-16	24-40	8-15	15-29	3-9	3-5	2-6
TRA	42-52	25-31	11-14	36-48	9-12	34-36	0	0	6-9
DAU	43-57	26-35	10-16	32-46	7-13	16-23	1-3	1-3	2-6
HUS	48-81	22-41	9-17	28-60	7-14	17-36	0	0	0-3

Abbreviations
DF dorsal fin rays, **AF** anal fin rays, **DS** dorsal scutes, **LS** lateral scutes, **VS** ventral scutes, **GR** gill rakers
poD plates between dorsal and caudal fins, **poA** plates between anal and caudal fins, **prA** plates between anus and anal fin, **AL** average length (cm), **ML** maximum length (cm)

1.3.Limitations of morphological species determination

The CITES identification guide (ECCC 2001) also showcases the main drawbacks of the morphological and meristic identification. Despite the fact that it is extremely well illustrated, the overall identification is complicated as for identification purposes species are grouped by geographic region of origin in the guide, while in farming, international transfers have resulted in a massive expansion of the range in aquaculture and on the markets. Therefore, the geographical origin has been rendered mostly meaningless.

An additional drawback for the identification is the fact that morphometric as well as some morphological characteristics are extremely difficult to be obtained in live animals. The shape of gill rakers, number of gill rakers, coloration of spleen, and even fin ray numbers require time and excessive handling of the fish which imposes difficulties even in anaesthetized fish. Additionally, meristic (countable traits and relative measurements), as well as many morphological characteristics, are meaningful only in statistical comparison as can be seen from table 1 showing large overlap in several of the countable characteristics. Therefore, a larger number of individuals of the same species are required to safely determine species by morphology.

1.3.1 Whole fish vs. parts

Intact live and dead fish are the most promising object to attempt identification since a variety of characteristics can be used to verify the potential alternative species.

Parts of fish
To identify the species in sturgeon carcasses is extremely challenging. Some species are easy to differentiate but in several similar species and especially in hybrids the differentiation requires the proper parts of the body that allow meaningful interpretation. While a definite species identification may not be possible in most cases, several characteristics can provide a sound indication of the species or the group of species in question.

Meat
Meat of sturgeons that is offered without skin is not feasible for identification of species or origin using morphological characteristics. Here the genetic species identification is the only means to verify the species in question.

1.3.2. Age limitations

Depending upon species as well as climatic conditions and feed availability, the majority of sturgeon species tend to mature after 8-20 years. The main relative growth takes place during this period, while the individual passes the transitions from yolk sac larvae (eleutheroembryo) hatching from the egg, first feeding fry, juvenile and subadult individual to maturing adult. During this grow out phase a variety of morphological features change allometrically. As a result, a variety of body proportions and in some species even morphological characteristics (e.g. HUS, which reduces its scutes during sexual maturation) change during these phases.

After maturation, the morphology of the species does not reveal substantial changes anymore. Since meristic characteristics depend upon measurements between certain morphological points, which change during allometric growth of the species, the morphometric characteristics do change with age/growth too. Up to now, no comprehensive identification key is available that considers these changes of characteristics over the lifetime of a sturgeon species.

1.3.3. Pure species vs. hybrids

The majority of identification keys focus on pure species – for good reason: pure species are the most conservative entities in expressing phenotypic characteristics, despite the remarkable variation that is observed within one species.

Hybrids

The reproductive barriers among sturgeon species are weak, as demonstrated by the massive production of interspecific hybrids in aquaculture through controlled reproduction (i.e. the cross between two – or even more – species including sturgeon and paddlefish).

In particular conditions however, hybridization can also occur in nature, when the range and the spawning conditions of the species overlap. Especially when sturgeon populations are largely diminished or the extent of their spawning migrations is restricted, hybridizations become more prevalent.

Sturgeon hybrids, even of different ploidy levels, are fertile and thus can reproduce. Natural hybrids are described for a variety of rivers such as the Danube, the Volga and the Ural rivers which comprise the crosses of the predominant species GUE, RUT, STE, and HUS. Sturgeon hybrids usually reveal intermediate characteristics between the parental species, while often the characteristics of the female dominate in the crossing, showing a Mendelian distribution of characteristics. As such, the identification of these specimens based on morphologic characteristics is complicated and requires an expert to perform it thoroughly, often supported by genetic analysis.

A variety of crosses between hybrids, backcrosses of hybrids with their parental species as well as between hybrids and other species have been described. These are mainly produced for aquaculture under the assumption that hybrid vigor would provide an improved performance compared to the pure species under production conditions. Starting with the BeSter (HUSxRUT) several hybrids have obtained an important share in aquaculture production such as SibSter (BAExRUT), GuBa (GUExBAE), and KalAm (DAUxSCH). Escapement into open waters has been described frequently. Since these hybrids reveal an admixture of the characteristics of parental species, the identification imposes major problems in F1 hybrids but the more so if backcrosses or F2...Fn hybrids are considered.

1.3.4 Determination of source

Morphology and morphometric can sometimes allow the discrimination of wild individuals from fish originating from aquaculture. Typical malformations are encountered regularly in farmed individuals and help to distinguish them from wild fish. Again, these characteristics are typical but neither must be present nor are they reliable indicators since fish stocked as juveniles from farmed origin and released into open waters might reveal the same characteristics due to early exposure to intensive rearing.

Typical traits of fish raised in **aquaculture** comprise:

- Bent or deformed pectoral fins
- Partially or totally missing pectoral and/or pelvic fins
- Absence of the septum (nasal bridge) between the nostrils (-> only 1 bigger nostril instead of 2)
- Abnormalities of the eyes like partially covered or missing eyes
- Shortened opercula

1.3.5 Further drawbacks on morphological identification

Eggs and larvae, early juveniles

Sturgeon eggs of different species have a similar appearance with a round to ovoid shape and a darkish brown, grey to black coloration. Also white to yellowish eggs are observed in rare cases. The eggs in sturgeons vary in size between 2 and 5mm diameter, egg size depending upon species, upon the nutritional status of the female, the age of the female and additionally includes an inheritable component. The oocyte is layered with a vegetal and an animal pole surrounded by a multi layered chorion. The eggs differ markedly in the number of micropyles (the passage for sperm through the chorion) between species from 3 to more than 50 but also reveal intraspecific and intra-individual variability (Detlaff, Ginzburg, Shmalhausen 1993).

Upon contact with water and sperm the corticoid reaction is initiated. Besides a swelling of the eggs associated with several changes in cortical structure, the eggs emit a layer of glucoproteins that render the egg adhesive.

The hatch from the egg takes place after 4-8 days as Eleuthroembryos with almost none of their adult characteristics. Sturgeon larvae of different species reveal overlapping characteristics. Differentiation between species increases during the development of the free embryo. Usually, the fish shows the principal characteristics of the adult after 45 days post hatch or more.

Caviar

As mentioned for the eggs, caviar – the salted product of sturgeon eggs – is almost impossible to determine to species level reliably. Size increases with level of maturity at which the fish has been processed. Colour changes with diet as well as a consequence of genetic properties. During times when the products of only three species of sturgeon dominated the caviar market, differentiation of species was more or less secure for experts due to the differences in size, coloration, and taste. The discrimination today is further hindered by the increasing number of species that are used for caviar production, including the utilization of hybrids. Again, visual inspection can provide first clues with regard to the species that might be encountered. But neither the exact species nor the origin can be determined based on morphological features.

While in wild fish only non-ovulated eggs are used for caviar production, in aquaculture, both ovulated and non-ovulated eggs are processed. The main difference morphologically is the presence of the follicle cells in the caviar produced from non-ovulated eggs. These follicle cells provide additional resistance and protection to the oocyte which allows further processing (separation from the gonad, washing and salting) without the need for additional stabilization. In ovulated eggs, the oocyte after the loss of the follicle cells has to be treated chemically to harden and to increase mechanical resistance. For this hardening process several methods can be applied. The discrimination of the two products is possible under a microscope when verifying the presence/absence of follicle cells. The germinal vesicle of the oocyte after ovulation is broken down and the chromosomes undergo a division blockage in metaphase II and as such can be used as an indication of the condition of the egg.

By-products

By-products like isinglass, swim bladders, oils, tissues etc. share the same problem with regard to the identification as any of the other body parts or derivatives. Only sturgeon leather and products made thereof can provide meaningful morphological indication to support species discrimination. Bony scutes, their shape, size and structure as well as that of dermal bones might reveal typical characteristics in several species (e.g. GUE, STE, STU and OXY) but due to the lack of a systematic comparative analysis are not suitable yet for species identification.

In general, it must be mentioned that the identification of a sturgeon species by morphology, even under optimal conditions requires a specialist with experience in this field of work. For laymen the potential to misinterpret characteristics is extremely large with respective outcomes for the result of the identification attempt.

- Identification of the wild Danube sturgeon species: https://youtu.be/8-As8zISvTQ
- Identification of exotic (Non-Danube) sturgeon species and hybrids: https://youtu.be/Z1LrMHGg4SM
- CITES Identification Guide in Ukrainian - based on Sturgeons and Paddlefish (c) Environment and Climate Change Canada, 2001
    - Full version: https://ua.danube-sturgeons.org/wp-content/uploads/2019/03/idguide_full.pdf
    - Short version (Danube species only): https://ua.danube-sturgeons.org/wp-content/uploads/2019/03/IDguide_short.pdf

2. Genetic methods for sturgeon species identification

Authors: Elisa Boscari, Leonardo Congiu, Arne Ludwig

While historically, morphological or biochemical methods were used for identification of sturgeon species (Ludwig 2008), both methods have major disadvantages if samples are processed (e.g. caviar and meat). To circumvent these drawbacks and for versatility reasons, the utilization of DNA-based techniques have become an important tool for species identification in nearly all animal and plant species under trade control over the past 25 years. In general, DNA analysis has several advantages: i) the genetic code is uniform; ii) DNA is thermo stable and can be extracted from all types of tissues even processed caviar and sturgeon meat; iii) only very small sample sizes are necessary; iv) results are reliable and verifiable between laboratories; and v) costs are comparably low.

Almost all species comprise various degrees of intra- and interspecific genetic differentiation. The amount and pattern of these divergences is determined by the formation of new genetic lineages through evolution caused by several factors including mutation, lineage sorting, genetic drift, migration and natural selection. Based on the specific goal of analysis, genetic markers can be used for the identification of i) species and hybrids; ii) populations; iii) sub-populations; iv) maternal and paternal lineages; v) families or even vi) individuals.

2.1 Mitochondrial DNA marker

What is Mitochondrial DNA?

Mitochondrial DNA (mtDNA) is located in mitochondria. Mitochondria are cellular organelles within eukaryotic cells generating most of the cell's supply of chemical energy. The mitochondria, and thus mitochondrial DNA, are passed almost exclusively from mother to all offspring independently of their sex. Unlike nuclear DNA, which is inherited in a recombinant mode from both parents to offspring, mtDNA is inherited in a clonal mode from mother to offspring.

Because the mutation rate of animal mtDNA is between 10x (vs. sex chromosomes) and 100x (vs. protein coding nuclear DNA) higher than that of nuclear DNA, mtDNA is a powerful tool for tracking phylogenetic ancestry through females (matrilineage) and has been used in this role to track the ancestry of many species back hundreds of generations.

