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AND NATURAL HABITATS

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# Technical Guideline for Sturgeon Population Monitoring

## Support Document for the Implementation of the Pan-European Action Plan for Sturgeons

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*Once the Guideline has been approved by the Commission, the results of this Guideline will belong to the European Commission who may decide to publish them in due course.*



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# 1 Executive summary

Sturgeon populations in European rivers and coastal waters have undergone a dramatic decline over the last 150 years. In addition to overharvest, the intensive developments of hydropower and river channelization have led to massive habitat loss and fragmentation affecting all stages of their life-cycle. As a consequence, all eight sturgeon species found in European waters are threatened with extinction (International Union for Conservation of Nature - IUCN) and are reported as being in "unfavourable" conservation status within the frame of the reporting under Article 17 of the Habitats Directive.

To improve this situation, the Pan-European Action Plan for sturgeons (PANEUAP) was adopted by the Standing Committee to the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) in the form of Recommendation No. 199(2018) and endorsed for implementation under the Habitats Directive and provides a guiding framework of actions to be implemented in sturgeon range countries by regional stakeholders including regional sea and river commissions.

The Action Plan requests all signatory countries to *"restore all existing sturgeon populations to "least concern" (IUCN) or "favourable" status and re-establish self-sustaining sturgeon populations as well as their life-cycle habitat in their historic range to an extent that ensures species survival and representation of the subpopulations where possible."*

The Action Plan stresses the importance for monitoring sturgeon populations as the foundation to design and evaluate any other conservation measured and ulimtaly evaluate the success of the whole PANEUAP itself. **Therefore, the purpose of this technical guideline is to specifically support the implementation of the Action Plan's Objective 5 to achieve "Timely and continuous detection of population sizes and changes in remaining wild stocks" namely to "design and implement monitoring programs" and to "secure regional coherence".**

Due to their ecology, late maturation, and long life-span, changes in sturgeon populations can only be assessed through long-term monitoring. The design of a coherent monitoring approach must be developed in each range country, for which priorities are derived from the population status and a national sturgeon action plan or conservation strategy. In catchments with shared populations, monitoring plans should be harmonized between range countries to allow the comparison of results. Population monitoring provides the essential means to evaluate and confirm the status of a sturgeon population and assess the resulting effects of any management measures such as:

- Effects of altered fisheries management including IUU fishing and bycatch
- Recovery of river continuity, including dam removal or fish passage facilitation
- Recovery of critical habitats
- Effects of supportive stocking

While several projects aiming at the recovery of sturgeon populations were implemented during the last 20 years, the implementation of a harmonized monitoring system and database allowing the evaluation of the effects of measures taken or to implement necessary adaptations is still lacking. This would be of major importance for migratory fish like sturgeon which render a transnational effort necessary to ensure comparable monitoring data in all range countries, regardless whether river or sea basins are concerned.
The current document serves as a technical guideline for administrators, monitoring experts and practitioners to plan and design future sturgeon population monitoring measures. The document comprises a five-step approach that addresses the critical issues required to assess any relevant changes in population status. Through a harmonized approach, it provides the basis for effective comparisons between measures applied in different projects or regions by:

**1) Assessment of sturgeon presence**

Rationale: The verification of presence is the first key piece of information to decide if a recovery strategy would be required. It must be taken into consideration that proof of absence is very challenging in such wide ranging and long-lived species. Since sturgeon might be present in certain habitats only intermittently while in the marine range, the identification of the respective population is almost impossible, requiring thorough information about population genetics and local substructures.
Methods: environmental DNA (eDNA), bycatch information, information from social media, side-scan sonar surveys.

**2) Identification of timing and habitat use**

Rationale: The determination of habitat utilization, its spatial and temporal characterization and the assessment of impacts is of essential relevance to verify the potential for recovery that the system offers. On top of that, knowledge about habitat use is paramount to effectively allocate resources for targeted population monitoring. This step represents the interlinkage between population and habitat monitoring.
Methods: Captures of different life-cycle stages using egg mats, D-nets, trammel/gill nets, side-scan sonar, telemetry tools, species distribution modelling.

**3) Identification of the genetic properties of a population**

Rationale: Assessing the genetic characteristics and diversity in a population gives important information about its viability or on the reproductive population size. Therefore, early life-cycle stages should be targeted. Moreover, the assessment of early life-cycle stages and spawning allows the detection of bottlenecks hindering spawning activity and early development in a population.

Methods: Captures of young life-cycle stages like eggs, larvae and Young of the Year individuals (YOY) through methods like egg mats, D-nets, and trammel/gill nets to obtain representative samples of the population. Tissue sampling and genetic analysis are applied to analyse population genetics and to determine effective population size for the respective cohorts.

**4) Determination of the recruitment into the reproductive/adult population and year class strength**

Rationale: Monitoring recruitment provides information on the growth and survival of early life-cycle stages to contribute to the reproductive/adult population. Mortality is highest for the youngest life-cycle stages and decreases as they grow. In sturgeon of the juvenile life-cycle stage, mortality is already reasonably low and the juvenile abundance allows to project future population development through the comparison of year class strength and the resulting adult population size. Annual assessment further helps to assess if bottlenecks for recruitment exist.
Methods: Capture of juveniles and subadults through trammel/gill nets or bottom trawls in the estuary or at sea on an annual basis, age determination of the fish, genetic assignment to the population in question, statistical modelling of recruitment.

**5) Data management**

Rationale: Ensuring quality and consistency of the data collection, proper storage as well as robust analysis of the monitoring data to be able to assess long-term trends in a population is vital. The geographical distribution of sturgeon populations necessitates internationally harmonized management, standardization amongst countries and institutions regarding data collection, and storage and analysis. These are essential prerequisites. Furthermore, the establishment of a shared database for the sturgeon monitoring data should comprise each conservation unit. For this purpose, all projects – in particular those funded with public money – should be obliged to contribute to such a database.
Methods: A harmonized data sampling protocol is to be established for the utilization in all projects dealing with sturgeon monitoring within a conservation unit (Annex 14.1).

This guideline provides a comprehensive overview about the possible methods and approaches for sturgeon population monitoring, including crucial considerations that should be taken care of in the planning phase of a monitoring program. Effective methods to target all life-cycle stages under different conditions are provided (Table 2). A collection of relevant literature in each chapter gives a solid base to properly plan a successful monitoring program. The goal of this guideline is to familiarize decision makers with both the necessity of sturgeon population monitoring and the resulting need of long-term solutions. It furthermore aims to guide practitioners through the important aspect of a properly planned monitoring program and provide information on how to successfully use different monitoring methods in the field.

The success of any population monitoring program aiming to assess the development of a sturgeon population, its recruitment, or the identification of bottlenecks is dependent upon rigorous implementation. This requires a clear definition of research questions and management objectives and the focus of all monitoring activities on answering them.

The results of monitoring actions need to be shared transparently with the public and relevant stakeholders involved in sturgeon conservation, including research institutions or stakeholders from the navigation, fishery, nature protection and/or water management sectors, both national as well as range wide.

Knowledge on the population status and the impacting factors are essential prerequisites for effective management of the populations in question and the habitats utilized. It is therefore mandatory to include sturgeon habitat monitoring into other existing monitoring approaches and obligations (e.g., in the frame of the EU Marine Framework Directive or the Water Framework Directive – where the composition, abundance, and age structure of fish fauna as biological elements for the classification of ecological quality have to be assessed).

Only a standardized data assessment makes monitoring data comparable between years and sites and thus is the basis of any long-term monitoring activity. Also, the design of the monitoring program needs to consider what kinds of data are necessary to answer the respective research questions and results in consistent data collection. An essential prerequisite for consistency is the availability of the resources required to perform the task. National governments and international organisations alike need to ensure necessary funding to support the implementation of monitoring actions. The combination of national sources with EU funding instruments such as LIFE, Horizon Europe, European Regional Development Fund (ERDF), the Cohesion Fund (CF) and the European Maritime, Fisheries and Aquaculture Fund (EMFAF) may provide good opportunities for beginning implementation but, in the longer term, such costs must be integrated into national budgets. The ministries of environment of EU Member States should therefore ensure that monitoring of threatened migratory fish species is included in their Priority Action Frameworks (PAFs), as references to the PAFs is the enabling condition for accessing funding from EU instruments.

**Without coherent population monitoring, one risks flying blind!**

# 2 Introduction

Eight sturgeon species are native to Europe's rivers and seas, and all are featured in the IUCN Red List of Threatened Species. Seven of the eight species have been assigned "critically endangered" status. Sturgeon, although threatened with extinction, are key indicators for the ecological integrity of rivers, as habitat for completing the life-cycle may cover entire catchments (Schiemer, 2000). Sturgeon are thus considered flagship species for many conservation actors for healthy and free flowing river systems.

Within the EU, all sturgeon species are protected under the Habitats Directive (92/43EEC) obliging EU Member States to **ensure that the species covered by the Directive are maintained, or restored, to a favourable conservation status throughout their natural range within the EU**. The monitoring of conservation status is an obligation arising from Article 11 of the EU Habitats Directive for all species (as listed in Annex II, IV and V) of community interest. The specific reporting obligation derives from Article 17, with the reporting for the conservation status assessment to be repeated every six years (last available report 2013-2018).

For the **EU Water Framework Directive** (WFD) aiming for all surface waters to reach good or higher ecological status or potential, monitoring is essential for assessing their current and future states. Good ecological status/potential is defined in Annex V of the WFD. It considers the quality of the biological community, the hydro-morphological characteristics and the chemical characteristics. Annex V sets out the requirements and lists the composition, abundance and age structure of fish fauna as biological elements for the classification of ecological status (in comparison to a reference state of fish composition). For some rivers, sturgeon need to be considered an integral part of the fish community, and information on their status will thus contribute to the assessment of the ecological status and will reflect the impacts of hydrological recovery measures. Article 15 specifically requires Member States to report on the progress of the water management measures in the respective River Basin Management Plan (RBMP) and the effectiveness of their measures in support of good ecological status. Unfortunately, the countries involved were free in the definition of the reference conditions and as such, migratory fish species such as salmon or sturgeon have been ignored in order to ensure the aim of the WFD would not be undermined by underachievement in these species.

The Bern Convention has established different types of reporting, although only one of them is compulsory under the terms of the convention. This is the **system of the "biennial reports"**, which all Parties making exceptions to the provisions of the Convention must submit to the Secretariat every two years in compliance with the strict terms and conditions spelled out in Article 9. These reports must contain a scientific assessment of the impact of such exceptions to the general obligation to protect the species and habitats covered by the Convention. Arguably, only population monitoring can provide a basis for any such scientific impact assessment.

Besides, the Groups of Experts set under the Convention also **monitor the implementation of both the Treaty and the Recommendations** adopted by the Standing Committee with regard to the conservation status of species or habitats, or specific conservation challenges.

Reflecting the high risk of extinction for this species group, a **Pan-European Action Plan for Sturgeons** (PANEUAP, (Bern Convention, 2018)) was adopted in November 2018 as a recommendation[1] of the Standing Committee to the Bern Convention to which all important European sturgeon range countries as well as both the EU and its Member States are parties. In May 2019, the EU Nature Directive Expert Group (NADEG) recommended the implementation of the PANEUAP to EU Member States. The PANEUAP was designed to serve as a framework of almost 70 actions that aim to *"restore all existing sturgeon populations to "least concern" (IUCN) or "favourable" conservation status (EU Habitats Directive) and re-establish self-sustaining sturgeon populations as well as their life-cycle habitat in their historic range to an extent that ensures species survival and representation of the subpopulations where possible."*[2]

The recommendation mandated the Secretariat of the Bern Convention to closely monitor the implementation of the PANEUAP and to coordinate the implementation of regular reporting on the implementation of the Action Plan at national level.

Since its adoption, the European Commission has followed the implementation of the PANEUAP closely and, in 2022, issued a service contract (ENV/2022/OP/0019) to support its implementation. The scope of the contract (the SCUTE project) covers the assessment of the implementation of the PANEUAP in 18 key sturgeon range countries, including 15 EU Member States (Romania, Bulgaria, Croatia, Slovenia, Hungary, Slovakia, Austria, Germany, Italy, Poland, Lithuania, Latvia, Estonia, France, and The Netherlands) as well as Serbia, Ukraine and Georgia. Existing knowledge about sturgeon habitats and migration obstacles in eleven key river basins including Danube, Rioni, Po, Vistula, Odra, Nemunas, Gauja, Narva, Elbe, Rhine and Gironde have been collected and displayed in maps.

Furthermore, the contract encompasses (1) a study about sturgeon bycatch and possible measures to avoid or mitigate it, (2) guidelines for sturgeon population monitoring, (3) guidelines for habitat monitoring as well as (4) guidelines for best practice *ex situ* breeding and release programs.

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[1] https://rm.coe.int/recommendation-199-2018-action-plan-sturgeon/1680a01895

[2] A species is categorized as "least concern" by the IUCN when it is not being a focus of species conservation because it is still plentiful in the wild. Species are in a "favourable" conservation status according to the EU Habitats Directive, when "*population dynamics data on the species concerned indicate that it is maintaining itself on a long-term basis as a viable component of its natural habitats, and the natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis*."

This document presents the guideline for population monitoring contributing explicitly to the **PANEUAP Objective 5** aiming at the "*timely and continuous detection of population sizes and changes in remaining wild stocks*".

In its framework, the PANEUAP lists actions towards the design and implementation of regular monitoring programs as well as securing the regional coherence of monitoring measures.

The guideline at hand complements the habitat monitoring guideline developed under the same service contract. The population and its habitats are ecological twins, and one cannot be sustained without the other. For a successful and sustainable conservation approach, measures targeting viable sturgeon populations within functional habitats are needed. Restoring populations, habitats or migration routes requires substantial resources and political will as well as a sound, knowledge base to make informed decisions concerning conservation priorities. This guideline will provide technical advice to directly support the PANEUAP's Action 5.1.2. to "*Define criteria and develop design of monitoring programmes for all life stages*" in order to establish a monitoring scheme that can be applied across borders by different range countries with jointly adopted and standardized methods. Through the adaptation of standardized methods, a monitoring program can possess a strong analytical or diagnostic power to obtain detailed knowledge of the population status, detect changes within a system, and provide the basis for any further management decisions.

## 2.1 Monitoring – a basis for decision making

Monitoring populations is the systematic, continuous and repeated observation, measurement and evaluation of fish population parameters or indices according to predefined goals and helps to address questions about the actual status of population numbers, the health and genetic diversity, age and sex structure, reproduction, and recruitment or mortalities (natural or anthropogenically induced). Only a regular and coherent monitoring effort will provide understanding of key factors affecting sturgeon distribution, reproductive success, and population viability. As such, they are essential to allow for timely detection of population trends and changes and support the identification of underlying causes and impacts for such developments. Population monitoring data are the prerequisite for science-based management decisions necessary for the maintenance and recovery of species, such as setting priorities on the best location, time and type of conservation measures, including decisions on:

- Changes of fishing regulations and practices concerning IUU fishing and bycatch
- Termination or prolongation of fishing bans
- Identification of best suited protection measures for life-cycle habitats including the creation of dedicated protected areas, or spatial limitation of specific activities

- Planning measures to restore key habitats or ecological corridors for migration
- The necessity and impact of *ex situ* measures such as supportive stocking activities

Population monitoring is required to assess the general success or failure of measures taken, keeping in mind the need to demonstrate impacts of conservation actions to policy makers and stakeholders as well as the general public and taxpayers, to secure public investment into conservation.

The status of long-distance migratory fish populations is an excellent indicator for the sustainability of management measures. With regard to habitat, this translates to the functioning of ecological corridors, the existence of sufficient spawning, feeding and wintering habitats, habitat accessibility and connectivity, as well as water quality. The dynamic interactions between sturgeon populations and their environment are critical and their assessments must be done in combination. Hence, the importance of identifying potential key life-cycle habitats through the documentation of habitat use by sturgeon and documenting adverse impacts upon them.

Ultimately, population and habitat monitoring are essential to evaluate and confirm the potential success or failure of measures undertaken in the frame of the PANEUAP, to *"restore all existing sturgeon populations to "least concern" (IUCN) or "favourable" (EU Habitats Directive) status and re-establish self-sustaining sturgeon populations <u>as well as their life-cycle habitat in their historic range to an extent that ensures species survival</u> and representation of the subpopulations where possible."*

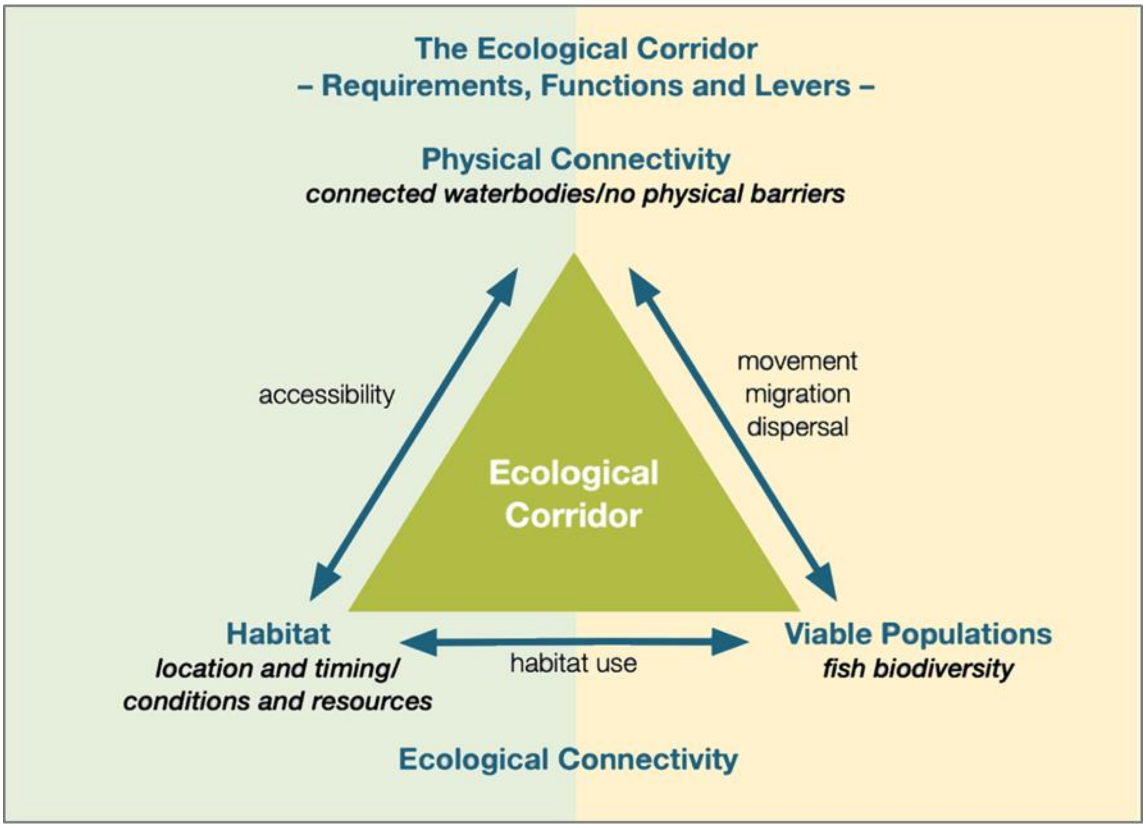


***Figure 1:*** *The ecological corridor for migratory fish (adapted after Haidvogl et al. (2021)). The left side is the main focus of habitat monitoring, the right side of population monitoring.*

The present guideline focuses on the right side of Figure 1 and will inform about the "Why?", "How?", "When?", and "Where?" of sturgeon population monitoring, including information about the analysis of collected data. Nevertheless, one should be aware of the relation between sturgeon and their environment and that the lack of specific environmental conditions and habitats will dictate the development of any population. Nevertheless, "Viable Populations" always depend on "Habitat" and "Physical Connectivity" in good quality. The monitoring of populations will always either be connected to monitoring one of the other levels or it will give at least information on either or both.