Mitochondrial DNA analysis is the prime standard of species identification in trade control. Mitochondrial markers are not only used in sturgeons and paddlefish; they are also in action in whales, sharks, big cats and many more species (Cooper et al. 2009; Dawnay et al. 2007; Dalton & Kotze 2011; Ahmed et al. 2018; Khedkar et al. 2014). About fifteen years ago, indirect methods (RFLP or AFLP patterns; SSCP; nested or semi-nested PCR – these methods were reviewed in detail in Ludwig 2008) were used for detection of mitochondrial DNA variation, because DNA sequencing was very costly and time consuming. Additionally, technical equipment was often not available especially in developing countries. However, in recent years sequence analyses became the most common technique for species identification. Sequencing is the analysis of the order of the four bases (cytosine, guanine, adenine, and thymine) of both types of DNA (mitochondrial and nuclear). The comparison of the sequences from different specimens results in the detection of species-specific differences (substitutions). In general these are compared to publicly available data banks (e.g. Genbank). The majority of these sequences comprise a valuable source for comparison, but some cases have to be treated with caution. A few sequences contain so-called “sequencing errors”, because these sequences were not edited before archiving. Additionally, false classification of reference specimens can also be risky, because it will result in an incorrect determination of species. An example for a false reference was published in 2014. Sequence comparison of eleven Acipenserid mitogenomes (a mitogenome is the complete mitochondrial genome of a species) available from GenBank showed that the mitogenome for SIN (EU719645) was either identified incorrectly or the result of undocumented hybridization with GUE (Dillman et al. 2014). This example demonstrated strongly the need for voucher specimens and reference materials for genetic analyses.

However, advantages and disadvantages of sequencing analysis reveal the highest diagnostic power of all DNA-based methods, and in contrast to the previous study (Ludwig 2008), the costs became relatively cheap. As such, sequencing is a powerful method for the screening of large numbers of samples today.

In sturgeon and paddlefish a huge set of mitochondrial reference sequences is available (see table A in Annex 1). In October 2020, complete reference mitogenomes were available for 23 out of 27 sturgeon and paddlefish species. Only for two important caviar producing species: *NAC* (which is currently under sequencing (Congiu L. pers. comm.)) and *PER*, complete reference genomes are not yet available, however many partial mitochondrial sequences exist for these two species (see Table A in

Annex 1). The Mitogenome for *KAU* was released very recently (Sheraliev et al. 2020). Reference mitogenomes are also missing for *FED* and *HER*. A reasonable amount of partial sequences from several mitochondrial genes is available for *HER* in Genbank; but no genetic information has been published for *FED* so far. None of the three species of the genus Pseudoscaphirhynchus is found in international commercial trade today.

2.2 Nuclear markers

The genome of each individual results from the fusion of haploid maternal and paternal genomes carried by the respective gametes. Consequently, at each locus, an organism has half of the alleles inherited from the mother and half inherited from the father. This biparental transmission makes nuclear genetic markers more informative for all applications in which the genetic contribution of both parents such as relatedness analyses or identification of inter- (between species) and intraspecific (within species) hybrids is relevant. These applications are especially useful for sturgeon since their ability to produce fertile hybrids, which can be backcrossed with one of the parental species or further hybridize with additional species, generates genomes with various degrees of admixed contributions. As an extreme example may serve the recent hybridization of GUExSPA (Káldy et al. 2020). The research in sturgeon species identification tools has been focussing on bi-parentally inherited nuclear markers with the aim of detecting hybrids only during the last decade.

It is not proposed to consider the studies that pioneered the use of nuclear markers using multilocus approaches such as RAPD, AFLP, extensively reviewed in Ludwig et al. (2008), as these are not used anymore due to their technical limits. For the purpose of species identification, the focus will be directed upon the approaches presently applied, that involve two classes of markers, the microsatellites and the Single Nucleotide Polymorphisms (SNPs), which can be used individually or in combination. Nuclear markers suitable for species and hybrid identification are also summarized in table B in Annex 1.

2.3 Microsatellites

Microsatellites (also called Simple Sequence Repeats - SSR; Short Tandem Repeats - STR; or Variable Number Tandem Repeats - VNTR) are tandemly repeated and usually non-coding motifs of one to six nucleotides with Mendelian inheritance pattern. At a given locus, the number of repeats is variable with a high-level length polymorphism and the different lengths are used for allele definition. Given their high variability, the widespread occurrence in eukaryotic genomes, and the easy analyses by PCR, they have for decades been the most common markers for the study of genetic differentiation at different levels of diversity (relatedness, population analyses, and species identification) in many eukaryotes including sturgeon and paddlefish (Ludwig et al. 2008).

One of the main advantages of microsatellites is their high variability: the presence of a high number of alleles per locus (for example compared to SNPs) makes microsatellites very informative for the study of genetic diversity. However, these markers also have some limitations. The main drawback is the need for a priori identification of the regions containing microsatellites and the need to design the primers for analysis. In sturgeons, compared to other species, this problem is partially compensated by the high number of loci already available and their high transferability across species. A second limitation of microsatellites is imposed by the limited comparability of the results among laboratories. Different analytical conditions, indeed, can cause small allele shifting so that the same individual analyzed in different laboratories may have slight differences in allelic size. In order to have a reliable allele sizing, all laboratories relying on this approach should include some reference samples with known genotypes in their sample set and subsequent intercalibration. Other problems such as misidentification due to allelic dropout, null alleles or wrong allele dosage estimation can also occur but they are locus-dependent and require specific statistical corrections during data analyses.

The use of microsatellites as diagnostic markers for species identification is generally based on the detection of alleles that are observed exclusively in a given species. But private microsatellite alleles are rare in sturgeon and paddlefish. Nevertheless, a prominent example of a valuable allele for species identification had been found for STE in Locus Afu-39 (formerly known as LS-39) (Jenneckens et al. 2001). All specimens of STE have fixed alleles of 111 bp. This allele can be used for STE identification and for hybrids with STE sturgeon involvement. But often “private or fixed” alleles are not available and in such a case also high-frequency alleles in the different species can be used in allocation procedures setting up a multilocus approach. In both scenarios, it is important to consider that the probability to observe a given allele in a genome depends not only on the allele frequency in the species but also by its ploidy level and this is valid not only for microsatellites but also for SNPs. For example, given one allele with a frequency of 50%, it will be observed with at least one copy in the 75% of diploid individuals and in the 93.7% of the tetraploid ones. Based on these criteria, several microsatellite loci have been proposed as diagnostic markers or multi-locus approaches for the detection of different sturgeon species (Barmintseva & Mugue 2013; Chassaing et al. 2011; Boscari et al., 2017).

However, microsatellites are also used for the detection of ploidy (Ludwig et al. 2001). This has specific importance for the identification of hybrids from parental species of different ploidy levels, using the fact that sturgeon species have different ploidy levels and therefore disomic and tetrasomic loci. A cross between a disomic species and a tetrasomic species produces trisomic hybrids.

2.4 Single Nucleotide Polymorphism

Single Nucleotide Polymorphisms (SNPs) are substitutions of a single nucleotide in the DNA of a species. They represent the most frequent case of genetic polymorphism. Such substitutions can be localized both on coding and non-coding regions and their characterization can be carried out through different approaches. Species identification based on SNP analysis has become increasingly important in recent years, especially thanks to the advent of massive sequencing methods that allow genotyping hundreds of individuals at hundreds or thousands of loci simultaneously. The advantage of these approaches is that they allow the identification of an applicable panel of loci, the so-called SNP calling procedure. Nowadays, it is no longer necessary to identify a panel of polymorphic loci before carrying out the genotyping but the two steps occur in a single process, the so-called genotyping-by-sequencing in which thousands of polymorphic loci are identified and simultaneously genotyped in hundreds of individuals. The large number of SNPs present in the genome and the ease of characterization represent the main advantage of SNPs over microsatellites.

In order to apply the SNPs in routine forensic analyses, SNPs with diagnostic power ("private" polymorphisms) must be identified in order to subsequently develop fast and cheap single locus analyses. Diagnostic SNPs can be searched either through the above-mentioned whole genome approaches based on next generation sequencing (Ogden et al. 2013; Havelka et al. 2017) or by focusing on highly polymorphic regions in the genome such as intronic sequences (Boscari et al. 2014, 2017a, 2017b). Since the use of diagnostic markers might be prone to analytical limitations and, in certain experimental conditions, the occurrence of false positive detection is possible, the utilization of more than one diagnostic SNP is very important to secure diagnostic vigour, whenever possible. For the same reason, a replication of each assay is strongly suggested.

Once the diagnostic SNPs have been identified, a specific locus detection test is generally developed based on the design of diagnostic PCR primers that give an amplification product only if the species targeted by the primer is present in the analyzed sample. The possibility to develop markers based on the presence/absence of diagnostic bands, therefore ensuring good transferability and replicability among laboratories, represents another important advantage of SNPs compared to microsatellites.

The use of nuclear SNPs for the identification of sturgeon species was first proposed as a single locus approach by Boscari et al. (2014). The authors have characterized a panel of diagnostic SNPs within an intronic region of the RPS7 gene thus setting up a PCR-based identification tool. The approach, combined with mtDNA, allows the detection of 5 species (NAC, FUL, STE, SIN and TRA) with a 100% accuracy and two among the most commercialized hybrids, Bester (HUS female x RUT male) and AL (NAC female x BAE male), with a 80% and 100% success, respectively. The use of intronic SNPs has been later extended to other intronic regions again in a single locus approach: the screening of introns of the gene RPS6 allows the characterisation of a diagnostic SNP fully suitable for HUS (Boscari et al. 2017a), while introns from the RPL4 and RPL5 genes combined with a microsatellites panel allow to setup a multi-locus approach for the differentiation between SCH and DAU with good overall diagnostic results (Boscari et al. 2017b). Havelka et al. (2017) have identified other diagnostic SNPs for RUT and HUS after a massive screening of loci obtained through Next Generation Sequencing. More recently, Havelka et al. (2019) isolated new SNPs shared by BAE and GUE through a ddRAD approach, thus allowing detecting at least one of these two species as paternal species in hybrids.

2.5 Discussion of genetic markers

Today, a large set of different types of genetic markers is available for sturgeon identification. Although nuclear markers (e.g. SNPs) receive increasing importance, mitochondrial sequence polymorphisms are still most often used in forensic analyses. The advantages of mitochondrial sequence polymorphisms lie in the fact that they are i) easy to handle; ii) reliable; iii) backed up by a huge set of experiences; and iv) widely accepted in court. When summarizing mitochondrial DNA based species identification of sturgeon and paddlefish, we can conclude that species-specific (diagnostic) mutations are available for nearly all target species. Diagnostic mitochondrial markers are only missing for PER. However, this is rather a phylogenetic issue than an issue of missing genetic data because the species status of PER is uncertain. Even if some authors support the status of a separate species, it is very likely a subspecies of GUE from a genetic viewpoint.

Taken together, mitochondrial DNA analysis is a powerful tool for pure-bred species detection. But the following limitations exist: i) a separation of GUE and PER is still not possible and ii) hybrids and introgressed species (historical hybridisation) cannot be identified based exclusively on mitochondrial DNA because of its maternal mode of inheritance.

In recent years, the occurrence of commercial products such as caviar or meat of hybrid origin has been increasing significantly. These hybrids opened new frontiers to commercial frauds in which, for instance, the hybrid caviar is sold as a product obtained from the pure-bred maternal species, in order to cheat standard controls exclusively based on mitochondrial DNA. Alternatively, pure-species wild caviar (illegal) is sold as being of captive hybrid origin (greenwashing). The large variety of hybrid lineages that involve more than two species, which have been produced for commercial and scientific purposes, increases the complexity of the genetic species analysis while nuclear markers have the power to detect first generation hybrids and their backcrosses with the parental species or crosses with additional species.

Genetic markers (mtDNA + SNPs and microsatellites) are powerful tools for purebred species identification as well as for hybrid detection and should be used in combination with each other as well as with mitochondrial sequencing. Based on available genetic methods, we can identify nearly all sturgeon and paddlefish species with a high degree of certainty. Only GUE and PER are problematic due to their unclear phylogenetic relationship. We strongly recommend checking the status of PER as an independent species. Nevertheless, more genetic markers would improve species identification as well as population and hybrid detection or even enable pedigree analysis for control of farmed fish.