Therefore, the guideline at hand provides guidance to responsible authorities and institutions on best practice approaches to design monitoring programs and to decide on funding priorities. Descriptions of methods and technologies, their purpose, advantages and disadvantages provide orientation and guidance for practitioners to develop individual solutions adapted to the conditions encountered to implement targeted methodological approaches, to address specific research questions, and to close existing knowledge gaps.

The technical chapters are complemented with a compilation of required resources, the timing and spacing of sampling efforts, main pros and cons, practical examples from the field of applied science and a compilation of key references on the respective topic for further reading and research.

# 3 Sturgeon population monitoring

Through the monitoring of specific life-cycle stages (Figure 2), specific bottlenecks affecting the population can be revealed, shaping the need of management efforts due to the fact that each life-cycle stage has different needs regarding their environment. If spawning success or the location and timing of spawning are of interest, the earliest life-cycle stages, such as eggs or larvae, should be targeted but the information could also be gathered by targeting spawning adults; however, the design of the monitoring program and the resources needed will differ completely. If monitoring long-term trends of the population or the efficacy of management actions is the goal, targeting juveniles might be necessary since they represent the recruitment into the population and are the earliest life stage with natural mortality rates that are low enough to permit future population predictions.

Due to the longevity of sturgeon species, their complex life-cycle, their use of diverse habitats (estuaries, marine shelf, rivers) that are often difficult to sample, and their current diminished populations with only a few individuals remaining, render the monitoring of sturgeon very difficult and stresses the need for rigorous and well-designed monitoring actions.

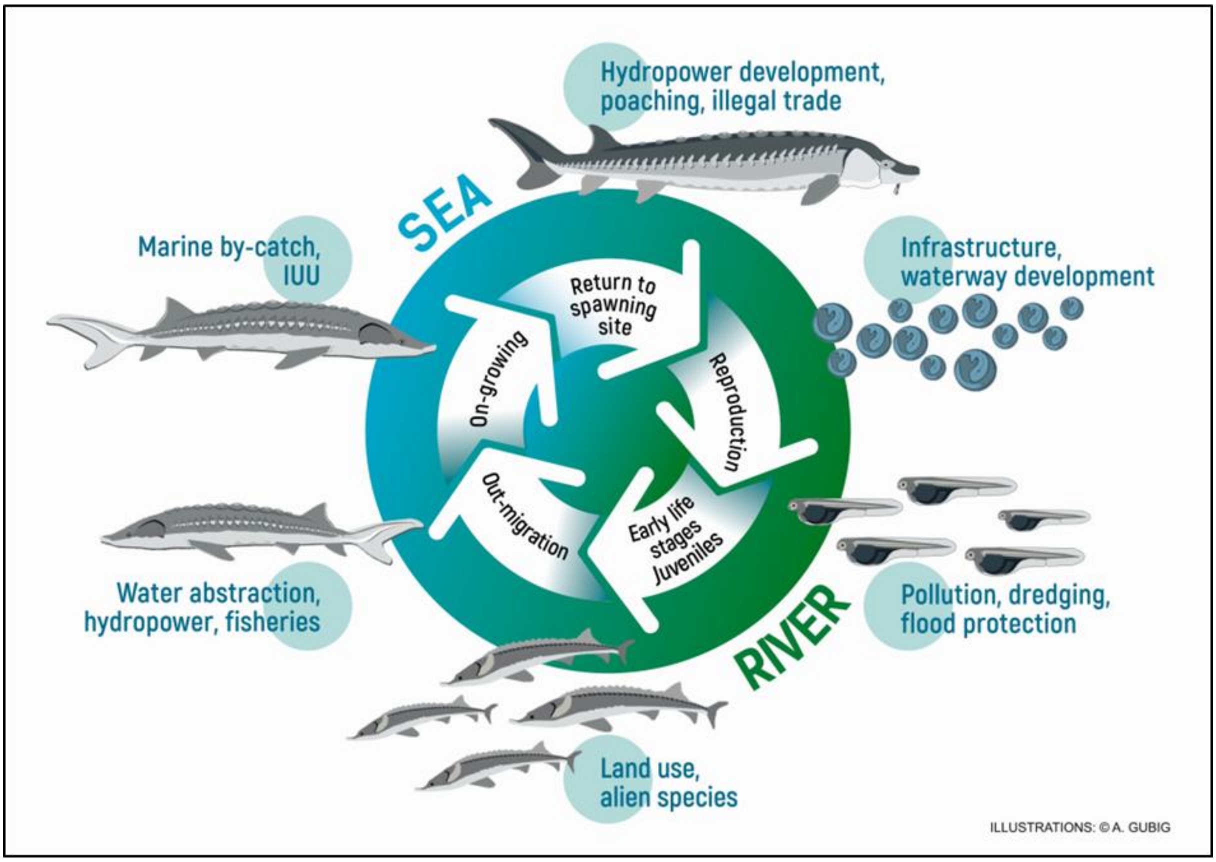


***Figure 2:*** *The sturgeon life-cycle and main threats (adapted after Friedrich et al. (2018)).*

## 3.1 Biological prerequisites

Sturgeon are a phylogenetically old group of species which were historically widespread across the Northern Hemisphere (Bemis et al., 1997; Haxton & Cano, 2016; Pikitch et al., 2005). They all have similar, but not identical, life-history strategies (Bemis & Kynard, 1997). Sturgeon are diadromous, sometimes migrating extensive distances to obligately spawn in fresh water (Bemis & Kynard, 1997; Hensel & Holčík, 1997; Rochard et al., 1990). The majority of species are anadromous, migrating between rivers and the sea, whereas others are considered potamodromous, fulfilling their whole life-cycle in freshwater. In general, sturgeon migrate upstream in rivers to spawn in fast-flowing waters (Bemis & Kynard, 1997). Maturity occurs late in life, a fact that is compensated by a long lifespan which can exceed 100 years. The age at maturity is variable between species but increases with latitude within a species. On average, this occurs between 6 and 27 years of age, with males becoming mature earlier than females (Bemis & Kynard, 1997; Holčík, 1989). The number of eggs a female can produce depends upon egg and body size. Between spawning events, the accumulation of sufficient resources to develop eggs may take several years. Therefore, the reproductive interval (e.g., spawning periodicity) is prolonged for several years, especially for females (Billard & Lecointre, 2001). Sturgeon are polygamous and polyandrous, broadcasting gametes over spawning substrate in fast flowing water.

Eggs are adhesive upon fertilization and attach to the hard substrate (Bemis et al., 1997; Bemis & Kynard, 1997; Billard & Lecointre, 2001). The fertilized eggs develop without maternal care into free embryos which utilize interstitial spaces for their development before emerging as feeding larvae (Bruch & Binkowski, 2002). Prior to external feed uptake, the larvae generally drift downstream into nursery habitats (Auer & Baker, 2002). Growth in the first year is fast but decreases with age (Bruch et al., 2009).

All populations of sturgeon have declined dramatically during the past 150 years as a result of overharvest, habitat loss through channelization of rivers, the construction of migration barriers, and pollution (Bemis & Kynard, 1997; Birstein, 1993; Hensel & Holčík, 1997; Lenhardt et al., 2006; Pikitch et al., 2005; Sandu et al., 2013). While efforts have been employed to facilitate recovery, given the level of fragmentation globally, full recovery may not be possible due to significant losses of habitat and genetic diversity (Haxton & Cano, 2016; Ludwig et al., 2009).

The eight species of sturgeon occurring in European waters and their respective protection status were identified in the PANEUAP. Table 1 provides an overview about their ecological traits and their current population status based on the latest IUCN assessment. A detailed summary about spawning characteristics of all species is given in the Technical Guideline for Sturgeon Habitat Monitoring (Reinartz R., 2024) developed in the frame of the same EC Service Contract 09.0201/2022/885601/SER/D.3.

***Table 1:*** *Overview about all eight European sturgeon species considered in the PANEUAP, including their morphological traits, age at maturity for females (F) and males (M), the spawning period based on (Holčík, 1989), their current distribution in Europe based on the PANEUAP as well as their IUCN Red List classification and the respective population trend. CR = critically endangered, EX = extinct, NT = near threatened, VU = vulnerable.*

| **Common name** | **Scientific name** | **Length** | **Weight** | **Age** | **Maturity** | **Spawning Period** | **Distribution** | **IUCN Red List 2011** | **IUCN Red List 2019** | **Population trend** |
|---|---|---|---|---|---|---|---|---|---|---|
| Russian sturgeon complex | *Acipenser gueldenstaedtii, A. persicus (colchicus)* | Max.: 2.4m; average: 1.3-1.6 m | Max.: 110 kg | >50 years | F: 10-16 years; M: 8-13 years | March-November (>8°C)*, Rioni: July (17-23.6°C)* | Black Sea and Lower Danube, Rioni (*A. colchicus*); unknown in Dnjeper, Don, Kuban | CR | CR | Decreasing |
| Adriatic sturgeon | *A. naccarii* | Max.: 2 m; average: 1.4-1.8 m | Max.: 90 kg | >50 years | F: 9-13 years; M: 6-8 years | February-March* | Po, single specimen in Adige, Livenza, Sile; unknown in Piave, Tagliamento, Buna | CR | CR | Increasing |
| ship sturgeon | *A. nudiventris* | Max.: 2.2 m; average: 1.2-1.5 m | Max.: 120 kg | >36 years | F: 12-18 years; M: 6-12 years | April-May (10-15°C | Unknown in Danube, Rioni | CR | CR | Decreasing |
| Atlantic/Baltic sturgeon | *A. oxyrinchus* | Max.: >4.3 m; average: 1.7-2.5 m | Max.: 370 kg | >100 years | F: 7-30 years; M: 5-24 years | May-July (13-22°C)** | In Baltic based on releases in Odra, Vistula, Prgolya, Nemunas, Daugava, Narva, Gauja, Pärnu | Globally NT, Baltic population CR/EX | Possibly EX and reintroduced in EU | - |
| sterlet | *A. ruthenus* | Max.: 1.2 m; average: 0.5-1 m | Max.: 16 kg | >25 years | F: 5-8 years; M: 3-5 years | April-May (12-17°C)* | Danube, Dnjeper, Dniester, Don, Kuban, Prut, Tisza, Sava, Mura | VU | VU | Decreasing |
| stellate sturgeon | *A. stellatus* | Max.: 2.9 m; average: 1.2-1.8 m | Max.: 80 kg | >35 years | F: 8-14 years; M: 6-12 years | May-June (17-23°C)* | In EU: Black Sea and Lower Danube, Rioni | CR | CR | Decreasing |
| European sturgeon | *A. sturio* | Max.: 6 m; average: 1.5-3 m | Max.: 850 kg | >60 years | F: 13-16 years; M: 10-12 years | May-June (in Gironde)*** | Stocking in Gironde-Garonne-Dordogne basin, and Elbe, Experimental releases in the Rhine, Ebro | CR | CR | Decreasing |
| beluga sturgeon | *H. huso* | Max.: 8 m; average: 2.2-3.5 m | Max.: 2000 kg | >100 years | F: 14-20 years; M: 10-16 years | April-May (9-17°C)* | Black Sea and Lower Danube, single specimen in Rioni; unknown in Kuban | CR | CR | Decreasing |

*(Holčík, 1989), **(Gessner et al., 2019), ***(Rosenthal et al., 2009)

## 3.2 Considerations in population monitoring and its purposes

Monitoring of populations in terms of status or trend is an essential prerequisite to employ effective management or recovery efforts and actions. Population monitoring will help accumulate scientific background information of populations. As such, monitoring helps to facilitate conservation or recovery measures for a species and to evaluate the outcome of implemented measures supporting adaptive management. For example, monitoring can reveal optimal flow and temperature regimes for successful spawning, which can inform hydropower operation. If a monitoring program reveals that the occurring sturgeon population is dominated by old individuals and no recruitment is occurring, it showcases the necessity of the re-establishment of the migration corridor or the functionality of spawning sites, altering river flows during critical periods such as spawning, or even the onset of a supportive stocking program to save the population. Targeted and well-planned monitoring with clearly defined objectives will help to identify solutions to support the most effective recovery measures and to allocate resources.

Ultimately, the aim of any monitoring program is to provide information that is based on good-quality scientific data, which can be used to assess the population status and trends of a species and if protection measures are necessary. The quality of monitoring data is the basis of the population assessment and therefore of any legally binding protective measures and, thus, decisive if a population is managed in accordance with its actual status.

Population monitoring is associated with the assessment of the age structure and size of a given population as well as with the recruitment of emerging year classes into the population. Monitoring the various life-cycle stages will provide different information about the population and their choice can reveal specific bottlenecks which should be targeted when managing the species. However, the life-cycle stage subjected to monitoring depends upon the objective of the study to achieve tangible results. Some life-cycle stages can provide highly variable results and therefore provide little information on the population status due to high natural mortality, though they might be essential when it comes to determine environmental impacts upon recruitment.

To maximize on the information gain from a monitoring program, adequate methods to answer specific research questions have to be chosen. In their review on fish monitoring, Radinger et al. (2019) concluded that the following aims are essential and must be clearly determined in order to conduct and establish an effective and reliable monitoring program:

- What should be monitored and how?
- How to allocate efforts within time and across sites?
- Establishment of criteria for data reliability
- Identification of practical constraints

Hence, the following aspects should be accounted for when designing a monitoring program:

- Clearly defined objectives and research questions
- Appropriate methodology for objectives and research questions
- Standardization and consistency ensuring spatial and temporal comparability
- Reporting
- Availability of permits
- Sustainability of human and financial resources for the time considered

The guideline at hand aims to give answers on all these aspects with regard to sturgeon population monitoring.

To implement a successful monitoring program, it must be ensured that all objectives can be met with the resources available. Lack in funding, employees, material, or time may lead to inconsistent or missing results and thus to an incomplete database. It is recommended to prioritise the research questions and concentrate the available resource to address top priorities in sufficient detail before adhering to others. Nevertheless, efficiency of sampling may be achieved by reviewing the collected data and the methods and techniques used based on previous years' sampling and by determining and adjusting the amount of effort required to detect change. Theoretically, effort should go down over time if the proper assessment techniques are employed and catches are suitable.

Due to the longevity of sturgeon, a population monitoring programme should ensure the availability of resources over the long-term in order to detect changes and trends at the population level. The successful recovery of the sturgeon population is challenging due to various aspects such as needed long-term efforts, international cooperation, high sampling effort, etc. But, without a long-term engagement in monitoring and supportive actions for habitat recovery, sturgeon populations may never recover.

## 3.3 Data collection and data storage

To effectively analyse collected data, proper documentation and storage are paramount. Hence, it is recommended to develop field protocols for data collection (see the Annex of this guideline for an example). Such protocols need to be digitalised and stored in a safe place, with at least one copy at different locations.

Nevertheless, for effective data storage and utilization of a population monitoring program, it will be necessary to establish a database in a long-term perspective. For example, the STURIO database was developed to store all collected data regarding the sustained European sturgeon population in the Gironde-Garonne-Dordogne system (Annex 14.3). The database contains several sublevels where specific data is stored. Data that is collected during the regularly conducted population monitoring in the estuary by a trawling campaign is stored in a specifically designed database (STURAT) (Lamour et al., 2024; Rochard et al., 2001; Roques et al., 2018). The regular monitoring has been a part of the national action plan since 2011, but was initiated much earlier. In addition, bycatch observations are collected and made available through a second database (STURWILD) (Charbonnel et al., 2023; Rochard et al., 1997), where information on hatchery fish, genetic assessments, or age classifications are stored.

Such a database is currently missing in most parts of Europe, but will be paramount for the management of sturgeon populations that use international waters.

# 4 Rationale for the standardization of population monitoring

While many thriving projects for the recovery of sturgeon populations have been initiated over the past 30 years, the harmonization of the recovery measures as well as the development of harmonized monitoring approaches that would ensure the comparability of the results obtained has not been achieved so far. This also includes the establishment of a joint database and a common and standardized analysis of monitoring data that facilitates the evaluation of the effects of measures taken to further fine tune the application of measures to effectively support population recovery.

The main prerequisite for such an assessment is the consistency of the information obtained and the continuation over time as well as the standardization of the approach taken. Only through standardized monitoring in space, time and gear, reliable and robust information about processes on the population level can be obtained despite spatiotemporal variation (Bonar et al., 2009; Guy et al., 2009; Pope et al., 2010). Following Pope et al. (2010), "*Standardized sampling is defined as sampling with identical gear during the same season (or set of environmental conditions) in the same manner over time or among fish populations*." These joint efforts are indispensable in jointly managed populations both in multinational rivers as well as in the respective marine basins to ensure a harmonization of measures to improve population status based upon the best available data sources.

Sound information on the effects of management measures aid the development of best practices to be applied on the remaining populations. The comparability of the conditions encountered, and the measures taken would be a prerequisite for steeper learning curves, resulting in more resource efficient conservation attempts, which would both require and facilitate an improved collaboration between projects.

## 4.1 Structured population monitoring outline – a five-step plan

To assess population trends and identify years or conditions with good and poor recruitment, a robust and reliable monitoring program is the only means to collect this information. Since sturgeon are long-lived and late maturing, changes in the reproduction and recruitment process should be assessed at the earliest possible opportunity to be able to recognize trends in population development as soon as possible to take supportive measures. However, monitoring of these early life-cycle stages should carefully evaluate their pros and cons to avoid additional pressure on the population through poorly planned monitoring actions.

For the assessment of changes on the population level, the numerical characterization of the abundance is a standard approach. Therefore, it is recommended to assess sturgeon populations based on a five-step plan which depends on the knowledge that is available on a population within a specific catchment.

**1. The first step** needs to be applied if no information about the occurrence of sturgeon, or only anecdotal information, is available. The assessment focuses on the proof of the presence or absence of the species in the area of interest. While information about presence can be collected accidentally through bycatches during commercial or recreational fishing or scientific monitoring activities, surveys focused on the objective should be conducted to obtain more detailed data on the numbers/abundance of the species present. The proof of absence is more complicated and at the very least more time consuming, considering the fact that if fishes are not caught does not necessarily mean they do not use the sampled area. False timing, inappropriate methods or simply the fact that the effort is insufficient to obtain information about rare species can lead to false conclusions about the population status. The knowledge about the occurrence of sturgeon in an area is the basis for further population monitoring.

**2. The second step** comprises the assessment of the time during which different (potential) habitats would be utilized by the different life-cycle stages. This assessment requires ecological knowledge of the targeted species and life-cycle stage. In case the knowledge base is not available, the goal of the second step should be to provide reliable information through a very thorough approach of identifying migration and habitat use over time. Information such as identification of spawning conditions, timing of outmigration or habitat use by YOY can be obtained, depending upon the life-cycle stage targeted. If identification of spawning success, areas, or timing are of interest, targeting eggs or larvae will become necessary. YOY sturgeon, compared to eggs and larvae, might provide indications about the suitability for the entire early development in a specific river system in a given year if mortalities and growth of the year class are assessed.

**3. Step three** addresses the genetic landscape of a population targeting early life-cycle stages, such as eggs, larvae or YOY. Targeting these life-cycle stages can yield important information about the genetic structure and diversity in the population, timing and site selection for reproduction, effective population size (e.g., effective number of breeders ($N_b$), provided the samples are representative), and reproductive success.

**4. In the fourth step**, the recruitment and year class strength of the offspring originating from the reproduction in a given year are determined. To ensure reliable results, this assessment must focus on life-cycle stages that reveal or are subjected to only limited and rather constant natural mortality to allow the forecasting of the future development of the population. Detailed information on the status of the species and their populations is derived from the annual results of the population assessment of juvenile individuals. Monitoring recruitment reveals survival under the existing conditions during the critical early life-cycle stages and if it supports and contributes to the population. Therefore, monitoring recruitment is important to predict population trends and identify adverse developments which subsequently serve the planning of management measures.

**5. The fifth step** addresses the quality and consistency of the data obtained since data management is a key element in monitoring of fish populations (Radinger et al., 2019). The assessment of relevant data, comprising relevant environmental parameters such as biotic and abiotic data, a sound and robust data analysis, as well as proper storage of monitoring data, are indispensable to allow the assessment of long-term trends. Data must be collected in the field with scientific rigor and, depending on the objectives, all relevant data needs to be identified before field work is conducted. The establishment of a sampling protocol that ensures the collection of these data is of valuable help to avoid missing out certain aspects. Collection methods are described in this guideline and an example of a sampling protocol is provided in Annex 14.1. All collected data must be stored in a database and should be added there immediately after field work.

Data acquisition as well as data storage, should allow access and exchange between research entities and between countries sharing sturgeon populations in order to be able to plan and implement conservation strategies at the population level which are transferrable to the landscape level. For a common species/population conservation unit, the establishment of a common database, ideally maintained and serviced by a regional stakeholder supported and trusted by all relevant parties involved (e.g., the International Commission for the Protection of the Danube River (ICPDR), International Commission for the Protection of the Rhine (ICPR), Baltic Marine Environment Protection Commission (HELCOM), etc.), is advised to ensure standardized data quality. Effective monitoring and management of sturgeon populations require long-term approaches and solutions due to the biology of the species (Challenger et al., 2020; Haxton, 2006; Nelson et al., 2020).

The five-step plan can thus be regarded as a tool to constantly evaluate the status of knowledge about a specific sturgeon population and to pointedly increase this knowledge based on research that unravels the most and important information to contribute to the positive development of the population. If a monitoring program covering all five steps is implemented, the knowledge base about a population will be sufficient to inform effective conservation measures. If only a few steps are sufficiently met, the knowledge base about a population is incomplete, which might result in uninformed and therefore inefficient management measures. Hence, it is highly recommended to work towards the implementation of a monitoring system that covers all five steps.

# 5 Animal welfare and handling of sturgeon

## 5.1 Animal protection legislation

Animal protection laws are a result of changing attitudes and social norms that have been developing over the years. From the anthropocentric view that humans are the crown of creation to which all other life forms would be inferior to the approach that all life forms possess a similar intrinsic value and are as such equal, the span between the antipodes is extremely wide. The legal framework over the years has reflected the above-mentioned changing attitude of societies towards scientific developments. Intensification of practices were a source of important changes in the use of animals, in agriculture and in food production, as well as in research and experimentation. The Conventions on the protection of animals elaborated at the Council of Europe were the first international legal instruments laying down ethical principles for the transport, the farming, the slaughtering of animals as well as for their use for experimental purposes and as pets. They have been used as a basis for and continue to influence all relevant legislation in Europe. Animal welfare is an issue of increasing importance in Europe, which is reflected by the work of the Council of Europe. Since 1988, work carried out at the Council of Europe has focused on the monitoring of the implementation of these different Conventions. The aim is to improve and harmonize – at the international level – the conditions for the use of animals in the different fields (animal husbandry, science, pet animals) concerned.

The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) appears most relevant for this guideline as it concerns the use of animals in procedures (experiments). Its provisions cover areas such as care and accommodation, conduct of experiments, humane killing, authorization procedures, control of breeding or supplying and user establishments, education and training, and statistical information.

Apart from the personal ethics background, norms and beliefs, animal welfare in science has a justification in its own, considering the fact that stress in animals reduces the quality of the results obtained. As such, considerate planning, selection of methods and careful handling should be self-evident principles in scientific work.

The legal framework is provided by the national laws addressing animal rights and the resulting processes which must be implemented for animal experimentation. Furthermore, species protection, habitat protection and general conservation laws provide a similarly important background, identifying the scope of the work and the prerequisites to be fulfilled to carry out such work. Since the laws and associated rules vary from country to country, they cannot be summarized sensibly, nor can they be repeated in detail here. It is emphasized that it is of highest relevance and utmost importance that the personnel involved in working with protected animals or in protected areas must be aware of the restrictions and the prerequisites they are subjected to by national laws and their implementation.

## 5.2 Permits

Most jurisdictions require permits for accessing and working in protected areas for the utilization of motorized vehicles to sampling/collecting macrozoobenthos and fish, especially when protected species could be affected. The handling and tagging of live endangered animals are subject to laws and the resulting requirements of permits. Furthermore, animal testing requires a permit which must be applied for and which requires detailed planning and a description of the experimental methods and the impact to the animals subjected to the tests, followed by detailed record keeping. Limitations may be regulatory (biopsies or blood sampling of threatened or endangered species may require permits), results based (high accuracy required) or a combination of these factors (Webb et al., 2019).

## 5.3 General considerations

Since sturgeon are endangered species and every individual is valuable for the future of the population that it represents, special attention has to be devoted to ensure safe and careful fish handling (Gessner et al., 2024). Animal welfare includes the safe handling and treatment of fish to ensure minimal impact resulting from the inevitable handling procedures. The processing and sampling of fish (e.g., methods, gear, and treatment) must apply best practice, taking into respect fish welfare during all stages of the operations. Scientific work and monitoring need to minimize the risk posed to single individuals and populations. Operating under the precautionary principle, especially when dealing with critically endangered species, should be self-understood.

As stress is cumulative, stress associated with handling and the number of consecutive handling events should be reduced in all circumstances. Reducing stress during each handling event should be done by assessing the importance of the procedure (e.g., "is there a less invasive technique that will provide the data needed with acceptable accuracy?"), the length of time of the procedure, and the environment under which the animal will be handled (e.g., temperature, air exposure, dissolved oxygen, water quality, direct sunlight, etc.). In addition to direct damage and reduced survival of individuals, handling during reproductive seasons may lead to animals stopping and/or aborting their spawning migration and in mass reabsorption of eggs in females as was described in the past. As a consequence, the cumulative effects may lead to reduced reproduction and recruitment, further endangering populations.

The following basic considerations are recommended (which also apply for other fish species):

- Ensure minimal time in nets, especially when water temperatures exceed 20 °C
- Strive for minimal handling time and handling impact
- Make sure handling fish takes place with wet hands/gloves and wet gear to minimize impact on the mucus layer of fish
- To handle fish >1 m, utilize a cradle/stretcher made from smooth and strong materials. Do not try to grab or even carry a fish holding it by the tail! This practice inevitably causes damage to the notochord of the fish
- During handling, continuous submersion in water and/or flushing of the gills largely reduces stress for the fish
- Adequately sized tanks for holding fish with aeration and continuous water exchange secure the fish in good condition. This is especially important when water temperatures exceed the optimal temperature for a given species. Nevertheless, water temperatures for holding should be close to waterbody temperatures to avoid thermal stress. Tank size depends on the expected maximum size of the target individuals but for large fish, a rectangular tank which constrains the fish may be less stressful
- Apply disinfectant (iodine solution or disinfectant spray (Methylenblue), refer to your veterinary referent advice) for treatment of wounds or injuries
- Release fish safely and immediately following data acquisition, ensuring gentle release: no dropping and no obstacles which could damage the fish, fish must be fully conscious if they were anaesthetized before, a sharp angle should be provided if the fish is released from a stretcher on a research vessel for it to be able to dive (Figure 18)
- Appropriate handling and removal – taking into account local regulations – of non-native sturgeon species upon the reliable identification of species
    - Local regulations must be checked a priori
    - If the species identification is not 100 % sure, the sturgeon should be released
    - At the very least pictures or tissue samples should be collected to identify the fish at a later date

The above-mentioned basics should be part of the briefing and safety training of personnel before active field work. Moreover, to ensure minimal handling time and handling impact throughout the entire process from catch to release, personnel should be trained before being involved in monitoring activities. It is recommended to provide working environments and fair payments to encourage long-term commitments of experienced personnel and to assign clear responsibilities to them (Directive 2010/63/EU on the protection of animals used for scientific purposes).

The safety of sturgeon being collected or handled is of paramount importance. When large fish are taken, the fish can do considerable damage to themselves, gear, and collectors, if not handled quickly and correctly. Therefore, always handle sturgeon with care and respect and prioritize the welfare of the fish during research and conservation activities.

Live sturgeon are a priceless and rare resource in sturgeon conservation and recovery, regardless if they are of wild origin or from conservation aquaculture. Make sure that your procedures of sturgeon handling and holding are in line with the best practices and highest standards, to not only ensure successful sturgeon research but, first and foremost, the well-being and survival of each individual sturgeon.

## 5.4 Safety during field work

When conducting field research, you are working in a potentially dangerous environment. You have the responsibility to your co-workers and to yourself to provide and ensure safe work procedures. Not only with regard to legal obligations and regulations, but also in line with common sense. Do not become inattentive and careless, even after long periods of successful field work. Stay safe and take care of others!

To ensure a successful monitoring campaign which is repeatable and applicable over a long term, some safety issues should be considered in order to avoid damage to people and gear in the field. The following aspects are recommendations to consider before, during, and after each sampling trip:

- A check of the weather forecast & river discharge
    - Unreasonable conditions should be avoided
- At least 2 people are to be assigned to a team
- Ensure communication with collaborators home base or emergency numbers
- Proper nautical equipment, life vests, rain gear, sturdy clothing & security shoes, gloves for handling sturgeon, lights when working at night
- Ensure sufficient hydration and sun protection
- Carry a first aid kit and ensure knowledge how to use it
- General alertness during field work
- Post-processing and cleaning of used gear and appropriate storage
    - Important to reduce risk of possible introductions of non-native species or diseases

Before conducting field work, necessary licenses for scientific monitoring must be obtained and available and should always be carried along in case of a control check by competent authorities.

# 6 Sturgeon monitoring methods by life-cycle stage

In the following chapter, different monitoring methods will be described, all based on the different life-cycle stages that can and should be targeted depending on the research aim (see Table 2 for an overview). Table 2 also gives rough estimations about the necessary effort and occurrent costs of different research aims and the respective methods. Costs usually increase with the amount of sampling and needed human resources, which often comprise the largest part of available budgets. An identification of needed resources before conducting sampling under the requirement that data quality is sufficient for scientific analysis and solid statistical inference is advised beforehand to keep costs at an optimal level. Cost estimations in Table 2 should be seen as comparison between research questions and respectively needed methods and analyses methods.

***Table 2:*** *Examples of research aims, which life-cycle stage to target and which sampling and analysis methods are appropriate. In addition, rough estimations for effort and costs associated with sampling and analysis (adapted after* (Haxton et al. (2023))*.*

| | | | | Sampling | | Analysis | |
|---|---|---|---|---|---|---|---|
| **Life-cycle stage** | **Research aim** | **Sampling method** | **Analysis** | **Effort** | **Costs** | **Effort** | **Costs** |
| All | Presence/Absence | eDNA | Metabarcoding / barcoding | Low | Medium | Medium | Medium |
| Eggs | Spatial assessment of spawning sites | Egg mats | Back-calculation of spawning event | High | High | Low | Low |
| | Temporal assessment of spawning, Timing of spawning | | Back-calculation of spawning event | High | High | Low | Low |
| | Effective number of breeders | | Kinship analysis | High | High | High | High |
| | Influence of environmental drivers on spawning | | Relationship of abiotic conditions and timing of spawning | High | High | High | Medium |
| Larvae | Spatial assessment of spawning sites | D-nets | Back-calculation of spawning event | High | High | Low | Low |
| | Temporal assessment of spawning, Timing of spawning | | Back-calculation of spawning event | High | High | Low | Low |
| | Duration and spatial extent of drift | | Spatial analysis of larval drift | High | High | High | High |
| | Effective number of breeders | | Kinship analysis | High | High | High | High |
| | Larval production | | Abundance/CPUE based on filtered cross-section or volume | High | High | Medium | Medium |
| | Growth rates | | Length/Age relationship, back-calculation of spawning event | High | High | Low | Low |
| YOY | Survival estimates | Gill/Trammel nets | CJS model, individual tags | High | Medium | Medium | Medium |
| | YOY Abundance/Year class strength | | Closed CMR model (e.g., Schumacher-Eschmeyer model) | High | Medium | Medium | Medium |
| | Effective number of breeders | | Kinship analysis | High | Medium | High | High |
| | Growth rates | | L/W relationships, growth models | High | Medium | Medium | Medium |
| | Habitat preference | | Assessment of habitat parameters (see Reinartz, 2024) | Medium | Medium | Medium | Medium |
| | Dietary composition | Gastric lavage | Food item identification, metabarcoding | Medium | Low | Medium | Medium |
| Juveniles | Survival estimates | | CJS model, individual tags | High | High | Medium | Medium |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Abundance | River: Gill/Trammel nets Marine/Estuary: Trawl, Gill/Trammel nets | CMR models | High | High | Medium | Medium |
| | Recruitment | | CMR models | High | High | Medium | Medium |
| | Growth rates | | L/W relationships, growth models | High | High | Medium | Medium |
| | Habitat preference | | Assessment of habitat parameters (see Reinartz, 2024) | High | High | Medium | Medium |
| | Relatedness, Genetic diversity | | Genetic assessment | High | High | High | High |
| Adults | Assessment of species distribution | Hydroacoustics | Occupancy modelling, counts | Low | Medium | High | High |
| | Population demographics | River: Gill/Trammel nets Marine/Estuary: Trawl, Gill/Trammel nets | L/W, L/F, Length/Age relationships, Fecundity, Sex ratios | High | High | Medium | Low |
| | Spawning Run Size | Gill/Trammel nets, hydroacoustics | CMR models | High | High | High | High |
| | Spatial assessment of spawning sites | | Count, Species identification | Medium | Medium | Medium | Medium |
| | Temporal assessment of spawning, Timing of spawning | | Count, Species identification | Medium | Medium | Medium | Medium |
| | Information on harvest | bycatch | Species identification, L/W, L/F, Length/Age relationships, CPUE | High | Medium | Low | Low |

## 6.1 Presence/Absence

Information about the presence or absence of sturgeon species can be obtained through scientific monitoring actions as well as through non-scientific information through bycatch in commercial or recreational fisheries. In the following chapter, identification of the presence of sturgeon species through bycatch and eDNA will be discussed. Bycatch, although not a monitoring method *per se* and difficult to quantify, as well as indigenous, traditional knowledge or stakeholder information can be extremely helpful as both can contribute additional information to implemented monitoring measures. The methodological approach in cases of stakeholder information largely depends upon their number and their willingness to cooperate and can range from individual interviews to systematic questionnaires, dedicated application to declare a bycatch, or group interviews. In any case, bycatch and eDNA sampling can provide indications about the presence of sturgeons and their habitat use.

### 6.1.1 Bycatch

Bycatch is the unintended and unwanted catch of non-target species in fisheries, be they commercial, recreational (angling), or scientific. Since bycatch *per se* implies compliance with existing rules, data on bycatch are easier to obtain than poaching information.

Bycatch can inform on the presence of sturgeon species in time and space, but the effort must be considered as certain fishing methods are applied only seasonally, while the catchability of sturgeon in the different gears is highly variable. Sturgeon bycatch predominantly occurs in trawl, gillnet, purse seine, longline or stow net fisheries, as well as recreational angling in marine, estuarine and river habitats. It provides key information about species occurrence and can add additional threads to sturgeon populations as high numbers of captures (Stakėnas et al., 2021) associated with mortality and injury rates can be reported (Doukakis et al., 2020; Place, 2006; Stein et al., 2004; Stakėnas & Pilinkovski 2019). However, those aspects are detailed in the Review of Bycatch Prevention and Mitigation Measures for Sturgeons (Rochard, 2024) and the focus of this document lies in the information that can be gathered through incidental capture. Data quality varies depending upon the willingness of reporting and if any reporting obligations were implemented. Even if a bias is caused as only presence data are reported, information about the occurrence, for example all over the species range or for several years can be collected and might allow for large scale habitat analysis (e.g., Charbonnel et al., 2023).

Monitoring of bycatch and, where possible, the associated mortality, is important to assess the impacts acting on a population. Therefore, it is recommended to collect the following data:

- Validation of the bycatch (species, size) in the form of pictures or videos
- Location (preferably GPS position), date and time
- Length, condition and status of fish (dead or alive, injuries)
- Tag number, if present

- Target species of the fishery
- If released or retained
- The gear used
- Fished depth and substrate

If fishermen are collaborating openly, it is valuable to obtain information about effort:

- In gillnets (mesh size, net length, net height, netting material and soak time, start and end position of gear, depth)
- For trawls (mesh size, mouth opening (width and height) and soak-time, start and end position of trawl, time of day, depth).