2.6 The challenge of reference samples

In the development of genetic tools for species identification, the selection of reference samples (RS) is a crucial phase, irrespective of the process used. RS must be certified as regards their purity and must be representative of the genetic variability of the species or population of origin.
There are different possible biases introduced using inadequate RS mainly due to: i) the lack of purity, ii) the non-representativeness of the genetic diversity of a species, and iii) the low number of reference species included in validation tests. All these points are discussed below.

Impure reference sample

Often, for the development of diagnostic markers, animals from aquaculture plants or in any case from captive stocks are used as RS. The history of these animals is generally known but in some cases, following tank transfers or the acquisition of new individuals from other plants, traces of their origin are lost. This generally does not represent a big problem since the species are morphologically recognizable. However, in aquaculture, pure individuals often coexist with hybrids of different degrees showing intermediate features which make the morphological-based identification problematic. This is especially true in the case of hybrids not of the first generation but descending from a hybridization event followed by several back-crosses. In these cases, the contribution of the introgressing species is retained in the population or in the captive stocks for several generations being progressively diluted. The result is that some animals that phenotypically are classifiable as pure species might have a certain fraction of genes from other species. If these animals are included in the reference dataset, the possibility to identify diagnostic markers decreases dramatically. This situation was hypothesized in the case of the study conducted by Boscari et al. (2017) on DAU and SCH. The authors have failed to identify a clear diagnostic marker to distinguish the two species, despite their belonging to different genera and their morphologically being very different. The production of hybrids between the two species is in fact very common in China and the two species are also known to occasionally hybridize in nature by being sympatric. The authors speculate that there has been some natural or artificial introgression events in the ancestry of some of the animals used as a reference for the two species.

Reference samples not representative of the variability of a species

Again, since animals often derive from stocks kept in captivity, it is possible that the individuals used as a reference for a species are not representative of its genetic diversity. In some cases, it is even possible that animals related to each other are used. In these cases, a genetic variant that should be identified as always present or always absent from a given species may not be and the use of this variant in forensic analysis could lead to false interpretations. For this reason, it is important to verify that the validation process of the identified markers has been carried out on the largest number of animals possible, with different origins, possibly analyzed by different laboratories.

An example of this type of bias is given by Zhang et al. (2013) in which the authors identified several reciprocal hybrids generated by switching the sire's and dam's species (BAE♀ × GUE♂ versus GUE♀ × BAE♂; BAE♀ × SCH♂ versus SCH♀ × BAE♂ and DAU♀ × SCH♂ versus SCH♀ × DAU♂) as genetically distinct. This is not genetically expected as first-generation hybrids between two species should group together regardless of the maternal or paternal species. It is conceivable that the group of hybrids analysed in this study are made by related animals and are not representative of their category.

Limited number or reference species

A third problem, often observed in published papers, is given by a limited number of reference species. A suitable species identification marker is expected to show one state in the target species and alternative states in all other ones. This condition can be verified only if all other species are included in the validation test but this is unfortunately often not the case. An example is given by a SIN marker that was proposed by Boscari et al. (2014) which, following further validation tests, proved to be partially shared with a part of the individuals of DAB, a species never before tested with this marker.

3. Specific chemical compound profiling (fatty acid)

Author: Markus Boner

What is fatty acid profiling?

Fatty acids are both important dietary sources and important structural components for cells in all biological organisms. The fatty acid composition of a tissue to a large degree reflects the animal's diet. The food sources can of course differ locally in nature and this can be used as a way of verifying origin (Zhang 2020), but the possibility of this verification is usually limited to certain initial situations. Modern qualitative and quantitative analysis of fatty acids is widely used in food technology or physiological research. The chemical analysis of fatty acids in lipids typically begins with breaking the fatty acid down into its original esters (triglycerides, waxes, phospholipids, etc.) and converting them to methyl esters, which are then separated by gas chromatography.

A wide array of scientific publications has demonstrated that the fatty acid profile delivers an opportunity to distinguish wild and farmed fish. One study has shown that wild and farmed salmon could be differentiated by the amount of linolenic and docosahexanoic acid (Molkentin 2015). Furthermore, linolenic acid might be used to differentiate between wild and farmed caviar as well (Gessner 2002). Other studies have stressed different fatty acids like the eicosapentaenoic acid (DePeters 2013), which was found to show a better differentiation quality with respect to wild and farmed caviar.

The possibility to differentiate between wild and farmed caviar is without doubt strongly dependent on the feed and feed ingredients and consequently, as this composition is variable, the relevant fatty acid for discrimination might be changing as well. However it could already be demonstrated in caviar that stearic and oelic acids are suitable for differentiating between wild and farmed caviar (Czesny 2000). Yet it remains a risk that the fatty acids in this study only led to the separation between wild and farmed caviar in this particular feed situation. Therefore, the overall information acquired from the entire fatty acid profile – instead of relying on only one discriminating acid - may result in a robust model for differentiation (Vasconi 2019).

Along with stable isotope analysis (see chapter 4), fatty acid profiling could deliver a second method to differentiate between farmed and wild products (e.g. caviar) and the method might even be suitable to differentiate between conventional and organic farming methods (Trocino 2012). The first evidence of this was provided in a study focussing on salmon where myristic acid shows significant differences between conventional and organically reared samples.

Unfortunately, fatty acid profiling is still a labour-intensive analysis and the routine working time is approximately 5 to 10 days. Normally the proteins are hydrolysed and the fatty acids are analysed after derivatization by gas chromatography.

However, new, upcoming technologies like ambient mass spectrometry, (e.g. DART-TOF-MS,) may improve routine analysis of fatty acids by massively cutting down on time and cost expenses. Direct Analysis in Real Time (DART), coupled with a time-of-flight (TOF) mass spectrometer (MS), is an established technique that yields highly definitive screening data leading to the identification of controlled substances present in a case sample. Sample preparation is quick and simple and run times are typically only a few minutes.

A hot Helium flow (350 to 450 °C) is used to extract the sample rapidly, transferring the chemical ingredients / compounds into a time of flight mass spectrometer. The result is a chemical fingerprint based on molecules of low molecular mass (<1000 daltons).

The biggest advantage of this technology is that almost no sample preparation is required to obtain the chemical profile of a suitable sample and it can be obtained within seconds.

After establishing a routine method, results including the statistical evaluation can be provided in less than an hour.

The DART-MS technology is used in various analytical fields (Black 2016) and may be suitable for fatty acid profiling as well. It has already been demonstrated that DART-MS can be adapted to detect a supplemental feeding with cereals in fish (Cajka 2013). In an in-house study (in process to be published), DART-MS was successfully applied for the first-time to analyse the profile of fat extract from caviar.

The chemical fingerprint of the extracted fat provided significant discrimination between wild and farmed caviar (figure 2) which shows the potential of this rapid method well.

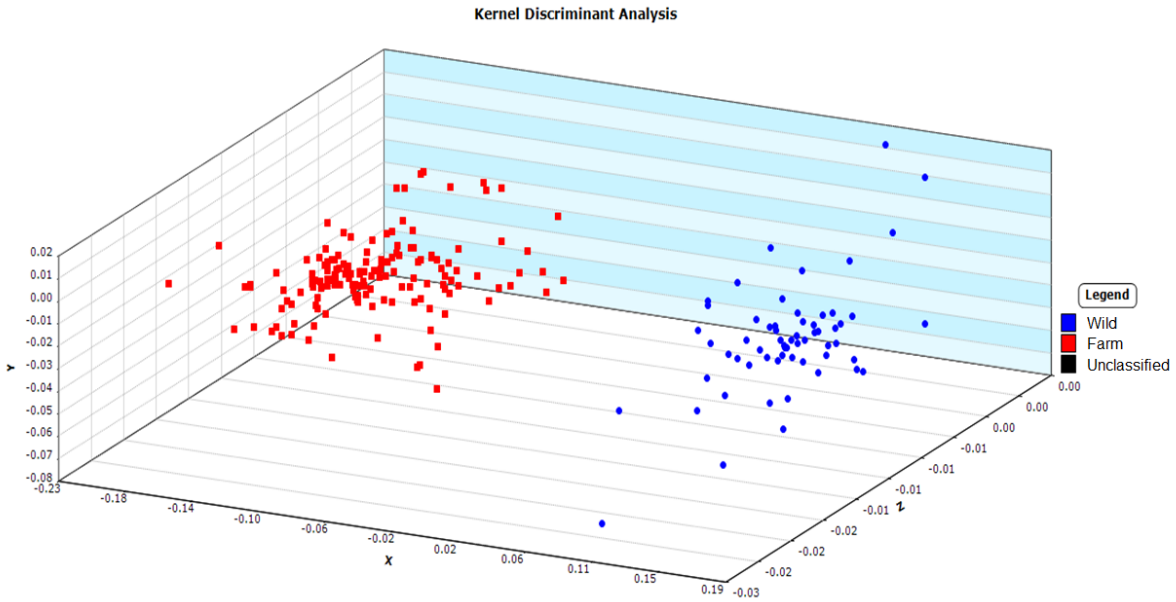

Figure 2: KDA Kernel discriminant analysis of the chemical fingerprint from the DART-TOF analysis.

Furthermore, it could be demonstrated that DART-MS in most cases delivers similar results when compared with stable isotope analysis when applied to test for the origin of caviar with respect to the production environment (e.g. wild vs. farmed). In that comparison more than 58 samples have been tested. Both technologies (Stable isotopes and DART-TOF-MS) delivered identical evaluations in 88 % of cases (51 samples). In three samples the DART-TOF-MS delivered no significant assessment, so the result could not be compared. Finally, in four cases (7 %) the DART-TOF-MS provided a conclusion contradicting that of stable isotope analysis. In these cases further background information (i.e. on the feed used in aquaculture) might be helpful to draw the right conclusions.

With regard to the high throughput of samples this method has a strong potential to deliver results at low cost. A price of approximately 20 to 40,- € is normal at present. Nevertheless the high investment of approximately €250.000 to establish a DART-TOF MS system in the laboratory must be taken into account.

4 Stable isotopes

Author: Markus Boner

What is stable isotope analysis?

Isotopes are atoms of the same element (which means they possess the same number of protons and electrons) that have different masses, due to the variable numbers of neutrons in the core of the atom. Isotopes exist for many elements. Most of these isotopes are, however, radioactive and only a few are stable and occur in different relative proportions in nature. These variations in abundance are mostly a result of kinetic fractionation effects of incomplete processes (e.g. biological processes, diffusion processes).

These small variations in bioelements can be measured by special isotope mass spectrometers (IRMS) by introducing light gases (H_2, N_2, CO, CO_2, SO_2) to the measuring system through combustion. Accordingly, these IRMS are usually operated in combination with combustion units like elemental analyzers (EA).

Today, the stable isotope method is a standard analytical tool to verify the authenticity of many materials (Primrose 2010). There is a wide array of publications available on the use of stable isotope analysis to determine the provenance of various products such as agricultural and animal products (Divelos 2012). Furthermore, the analysis of stable isotopes is an established method to study animal migration patterns (Hobson 1999) and aquatic food webs (Middleburg 2014), and has already been successfully applied in criminal investigations (Meier-Augenstein 2010).

The basis of any origin analysis is the fact that in nature different patterns of these isotopes occur in typical patterns in the tissue samples depending on geographic origin. These patterns can be used for verification of origin. In the case of stable isotopes, these patterns arise in nature through incomplete processes.

By way of example, one of the broadest incomplete processes in nature is the global water cycle. Therefore, the mean isotopic value of hydrogen and oxygen in precipitation are primarily dependent on the annual temperature of specific locations (Dansgaard 1964) and secondarily on other less influential factors such as altitude, latitude and the continental effect (Araguas 2000). Consequently, there is great geographic variability in isotopic patterns in water (Bowen 2002). These location dependent water isotope signatures are reflected in the tissue water of plants and can be used to track the origin of animals (Boner 2004) and their products (Lesley 2010).