The availability of these data allows the assessment of Catch-per-Unit-Effort (CPUE) to learn about gear efficiency, to assess habitat use in a given area, to determine mortality risk, etc. Due caution is recommended since the data provision usually is neither exact nor complete and the used gear can be very selective regarding size and, hence, life-cycle stages.

To increase the response of bycatch reporting, it is crucial to create a trust basis and acceptance for respective aims and objectives. This means facilitating the understanding of the value of data quality for the understanding of processes at the population level.

In order to check the quality of bycatch data, it might be necessary to conduct bycatch analysis through self-led surveys in hot spot areas, using the same gear as commercial fishermen and compare these data to published bycatch reports. Also, additional scientific monitoring will be necessary to complement the qualitative nature of bycatch information with quantitative information. Due to the inconsistency in data quality, detections of population trends cannot be expected from bycatch surveys.

### 6.1.2 eDNA

Environmental DNA (eDNA) is a useful method to confirm the presence of species within a waterbody. While traditional fishing methods require substantial effort, especially at low population levels, eDNA sampling might still be able to detect rare species. Furthermore, it is less invasive than traditional sampling methods which require individuals to be captured, inducing handling stress (Pfleger et al., 2016). The technique capitalizes on species continuously expelling their DNA into the local environment through the sloughing of skin or scales, excretion, and/or the release of mucus (Taberlet et al., 2012), and has proven to be effective for sturgeon (Bergman et al., 2016; Meulenbroek et al., 2022; Pfleger et al., 2016; Stoeckle et al., 2017; Xu et al., 2018; Yusishen et al., 2020).

***Figure 3:*** *eDNA sample with the sample filter in the red circle. The water flows in the direction of the black arrows (© BOKU, P. Meulenbroek).*

### 6.1.3 Purpose

The main purpose of eDNA sampling is the evaluation of presence or absence of sturgeon species in an area of interest. Through eDNA, this information can be collected for a wider area with relatively low effort and costs compared to many other methods. In terms of sturgeon monitoring, eDNA is recommended as a first step if the presence of sturgeon is unknown.

### 6.1.4 Sampling site

The sampling area should be chosen in order to maximize the possibility to collect sturgeon DNA. Moreover, to make different samples comparable, the same approach should be used within a given river basin. Relevant areas can either be identified through historic evidence, habitat suitability or expert judgement. Since DNA degrades over time, sampling sites should be chosen to provide a certain overlap of persistence of eDNA. In general, decay of DNA is fastest in lotic environments and transport distances can be between hundreds of meters to greater than 100 km (Harrison et al., 2019). While Villacorta-Rath et al. (2021) reported detections >20 km in a nutrient poor and clear stream, Deiner & Altermatt (2014) suggest to take samples every 5-10 km. Pont et al. (2018) detected DNA of whitefish (*Coregonus lavaretus*) up to 60 km below the outflow of Lake Geneva in the Rhône River. Thus, the interdependence of each sampling site should be considered in data sampling and data analysis. In their supplemental material, Pont et al. (2018) provide a diagram showing simulated maximum detection distances of eDNA depending on water depth and water velocities, which can be useful for designing surveys. Nevertheless, since eDNA is a rather new approach, a variety of questions remain open and research in the field is still ongoing and many more trials or experiments need be done, especially considering the transport length and quantification of DNA.

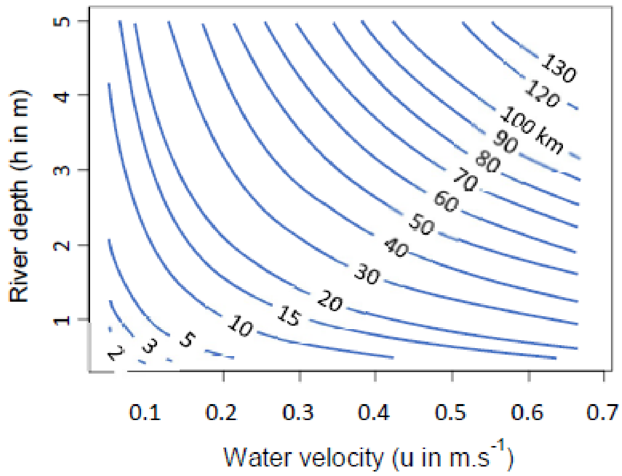


***Figure 4:*** *Simulated maximum detection distances of eDNA (after Pont et al., 2018).*

### *6.1.4.1 Timing of sampling*

The timing of sampling should be selected to increase the probability that the target species is present in the habitat sampled (e.g., corresponding to the ecology of the species, breeding season for anadromous species). If time of presence is not entirely clear, repeated sampling on a weekly or bi-weekly schedule is recommended. Furthermore, for the selection of sampling time, the activity of the target species should be considered. During extreme water temperatures in winter or summer, sturgeon are less active and the amount of DNA expelled might be less. It is beneficial if activity levels are high. As such, migration, reproduction or foraging periods are to be preferred over the wintering periods. Unsuitable flow conditions such as high discharge or high turbidity should be avoided since detection of target DNA can be adversely affected.

### *6.1.4.2 Materials & Methods*

Water samples, either multiple small samples (1-2 L; e.g., Bergman et al., 2016; Pfleger et al., 2016; Stoeckle et al., 2017; Xu et al., 2018) or less larger samples (~30 L; e.g., Meulenbroek et al., 2022; Pont et al., 2018), are obtained from a site of interest. In order to detect rare species, it is recommended to take several replicates at one sampling site to maximize the probability of the sample containing DNA from the target species. The collection of water samples varies depending on the sampling protocol and can be conducted using buckets (Stoeckle et al., 2017) or peristaltic pumps (Bergman et al., 2016; Meulenbroek et al., 2022). It is of utmost importance that cross contamination between samples is avoided, thus requiring new and DNA free material is used for each sampling event. Water samples are filtered using filters with pore sizes between 0.22 µm (Bergman et al., 2016) to 1.5 µm (Pfleger et al., 2016).

Depending on the sampling protocol, sufficient sample volumes and replicates at a given site need to be taken in order to ensure the detection of rare species.
It is recommended to take at least two samples at the same location for the detection of rare species (Meulenbroek et al., 2022). For large rivers, sampling along a transect oriented upstream from side to side should be considered to cover all the width and, thus, all the potential species signatures (Figure 5).

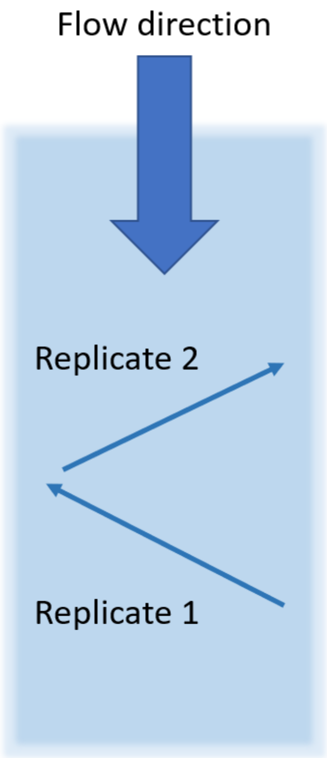


***Figure 5:*** *Approach to take eDNA sample replicates in a large river. Each consecutive replicate should be situated upstream of the preceding to avoid contaminations (© INRAE, M. L. Acolas).*

Also, it is paramount that samples are not contaminated with DNA from other species or – even worse – of the target species as wrong results could lead to wrong management decisions. Hence, contamination of used gear (waders, jackets, boat, buckets, etc.) has to be avoided, and, if it was in contact with possible target species, it should be cleaned properly before use in an eDNA survey. In running waters, samples should never be taken downstream of the sampling person or the boat. New and clean gloves should be used when working with the filter containing the sample and the protocol given by the processor of the samples should always be followed.
In order to take an eDNA sample using a peristaltic pump, all elements are assembled, and the coarse filter is attached to a pole or stick which is held into the water. The water flows through the coarse filter and the tube through the filter capsule into a bucket. The bucket is used to measure the amount of filtered water (Figure 3).

To conduct successful eDNA monitoring and to ensure the collection of uncontaminated samples, it is recommended to follow the instructions of the laboratory staff in charge of the processing of samples.

Sampling kits usually contain the following:

- Filter capsule for sampled water
- Coarse filter and disposable tube, if a pump is used
- Two pairs of disposable gloves per sample

#### *6.1.4.3 Analysis*

DNA is extracted using an Isolation Kit which varies between manufacturers (see Bergman et al., 2016; Meulenbroek et al., 2022; Pfleger et al., 2016 for different examples) or by centrifugation and amplified in a polymerase chain reaction (PCR) using species-specific molecular primers (Strickland & Roberts, 2019; Yusishen et al., 2020). A logistically simpler technique is loop-mediated isothermal amplification (LAMP), which is a field based, 'real-time' rapid assessment (Lee, 2017) or qPCR (Chancerel et al., 2023). This technique will confirm the presence of a species without the lag time of the laboratory PCR technique. Multiple runs of the analysis should be conducted on a sample as detection by PCR or LAMP is not perfect.

In general, results for eDNA samplings are presented as number of positive DNA reads in a sample, from which a rough relative abundance or species distribution for a specific site can be obtained if metabarcoding is used (Meulenbroek et al., 2022). Nevertheless, relative abundance in relationship to detection strength is not necessarily linear, which always needs to be considered when interpreting results. Areas with a large amount of sturgeon DNA reads could be good starting places for further investigation though.

In order to assess environmental influence during the sampling and to increase the interpretation of eDNA results, it is recommended to collect additional environmental data for each sample during eDNA sampling (Harrison et al., 2019):

- **Date (season)**
- **GIS waypoints**
- **Water temperature**
- **Flow velocity/discharge**
- **Water body width and depth**
- **Salinity**
- **Turbidity**
- pH
- Microbial growth (such as chlorophyll a or organic matter)
- Substrate type
- Nutrient levels (such as chlorophyll a or organic matter)
- Geomorphological features (stream slope, average stream-scale form, longitudinal roughness etc.)

The assessment of variables in bolt is highly recommended.

#### *6.1.4.4 Drawbacks*

Sampling eDNA will not yield information on population parameters and does not identify life-cycle stages or quantity of the species with any certain level of confidence (e.g., Roussel et al., 2015).
eDNA can only confirm the presence or absence of the species, which is used to plan the next stage of assessment (e.g., identify areas of high probability to set egg mats or larval drift nets to assess that life-cycle stage). However, if a species was not detected, it does not necessarily mean it is not present (Roussel et al., 2015). Especially for rare species, dilution may play a considerable role (Meulenbroek et al., 2022). Contamination of eDNA samples, on the other hand, could lead to false positives. Moreover, inflows of water treatment plant or discharge from hatcheries might lead to the detection of species not present in the river itself (Pont et al., 2021).

### 6.1.5 Social Media

Information about accidentally captured sturgeon can be obtained from various social media channels or homepages of fishing clubs/magazines as well. Fishermen often tend to post pictures from recent catches. Usually, a great deal of information can be gained from the picture itself or the comments below it (species, approximate size, life-cycle stage, area, etc.). For further information, the author of such social media posts should be approached individually. Even though information collected in that way is highly scattered and sporadic, valuable information about regions not targeted through any monitoring measures can be obtained and therefore new areas for targeted monitoring measures can become available.


***Figure 6:*** *Data provision via social media on the capture of a sterlet in the Lower Drava with the angling rod. In this area, no regulated sturgeon monitoring is currently implemented.*

### 6.1.6 Case Example Presence/Absence – Danube eDNA

*Meulenbroek et al. (2022) conducted an eDNA study during the Joint Danube Survey in the Danube with the goal to develop a reference database for Danube sturgeon species, validate eDNA metabarcoding primers ex situ and to use them in situ to provide information on the distribution and the relative abundance of sturgeon species in the Danube. Therefore, 29 samples were taken in the Danube, as well as samples from 18 tributaries. The spacing between sampling sites was chosen in order to minimize the chances that DNA from upstream sampling sites could be transported to downstream sampling sites. At each site, two surface samples of about 30 litres were taken using a peristaltic pump, 0.45 µm filter capsules and sterile disposable tubing, either via wading or from the boat in deeper parts, sampling from shore to shore.*

*Tissue samples from Acipenser stellatus, A. gueldenstaedtii, A., ruthenus, A., nudiventris, A. baerii, A. transmontanus and H. huso acted as reference samples for the database. Non-native sturgeon samples were obtained since they are known to occur partly in the Danube itself or DNA present due to runoffs from hatcheries.*

*In the Danube, three hotspot areas of A. ruthenus occurrence were detected and, in total, 14 of 29 samples were positive as well as two samples from tributaries, namely in the Inn and Tisza. A. stellatus was detected only in the delta and A. gueldenstaedtii in the Inn, which originated from rearing ponds upstream of the sampling site. Other sturgeon species could not be detected during the field survey.*

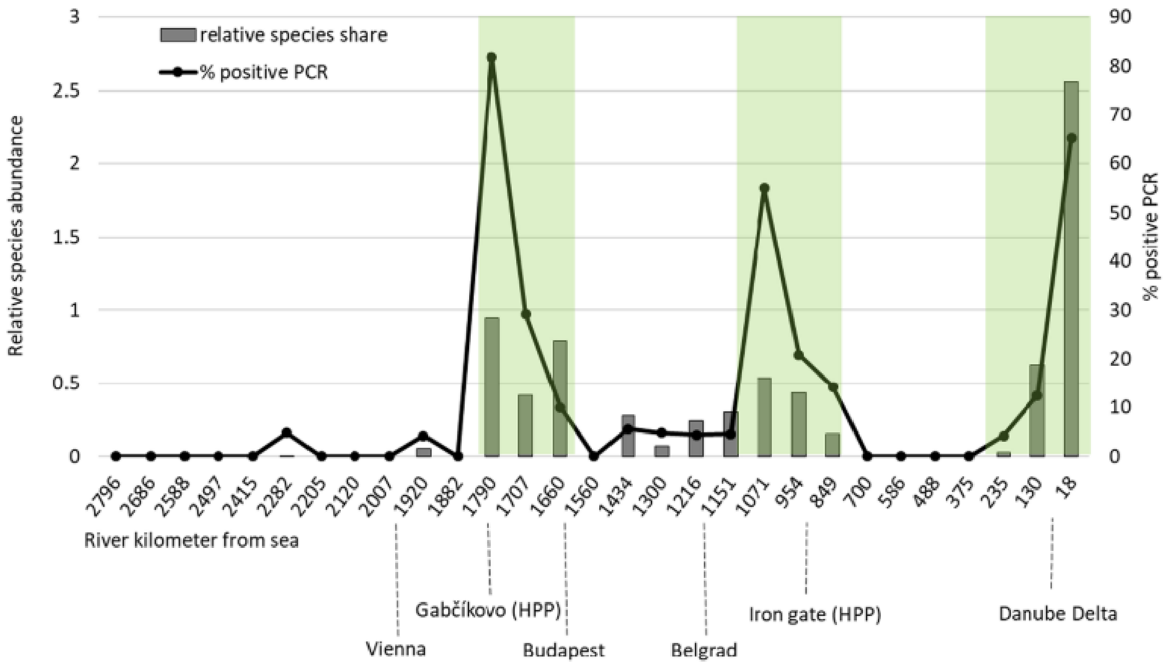


***Figure 7:** Relative species share and percentage positive PCR detections for Acipenser ruthenus along the River Danube from source to sea (green boxes indicate cluster of positive sites in 3 sections of the Danube). See Meulenbroek et al. (2022).*

*As shown through this study, eDNA proved to be a useful method to confirm areas where sturgeon were present, even in a large river such as the Danube. This information is highly valuable and provides hotspots for further monitoring actions. The population downstream of the hydropower plant (HPP) Gabčíkovo is currently targeted through a net fishing campaign aiming to obtain first catch-based estimates of the populations of sterlets. In the course of the LIFE Living Rivers project, a telemetry study is planned to collect insights into the movements and behavior of the sterlets in the Gabčíkovo area. Those results will be the basis for the construction of fish passes at the HPP.*

## 6.2 Eggs

Both egg mats and D-nets can be used to sample sturgeon eggs. However, egg mats offer the potential to obtain live sturgeon eggs while D-nets, due to reduced hydraulic pressure, often result in severely impacted eggs. This is especially important if collected eggs are raised after collection or staging of collected eggs is of interest.

### 6.2.1 Purpose of sampling

Sampling egg deposition is an effective means to determine if sturgeon are using a spawning location, prove reproduction, assess the timing of spawning, egg survival, and the effective numbers of breeders through genetic analysis (Chiotti et al., 2008; Paragamian et al., 2002; Paragamian & Wakkinen, 2002; Poytress et al., 2015; A. Smith et al., 2017). It can also be an effective means to determine if management actions (e.g., flow regimes, spawning site restoration) are working in terms of providing suitable spawning conditions for sturgeon.

The abundance of eggs does not necessarily correlate with a strong year class or recruitment (Dumont et al., 2011). The number of eggs deposited annually can be highly variable due to spawning periodicity, potential spawning synchrony and efficacy of egg mats (e.g., detection probability) and, therefore, monitoring egg density could provide highly variable results that are difficult to quantify. The survival of eggs and early life-cycle stages is low and is impacted by a large variety of environmental factors (Caroffino et al., 2010; Gross et al., 2002; Vélez-Espino & Koops, 2009) and therefore not suitable to assess population trends.

An estimate of the effective numbers of breeders ($N_b$) could be determined through genetic analysis (Blankenship et al., 2017). However, estimates of $N_b$ may be skewed given a large number of eggs captured may only originate from few females if systematic sampling across spawning areas is not conducted (Blankenship et al., 2017) and eggs of different females may not be detected.

### 6.2.2 Egg mats

The presence of sturgeon eggs deposited within an area can be assessed by the use of eggs mats. There are variations to the design of egg mats, but the principles remain consistent.

Usually, they are made out of weighted metal frames or concrete blocks containing rough material such as latex coated horsehair or filter foam. Further examples composed of 39 × 19 × 9 cm concrete cinder blocks wrapped with 75 x 35 cm of an industrial air filter material (e.g., latex-coated horse hair or fiberglass) (Gillespie et al., 2020), furnace filter wrapped around a 38 x 24 x 0.5 cm steel frame secured by 5 x 2.5 cm binder clips (Hunter et al., 2020), or 76 x 91 cm pieces of latex-coated animal hair mounted to an angle-iron frame on both sides (McCabe & Beckman, 1990). These mats are systematically or strategically placed on spawning areas in efforts to capture drifting eggs broadcast by females. Hence, the selection of a sampling site needs particular attention during the preparation. Potential spawning locations, timing and flow directions have to be considered.
Egg mats are marked with small buoys, attached to the mat by a rope to facilitate retrieval. The length of the line and the size of the buoy should be adequate to the sampled depth and flow velocities. Excessively long lines and oversized buoys increase the lift exerted by the current, forcing the egg mats to move. The weight and shape of the egg mats have to match flow conditions at the sampling site. If flow velocities are too high, flat egg mat suspension increases stability and the weight of the egg mat needs to be adapted or anchors should be used in the front of the egg mats to additionally secure them (Seesholtz et al., 2014).

#### 6.2.2.1 Sampling site

As mentioned above, due to the stickiness of the eggs and the low probability to capture occasional single eggs drifting downstream, the sampling area should be immediately downstream of the (presumed) spawning areas.

#### 6.2.2.2 Timing of sampling

The only possible time to successfully deploy egg mats is during spawning time of the target species (based on species ecology). The start of a sampling campaign can be oriented at abiotic parameters such as water temperature or, if available, information from telemetry studies when spawners move to spawning grounds (Chiotti et al., 2008; Poytress et al., 2015).

#### 6.2.2.3 Materials & Methods

Constructing egg mats is rather simple (Figure 8). Among the most important considerations are that the egg mat does not drift off and therefore needs a heavy base (a concrete or steel frame) and rough material (rubberized horsehair) to entrap the eggs. In order to make egg mats visible for retrieval or possible occurring shipping, a rope with a marker buoy should be attached. If necessary, an anchor attached to the egg mat with a rope can help to maintain the rig in position if unfavorable flow conditions occur.

***Figure 8:*** *Egg mat used to collect drifting eggs that stick to the coarse material (© IGB, J. Gessner).*

### *6.2.2.4 Analysis*

The date of spawning events can be back-calculated using the developmental stage of collected eggs and water temperatures (McCabe & Beckman, 1990; Seesholtz et al., 2014). Sturgeon species can be identified through DNA barcoding (Plough et al., 2018). Spatial distribution of spawning could be determined if egg mats are systematically placed and correlated with environmental variables to help determine site selection (Gillespie et al., 2020; Paragamian & Wakkinen, 2002; Smith et al., 2017; Sulak & Clugston, 1998).
Egg densities can be estimated (Bouckaert et al., 2014); however, interannual catches are highly variable (Caroffino et al., 2010; Poytress et al., 2015), which may be attributed to spawning periodicity, spatial deposition (Gillespie et al., 2020; Sulak & Clugston, 1998), or environmental conditions.

### *6.2.2.5 Drawbacks*

The number of sampled eggs can vary substantially from single eggs (Poytress et al., 2015; Sulak & Clugston, 1998) to several thousand (Chiotti et al., 2008) depending upon the timing and site chosen and the effectiveness of the reproduction. Egg retention could also diminish over time due to predation or scouring by water currents (Caroffino et al., 2010; Sulak & Clugston, 1998). Additionally, if sturgeon abundances are low and spawning grounds are unknown, sampling of eggs can range from extremely challenging to impossible and extremely intensive efforts might be necessary in order to sample a small number of eggs.
It may be necessary to remove egg mats during flood events to avoid losing them (Poytress et al., 2015).

### 6.2.3 Case Example: Eggs – Kootenai white sturgeon

*In their study,* Paragamian & Wakkinen (2002) *attempted to determine the temporal distribution of white sturgeon (Acipenser transmontanus) spawning as related to natural and man-made variations in flow and temperature.*

*After the construction and filling of the Libby Dam and its reservoir in 1974, the Kootenai River downstream of the dam changed massively, only retaining 10-20 % of its natural flow during spawning season, the water temperature range narrowed and the river became less productive, resulting in the limitation of recruitment of the Kootenai white sturgeon population and their assignment as endangered species in 1994.*

*Several years were spent finding spawning areas and the optimal location for placing the egg mats. For the site survey, egg mats were placed every 500 m along 15-30 km long stretches each year. Since no eggs were collected in shore areas, these were skipped after the second year. The authors thereafter used telemetry data to ascertain spawning areas* (Paragamian et al., 2002)*. Nevertheless, each season, between 70-100 egg mats were deployed for approximately nine weeks and checked daily for the presence of eggs. Collected eggs were preserved, aged, and the respective spawning event was back-calculated.*

*Through this extensive effort, it was able to narrow the temperature range of high spawning probability to 9.5-12.5 °C and to clearly show that spawning took place during rising temperatures and ceased when temperatures dropped by 0.8 °C or more. Even though no clear patterns for flows were detected, the majority of spawning events occurred during elevated flows >600* $m^3/s$*. In general, the most favorable conditions for spawning were stable water temperatures and flows. Thus, based on the results gathered through collecting and analysing eggs, the authors were able to suggest an optimization of the management regime for the Libby Dam to provide suitable conditions for white sturgeon spawning to occur.*

### 6.2.4 D-nets

D-nets can be used to assess egg deposition by collecting downstream drifting eggs during and immediately post spawning. LaHaye et al. (1992) set D-nets (0.5 m diameter, 1.5 m long, 500 *µ*m meshsize) downstream of spawning areas for short durations (10-20 mins) to prevent clogging by floating debris. River size and flow require consideration as under different conditions much larger nets might be required. The main drawback of the technique is the fact that the majority of the eggs sampled in D-Nets are not viable due to mechanical stress (LaHaye et al., 1992).

## 6.3 Larvae

Free embryos and larval sturgeon can best be sampled during their drift phase. Drifting larvae are dispersed in the water column. While the preponderance of drifting lake sturgeon (*Acipenser fulvenscens*) larvae was sampled in the upper portion (Caroffino, Sutton, & Daugherty, 2009), larvae of the Russian sturgeon, stellate sturgeon and sterlet were reported to drift mainly in the lower portion (Kalmykov et al., 2010).

The magnitude of larval drift interannually can be highly variable (Caroffino et al., 2010; Friday & Haxton, 2021) and survival can be extremely low (Caroffino et al., 2010; McDougall et al., 2020; McDougall, Pisiak, et al., 2014). Therefore, larval sampling is of limited use for the assessment of population status given that large numbers of larvae in the drift does not necessarily equate to year class strength (Dumont et al., 2011; Friday & Haxton, 2021). However, monitoring larval drift can provide information about the survival of fertilized eggs, provided that an assessment of egg deposition was carried out, the duration of development, effects of flow manipulation and the effective number of breeders ($N_b$) through genetic analysis.

### 6.3.1 Purpose of sampling

The collection of larvae serves similar purposes as the collection of eggs:

- Proof of reproduction
- Determination of spawning habitat choice
- Duration of development
- Verification of survival (requires quantitative sampling of eggs)
- Assessment of effective population size
- Confirmation of suitable river conditions

### 6.3.2 D-Nets


***Figure 9:*** *D-nets for larvae and eggs used in the Danube in Romania with an area of 0.44 m², 4 m long and a mesh size of 2 mm (left) and a captured beluga sturgeon larva (right, © DDNI, M. Paraschiv).*

D-framed nets consist of a metal frame, a net with small mesh size and usually a detachable collection bucket. The nets are placed into the current below spawning sites to fish for drifting larvae post hatch. Rectangular shaped nets were used as well, for example if nets need to be stacked to sample the whole water column (Caroffino, Sutton, & Daugherty, 2009). Examples of dimensions are given in Table 3.

***Table 3:*** *Measures of different D- nets, deployed depths and target species. (Caroffino, Sutton, & Daugherty, 2009) stacked seven nets. Therefore, the area in brackets is given for all seven nets and the area without brackets belongs to a single net.*

| **Source** | **Shape** | **Width (cm)** | **Height (cm)** | **Area ($m^2$)** | **Length (cm)** | **Meshsize (mm)** | **Cod end** | **Deployed Depth (m)** | **Target species** |
|---|---|---|---|---|---|---|---|---|---|
| Smith & King, 2005 | D | 85 | 55 | 0.57 | 250 | 1.6 | Detachable collection bucket | <1.7 or >1.7 | *A. fulvescens* |
| Tucker et al., 2021 | D | 85 | 55 | 0.57 | 250 | 1.6 | Detachable collection bucket | <2 | *A. fulvescens* |
| Auer & Baker, 2002 | Rectangular | 81 | 58 | 0.47 | 300 | 0.95 | Detachable collection bucket | - | *A. fulvescens* |
| Auer & Baker, 2002 | D | 76 | 54 | ~0.5 | 317.5 | 1.6 | Detachable collection bucket | - | *A. fulvescens* |
| Lawrence et al., 2020 | D | 76 | 54 | ~0.5 | 317.5 | 1.6 | Detachable collection bucket | - | *A. fulvescens* |
| Hunter et al., 2020 | D | 76 | 54 | ~0.5 | - | 1.6 | - | 10.2-17.3 | *A. fulvescens* |
| Hunter et al., 2020 | Conical | 0.3 | | ~0.07 | - | 0.75 | - | 1-16 | *A. fulvescens* |
| Caroffino, Sutton, & Daugherty, 2009 | Rectangular | (7x)70 | (7x)20 | (0.98)0.14 | 240 | 1.6 | Straight stitch | <0.8 | *A. fulvescens* |
| Gessner & Skora, pers. comm. | Square | 100 | 100 | 1 | 550 | 1 | Detachable collection bucket | <2 | *A. oxyrinchus* |
| Onără et al., 2011 | D | - | - | 0.4-0.72 | 400-600 | 2 | Bindable | 12-15 | *A. ruthenus, H. huso* |

The mesh size of the detachable collection bucket can be smaller than that of the net (<1 mm (Smith & King, 2005; Tucker et al., 2021). Sturgeon larvae decrease in diameter upon absorption of the yolk sac; therefore, the use of smaller mesh sizes can be beneficial in order to prevent larvae from strangling in the mesh.

***Figure 10:*** *Deployed D-net close to the shore and the opening of the detachable collection bucket into a collection box (© IGB, J. Gessner).*

In the Lower Danube, pairs of two D-nets were used to catch drifting sturgeon larvae and to identify spawning habitats. The D-nets were deployed in the main current where drifting larvae were expected.

### *6.3.2.1 Sampling site*

D-nets are usually placed downstream of spawning areas. If several nets are used, they can be set parallel to shore (Tucker et al., 2021). D-nets are often benthic sets in shallow areas in the thalweg downstream of the spawning shoal (Friday & Haxton, 2021; Tucker et al., 2021), however, they can be set in deep areas in the mid-channel as well and be secured either by using piles and anchors (Hunter et al., 2020; Onără et al., 2011; Roseman et al., 2011) or attached to bridges (Auer & Baker, 2020) if navigation allows. Nets at the shore can be fixed using anchors or poles (Caroffino, Sutton, & Daugherty, 2009). Nets can be placed stacked on top of each other at different depths to sample larval drift in the entire water column (Hunter et al., 2020) by being suspended by a sufficiently large-anchored buoy or from a boat. In some studies, plankton nets have also been used for sampling larval drift of shovelnose sturgeon (*Scaphirhynchus platorynchus*) (Goodman et al., 2013). Setting several D-nets at one sampling site is recommended to increase chances of collecting drifting larvae (Figure 11).

The location of the nets largely depends on the knowledge about spawning sites and local currents as well as the drift behaviour of the respective species. If these factors are unknown, an experimental approach of net sets can be undertaken in order to find areas where drifting larvae can be collected (see case example or Hunter et al. (2020)).

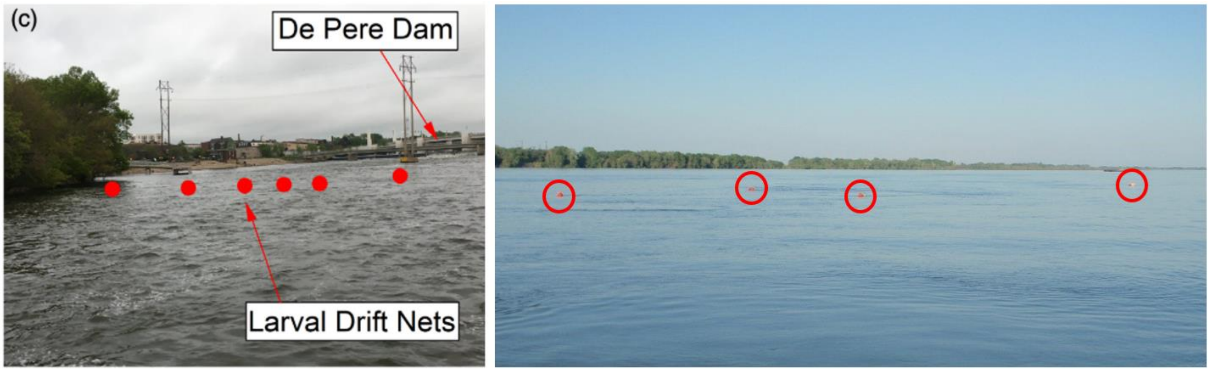


***Figure 11:*** *D-nets set perpendicular to the shore (left, adapted after Tucker et al., 2021), or in pairs in the mid-channel (right, © DDNI, M. Paraschiv).*

### *6.3.2.2 Timing of sampling*

Sampling with D-nets targeting drifting sturgeon larvae should generally be started when there is an estimated 50% larval development based on a thermal index model and can be continued until there is zero catch for several days (Friday & Haxton, 2021; Tucker et al., 2021). D-nets in general are set in the evening and picked up the next morning (Auer & Baker, 2002) or even several hours after dusk (Auer & Baker, 2020; Lawrence et al., 2020; Smith & King, 2005; Tucker et al., 2021). Depending on the amount of floating debris encountered, it can be necessary to check the nets at hourly intervals (Smith & King, 2005) to prevent them from clogging.

### *6.3.2.3 Materials & Methods*

- D-shaped steel frame with attached net (~3-5 m long, mesh size 0.75-2 mm, depending on the species)
- Net opening between 0.4-1.0 $m^2$
- Iron/wooden poles for mounting, hammer
- Anchor with rope attached to D-net
- Flow meter
- Rope with marking buoy
- Trays to collect the catch from the cod end

### *6.3.2.4 Analysis*

Larval sturgeon can be identified macroscopically as well as by barcoding (Boley & Heist, 2011), especially when multiple species are spawning at a location. An estimate of larval drift abundance can be determined using the number of larvae sampled, the area of the river sampled by drift net, and the total cross-sectional area of the river (Tucker et al., 2021). However, the abundance of drifting larval sturgeon is difficult to estimate due to variation in flow, river morphology, and sampling gears (Caroffino et al., 2010). Additionally, since drift is not uniform across the river or throughout the water column (Tucker et al., 2021), larval drift estimates may be overinflated. To estimate the volume of water filtered, flow velocity and, subsequently, the discharge should be measured with a flow meter that can be attached in the center of the D-net (Auer & Baker, 2002).

### *6.3.2.5 Drawbacks*

The number of larvae captured in larval drift samples can be very low (Onără et al., 2011; Tucker et al., 2021) and can vary greatly between years (Auer & Baker, 2020; Benson et al., 2006; Dumont et al., 2011; Friday & Haxton, 2021) while drift can vary in duration interannually (Benson et al., 2006; Friday & Haxton, 2021).

## 6.3.3 Case Example Larvae – Sturgeon River lake sturgeon

*Auer & Baker (2002) collected larvae of lake sturgeon to determine the extent and duration of larval drift and the instream location of larvae and their relation to water flow, aiming to suggest a period of time in which interferences such as sea lamprey chemical treatments, fish stocking, and road construction can be conducted with the least consequences for the first life period of lake sturgeon.*
*After the identification of spawning activities, D-nets were deployed eight to ten days later. Sampling started at a site either 14 or 26 km downstream of the spawning location. When no larvae were collected at one site, D-nets were moved past the next downstream site and installed leapfrog style. In total, six sites extending over 61 km were sampled in this manner, usually overnight. During 1996, sampling took place at only one site to define drift across the river with four equally distanced nets in low flow to high flow areas. In 1997, D-nets were set further at the downstream locations only to find larvae further downstream.*

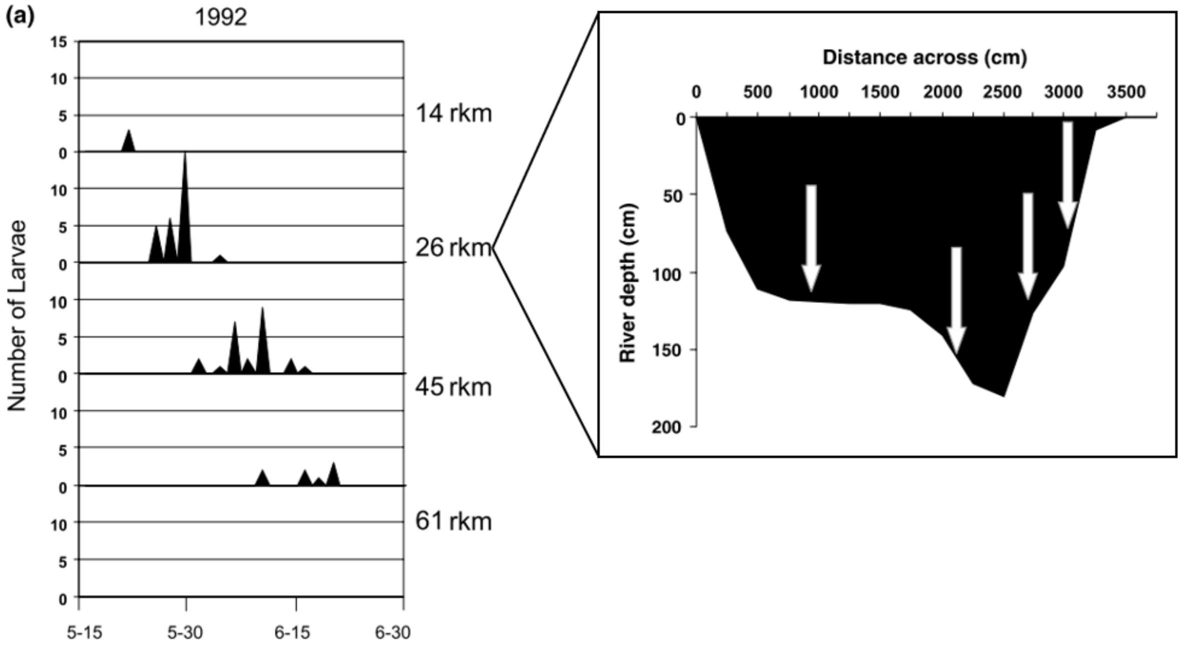


***Figure 12:*** *The picture on the left side shows the number of collected larvae at the subsequent sampling sites in downstream direction between May, 15th and June 30th in 1992. The river cross section belongs to the sampling site at rkm 26 (adapted after Auer & Baker (2002)).*

*The authors were able to collect almost 1000 larvae during the entire study, showing a consecutive downstream drift (Figure 12), with the larvae spreading out and fewer larvae were captured the further downstream the sampling took place.*

*Most larvae were collected in slow currents over sandy substrate with captures increasing between 21:00-24:00 h.*

*With an adaptive design in D-net sets, the authors were able to collect valuable information about the spatial nature of lake sturgeon larval drift and could determine potential nursery habitats in the lowest parts of the Sturgeon River. This kind of study requires sampling to continue over years. This was especially true in this study as flow conditions were unfavourable during two years and only a few spawning females were present in two other years. Therefore, only very few larvae were captured, which can pose difficulties on the interpretation of the data. With the resulting findings, the authors were able to suggest critical time windows where lake sturgeon larvae are most vulnerable and any human interference should be avoided. The drift study further showed an important nursery habitat, providing strong arguments for its protection based on monitoring data.*

## 6.4 Young-of-the-Year (YOY)

Sampling of YOY sturgeon can be conducted using several methods but interspecific differences in the behavior might require adaptation of methods since not all species might be susceptible to all methods.