Most studies using stable isotopes for authentication purposes focus on terrestrial plants and animals. This is due to the fact that the origin application of hydrogen and oxygen is linked with fresh water isotope patterns. The sea water pattern only shows small variability world-wide and is hence less effective in the use of geographic provenance testing. In certain instances, D/H and $^{18}O/^{16}O$ stable isotope ratios have been successfully used to verify the provenance of freshwater fish (Soto 2016). As sturgeons migrate to freshwater to spawn, the stable isotopes of oxygen and hydrogen can be applied to them, and to the derived product, caviar, in origin verification studies (figure 3).

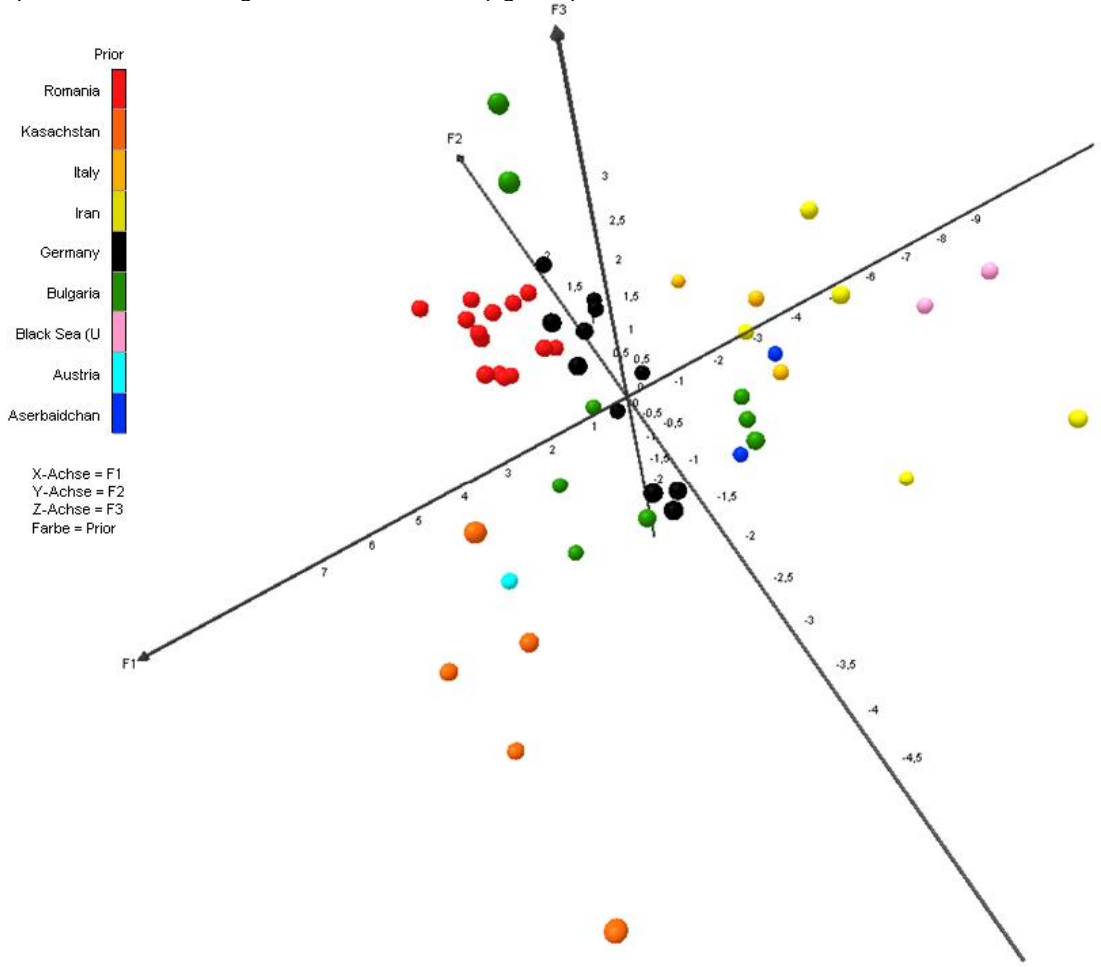

Figure 3: Agroisolab Database of sturgeon: D/H and $^{18}O^{16}O$ isotopic ratio of the tissue water and the organic fraction.

Nevertheless, there is still a need for further origin parameters. Further information may be available in stable strontium isotopes ($^{87}Sr/^{86}Sr$) which have been successfully used to track the origin of salmon (Barnett-Johnson 2008).

Unfortunately, strontium isotopes are highly affected by the processing of sturgeon and caviar (e.g. salt effect). Nevertheless, new models have been developed to minimize the processing effect and finally to link the sturgeon sample with the origin (Tchaikovsky 2019).

Further stable isotopes of the bio elements of carbon, nitrogen and sulphur measured in animal tissue reflect the available feed and, therefore, indirectly the origin of an animal or an animal product. This indirect origin verification has been further validated for fish (Kim 2015).

The vast majority of the available studies have concentrated on distinguishing farmed from wild fish, or organic from conventionally farmed fish due to the logic that diet in the wild and feed in farm production is expected to differ considerably.

The stable isotope ratios of carbon and nitrogen provide relevant information on the production system. Carbon and nitrogen isotopes have proven suitable to differentiate salmon of wild, organic and conventional-farmed origin (Molkentin 2007) and shrimps from wild-caught and aquaculture production systems (Gamboa-Delgado 2014).

Unfortunately for sturgeon and their product caviar, the latest research (European sturgeon database) has shown that, considering the current state of knowledge, nitrogen and carbon isotope ratios deliver only a slight differentiation possibility with

respect to the farming system. However **for caviar and sturgeon meat, the combination with sulphur isotopes results in a high discrimination power for wild and farmed origin** (figure 4).

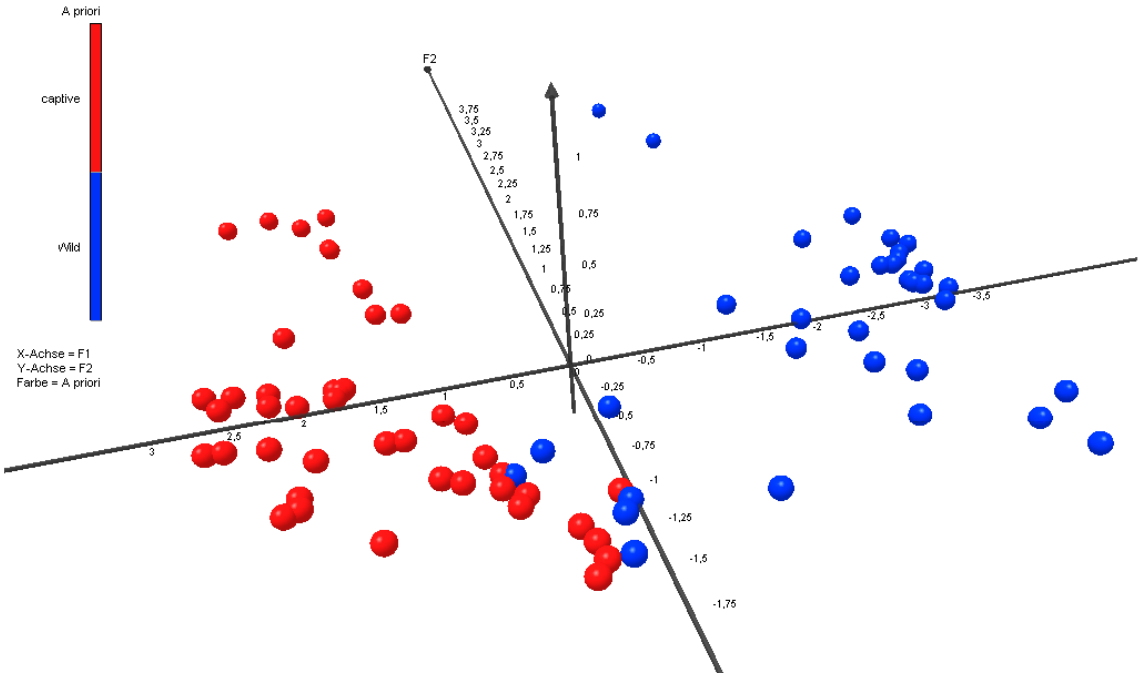

Figure 4: PCA-Differentiation of caviar from wild / farmed origin using $^{34}S/^{32}S$, $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotopic ratio; current project result (n=82).

The most important parameter for the discrimination is the stable isotope of sulphur, which has rarely been used in aquatic studies to date. Nevertheless, some studies have already shown the potential for authentication (Hesslein 1991, Kiyashko 2011).
The low discrimination power of the stable isotopes of nitrogen and carbon might be linked to the complexity of sturgeon feed, as well as the varying feeding conditions. Similar to results summarized in the European sturgeon database, the latest studies agree that the stable isotope ratios of nitrogen or carbon are often not sufficient to determine the production method (farm / wild) of fish and the combination with further parameters e.g. fatty acid profile is helpful (Molkentin 2015, Vasconi 2019).
Another powerful development of the stable isotope method could be the analysis of the stable isotopic ratios in amino acids. The latest results show a significant differentiation of wild and farmed salmon (Wang 2018).

In summary, stable isotopes deliver a wide range of information on the origin and the production method (wild / farm). However, stable isotope technology is only applicable if the diet of fish in the wild is different to commercial feed used in aquaculture. Therefore, it is important to keep in mind that the method cannot actually discriminate wild-caught from captive-bred sturgeon but sturgeon fed with natural versus commercial feed (e.g. American Paddlefish from an aquaculture facility in Ukraine was determined as wild-caught as it feeds on natural food only). The stable isotope analysis should also always be linked to a genetic analysis of species (otherwise e.g. a dish sold as sturgeon, with the fish identified as wild-caught, could actually be from a species that is not protected, such as the European Catfish, which was legally caught in the wild).

Finally, it should be stressed that **the stable isotope method requires a geolocated reference database.** The reason is simple: the method uses environmental and feeding effects to verify the authenticity which have to be verified for further discrimination.

Routine implementation of this work by supervisory bodies remains challenging, however, as the technology is still only available in a few laboratories worldwide. The analysis of stable isotopes requires a high degree of expertise, results take between 5 to 10 working days and rush samples (48 h) are highly challenging. The price for analysis remains expensive (100 to 400.- €) and further development like the detailed analysis of the amino acid profile will raise the price up to 500 to 700.- € per sample.

Furthermore, the combination of the abovementioned stable isotope analysis of the bio elements (COHNS) with further heavy isotopes like strontium may be helpful to improve the origin check as well. Nevertheless the price to analyse only one heavy isotope is still high. An analysis of strontium isotopes (^{87}Sr / ^{86}Sr) typically lies in a range above 300.- €.

Despite these shortcomings, the stable isotope method is, at the moment, the leading standard method for authentication due to its potential for universal application to a broad range of products (e.g. ivory database; http://ivory.org).
It has been accepted in various court cases as an analytical proof (Camin 2017). **The method has been used for caviar court cases** as well, where it has provided evidence for illegal trading (Oct. 2013: AKZ: 231 Ls 700 Ls 2870/11, Germany).

5. Perspectives on improving traceability to minimize fraud and illegal trade

More than 20 years ago, the universal labelling and registration system for the identification of caviar was established at CITES CoP11 in 2000. It has undergone some revision since then and is currently set out in CITES Resolution Conf. 12.7 (Rev. CoP17).

However, despite the apparent successes of the labelling system since its introduction and despite the increasing caviar production from aquaculture - comprising more than 95% of legal international caviar trade -, it remains a fact that wild sturgeon stocks are further declining dramatically, and poaching and illegal trade in caviar (and meat) are continuing to be reported by experts in range States of the Caspian and the Black Sea, the Lower Danube, the Russian Federation and the USA.

Until today no effective evaluation of the strengths and weaknesses of CITES Resolution Conf. 12.7 and its impact on the protection of wild sturgeon has been carried out officially. As a consequence, the risk of illegal trade in sturgeon products remains acute. This is reflected in the prevailing expert opinion, that illegal caviar and other sturgeon products are still widely available on markets, especially in domestic trade, and continue to fuel poaching threatening wild populations. It cannot be excluded that these domestic sources also fuel the illegal international trade.

The fact that decreasing amounts of illegal caviar have been documented in international trade over the past 20 years (Van.Uhm & Siegel 2016, Harris 2018) is often attributed to the effective implementation of the Resolution. However, several reasons could be contributing to the observed trend. Firstly, the massive decline of sturgeon populations in the core range of the commercial species (Ponto-Caspian Region) reduces their availability. Secondly, it is observed that the attention of enforcement authorities in some regions (i.e.EU) has shifted to new focal areas, such as illegal timber, and thus reduced the efforts in customs controls. Unfortunately, no correlations between enforcement efforts and detected sturgeon seizures are available publicly. Thirdly, it must be suspected that the illegal trade has adapted to the regulations and has established new means of transport (wild caviar labelled as farmed, alternative means and routes of transport, etc.) or else is taking place in the countries of origin for domestic consumption. It should also be noted that the possible reasons vary between geographic regions. A more in depth study on sturgeon commodities and their origin in trade would be highly desirable to systematically address the issues related to the impact of illegal trade and the required actions.

Over the last decades, sporadic initiatives to strengthen the traceability of sturgeon products in trade have failed, since several states objected to an improved approach towards increased traceability. The main concerns focused on the lack of evidence that illegal caviar trade still imposes a risk for wild sturgeon populations, as well as on the costs and administrative efforts associated with any refinement of the existing methodology. However, the limited research available on this topic has revealed mislabelling or fraudulent labelling of caviar through application of DNA and stable isotope testing of otherwise apparently correctly labelled samples in trade (Jahrl et al. 2021). This prompts credible concerns that the caviar labelling system is being circumvented and, moreover, that caviar from wild-caught sturgeons may be laundered through labelling as aquaculture caviar. Wild caviar from poached sturgeons in several range States can be obtained much more cheaply than caviar produced through a legal - time and resource intensive - aquaculture production. The lack of control procedures to verify whether or not the actual content of a container corresponds with the label poses a minimal-risk / high-gain opportunity for such fraud.

It is generally acknowledged that food fraud jeopardises the sustainability of food systems. It deceives consumers and prevents them from making informed choices. A zero tolerance policy with effective deterrents is crucial in this regard[7]. Combating food fraud has become a high priority in Europe. As such, the European Commission plans to scale up its fight against food fraud to achieve a level playing field for operators and strengthen the powers of control and enforcement authorities, as described in the text box below.

Example: Europe's Farm to Fork Strategy[8]

The recently adopted EU strategy "Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system[9]" is at the heart of the European Green Deal. It aims to ensure that the food chain, covering food production, transport, distribution, marketing and consumption, has a neutral or positive environmental impact. Along with other factors such as climate impact, animal health and welfare, as the aim to reverse the loss of biodiversity, are specifically mentioned. The strategy further aims to make the EU food system a global standard for sustainability and declares that such a transition to sustainable food systems requires a collective approach involving public authorities at all levels of governance (including cities, rural and coastal communities), private-sector actors across the food value chain, non-governmental organisations, social partners, academics and citizens.

The strategy also aims towards an accelerated shift to sustainable fish and seafood production, while the proposed revision of the EU's fisheries control system 27 will help to limit fraud through an enhanced traceability system. The mandatory use of digitalised catch certificates will strengthen measures to prevent

[7] Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52020DC0381.
[8] Available at: https://ec.europa.eu/food/farm2fork_en.
[9] Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52020DC0381.

illegal fish products from entering the EU market and, through the support of the European Maritime and Fisheries Fund for sustainable seafood farming, the Commission envisages adopting EU guidelines for Member States' sustainable aquaculture development plans. Regulation (EU) 2017/625[10] provides a framework for all official controls along the agri-food chain and on its basis National Reference Centres for Food are being established in the European member countries at present. Such centres can play an important role in risk-based controls and the application of forensic methods and thus significantly reinforce defences against food adulteration. This EU policy thus provides support to tackle issues in sturgeon production and trade, in particular since the EU Fork to Farm Strategy "[…] is implemented in close coherence with the other elements of the Green Deal, particularly the Biodiversity Strategy for 2030".

While it is difficult to quantify the ultimate conservation impact of the observed abuses in sturgeon trade, it is obvious that, with many sturgeon stocks at critical levels, any fraud poses a conservation risk. As such it is highly recommended to the Conference of the Parties to consider appropriate measures that render fraud more difficult and finally couple control efforts with application of forensic techniques.

In other developments, at its 27th meeting in 2014, the Animals Committee requested the CITES Secretariat to commission a study that compared the traceability systems then in use in CITES. That study is available in SC66 Inf. 12. With regard to the universal caviar labelling system, the study identified several issues that needed to be addressed. A report from TRAFFIC (Harris et al. 2018), published as AC30 Inf. 33 in June 2018, also supports these findings. The Vienna Declaration of the 8th International Symposium on Sturgeons[11] in 2017 drew the same conclusion in Recommendation 18.

Annex 2 to this document lists current shortcomings to the CITES labelling and highlights examples showing that the current labelling requirements are not followed, that they are not sufficiently forgery- proof to avoid illegal caviar entering the market with falsified labels, and that the current wording in Resolution Conf. 12.7 (Rev. CoP17) is insufficiently clear, resulting in a **vast diversity in designs of labels and positioning of codes, barely readable codes or codes that are easily tampered with, lack of evidence of any opening, etc**. Inadequacies may be summarised as follows:

a) Caviar labels have **very little uniformity in terms of their design and quality** (including the positioning of the CITES code), causing difficulties for authorities to recognize and verify the authenticity of labels during inspections/controls;
b) Frequently, the **requirement that labels are non-reusable and that they should either seal the container or that the container should allow visual evidence of any opening or tampering is not met**;
c) In some cases, the **code is difficult to locate or read** (e.g. print is very small or of poor quality or becomes unreadable when the label is exposed to water);
d) Where the **production and issuance of labels is not centralized** or supervised by a central body (e.g. the Management Authority), there is no scope for an overview of the number of labels issued compared to the caviar production volume per species;
e) **Labels lack security features** or the labels are otherwise of **poor quality/design**, thus facilitating forgery of labels.

All the above mentioned points render controls and **enforcement** of the legal prerequisites very challenging. It is further handicapped by complicated, inappropriate and diverse implementation of regulations with regard to bookkeeping, unclear competencies and split responsibilities (veterinary services, CITES enforcement and consumer protection) as well as a tedious verification of production, processing and trading data.
In order to contribute to an informed discussion by the CITES Working Group on Sturgeon and in order to build consensus for the improvement of transparency and traceability of sturgeon products in trade, the authors have outlined low level recommendations to improve current regulations but also discuss the general need to improve traceability systems.

5.1 Suggested adjustment of current labelling in Resolution Conf. 12.7 (Rev. CoP17)

A better definition is suggested to clarify the current definition of "non-reusable label" in Resolution Conf. 12.7 (Rev. CoP17) and to ensure that the labels in use fully conform to those provisions - addressing points a), b) and c) above; The suggested refinement in italic would read:
"Non-reusable *water-resistant* label: any label or mark *that remains intact and legible even in humid conditions, and* that cannot be *opened or* removed undamaged or transferred to another container. ~~If the~~ *This* non-reusable *water-resistant* label ~~does not~~ *must directly* seal the primary container, ~~caviar should be packaged in a manner that permits~~ *permitting* visual evidence of any *prior* opening of the container."

Additional measures are suggested to be included into Resolution Conf.12.7 in order to improve the security of the labelling system, reduce fraud and ease the responsibility of management authorities (addressing points d) and e) above), by requiring:

i. That marking should be more standardized and particular specifications for the design of labels are given (as already noted in Resolution Conf. 12.7 (Rev. CoP17));
ii. That labels are centrally produced by companies authorised by the national CITES Management Authority;
iii. That they incorporate a universal security feature such as a hologram; and
iv. That they incorporate a sequential serial number to enable controls if labels are missing or duplicated; with a complete list of labels produced and their CITES codes and the sequential serial numbers issued to registered producers and (re-

[10] Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32017R0625.
[11] Available at: http://www.wscs.info/news/news/sturgeon/vienna-declaration.aspx.

)packagers during this year provided to the relevant CITES Management Authority to assist the detection of fraud, for example by checking against bookkeeping, import or export permits after each calendar year.

5.2 Improving traceability along the production, processing and trade chain

As defined by CITES, traceability is the ability to access information on specimens and events in a CITES species supply chain. In order to establish a safe, transparent and easy to monitor system to trace sturgeon products throughout the production and trade process, the weaknesses of the current system must be analysed thoroughly and the respective conclusions from these analyses must be incorporated in a best practice solution, in cooperation with the intersessional working group on traceability.

Currently, there is no system in place that allows an easy tracking of fish in aquaculture from an early life-cycle phase to the final product. As for instance unique tagging is not a mandatory prerequisite and thus repeated transfers from one farm to another during the outgrowing period render the follow up for individual fish almost impossible as long as they are anonymous in production.

The establishment of a system comprising the utilization of a uniform passive integrated transponder (PIT such as NFD, RFID, or similar) tagging system with non-reprogrammable tags, associated to the individual collection of genetic information stored in an accessible and searchable biological library (for example genotypes or tissues) would allow to trace the individuals from fingerling to processing and throughout trade. It is suggested that such a library should be managed by a central management structure or consortium, as the integrity of sample libraries is critical to apply scientific methods to disrupt illicit supply chains and promote due diligence. The set-up of the World Forest ID[12] for reference samples in timber species may serve as one example. It has established protocols and tools for sample collection and ensures that all forest source material in the collection will be subject to the highest levels of scrutiny.

For sturgeon, such a traceability system would further collect information on rearing history, including transfers between farms, weight gain, health data, and treatment information, to the time when they enter a processing process and is applied here for any type of products. The process allows the registration of the amounts of the products, their destination and associated information in a database. Any sturgeon product (either as live fish, meat, roe, or side products) in this case is associated with the coded information from the database. As such, traceability of the individual is ensured throughout the production and trade process.

Simple algorithms can be applied to automatically verify the plausibility of the subsequent production and trade steps due to the genetic information that is underlying the PIT Tag information and thus detect products suspicious to fraud. As such, the producer is not only able to verify the production process, but also to use the data gathered in the design and modification of the production process. It is essential, though, that the production data are accessible at different levels for different purposes, and separation of these levels must be ensured to increase trust in the system. For instance, a total data overview for the respective producer only, a second layer of data available to the enforcement agencies (customs, CITES control, customer protection), and a third layer that is accessible for the consumer to obtain information about the end product.

For enforcement agencies, this model would allow a reliable and easy access via mobile devices (e.g. smartphones) to conduct checks on the information provided on a product, through plausibility checks with the system. Any deviation between the information in the system and the information provided on the article can then lead to further investigation or testing with forensic techniques

For large buyers, such as airlines and cruise lines, the system adds to the safety of the products, reduces the bureaucratic load in maintaining records on the product and provides for consumers to be informed accordingly. The data acquisition and access are depicted in figure 5.

[12] For more information, see: https://worldforestid.org/.