For example, YOY lake sturgeon often select shallow habitats (Benson et al., 2005b) and may be easy to detect through visual observation, thereby providing the opportunity to count or capture fish with a dip net (Benson et al., 2005a; Holtgren & Auer, 2004; Mann et al., 2011) in clear water systems. Surveys can be conducted by wading (Benson et al., 2005a; Caroffino, Sutton, & Lindberg, 2009) or from a boat (Mann et al., 2011). YOY Gulf sturgeon (*Acipenser oxyrinchus desotoi*) were reportedly captured by dip nets or by hand during snorkeling as well (Carr et al., 1996). Day and night electrofishing and snorkeling were less effective for YOY lake sturgeon (Benson et al., 2005a) and might be considered less effective for sturgeon in general.

However, the sampling of YOY sturgeon of other species seems to be more difficult and requires other methods. In the Danube River, driftnet fishing with trammel nets proved to be effective to target YOY beluga, stellate sturgeon, Russian sturgeon, and sterlet (Margaritova et al., 2021; Mihov et al., 2022; Paraschiv et al., 2006; Paraschiv & Suciu, 2005). Bottom trawls have proven ineffective (Benson et al., 2005a) or highly variable among years with low catches (Counihan et al., 1999). In the Danube, sporadic captures of sterlet using an electrified bottom trawl (Szalóky et al., 2014) were reported (Tibor Erős, pers. comm.). Set-lines and stow nets were considered ineffective to capture YOY lake sturgeon (Benson et al., 2005a). In the Oder River, sturgeon are regularly captured in stow nets in fall at lower water temperatures (Jörn Gessner, pers. comm.). In general, active methods seem to be most effective, which may be attributed to lowered activity and restricted ability of escapement by YOY sturgeon.

Here, only the methods which are considered the most effective are described. However, it is suggested to test the effectiveness of different gear types before deciding on standard methods to be applied. For a cautionary approach, it is preferential to fish in the immediate river reach rather than on the banks of the river mouth area to prevent the catch of migratory individuals from neighbouring rivers, if these exist.

### 6.4.1 Purpose of sampling

The assessment of YOY sturgeon in the summer and fall after hatch is of high interest to determine the feeding grounds of these life-cycle phases and to detect overlap in the use of nursery grounds between species, especially in less well studied systems, or to determine changes in the ecosystem over time.

- Assessment of populations structure
- Identification of YOY feeding habitats
- Collection of genetic samples
- Assessment of year class strength of YOY
- Effective number of breeders

### 6.4.2 Trammel/Gill nets

When using nets for sampling, it is recommended to check the sampling site for snags before deployment or even clean sites from snags before starting sampling. It is paramount to **avoid ghost nets at all costs(!)** as they most likely will cause massive deaths of sturgeon (Kappenman & Parker, 2007) and other fish. An emergency plan to retrieve snagged nets should always be at hand and necessary material to retrieve snagged nets should always be available on site.
Some prerequisites that should be considered when sampling with nets:

- Short time periods in summer (high water temperatures and increased danger of mortalities)
- Used anchors should be heavy enough to ensure static nets stay at sites where they were deployed
- Used ropes need to be long enough to ensure the floats are on the surface despite strong currents. However, they should not be too long because flotsam can entangle and create drag, which could submerge the floats (Roseman et al., 2011) or move the net
- Floats should not be too large in order to minimize drag but they should be large enough to avoid being submerged
- Static nets should be marked and labelled as research gear

Trammel nets with three layer or one-layered gill nets can be effective in capturing YOY sturgeon or older individuals. The use of gill nets proved to be relatively effective for targeting age-0 and age-1 sturgeon (Hale et al., 2016). The gill nets used were 91.5 m long and 2.4 m height, with two panels consisting of 5.1 cm-stretch mesh and two panels with 7.6 cm-stretch mesh. The nets were constructed from 0.33 mm-diameter, clear monofilament, and each had a lead line (29.5 kg per 182.9 m) and a foam core rope 1.3 cm in diameter with floats every 4.57 m.

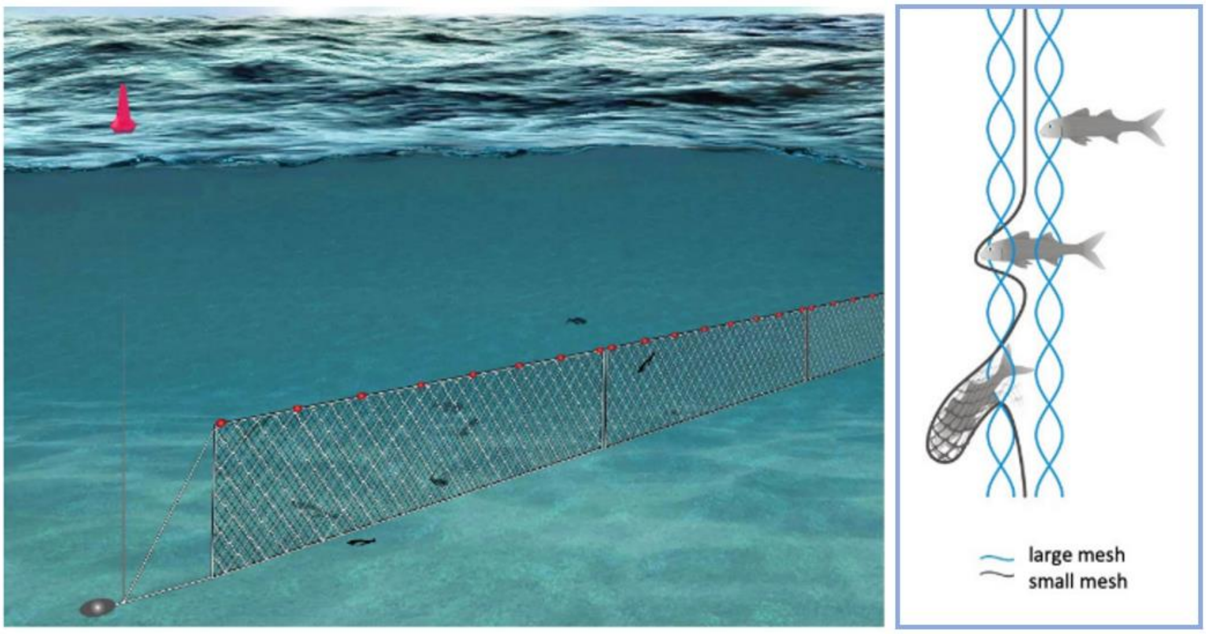


***Figure 13:*** *Schematic drawing of a trammel net and operating mode (adapted after He et al. (2021)).*

In general, lead lines with weight between 10-20 kg/100 m and float lines with a buoyancy of at least 3 kg/100 m are sufficient for most situations in running waters. If more buoyancy is necessary, for instance in strong currents, additional floats can be attached. Nets can be anchored parallel to the river flow and set diagonally when flows permit (Hale et al., 2016). YOY catches using this technique can be highly variable among years (Counihan & Chapman, 2018). However, YOY sturgeon are generally sampled in low numbers by gillnets (Counihan & Chapman, 2018; Haxton et al., 2014; Haxton & Friday, 2020; McDougall, Barth, et al., 2014) making it difficult for quantification purposes.

### *6.4.2.1 Static nets*

Static nets are held in position by anchors (appropriate weight for sampling location) and made visible with floats at the surface. Net deployment should allow the answering of relevant research questions. In general, nets should be deployed in a way to maximize the probability to capture the target. Hence, in rivers, nets are often deployed perpendicular to the current if the current allows. Alternatively, if the current is too strong to allow the net to fish properly, it can be fished in an angle to the shore that depends on the direction from where the fish migrate (up- or downstream) and on current intensity (Figure 14).

For safe and fast deployment of the net, the gear should be prepared properly. Especially obstacles that could entangle the net (jacket cords, parts of the boat, etc.) should be removed or covered. Anchors and floats should be attached at both ends and all lines have to be detangled. For setting the net, the boat should begin to move backwards slowly but consistently to feed the net from the bow of the boat (Figure 14). When the net is fully played out, it can be pulled tight while continuing going backwards. During net deployment, it is important to check that the net is not twisted! The boat driver is responsible for the final position of the net and must take extra care with regards to boat operation in running waters (see Jones & Yunker, 2010).

***Figure 14:*** *Net deployment and possible net locations (© BOKU, H. Eichhorn).*

When lifting a net, working against the current and starting with the downstream end of the net is recommended. However, if the boat is handled carefully and the current permits, retrieving the net in the direction of the flow works as well. One person at the ship's bow grabs a float and removes the net, pulling at the lead and the swimming lines together while the boat slowly motors upstream. It is recommended to immediately detach anchor and float in order to prevent these from getting entangled in the net. A third person can remove captured fish as soon as they are landed in the boat. If snags occur when beginning to retrieve a net, the respective end can be left in the water while retrieval from the other end of the net might loosen the snag.

#### *6.4.2.2 Drifted nets*

When a gill or trammel net is fished from a drifting boat in the main channel, the channel border, or tailwater habitats (Koch et al., 2009), it is important to check that no snags are in the area to be sampled. A drifted net can be fished either from two boats or from one boat and a drifting device (water anchor) on the other end of the net. The floating device has to be large enough to create sufficient drag to be capable of moving the net. Usually, large wooden pieces or floats are suitable. Weights on the lead line need to be heavy enough to ensure bottom contact while being dragged over the bottom by the floating device, while at the same time depending on current velocities. Adaptations depending on flow situations might become necessary. Wooden ''mules'' can be attached to both ends of the net to help the net drift more efficiently and to prevent the net from closing while being deployed. Drift nets are usually drifted downstream, perpendicular to the thalweg. The deployment of a drifting net is similar to the deployment of a static net described above. When fishing a drifting net between two boats or with a float, both need to move at the same speed as soon as the net is entirely deployed. When pulling the net with two boats, the weights attached to the net can be heavier. It is recommended to attach a float at the end of the line that connects to the net to the boat ensuring retrievability if it gets snagged during drifting and the line needs to be released for safety reasons. When fished from two boats, the net is retrieved by only one boat.

#### *6.4.2.3 Sampling site*

In order to capture YOY sturgeon, it is important to find sites where they reside, usually feeding areas (Paraschiv & Suciu, 2005). One approach could be figuring out where potential feeding or nursery habitats are, depending on food availability (sand/clay substrate and deposition/sedimentation areas (Margaritova et al., 2021; Mihov et al., 2022; Radu Suciu, pers. comm.)). As mentioned above, the cleaning of the sampling area from larger debris and driftwood might be necessary to avoid snags.

#### *6.4.2.4 Timing of sampling*

When to target YOY sturgeon depends on the species, the sampling location and annual water temperature regimes, subsequent spawning times and the behavior of the species. Targeting YOY during their outmigration from rivers usually is successful (Hale et al., 2016; Margaritova et al., 2021; Mihov et al., 2022; Paraschiv et al., 2006; Paraschiv & Suciu, 2005; Rochard et al., 2001) and is therefore recommended.
For the Danubian species spawning in spring between April and June (Holčík, 1989), YOY sturgeon are targeted in summer because at this time they are large enough to be captured with nets and are still present in the river. While beluga sturgeon grow faster (up to 200 mm at an age of already 8 weeks (Paraschiv & Suciu, 2005)), sterlet and stellate sturgeon were mainly captured beginning in July through August, with sizes ranging between 100-300 mm (Mihov et al., 2022). YOY of the European sturgeon grow to >300 mm until their second winter (Rochard et al., 2001). Sampling with drifted nets is usually conducted during daytime and shipping traffic has to be considered thoroughly.

However, if static nets are used, the deployment time should depend upon the water temperature to prevent the fish from being stressed or even from suffocating. Especially with elevated water temperatures, an increased metabolism leads to substantially shortened time until the fish dies from oxygen deficiency. Nets can be deployed for 10-12 hours at water temperatures <15°C but deployment time should be reduced to around 4-5 hours at water temperatures >15°C.

#### *6.4.2.5 Materials & Methods*

The following list of material is a suggestion for the successful sampling of YOY sturgeon with nets. A minimum of two people should be involved in sampling with nets.

- Net storage device (bucket, canvas, etc.)
- Gill nets
    - ~20 mm stretched mesh
    - 1-3 m high

  - 25-100 m long
  - 0.2 mm multifilament (Monofilament or multimono)
- Trammel nets
  - 100-200 mm outer mesh size (0.3-0.7 mm multifilament)
  - ~20 mm inner mesh size (0.2-0.3 mm multifilament)
  - 1-3 m high
  - 25-100 m long
- Anchors/weights
- Floats
- Ropes to attach the floats at the net (long enough to match the predominant depth!)
- Large watertight container for captured fish with air pump, bucket, dip net
- Material for collecting biotic data (see chapter 7)
- GPS for site determination
- Echo sounder is recommended
- Large drift float (for drifted nets from one boat)

In order to ensure good quality of the sampling gear, nets should be handled and stored appropriately. There are several ways to store the nets after deployment. However, all nets have to be cleaned and detangled immediately after lifting or, if several nets are hauled in, after return to the bank. Storage is to be carried out by hanging in a dark and dry environment with no exposure to direct UV light. Repairs should be carried out after each fishing trip to have the nets ready for the next deployment. Disinfection might be necessary when different rivers are sampled with the same gear in order to avoid spreading of non-native species or diseases.

#### *6.4.2.6 Analysis*

A main target of sampling YOY sturgeon is the assessment of genetic diversity in the spawning population. Knowledge of the genetic characteristics of a sturgeon population is important to understand underlying process such as adaptations, heterogeneity, or selection processes. These results are to be used for the management of populations in large (Kjartanson et al., 2023) and small management units (Whitaker et al., 2020).

It has been suggested that cohort strength may be related to YOY abundance given the reduced mortality rates once the first winter has been survived (e.g., bony scutes (Caroffino et al., 2010)). However, variation in interannual catches of YOY sturgeon (Caroffino, Sutton, & Lindberg, 2009) along with highly variable mortality at this life stage (Gross et al., 2002; McDougall, Pisiak, et al., 2014; Schueller & Hayes, 2010; Vélez-Espino & Koops, 2009) render it difficult to correlate YOY abundance with year class strength and project population trends (Haxton & Friday, 2020).

Larger YOY can be marked with PIT (Passive Integrated Transponder) tags (Mann et al., 2011) which can be used to estimate abundance through Capture-Mark-Recapture (CMR) techniques. YOY abundance estimates may take considerable effort as catches can be low (Holtgren & Auer, 2004). Caroffino, Sutton, & Lindberg (2009) were able to sample sufficient numbers of YOY sturgeon for an abundance estimate with relatively tight confidence intervals. These abundance estimates declined over the course of the season (Caroffino, Sutton, & Lindberg, 2009; Counihan et al., 1999), with lower catches reported in the fall (Benson et al., 2005; Holtgren & Auer, 2004) due to a variety of reasons related to environmental conditions, spawning periodicity, etc. Therefore, sampling time should be considered to maximize catches (see Mihov et al., 2022; Paraschiv & Suciu, 2005).

#### *6.4.2.7 Drawbacks*

In order to effectively target YOY sturgeon, knowledge about feeding areas is important as the fish can be targeted and recaptures can be increased to facilitate the assessment of group sizes (Paraschiv & Suciu, 2005). However, if feeding areas are not known or are inaccessible, more effort might be necessary to establish YOY monitoring.

### 6.4.3 Case Example Young-of-the-Year – Bulgaria

*In the Danube River, sturgeon populations are currently at their all-time low with single species having been classified as functionally extinct. Mihov et al. (2022) investigated whether sturgeon are still spawning in the Bulgarian section of the Danube and to estimate YOY sturgeon abundances during their downstream migration. Therefore, the authors used 100 m x 2 m bottom drifting trammel nets with 20 mm inner mesh size to capture YOY sturgeon at the currently only known nursery site in Bulgaria during their downstream migration. The fish were targeted over summer, when they were large enough to be captured with trammel nets (100-300 mm). One drift lasted approximately 45 minutes. Captured fish were measured, weighted and tagged.*

*During the eight-year sampling campaign, 713 sturgeon belonging to four species (beluga sturgeon, stellate sturgeon, Russian sturgeon, and sterlet) were captured. However, captures were highly variable, with two thirds of the catch occurring in a single year while in other years only single individuals could be obtained, while some species were not caught at all. In total, only seven beluga sturgeon and one Russian sturgeon were captured. Nevertheless, the authors were able to show temporal reproduction of all remaining sturgeon species in the Lower Danube while highlighting the interannual variability. Whether the inconsistent results were related to variable spawning success or to insufficient attempts remains unknown. Through captures of YOY sturgeon, areas that hold spawning places can be identified by back-calculating the hatch date based on the size of captured fish and water temperatures. Those areas can be further investigated by the approaches mentioned earlier to be protected. Stomach contents of the same captured fish were analysed (Margaritova et al., 2021), yielding important information about food requirements of this early life-cycle stage, helping to locate potential habitats by targeted surveys.*

### 6.4.4 Stow nets (Hamen)/Fyke nets (Grossreusen)

Stow nets are used in rivers or estuarine areas with strong currents where targeted fish are drifted into the nets (He et al., 2021). They are extensively used in the lower river sections in the Baltic Sea tributaries where they are fished with 8-10 nets side by side, covering up to 30% of the river width, mainly targeting eel and other downstream migrating fish during the fall (Gessner & Arndt, 2006). While no captures of YOY lake sturgeon were reported when using a similar technique (Benson et al., 2005), reports of bycatch of Baltic sturgeon (*Acipenser oxyrinchus*) in large numbers indicate the suitability of this method to capture YOY and juvenile sturgeon (Stakėnas et al., 2021; Jörn Gessner & Gerd-Michael Arndt, pers. comm.).