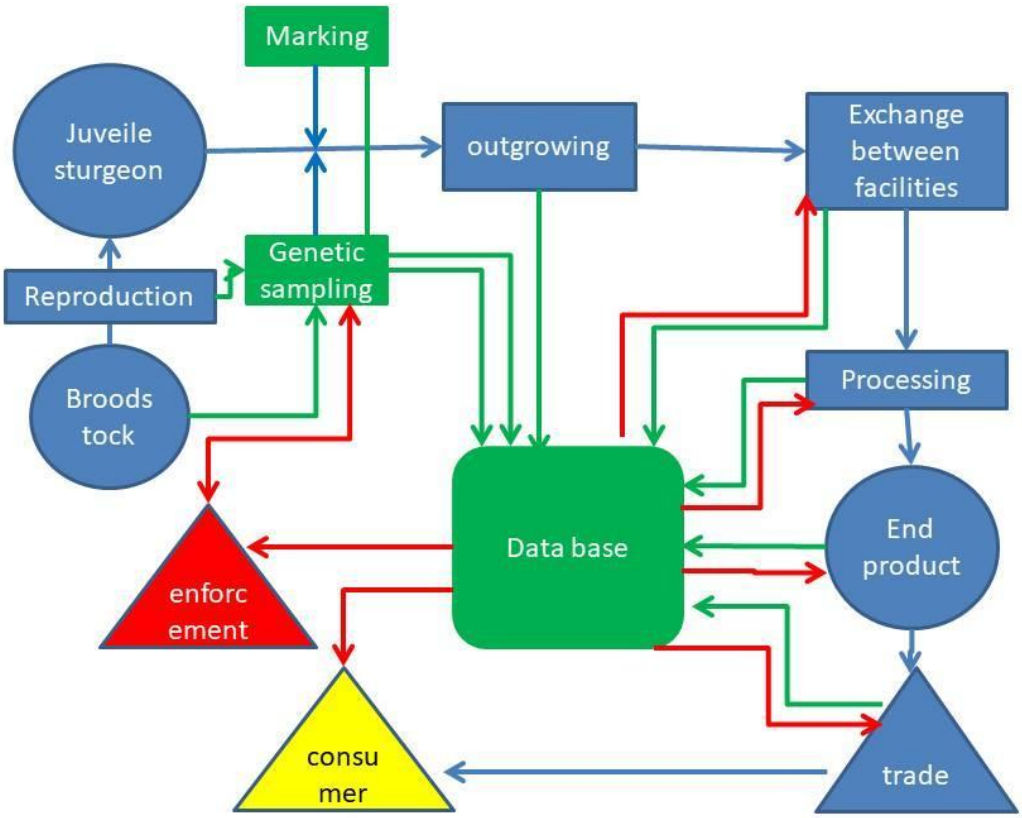

Figure 5: Principal interaction and data flow between different production levels and a central database © Geneusbiotech b.v.

Open questions relate to data security, the hosting of the data bank and the support of the different stakeholder groups (fish farmers, processing companies, traders and wholesalers, retailers, consumers and enforcement agencies) for the application of the system.

An approach comparable to block chain technology would for example offer a possibility to transfer forgery-proof information from a decentralised database used by numerous participants. Coupled with smart packaging of products such as centralised produced QR codes, magnetic ink, holograms, seals or readable track tags, this could provide another layer of security. The production and printing of QR codes should be in a centralized way, so smartphone apps can easily identify fraudulent produced codes. Further advancements on RFID technology (Radio-frequency identification), resulting in lower costs for its application, might also present an ideal method in the future, as it can already be incorporated into the container during the manufacturing process. Such smart packaging options would avoid many of the abovementioned shortcomings of the current label and could easily be integrated in a consumer friendly appearance, also valuable for marketing.

An approach comparable to block chain technology would for example offer a possibility to transfer forgery-proof information from a centralised database used by numerous participants. Coupled with smart packaging of products such as QR codes, magnetic ink, holograms, seals or readable track tags, this could provide another layer of security. Smartphone apps can easily identify fraudulent produced codes by validating with the database. Further advancements on RFID technology (Radio-frequency identification), resulting in lower costs for its application, might also present an ideal method in the future, as it can already be incorporated into the container during the manufacturing process. Such smart packaging options would avoid many of the abovementioned shortcomings of the current label and could easily be integrated in a consumer friendly appearance, also valuable for marketing.

Although it is acknowledged that any change towards such a system will impose additional efforts by producers, the resulting advantages to producers, traders and consumers need to be emphasised. One option could be the utilization of the system as a watermark for legal products in trade, replacing the current, labour and time intensive paper-based CITES application process. The transition to a sustainable sturgeon aquaculture and trade system is also a huge economic opportunity since “citizens’ expectations are evolving (especially in Europe) and driving significant change in the food market. This is an opportunity for fishers and aquaculture producers, as well as food processors and food services.” [13] Such a transition will allow them to make sustainability their trademark, turn it into a competitive advantage and at the same time contribute to the protection of biodiversity - the wild sturgeon populations, which form the natural resource of their whole business model.

[13] Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52020DC0381.

6. Summary table and recommendations

This study provides an update of the identification guideline for sturgeon products in trade (Ludwig 2008) and reflects the developments in the forensic methods but also the results of surveys on illegal trade over the last 15 years. It is intended to support enforcement agencies in their attempt to detect illegal trade. Furthermore, the study outlines potential means of increased traceability and ultimately efficiency in enforcement.

While acknowledging the impact of CITES Resolution Conf. 12.7 on international sturgeon trade, its effectiveness with regard to the protection of wild sturgeon and paddlefish populations remains unevaluated and the risk of illegal trade in sturgeon products is still considered acute. This is reflected by the prevailing expert opinion and results of market surveys that illegal caviar and other products are still widely available on markets, especially in domestic trade, and continues to fuel poaching and threatening wild populations.

Based on these findings it must be noted that there is a need for increased control efforts along the sturgeon trade chain, applying state-of-the-art forensic analysis. Consistent and regular controls of sturgeons and their products, should - in cases of substantiated suspicion - use combined genetic and isotope analysis, in order to reliably detect illegal harvest and trade. Since DNA analysis is not suited to differentiate wild from farmed products, this method must be combined with other techniques such as isotope analysis. Utilizing appropriate methods allows verification of the species or hybrids the product originates from, the source (wild/farmed) as well as the geographical origin. The capacity of national institutions to carry out the suggested forensic methods should be provided. Alternatively, multinational collaboration can be envisaged. These controls must specifically include sturgeon products that are declared to derive from aquaculture, even when bearing CITES labels, since past surveys have repeatedly shown mislabelling or inaccurate declaration of source.

Particular attention needs to be paid to the improved implementation of the universal caviar labelling system.
CITES Management Authorities should strictly require appropriate quality (e.g. water- and damage-resistance) and design of the CITES caviar labels of companies producing or repackaging caviar, also ensuring that the CITES code is readable and easy to locate, and the labels are affixed in a way that provides visual evidence of any compromised integrity of the package and prevent its repeated use (see chapter 5.1 and Annex 2).
CITES Management Authorities in Parties who are not yet implementing the CITES caviar labelling provisions for domestic trade (such as Ukraine, China, Russia, the USA inter alia) should fully revise relevant national legislation to implement CITES Resolution Conf. 12.7 (Rev.CoP17).

Improving the traceability of sturgeon products in trade should be investigated future target along the points raised in chapter 5.2, making reference to the current shortcomings of the labelling requirements and suggesting new technologies to enhance transparency in trade, ease controls of products and increase consumer safety through an anti-counterfeit approach.

The following table provides an overview on the different methods described in this publication. It explains the scope of each method (which forensic technique can be used to identify the sturgeon species or hybrid, its relatedness, its geographic origin or the production methods (wild caught versus farmed)).It provides advantages and restrictions, estimates on costs and technical capacity needed.

Table 2: Summary of forensic methods, their advantages, restrictions and costs implications

Method	Application for sturgeon identification (scope)	Advantages (in general or in comparison)	Restrictions/ limitations	Cost / sample	Equipment needed + associated costs	Required skills of personnel	Comments
Stable	Verification of geographic	Already tested and	Stable isotope	Price for	EA	Analysis	Currently the leading

isotope composition analysis	origin: stable isotopes of hydrogen and oxygen are used to determine geographic provenance in freshwater fish. Verification of production method wild caught versus farmed: Nitrogen and Carbon isotopes to determine wild versus farmed origin; Sulphur has highest diagnostic power for discrimination wild versus farm .	applied for caviar and sturgeon meat Accepted as analytical proof in court cases (e.g. in Germany, Europe*)	analysis of seizures/samples is only available in a few laboratories world-wide. Results take 5-10 working days; rush samples (48hrs) are highly challenging; Discrimination wild caught versus farmed actually identifies the feed, not the fish itself	analysis of 1 sample remains expensive (100-400 €)	(Elementanalyser) in combination with IRMS (Isotopic Ratio Mass Spectrometry) Manufacturer: Thermo Finigan, Elementar, Nu Instruments IRMS: 100.000 -> 140.000 € EA: 40.000 to 80.000 €	requires high degree of expertise.	standard method for authentication due to its application to a wider range of other products (food, Ivory, timber, ..). Several official food laboratories in Europe are using IRMS to detect fraud samples in the market. e.g. Germany: 6 official labs. Stable isotopes are recommended in European guidelines to track the origin (e.g. timber). Furthermore UNODC guidelines like ivory, timber note IRMS for origin tracking.
Fatty acid profiling	Distinction of production method (wild versus framed) depends upon feed and feed composition	Standardised Gas Chromatography method.	Fatty acid composition of eggs or tissue depends on the residuals of feed composition, can cause unclear results in extensive rearing on natural diets.); Species specific variability in composition not fully known; Can be manipulated by addition of FA mix after processing; Sensitive to decay during storage.	Depending upon lab specialization and throughput	Specialized equipment (HPLC, GCMS) needed, medium expensive 30,000-80,000€	Trained personnel with experience in the method required, intercalibration between labs useful, harmonization of methods required	Still research needed, which additional fatty acids could be relevant

	DART-TOF-MS Direct Analysis in Real Time (DART), coupled with a time-of-flight (TOF) mass spectrometer (MS)	Almost no sample preparation is required to obtain the chemical profile of a suitable sample and it can be obtained within seconds	See above	Lower costs per sample (20-40 €)	High investment costs (approx. 250.000.- € for laboratory system (DART MS)	Analytical chemistry	DART-MS was successfully applied for the first-time to analyse the profile of fat extract from caviar. Routinely used to verify the species of timber in USA
Overview of DNA analyses	Determination of sturgeon species (populations, hatchery stocks, individuals)	Genetic code is uniform; DNA can be extracted from all types of tissues of living specimen even processed caviar and sturgeon meat; DNA is thermally stable (processed samples); only very small samples are needed; results are reliable and verifiable between laboratories	Calibration between labs is recommended for nuclear DNA; References are available in GeneBank	Costs are rather low (depending on the question of interest between 20-100€)	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Routinely used to verify the species of origin and hybrid status since years; Used in species identification not only in sturgeon worldwide; Huge panel of experience
Mitochondrial DNA analysis	Determination of the maternal sturgeon species from tissue of live specimen, or processed samples (meat/caviar)	Complete reference mitochondrial genomes are available for 22 out of 27 sturgeon and paddlefish species; Cheap, fast and reliable; Produces often results even in samples of poor quality; Worldwide accepted method by court	DNA sequencing needs good and reliable reference databases: No complete reference genomes have been archived for two important caviar producers NAC and PER; it is not possible to distinguish GUE and PER based on genetic markers ; hybrids and introgressed species (historical hybridisation) cannot be identified based on mDNA only	low costs 10-20€	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment) Notable, equipment is the same in any of genetic methods.	Advanced knowledge of molecular genetic methods; trace lab experience	Prime standard of species identification in trade control not only in sturgeon and paddlefish; Diagnostic reference sequences of all sturgeon and paddlefish species are available in public data bases