Fyke nets are large static nets which are fished in no or low currents. They utilize a guiding weir to direct the fish into a collection chamber similar to the stow net which is comprised of 3-4 chambers, separated by hopper-like sections of netting ending in a small mesh cod end. The nets maintain position in the water while the cod end is lifted and emptied. The fish are collected in the cod end, which needs to have a suitable mesh size depending upon the targeted size of the YOY sturgeons. The only potential impact on the fish could be suffocation due to too large nets or debris being collected in the net over 12 h of fishing. As such, low mortalities were reported especially in fyke nets (Stakėnas & Pilinkovskij, 2019). Captured fish can be collected from the cod end alive.

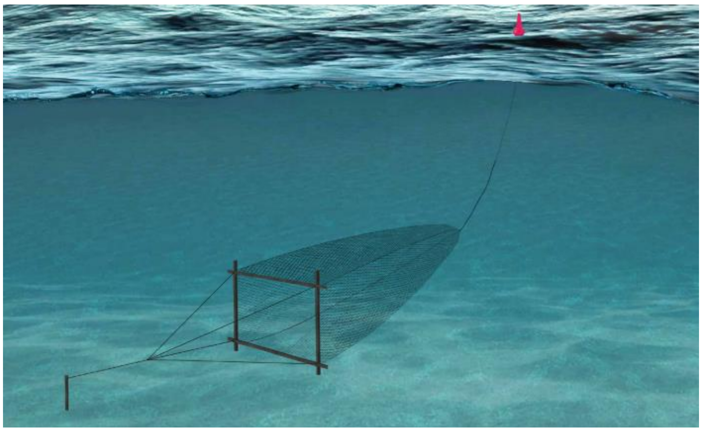

***Figure 15:*** *Schematic drawing of a stow net (adapted after He et al. (2021)).*

#### *6.4.4.1 Sampling site*

Since stow nets require current to operate effectively, appropriate areas should be selected. Stow nets can be deployed in the thalweg of the river, aiming to catch downstream migrating fish.

Large fyke nets were successfully used in coastal areas and lagoons (Stakėnas & Pilinkovskij, 2019). As for drift net fishing, with fyke nets feeding areas should be targeted. In Lithuanian lagoons, captures of juvenile Baltic sturgeon were associated with sandy and silty substrates (Stakėnas & Pilinkovskij, 2019).

#### 6.4.4.2 Timing of sampling

When targeting YOY sturgeon with stow nets in lagoons or lower parts of rivers with gentle flows, the timing should comprise the time of the year when YOY out-migrate. Stakėnas & Pilinkovskij (2019) reported most captures of young (YOY and just over one year) Baltic sturgeon during April-June, at times when they were most actively feeding. In the Danube, YOY sturgeon are captured with trammel nets until September, between 100-400 km away from the estuary (Mihov et al., 2022; Paraschiv & Suciu, 2005). Hence, targeting YOY in the estuary could be conducted from late Summer throughout the autumn.

#### 6.4.4.3 Materials & Methods

To increase chances of capturing YOY sturgeon, a set of 4-5 stow nets with ~8 m length, ~1.5 cm stretched mesh nylon multifilament, 1.8-4 m² net opening, 3 rings and a 1 cm cod end should be employed. Stow nets are fixed with poles, rammed into the substrate, and anchored upstream, depending on the current. To keep the mouth open, the nets are tied to the frames of the hopper sections (Pravin et al., 2011).

#### 6.4.4.4 Drawbacks

The installation of stow nets requires high personnel expenditures, and, depending on the sampling site, the use of a larger ship may even become necessary (Collas et al., 2021). Moreover, it is a passive method, requiring YOY to be drifted into the net. Thus, if the wrong site is chosen or the net only covers a small proportion of the river, extensive sampling may be necessary to capture sturgeon.

### 6.4.5 Beach Seine

Seining may be effective for sampling YOY sturgeon given their proclivity to sandy, shallow areas (Holčík, 1989). Thus, seines can be used to capture early life stages of sturgeon in slow flowing sections of rivers and in the delta. A beach or bag seine (a beach seine with a bag to concentrate fish) can be used and is most efficient when the substrate is smooth and clean. For being effective, the lead line must maintain contact with the substrate at all times while the floatline must not be submerged. According to Guy et al. (2009), the standard bag seine is 9.1 m long by 1.8 m high and is comprised of 6.4 mm Delta knotless mesh. The center of the bag is in the middle of the net at around 4.6 m, measuring 1.8 x 1.8 x 1.8 m of the same mesh type. The lead line is 29.5 kg lead-core line; the float line has a floating core with additional polyvinyl chloride sponge floats (50.8 mm dia. x 38.1 mm long) placed every 30 cm. The brails are made of 2.5 m long wood.

However, if available, longer seins can be used but mesh sizes must fit the size of the target fish. Fadaee et al. (2006) marked sturgeon collected by commercial fishermen with beach seine with a length of 1000 m and a depth of up to 10 m in coastal waters of Iran.

The seine is an active method, where one end is held or anchored at the shore. The other end is moved upstream where it is pulled into water in a sweeping 180° arc, and returned with the current downstream back to the anchored end by 3-5 people. If large seines are used, the use of a boat to move the net into the water and a winch to retrieve the net will be necessary. The lead line should remain on the river bottom and the float line at the surface at all time during the haul (Guy et al., 2009). Fish are trapped between the net and both ends. The net is pulled towards the shore until all fish can be collected and transferred into a storage container. Numerous seine hauls should be conducted in suitable areas to target YOY sturgeon.

***Figure 16:*** *Beach seine sampling in a shallow shore habitat (© IGB, J. Gessner).*

#### *6.4.5.1 Sampling site*

To conduct successful seining, obstacles such as large stones and tree trunks should be avoided, and water depths should not exceed the height of the beach seine. When dragging the seine in a downstream direction, the operators need to make sure they are pulling the seine faster than the flow velocity to avoid the seine folding inwards (Guy et al., 2009). Hence, flow velocities should not exceed the speed at which a haul can be safely conducted.

#### *6.4.5.2 Timing of sampling*

Seining should be conducted during summer or autumn where YOY fish are still available and water levels are generally low. Seining can be conducted during the day or night as long as safety standards can be met.

#### *6.4.5.3 Materials & Methods*

Seining only requires a functional seine of which the dimensions are adapted to the sampling area. For handling, a team of 3-5 (or more) people, depending upon the size of the seine and the equipment necessary to take care of sampled fish (see chapter 7), is recommended.

#### *6.4.5.4 Drawbacks*

Seining is only suitable if areas where sturgeon occur are accessible and not to deep. If deep water dominates or flow velocities are too high, different methods have to be applied.

## 6.5 Juveniles and subadults

Juveniles may be the best stage for monitoring population status. The presence of juveniles corroborates recruitment occurring in the population. For the most part, changes in juvenile abundance would be detectable sooner than the adult stage (Nilo et al., 2006). Aging of this life stage is more reliable than of adults using standard methods for aging (Bruch et al., 2009) and, therefore, more reflective of the true response of the sturgeon population (e.g., bias is not introduced by understating true age of adults). Finally, survival of juvenile sturgeon (e.g., >1 + years) is relatively high and constant (Gross et al., 2002; McDougall et al., 2020; Vélez-Espino & Koops, 2009), therefore an index of abundance, or changes to their abundance, could reflect future population trends (Haxton & Friday, 2020; Jager et al., 2002; McDougall, Pisiak, et al., 2014). Nevertheless, the timescale considered when sampling juvenile sturgeon and subsequent interpretations must always account for the biology of the sampled species (Haxton & Friday, 2020).

### 6.5.1 Purpose of sampling

- Monitoring of reproduction efficiency and recruitment into the population
- Genetic characteristics
- Assessment of population size and condition of population

Methods of population assessments can vary depending upon the behavior of the fish and their life-cycle habitats. Both passive and active fishing methods can be applied but the analysis must take the drawbacks into consideration. Active methods have the advantage of being independent of the activity of the targeted fish. Among these, trawling in open waters, be they coastal or lacustrine, is the most commonly applied method.

### 6.5.2 Standardized Trawls

Since the number of sturgeon in coastal waters has been significantly reduced over the past decades, zero-inflated catch results must be expected. The assessment of the catchability of sturgeon in relation to trawling speed, water depth, distance of the net to the boat, net shape, material, size, and operation duration are critical information to obtain reliable data.

Ideally, the sampling follows a randomized approach with regards to the start- and endpoint of a trawl, the direction, the timing etc. to avoid any bias by previous experience and expectations. The gear, material, and the application of the trawl (boat, engine, speed, distance from boat at depth, duration) in the survey is highly standardized and as such, reproduceable.

#### *6.5.2.1 Materials & Methods*

Sizes of trawl nets reported in the literature differ. For example, in the Caspian Sea, 9 m and 24.9 m trawl nets are used for sturgeon (Khodorevskaya & Krasikov, 1999). Smaller trawl nets (e.g., 4.9 m or 3.6 m) were used in rivers (Kennedy et al., 2007; Steffensen et al., 2015). In France, trawl nets with 12 m width, 4 m height and a mesh size between 20-60 mm (Lamour et al., 2024), or with 13 m width, 3.5 m height and a mesh size of 70 mm (Rochard et al., 2001) were used. Other options reported are small-mesh benthic trawls using a North American standard of a 3.2 mm heavy Delta-style mesh cover or large-mesh trawls with 19.2 mm mesh made of number 9 spectra twine on the top and number 12 spectra twine on the bottom (Guy et al., 2009). Mesh size has an effect on the drag of the net as well as on the size of the fish caught. For surveys, small bottom trawls adapted to the environment with a horizontal opening of maximum 30 m and vertical opening of maximum 10 m are recommended. The mesh size of the trawl should be at least 12 mm or optimally 16 mm in the cod-end.

The ship needs to be strong enough to pull the respective trawl and to provide sufficient space for a live well of twice the dimensions of the largest fish expected with continuous water exchange (Figure 17). Fish should be handled in a sufficiently sized stretcher with steel handles (Figure 18). Two support stands should be used to fix the stretcher for handling and sampling. A hanging scale is used to determine the weight of larger fish while being supported in the stretcher. An adapted system to release the fish while avoiding injuries should be implemented, especially for large fish (Figure 18).

Nets are trawled using large vessels. Khodorevskaya & Krasikov (1999) used trawling wires of 80-100 m length at towing depths between 3-15 m, and of approximately 150 m length at towing depths between 25-50 m and approximately 450 m at depths below 50 m at a towing speed of 2.5 knots. Trawling was reported more effective when tidal cycle and heading direction favour higher boat speeds (Rochard et al., 2001).


***Figure 17:*** *Boat for trawling (left, © INRAE, R. Le Barh) and deployment of a benthic trawl from an approximately 14 m long research vessel (right, © INRAE, M. L. Acolas).*

The effective duration of each trawl should be 30 minutes exactly, to standardize effort, minimize damage to the fish and facilitate the safe release of the fish.

The gear must maintain contact with the bottom throughout the 30 minutes' survey trawl. The effort needs to cover a fixed proportion of the shelf area of relevant depths (~10-65 m).

***Figure 18:*** *Properly sized stretcher used for releasing sturgeon from a large research vessel (© INRAE, R. Le Barh).*

The required equipment is comprised of:

- Research vessel (strong enough to maintain trawl speeds between 2.5-4.5 knots)
- Bottom trawl with max. 30 m horizontal and max. 10 m vertical opening
- Sufficient length of trawling wires to cover the relevant depths
- Live well for large fish with water supply, stretchers, measuring tape and hanging scale adapted

A trawler of sufficient size usually is equipped with a crew of 3+ people. In addition, the team to run the sampling should be comprised of 2-3 people, including the scientific lead of the monitoring.

#### *6.5.2.2 Drawbacks*

Trawling is an expensive method as a research vessel, large trawl nets, and a large crew are required. Moreover, bottom trawling bears the risk of becoming snagged or the trawl being lost (Dettmers et al., 2001).

### 6.5.3 Trammel/Gill netting

Juvenile sturgeon are vulnerable to gillnets (Haxton et al., 2014; McDougall, Barth, et al., 2014; Pratt et al., 2014). Stakėnas et al. (2021) reported the highest bycatch of juvenile Baltic sturgeon (mostly 1-2 kg) in gill nets with mesh sizes between 50-70 mm. In order to target juvenile lake sturgeon of different sizes and ages, stretched multimesh monofilament nets with mesh sizes of 25.4, 50.8, 76.2, 127.0, and 152.4 mm (McDougall, Barth, et al., 2014) or North American standard gillnets with 38, 51, 64, 76, 89, 102, 114, and 127 mm stretched mesh sewn together in random order were used. Those nets were 1.8 m deep and had a length of 24.8 m (Haxton et al., 2014). Alternately, nets with different mesh sizes can be combined to form a single continuous net.

#### *6.5.3.1 Materials & Methods*

Trammel nets can also be an effective means to sample juvenile sturgeon (Grohs et al., 2009; Hamel et al., 2014; Hammen et al., 2018). Shovelnose sturgeon between 233-850 mm total length (TL) were sampled using trammel nets that were 30.5 m long consisting of 13.6 kg lead-core line and 12.7 mm foam-core float line, outer mesh sizes of 304.8 mm (number 9 multifilament nylon twine) and 1.8 m deep and a single 2.4 m deep panel of inner mesh of 50.8 mm (number 139 multifilament nylon twine). Stationary 25 m x 2.5 m trammel nets with inner mesh sizes of 40 mm and outer mesh sizes of 200 mm, deployed in deep pools, were used in the Upper Danube for sterlet in size classes from 540-920 mm total length (Neuburg & Friedrich, 2023).

See also section 6.4.2.5.

#### *6.5.3.2 Drawbacks*

In comparison to benthic trawls, net fishing is a rather cheap method. However, since static nets are a passive method, juvenile sturgeon have to be active to move into the nets in order to be captured. When sturgeon are scarce, the effort required to catch sufficient numbers may be fairly significant.

### 6.5.4 Sampling site

Site selection is carried out prior to sampling and should follow the principles of random sampling to avoid bias for aggregation areas. Sites should include all known depths where the target species is present. During trawls, the vessel should move in random directions wherever possible. Individual trawls are documented using GPS data of the start and endpoint upon bottom contact.

In Gulf sturgeon, juvenile capture was more successful at river mouths than within the river (Novak et al., 2017; Peterson et al., 2016; Sulak & Clugston, 1998). Juvenile Gulf sturgeon up to 1000 mm TL stayed at the river mouths and did not migrate out with subadults and adults (Peterson et al., 2016; Sulak & Clugston, 1998). Juvenile Russian sturgeon were caught in trawls and gill nets in the river mouth and pre-estuary regions in the northern Caspian Sea (Levin, 1971). Russian, stellate, beluga and ship sturgeon juveniles were sampled near the mouth of the spawning river and ~25 km out (Zakharyan, 1972). However, Rochard et al. (2001), Lamour et al. (2024) as well as Holostenco et al. (2013) reported specific areas where juveniles aggregate and other areas where fewer captures occurred. Similar patterns of preferential areas were observed for juvenile Atlantic sturgeon (Novak et al., 2017) and Gulf sturgeon (Peterson et al., 2016) in telemetry surveys.

### 6.5.5 Timing of sampling

While Rochard et al. (2001) and Lamour et al. (2024) were able to capture juvenile European sturgeon during each season in the Gironde estuary, Holostenco et al.

(2013) and Maximov et al. (2014) reported the most captures of juvenile stellate, beluga and Russian sturgeon in summer and fall (only stellate sturgeon). Rochard et al. (2001) reported the highest CPUE in summer as well. In the Curonian Lagoon as well as in the Baltic Sea in Lithuania, the most captures of Baltic sturgeon were reported for autumn, but the results are based on commercial fishery and hence depended on the fishing effort of commercial fishermen, which is often not comparable between seasons (Stakėnas et al., 2021). Atlantic sturgeon tended to use the Saco River estuary between spring and autumn, emigrating until November, probably to wintering areas outside the estuary (Novak et al., 2017). Hence, targeting juvenile sturgeon might be most effective during summer and autumn when they are actively feeding.

### 6.5.6 Analysis

Effort is considered the time of trawling and distance trawled. For bottom trawls, CPUE is expressed as the number of fish per time increment of trawling (Guy et al., 2009). Alternatively, net hours or net length per hour for gill or trammel nets. For trawl surveys, as a prerequisite for the calculation of the CPUE, the effective net opening (width x height) must be determined.

Catchability ($q$) is not constant (Gordoa & Hightower, 1991; Kotwicki et al., 2014) and can be influenced spatially (Casey & Myers, 1998; Godø et al., 1999; Kotwicki et al., 2013; Zhang et al., 2020), temporally (Kotwicki et al., 2013), diurnally (Casey & Myers, 1998; Huse et al., 2001), seasonally (Casey & Myers, 1998), with varying abundances of target species (Kotwicki & Ono, 2019), gear type (Cadrin et al., 2016; Kotwicki & Ono, 2019), species (Fraser et al., 2007; Young et al., 2019), body size (Fraser et al., 2007, 2008; Heino et al., 2011), fish behavior (Cadrin et al., 2016), and with technological advancements (Eigaard et al., 2014). Variability in catchability leads to inaccuracy of abundance estimates.

For example, if catchability is held constant at true low population abundances, estimated abundances will be overinflated (Cadrin et al., 2016; Kotwicki & Ono, 2019) which could propagate into overzealous fishing limits and rebuilding targets (Cadrin et al., 2016). Catchability during trawling surveys should ideally be re-evaluated to reduce stock abundance uncertainties (Cadrin et al., 2016). Moreover, catchability should be evaluated for each size class of each species to provide better accuracy in estimates at a given sample site (Fraser et al., 2007). For the assessment, either the set of pingers hooked up to the depth finder or a submerged video-camera can be utilized, provided that visibility is sufficient. Catchability would be the relationship between the number of fish caught divided by the number available to be caught. At least 20 trawls are considered the minimum to effectively assess catchability. An alternate means of determining catchability would be by repeated sampling (e.g., trawling the same section of coastal shelf repeatedly in both directions on the same or a closely adjacent track) and estimating detection probability (MacKenzie et al., 2017).