Nuclear DNA markers 2 classes of markers that can be used individually or in combination:	Determination of the sturgeon species (both parental species) Used to recognize the genetic contribution of both parents such as relatedness analyses (strain identification) or detection of hybrids	The possibility to characterize paternal contribution allows to test for hybridization and for relatedness, not feasible with mitochondrial DNA only	Limited number of nuclear markers available but their number increases fast	Cost depends on complexity of hybrids (2 or more species involved; back crosses with paternal species etc.) but ranges between 20-100 €)	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Frequent hybridisation in sturgeon species opened new frontiers to commercial frauds in which, for instance, the less valuable hybrid caviar is sold as product obtained from the pure maternal species, in order to cheat standard controls exclusively based on mtDNA. By the same time, caviar from poached sturgeon is offered as "hybrid" blurring the origin.
Microsatellites	Markers of genetic differentiation at different levels of diversity (relatedness, population analyses, and species identification, hybrid detection)	High variability (number of alleles per locus) makes microsatellites very informative for the study of genetic diversity; Sturgeons have different levels of ploidy. These differences can be used for species/hybrid detection based on microsatellites.	Lower comparability of results between different laboratories Intercalibration highly recommended; misidentification due to allelic dropout; null alleles or wrong allele dosage estimation	20-50 €	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Should not be used as stand-alone method for species identification but produces valuable data for hybrid detection and for validation of mt and SNP analyses. All laboratories relying on this approach should include some reference samples with known genotype in their sample set.
Single Nucleotide Polymorphisms (SNPs)	Prime standard for hybrid detection but also useful at different levels of diversity, (relatedness, population analyses, and species identification)	Advent of massive sequencing methods allow simultaneous analysis of hundreds of samples good transferability and replicability among laboratories	As regards their applicability in routine forensic analyses, SNPs with diagnostic power ("private" polymorphisms) must be identified in order to then develop fast and cheap single locus analyses	20 – 100 € depending on number of SNPs	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Gains increasing importance because Next-Generation Sequencing techniques produce tons of SNPs annually; Validation of private SNPs difficult sometimes because of secure reference samples

Morpho-logical identification	Identification of sturgeon species	Easy, low-cost and time efficient; can be applied if sturgeons are encountered on a vessel, on the bank of a river, in a farm or in trade; Mainly applicable for intact fish	Not reliable for parts of fish; not suitable for meat or caviar Only reliable for specimen older than 45 days, difficult for some species (e.g. Beluga sturgeon) until sexual maturity Morphometric and some morphological characteristics can be difficult to be obtained in live animals or are meaningful only in statistical comparison. Therefore, a larger number of individuals of the same species is ideal to safely determine species; Identification of hybrids occurring in nature and in aquaculture is complicated	Only personal salary	Measuring tape, sliding calliper, magnifying glass, stereo photography	The identification of a sturgeon species by morphology, even under optimal conditions, and especially of hybrids requires an experienced specialist	CITES ID Guide in EN, FR, ES (EEEC) and UA (WWF) Only reliable for adult fish; Entire fish is necessary Not useful for hybrids because variation of morphological traits in hybrids is unknown
	Wild caught versus farmed: discrimination due to typical malformations that are encountered regularly in farmed individuals		Malformation may but do not have to occur in aquaculture and can only serve as a hint but no proof				Unreliable, limited to the utilization as a pre-screening

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ANNEX - 1

Table A: Overview of complete mitogenomes and mitochondrial reference sequences available for sturgeon species.

Species	Mitogenomes	Cytochrome b/ compl	D-Loop/ compl
BAE	4	75/5	210/4
BVI	10	18/11	97/13
DAB	2	5/2	3/2
FUL	5	13/6	31/5
GUE	2	110/22	132/5
MED	1	11/2	8/2
MIK	1	9/2	4/1
NAC	-	24/2	16
NUD	2	11/3	15/2
OXY	6	39/7	144/6
PER	-	28/3	50
RUT	1	65/3	194/2
SCH	7	22/9	16/7
SIN	2	8/3	59/2
STE	1	69/10	105/2
STU	1	26/2	62/46
TRA	1	19/3	28/4
DAU	4	11/4	6/5
HUS	1	66/11	56/30
FED	-	-	-
HER	-	6/3	4
KAU	1	12/5	5
ALB	11	18/14	28/17
PLA	15	33/19	41/20
SUS	3	9/4	6/3

SPA	6	31/7	18/16
GLA	1	7/4	5/2

Table B: Summary of nuclear markers available for the sturgeon species ID and information addressing their efficiency and cross-species tests.

Concerning the column “Efficiency” where the probability to detect the target species is estimated for each marker, the frequency of diagnostic alleles and the ploidy of the species have been considered for the calculation.

Species	Reference	Marker	Locus	Criteria	Efficiency	Cross-species tested	Reference samples	Comments
BAE	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	60.6%	FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS	-	Shared with RUT
			Vimentine	Diagnostic PCR product	78%	GUE, PER	-	-
	2	SNP	Unknown	Diagnostic PCR product	99%	FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS	-	Shared with GUE and highly present in PER
BRE	-	-	-	-	-	-	-	-
DAB	-	-	-	-	-	-	-	-
FUL	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS	-	-
GUE	2	SNP	Unknown	Diagnostic PCR product	96%	BAE, FUL, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS	-	Shared with BAE and highly present in PER
PER	-	-	-	-	-	-	-	-
MED	3	Microsatellite	An20	Species-specific range: 185-197	-	BAE, GUE, MIK, NUD, PER, RUT, SCH, STE, DAU, HUS	-	-
					100% (F)			
			AfuG51	Diagnostic allele: 296	100% (F)			
			AoxD161	Diagnostic allele: 114	97% (F)			
			AoxD165	Species-specific range: 230-254	Low (F)			

MIK	-	-	-	-	-	-	-	-
NAC	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, FUL, GUE, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS	-	-
	4	Microsatellite	LS54, Aox23	specific allelic range	100%	OXY, STU	Possibly related	Low number of species compared
			LS19, LS68 AoxD161	SNPs on flanking region	100%			Need of sequencing; Low number of species compared
NUD	3	Microsatellite	An20	Diagnostic allele: 153	100% (F)	BAE, GUE, MED, MIK, PER, RUT, SCH, STE, DAU, HUS	-	-
			AfuG51	Diagnostic allele: 272	100% (F)			
			AoxD165	Diagnostic allele: 196	74% (F)			
OXY	4	Microsatellite	Aox23, AoxD161, LS68	SNPs on flanking region	100%	NAC, STU	Possibly related	Need of sequencing; Low number of species compared
RUT	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	96%	BAE, FUL, GUE, NAC, PER, SCH, SIN, STE, TRA, DAU, HUS	-	Shared with BAE
	5	SNP	Unknown	Diagnostic PCR product	100%	BAE, GUE, MIK, NAC, PER, SCH, STE, TRA, DAU, HUS	-	-
	3	Microsatellite	An20	Diagnostic allele: 177	>80% (F)	BAE, GUE, MED, MIK, NUD, PER, SCH, STE, DAU, HUS	-	
			AfuG51	Diagnostic allele: 252	>91% (F)			Partially shared with DAU
SCH	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, FUL, GUE, NAC, PER, RUT, SIN, STE, TRA, DAU, HUS	-	Shared with DAU
	3	Microsatellite	An20	Diagnostic allele: 137	100% (F)	BAE, GUE, MED, MIK, NUD, PER, RUT, STE,	-	-

			AfuG41	Species-specific range: 185-197	Moderate (F)	DAU, HUS		
	6	SNP	RPL8 (Intron RP5)	Diagnostic PCR product	100%	DAU	-	Partially shared with DAU; To be used in combination with RPS7 tool (1)
SIN	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, FUL, GUE, NAC, PER, RUT, SCH, STE, TRA, DAU, HUS	-	-
STE	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, TRA, DAU, HUS	-	-
	3	Microsatellite	An20	Diagnostic allele: 141	85% (F)	BAE, GUE, MED, MIK, NUD, PER, RUT, SCH, DAU, HUS	-	-
			AfuG51	Diagnostic allele: 288	86% (F)			
STU	4	Microsatellite	AoxD161	Specific allelic range	100%	NAC, OXY	Possibly related	-
			Aox23, AoxD161, LS68	SNPs on flanking region	100%			Need of sequencing; Low number of species compared
TRA	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, DAU, HUS	-	-

DAU	1	SNP	RPS7 (Intron RP1)	Diagnostic PCR product	100%	BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, HUS	-	Shared with SCH
	3	Microsatellite	AoxD165	Private allele: 206	73% (F)	BAE, GUE, MED, MIK, NUD, PER, RUT, SCH, STE, HUS	-	-
			AfuG41	Absence of amplification	100% (F)			
			An20	Diagnostic allele: 169	93% (F)			
	6	SNP	RPL8 (Intron RP4)	Diagnostic PCR product	100%	SCH		Partially shared with SCH To be used in combination with RPS7 tool (1)
HUS	6	SNP	RPS6 (Intron RP2)	Diagnostic PCR product	100%	BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU	-	-
	5	SNP	Unknown	Diagnostic PCR product	100%	BAE, GUE, MIK, NAC, PER, RUT, SCH, STE, TRA, DAU	-	-
	3	Microsatellite	An20	Diagnostic alleles: 145, 149	83%, 45% (F)	BAE, GUE, MED, MIK, NUD, PER, RUT, SCH,	-	-

			AoxD161	Diagnostic allele: 98	98% (F)	STE, DAU		
			AoxD165	Diagnostic allele: 178	96% (F)			
			AfuG41	Species-specific range: 249-277	Moderate (F)			

ANNEX - 2

Shortcomings of current labelling system – Possible solutions & examples

1. Main shortcomings of the current CITES labelling system

1.1. **Lack of centralised production of CITES labels:** In most countries no central body but producers themselves are responsible for the production of the labels, resulting in a lack of quality control and a wide diversity of labels, frequently of poor quality (see 2.2) as well as a lack of control of the amounts of labels in circulation. The latter renders it **impossible to compare quantities of caviar produced with the amount of labels in circulation.**

1.2. **Difficulties in verification of CITES label authenticity:** Caviar labels are extremely **diverse in design, quality and positioning of the CITES code**, causing difficulties for authorities (and traders and consumers) when attempting to verify the authenticity of labels during inspections/controls or purchase (see all examples below).

1.3. **Poor quality and design of CITES labels:**

- The code is often **unreadable**, due to very small font size or poor quality print (see Examples 4.2.1, 4.2.2, 4.2.5, 4.2.6, 4.2.7, 4.2.8, 4.2.9).
- The code is **difficult to locate**, often being positioned in other text (see Examples 4.2.2, 4.2.5, 4.2.6).
- Often labels are **not water resistant** and codes get unreadable when stored on ice (see Example 4.2.3); also manipulation of codes with easily available substances such as alcohol or acetone is possible.
- Labels have shown to be **re-usable** and could be removed undamaged (see Example 4.2.4 and several of those where the CITES code is on a sticker on a sealing banderol, not on the banderol itself).

1.4. **Sealing of containers /visual evidence of any opening of containers:** currently the definition states: *"any label or mark that cannot be removed undamaged or transferred to another container, which may seal the container. If the non-reusable label does not seal the primary container, caviar should be packaged in a manner that permits visual evidence of any opening of the container*. "

- This is interpreted in different manners: containers are often sealed with a banderol, but the **CITES code is on a separate sticker** (see Examples 4.2.5, 4.2.6, 4.2.7, 4.2.8) **or not attached** to it at all (see Example 4.2.1), or the caviar is packaged in a way that does not make clear whether CITES requirements are fully met (see Examples 4.2.1, 4.2.2).
- Labels do not seal the container (see Examples 4.1.3 right and 4.2.9, 4.2.1) or can be opened without being torn or otherwise damaged (see Example 4.2.4) so that there is **no visual evidence of opening** the container.

1.5. **Missing security features:** currently, labels can be printed on **any paper**, with **any printer** and **do not require any security features** resulting in an easy production of fraudulent labels by simply printing or copying labels by any third party (e.g. not registered facilities, poachers or illegal traders).

1.6. **CITES codes do not provide all required information:** although the minimum information to be included in the respective codes on CITES labels are defined in Conf. 12.7 (Rev. CoP17), there is no control of the integrity of the codes that are actually applied on the labels and containers, and the definition of the lot identification number seems insufficient for effective traceability.

- codes are sometimes incomplete, especially lot numbers have been found to be missing
- producers have been found to use identical codes (including standard species codes) for different caviar types they sell in a certain period of time (see Examples 4.2.9.4, 4.2.9.6)
- lot identification numbers are not defined sufficiently by CITES: most producers use an individual number for each harvested sturgeon, some use the same lot identification number for all sturgeons - even from different species - harvested on the same day (see Examples 4.2.9.3 and 4.2.9.4 - all with lot identification number 0072 though found for sale nearly a year apart -, 4.2.9.6, sample codes in laboratory report 5.7.1 - both the Beluga and the Sevruga caviar had lot identification number 0001); in all these cases there is no reliable retracing of origin.

1.7. **Lack of random testing of caviar samples with apparently correct CITES label and code:** control of the codes and product identity (species/origin) is not possible without DNA/isotope analysis so that mislabelling and laundering cannot be detected without testing.

2. Suggested possible solutions

2.1 Issuing of CITES labels:

Labels are **issued only by an authorised body** (e.g. CITES Management Authority or printing company authorized by CITES Management Authority) including security features and following agreed uniform design parameters.

2.2 Improved quality and design of CITES labels:

If labels cannot be produced by a centralised body, label specimens must be presented to CITES Management Authorities, who should only accept them if basic requirements are met (**minimum print quality and readability of codes, non-reusability, resistance to water, presence of security feature, better positioning on container, correct code system**).

2.3 Security features for CITES labels:

These should be either on the label itself or at least on marks/stickers issued by an authorised body: such as holograms, unique security numbers, security prints, security cuttings (to ensure damage to the label in case of opening), or thermal transfer printing; **this security feature must be only available to registered producers or registered (re-)packagers.**

2.4 Sealing guidelines must be clarified:

The label with the requested codes must directly seal the primary container, permitting visual evidence of any prior opening of the container.

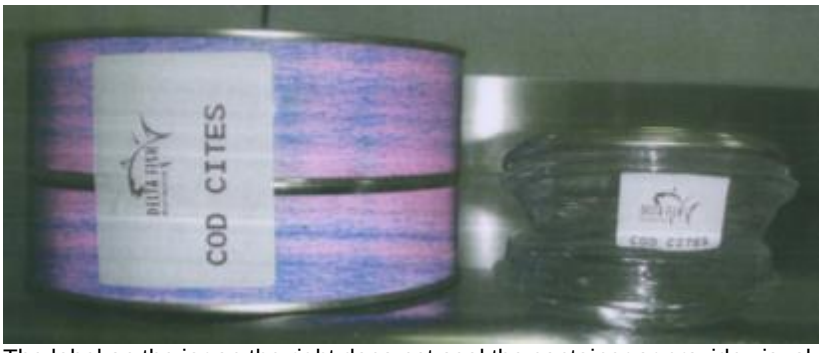
3. Examples

3.1 Examples of CITES label specimen, as accepted by CITES Management Authorities, which are prone to forgery due to poor quality

Example 3.1.1

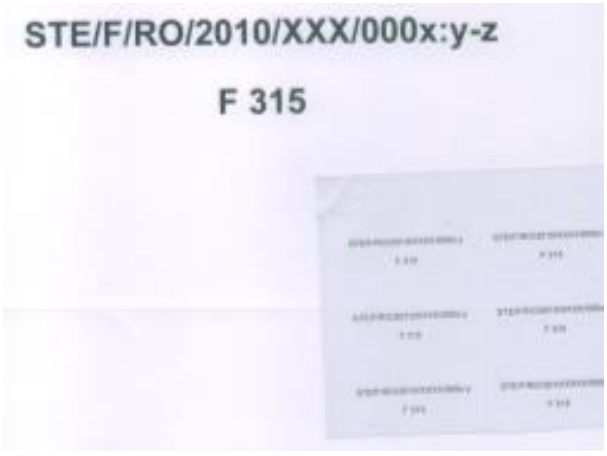
These labels were found falsified in a court case in Germany and in journalist investigations (see Chapter 5).

Example 3.1.2



The label on the jar on the right does not seal the container or provide visual evidence of any opening. Caviar jars of this brand without CITES label or with labels not sealing the container have been found repeatedly on Bucharest airport and in shops (see Examples 4.2.9).

Example 3.1.3



These labels seem to be printed on very simple paper stickers without any additional features and could be reproduced easily by anyone.

3.2 Examples of CITES labels for sturgeon caviar in trade to illustrate difficulties with current labelling requirements and their implementation

Example 3.2.1

-> container with code could be reused; code not connected with banderol permitting visual evidence of opening
-> code difficult to read

Example 3.2.2

-> through way of packaging it is not clear whether label with code is sealing the container or permitting visual evidence of opening
-> CITES code in very small writing within other text

Example 3.2.3

-> label of very bad quality, dissolved in ice, making CITES code unreadable

Example 3.2.4

-> label can be lifted undamaged (very solid material that does not break) and tin can be opened without visual evidence

Example 3.2.5

-> CITES code in very small writing within other text

Example 3.2.6

-> CITES code in very small writing within other text

Example 3.2.7

-> CITES code in very small writing (less than 1mm high), hard to decipher (e.g. year of harvest)

Example 3.2.8

-> CITES code in very small writing, hard to decipher

Examples 3.2.9

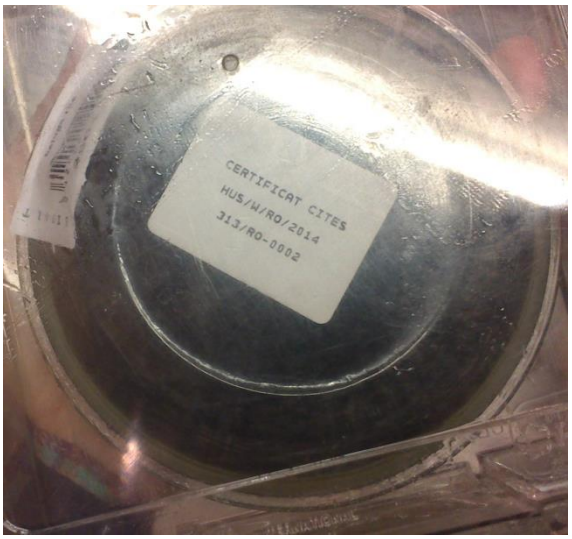
3.2.9.1 Duty free area of Bucharest Airport Otopeni (Henri Coandă); 7 March 2014

3 glasses of Beluga caviar and 4 of Sevruga caviar, in security boxes, **none of which bore CITES labels**, just adhesive tags with content (e.g. “sevruga roe”), bar code, price and the note “EXPORT”.

Confronted with these findings, the company claimed this to be a mistake and the duty free shop must have put the price tag over the CITES label (which was definitely not the case).

3.2.9.2 Duty free area of Bucharest Airport; 9 April 2014

In April 2014, caviar glasses bore very simple labels (not affixed according to CITES labelling requirements as not sealing the container or permitting visual evidence of opening) with CITES code HUS/W/RO/2014/313/RO-0002, classifying the caviar as Beluga caviar from wild caught sturgeons from Romania (while a zero export quota for sturgeons from NW Black Sea and Lower Danube River in place and trade in caviar from wild caught sturgeons banned by national legislation).



According to information from the investigating authority (National Environmental Guard Romania), the caviar originated from seizures and was commercialised by the Romanian Police (however, for CITES goods App. II having been seized and re-entering legal trade, the source code used is supposed to be „I”, not “W”).
On 30 April 2014, there was still Beluga caviar with source code “W” for sale at the airport.

3.2.9.3 Duty free area of Bucharest Airport; 5 May 2014

In May 2014, caviar glasses bore again very simple labels (not affixed according to CITES labelling requirements) with CITES code HUS/C/RO/2014/RO-0002/0072. This time, according to the CITES code, the caviar derived from captive bred Beluga sturgeons from Romania. However, there was no production of Beluga caviar in Romania at that time.

3.2.9.4 Duty free area of Bucharest Airport; 20 April 2015

In April 2015, there was again Sevruga and Beluga caviar for sale, bearing CITES labels (very simple and not affixed according to CITES labelling requirements) with CITES code. Both the container with Sevruga as well as the one with Beluga caviar had identical CITES codes (which was exactly the same code as of the Beluga caviar in May 2014; see above), classifying the species as from Beluga sturgeon (standard species code: HUS)
-> Sevruga: HUS/C/RO/2014/RO-0002/0072 (price tag at side of the glass declaring “sevruga roe”)
-> Beluga: HUS/C/RO/2014/RO-0002/0072 (price tag at side of the glass declaring “beluga roe”)
There is still no production of Beluga caviar in Romania. Upon request, the company stated that they always used the code given as an example in Government Decision no 1191/201, changing only the year of packaging, lot identification number and plant registration code.

3.2.9.5 Supermarket Cora, Bucharest; 28 October 2016

Very simple labels and not affixed according to CITES labelling requirements; CITES code RUT&HUS/C/RO/2016/RO-0002/0009.

DNA analysis showed that the sample was also mislabelled – the caviar was actually pure *Huso huso*, while the species code defines it as a hybrid of *Acipenser ruthenus* and *Huso huso*.

3.2.9.6 Supermarket Auchan, Bucharest; 18 August 2017

Very simple labels and not affixed according to CITES labelling requirements. Three different trade names (Osetra, Imperial, Beluga) and prices, but identical CITES codes on back (RUT&HUS/C/RO/2017/RO-0002/0017) and lot numbers on front.

All three samples are mislabelled, originating not from a hybrid of *Acipenser ruthenus* and *Huso huso* but from a hybrid with *Acipenser naccarii*.

ANNEX - 3

Table C: Standard codes for identification of Acipenseriformes species, hybrids and mixed species

Species	Mitogenomes
Acipenser baerii	BAE
Acipenser baerii baicalensis	BAI
Acipenser brevirostrum	*BVI*
Acipenser dabryanus	DAB
Acipenser fulvescens	FUL
Acipenser gueldenstaedtii	GUE
Acipenser medirostris	MED
Acipenser mikadoi	MIK
Acipenser naccarii	NAC
Acipenser nudiventris	NUD
Acipenser oxyrhynchus	OXY
Acipenser oxyrhynchus desotoi	DES
Acipenser persicus	PER
Acipenser ruthenus	RUT
Acipenser schrenckii	SCH
Acipenser sinensis	SIN
Acipenser stellatus	STE
Acipenser sturio	STU
Acipenser transmontanus	TRA
Huso dauricus	DAU
Huso huso	HUS
Polyodon spathula	SPA
Psephurus gladius	GLA
Pseudoscaphirhynchus fedtschenkoi	FED

Pseudoscaphirhynchus hermanni	HER
Pseudoscaphirhynchus kaufmanni	KAU
Scaphirhynchus albus	ALB
Scaphirhynchus platorynchus	PLA
Scaphirhynchus suttkusi	SUS
Mixed species (for ‘pressed’ caviar exclusively)	MIX
Hybrid specimens: code for the species of the male x code for the species of the female	YYYxXXX

Source: CITES Conf. 12.7 ; https://cites.org/sites/default/files/document/E-Res-12-07-R17.pdf