### 6.5.7 Case Example: Juveniles – Gironde estuary European sturgeon

*The Gironde-Garonne-Dordogne system in France shelters a European sturgeon population, which was largely sustained through conservation stocking programs (stocking between 2007-2015). The estuary has been used by juvenile European sturgeon for several years as nursery habitat and migration corridor and has been monitored regularly since 2009 to evaluate population health and stocking efficiency. However, habitat use and drivers of habitat choice are as of yet not well understood.*

*Lamour et al. (2024) analysed habitat selection of European sturgeons in the estuary and the influence of environmental factors based on trawling surveys (1022 trawl tows) in the Gironde estuary and subsequent captures of 452 sturgeons (fork length (FL) between 25.5-154 cm) between 2010 and 2018. Every two months, approximately 20 trawl tows distributed over 20 sampling rectangles were conducted to allow for a homogeneous coverage of the area, using a 21 m long bottom trawl with a decreasing mesh size (60-20 mm) and a maximum opening of 12 m in width and 4 m in height. Sampling rectangles were delineated in the mesohaline and polyhaline sectors of the estuary but limited in space by the navigation channel and the presence of wrecks and shallows. The average duration of one trawl was 30 ± SD 8 min and their average length was 3.9 ± SD 0.7 km. For analysis, European sturgeon individuals were categorized into two groups:*

*1) estuarine dwellers (ED; FL <68 cm) using mainly the estuary, and*

*2) sea explorers (SE; FL ≥68 cm) which could accomplish migration at sea.*

*The authors conducted hotspot analyses on a seasonal basis to localise ED and SE concentrations and explored environmental variables as potential drivers.*

*ED and SE were captured in mesohaline and polyhaline parts of the estuary during all seasons but densities varied seasonally. Highest densities were observed in autumn for SE and for ED during all seasons except spring. Both groups used common areas located in the downstream part of the estuary (overlap from 26 to 33%) except in autumn (12% overlap). The main abiotic drivers for habitat choice (76 % explanation) during all seasons were water temperature and water column height, salinity and concentration in suspended matters, and bottom current velocity.*

*Habitat selection was observed for SE in all seasons and for ED in summer and winter. Since ED are mainly using the estuary, they are most likely accustomed to the range of abiotic variables characterising the estuary, though other factors are probably involved in spring and autumn. During summer and winter, ED occupied both downstream habitats and upstream areas with lower salinity but higher water temperature. SE were encountered mainly downstream in deeper areas with higher salinity and lower water temperature than in the rest of the estuary, except in winter, when areas with higher water temperatures were chosen. In summer, water temperature seemed to be the limiting factor for SE, since areas with the lowest water temperatures were chosen and lowest densities were observed.*

*The authors were able to show overlaps of downstream hotspots between the stocked population and ancient wild cohorts, while former upstream hotspots disappeared most likely due to environmental changes during the last few decades. Nevertheless, the results highlight important areas for both groups and are valuable for designing conservation measures.*

## 6.6 Adults

### 6.6.1 Purpose of sampling

Sampling for adults is performed to estimate the size of the spawning population or to find important spawning, wintering or feeding habitats. Long-term monitoring of population trends yields information about the development of the population and could potentially reveal occurring bottlenecks. Nevertheless, monitoring the recruitment into the population appears to be more effective in detecting changes at the population level also due to the longevity and thus slow response of adult fish.

Monitoring adult sturgeon has its challenges. The temporal lag between a management action and a response would be extremely extended, making long-term monitoring both expensive and potentially lacking statistical power to detect a change, unless the population response was drastic. Moreover, monitoring a spawning population downstream of a barrier may provide the misconception that the population is healthy given their longevity; recruitment issues may go unnoticed until the population has declined.

However, adult sturgeon can be monitored by multiple means. Commercial fisheries (Mailhot et al., 2011), bycatch (Dadswell et al., 2016, 2017; Stokesbury et al., 2014) and recreational fisheries (Baker & Borgeson, 1999; Bruch, 2008; Dieterman et al., 2010) are good sources of biological samples and may be the only source of data for some stocks (Iorga et al., 2011). If the samples are based on harvested fish, it means the fish is lost to the population and only represents what was there. Monitoring adults potentially yields important information about the timing of migrations, the population size, habitat use and spawning frequency.

### 6.6.2 Hydroacoustics

The most common hydroacoustic techniques to monitor sturgeon populations have been side-scan sonar or dual-frequency identification sonar (DIDSON). However, side-scan sonar surveys appear to be the most frequently used method recently.
Hydroacoustics provide an alternate, non-invasive tool to encounter, enumerate and monitor sturgeon in rivers through sonar technologies that produce sound waves. Initially, these technologies were used to map underwater features and collect information about underwater environments through the reflections of sound waves to map habitats as well as for the identification of habitat suitability (Kaeser & Litts, 2010; Walker & Alford, 2016). Fish can be identified mainly due to shape information based on their acoustic shadows or silhouettes (Langkau et al., 2012). Those shadows can be measured using an imaging software (e.g.,

Hughes et al. (2018)). Hydroacoustics are used to prove the presence of sturgeon or to count sturgeon at spawning or wintering areas. In general, the assessment of large fish (>1 m) is easier and species identification remains difficult when more sturgeon species coexist in a system. Inferences are made based on fish size or the timing of migration if the species differ significantly (Auer & Baker, 2007). Observation of spawning fish in deeper water can be facilitated by visual sonar techniques while, due to the limited visual angle, these methods do not provide an overview but rather sectoral images. Highly turbid water might limit the applicability due to deferred signals. Nevertheless, hydroacoustics were successfully used in moderately turbid water (Hughes et al., 2018) and can facilitate the planning process for other monitoring efforts (setting egg mats, locations for net fishing) because large areas can be surveyed with reasonable efforts (Poytress et al., 2015). Flowers & Hightower (2013) surveyed six river systems for the presence of white sturgeon and successfully detected them in five. Also, similar results of population estimates were obtained with less time spend through hydroacoustic survey when compared to CMR surveys (Hughes et al., 2018; Mora et al., 2015).
Echo sounders with side-scan function produce images of the water column and the bottom on both sides of the boat. The range of the image can usually be set and depends on the water depth, whereby it is usually lower in shallower water and objects at the edge of the image get blurred. Most side-scan devices support different frequencies; nevertheless, higher frequencies result in better resolution images and are usually used for fish identification (Kazyak et al., 2020). Reported frequencies ranged between 600 kHz (Thomas & Haas, 2002) to 1600 kHz (Kazyak et al., 2020) and swath widths of 50-70 m (25-35 m on each site of the boat) were covered (Andrews et al., 2020; Flowers & Hightower, 2013; Johnson et al., 2016; Kazyak et al., 2020) but this depends on the water depth. Optimal survey speed is between 4.5-10 km/h (Kazyak et al., 2020) and ideally lies around 8 km/h (Kaeser & Litts, 2010). Echo-sounders were used to identify and count Chinese sturgeon (*Acipenser sinensis*) in the Amur and Yangtze Rivers (Gao et al., 2016; Zhang et al., 2014). A 16 km long reach was covered by zigzagging in a downstream direction. Sturgeon were identified based on the characteristics of the returned acoustic signal.

Also, side-scan sonar can be used to enumerate larger sturgeon (>1 m) within an area. Usually, fish which are counted in transects abundance at the respective sites can be modelled (Flowers & Hightower, 2013; Hughes et al., 2018). Consecutive passes over one transect can improve within-site variation of counts (Hughes et al., 2018). Most estimates obtained through side-scan surveys represented the adult population as individuals <1 m are difficult to identify confidently. However, Andrews et al. (2020) were able to encounter shortnose sturgeon (*Acipenser brevirostrum*) of lengths <1 m (25-149 cm) and able to obtain reliable population estimates in the Saint John River.

Crossman et al. (2011) were able to count white sturgeon and reliably estimate the sizes of encountered fish using a stationary dual-frequency identification sonar (DIDSON) at distances of around 20 m. A mobile approach with consecutive passes over transects using DIDSON was used for green sturgeon (*Acipenser medirostris*) and to estimate sturgeon density within the sampled area (Mora et al., 2015).

Auer & Baker (2007) used a stationary split-beam echo sounder operating at 200 kHz and a 4x10° elliptical-beam transducer with a near field range of 1.7 m to estimate the number of sturgeon migrating to a spawning area. The mounted unit, however, requires sturgeon to pass this river section and is limited to small rivers (~30 m width and max. 3 m depth in this study). Species identification was based on experiments to discriminate between reflected signal strengths. The stationary split-beam sonar enabled the monitoring of the direction the sturgeon were moving.

### *6.6.2.1 Sampling site*

In general, hydroacoustic techniques can be used in many different sized rivers to detect and identify sturgeon. However, sites surveyed contained depths of around 10 m or less but usually >2 m (Andrews et al., 2020; Hughes et al., 2018; Vine et al., 2019). Ideal conditions represent shallow, slow flowing water over sandy bottom or other fine substrates, but problems might occur when complicated or large substrates are prevalent and strong currents occur since air bubbles can inhibit the efficiency of the device (Hughes et al., 2018). A manual check of pictures might be necessary to differentiate between acoustic shadows from fish or other obstacles, such as sand dunes (Andrews et al., 2020).

Ideal conditions for stationary set ups are similar, including soft, sandy substrate, and laminar flow to reduce production of air bubbles interfering with acoustic signals, and an unobstructed (e.g., boulders, debris) cross section of the river (Auer & Baker, 2007) with a gradually descending slope (Figure 19) to cover the whole water column (Crossman et al., 2011).

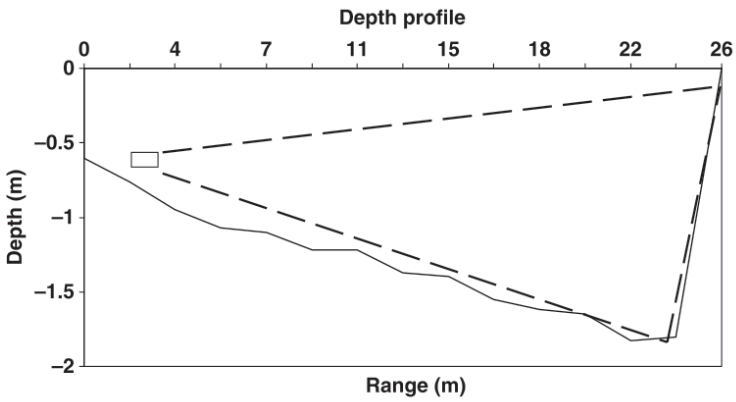


***Figure 19:*** *Ideal profile of the river bottom for stationary hydroacoustic surveys. Gradually descending slopes exclude areas with acoustic shadows where detections are impossible to realize (adapted after Auer & Baker (2007)).*

### *6.6.2.2 Timing of sampling*

When the goal of hydroacoustic surveys is to count sturgeon and to get abundance estimates, time windows when the respective species aggregate should be chosen.

Andrews et al. (2020) targeted shortnose sturgeon in their wintering habitat and were able to obtain estimates that were comparable to estimates from CMR surveys. Spawning time was used to count sturgeon as well (Gao et al., 2016; Johnson et al., 2016; Mora et al., 2015).

#### *6.6.2.3 Materials & Methods*

To perform side-scan surveys, a side-scan device mounted on a boat is necessary and it has to be linked to a GPS to collect position data. The hydroacoustic device is usually mounted on the hull of the boat or from a pole within the first meter below the water column. When a tow fish unit is used, deployment should be at a depth of 10-20 % of the side-scan range coverage off the bottom.
Stationary mounted systems like a DIDSON may require a more sophisticated setup, including material to install the device and a power source if the survey is planned for a longer time period, but the setup allows monitoring over a period of time in a given river section or bypass channel.

#### *6.6.2.4 Analysis*

With large numbers of images of transects being collected, the difficult and time-consuming data analysis and subsequent counting of fish needs to be carried out. Even though machine learning tools can be used to count fish (Andrews et al., 2020), they have to be calibrated, and manual checks might be necessary to distinguish between fish and obstacles. For this purpose, decoys submerged in the transducer sampling area can be valuable to have clear reference sizes at hand to calibrate the results (Dewayne Fox, pers. comm.).

Estimates of population size can be obtained through N-mixture models (Hughes et al., 2018; Vine et al., 2019) or based on sturgeon densities and subsequent relation of sampled area to total area of the study site (Mora et al., 2015). N-mixture models were developed to assess spatiotemporal variation in abundance for small populations or populations with low detection probabilities due to their secretive habits (Royle, 2004). Identification of sturgeon presence over a large scale can be obtained through Occupancy models (Flowers & Hightower, 2013). Occupancy models aim to estimate the proportion of sites occupied by the species of interest based on a sampling method involving multiple visits to a site, allowing estimates of detection probabilities and proportion of sites occupied (Mackenzie et al., 2002). Simple counts were used to identify spawning habitats and detect areas of higher abundances (more counts) of white sturgeon (Johnson et al., 2016). Comparisons with CMR surveys yielded similar results between hydroacoustic estimates and CMR estimates (Andrews et al., 2020; Hughes et al., 2018; Mora et al., 2015).

In addition to abundance estimates, habitat use and size distribution, hydroacoustic surveys usually provide an efficient tool to precisely map surveyed habitats (Kaeser & Litts, 2010).

#### 6.6.2.5 Drawbacks

While hydroacoustic techniques offer possibilities to obtain population estimates of large individuals, demographic data such as growth curves or sex ratios cannot be determined (Hughes et al., 2018). Moreover, to confidentially detect and identify sturgeon, the observers need to be experienced. Fish that lie on the ground and do not have any acoustic shadow might be hard to identify, especially in areas with hard substrates and many other obstacles. Detection probability is influenced by sturgeon orientation relative to the transducer (angled or parallel), when hidden in the hydroacoustic shadow of objects and if sturgeon are located directly below the sonar (Hughes et al., 2018). Also, the collection of several hydroacoustic recordings requires a large amount of data storage space.

### 6.6.3 Other Methods

Sturgeon congregate when spawning, which can increase sampling success (Bruch & Binkowski, 2002). However, invasive monitoring methods, such as netting for ripe spawners, may deter these fish from spawning due to stress and thus negatively affect population development. While some lake sturgeon populations spawn in shallow waters (Bruch & Binkowski, 2002) where visual observations and counts of spawning fish can be made (Tucker et al., 2021), spawning in many species occurs in deep (Bouckaert et al., 2014) or turbid waters (Tucker et al., 2021), where visual observations are not possible. Visual observations, where possible, should be conducted around noon when the sun permits maximum viewing opportunities due to light penetration (Tucker et al., 2021). Observations can be conducted from the shoreline, boat or by use of a drone.

Spawning sturgeon are captured on site using a variety of different techniques including large trapezoid dipnets (Smith & Baker, 2005; Tucker et al., 2021) or short set large mesh gillnets (Dumont et al., 2011; Haxton, 2006). Trammel nets and gill nets are widely used to capture adult sturgeon and proved to be effective when comparing gear types (Spindler et al., 2009). Multimesh monofilament gill nets (2 m high and 60 m long, 30 m of 203 mm stretch mesh, 20 m of 254 mm and 10 m of 305 mm) set parallel to the flow for 60-90 minutes have been effective to capture lake sturgeon between 66-184 cm (Dumont et al., 2011). Nets have also been set diagonally or perpendicular to the flow when conditions permitted, or in back eddies (Haxton, 2006). Outside of the spawning period, adult sturgeon could be targeted by extra-large mesh gillnets in feeding habitats (Baker & Borgeson, 1999; Haxton et al., 2014; Haxton & Friday, 2019). A standardized, randomized netting protocol using multimesh gillnets incorporating panels of 204, 230, 255, and 306 mm stretched mesh sewn together in random order, 2.13 m deep with a total length of 24.8 m, was used to provide an index of relative abundance of adult lake sturgeon across multiple rivers in North America. Nets were set with anchors perpendicular to the shore where flows permitted, or on an angle (e.g., 45°) when flows were deemed substantial enough to impede the effectiveness of a perpendicular set and nets were deployed for an average of 22 hours (minimum 18 hours, maximum 26 hours) (Haxton et al., 2014, 2018).

In the Lower Danube, adult spawning sturgeon are captured using trammel nets with 60-100 mm inner and 400-500 mm outer mesh sizes that are drifted in the main channel using a boat and a floating device ("water anchor") to be pulled by the current (Marian Paraschiv, pers. comm.). In the Upper Danube, adult sterlets are captured with stationary trammel nets with 40 mm inner and 200 mm outer mesh sizes (Neuburg & Friedrich, 2023).

Trawling is a means to sample sturgeon in large waterbodies (Dadswell et al., 2016; Khodorevskaya & Krasikov, 1999). Otter trawls, however, were found to be a highly inefficient assessment technique to capture pallid sturgeon (*Scaphirhynchus albus*) in the Mississippi River as the fish were inhabiting dune structures in the main channel, which were not effectively fished by the trawl (Steffensen et al., 2015).

Another method used mainly in North America, is the setting of baited setlines 100 m in length for subadults and adult sturgeon in early spring to late summer, containing 25 springers with 7/0 saltwater hooks. Setlines are deployed for 24 to 48 h intervals at water depths of 2 to 16 m, and baited with a variety of baits (Bauman et al., 2011). Setlines were found to be more effective at sampling white sturgeon than gillnets (Irvine et al., 2007; Steffensen et al., 2013) or boat electrofishing (Irvine et al., 2007). Setlines have versatility as they can be set deep and in currents where other assessment techniques may not be as effective. Hughes et al. (2018) used baited setlines with 30 m mainline of 0.79 cm double-braid nylon rope, rigged with two springers with offset circle hooks size 16/0, 14/0, and 12/0 respectively. Hooks were spaced 4 m apart to capture white sturgeon between 60-229 cm fork length. All lines were set in the mid-channel, secured with 10 kg weights at each end and equipped with a surface buoy.

Li et al. (2007) counted shortnose sturgeon in their wintering habitat using cameras which were lowered through holes drilled in the ice.

### 6.6.4 Case Example Adults – White sturgeon side-scan sonar

*In their study,* Hughes et al. (2018) *aimed to estimate white sturgeon abundance using side-scan sonar and to compare their results to standard capture-mark-recapture data in the Snake River, Idaho. The authors used a side-scan sonar unit operating at 1200 kHz with a 30 m range (60 m swath) mounted to the boat 0.7 m below the surface. Boat speed was kept at 6-8.3 km/h for optimal side-scan imaging. To count white sturgeon, the whole stretch was divided into 36 sections of 1.6 km length. All suitable habitats >1.8 m depth were surveyed three times to generate three independent counts. When a habitat was too wide to be covered with the 60 m swath, parallel transects were conducted with slight beam overlap to mosaic the collected imagery. The side-scan survey was conducted over six days in early February, the capture-mark-recapture survey was conducted for 24 days between late February to mid-April using baited setlines.*

*During the side-scan survey, the authors counted >110 white sturgeon. While in half of the sections, no sturgeon was encountered, around two thirds of the counts were made in only six sections towards the upstream end of the whole stretch,* *highlighting the efficiency of the method in detecting aggregation areas of sturgeon with relatively low effort. The abundance could be estimated to around 140-150 individuals, which was lower than the estimate of 219 individuals through the capture-mark-recapture study. The authors explained it through a theory of a possible overinflation of the CMR estimates which may have been caused through "trap-shy" effects during sampling.*

*Using a side-scan sonar helped to survey a large area and aggregations of sturgeon could even be restricted to only a smaller part of the whole area, helping to efficiently allocate future efforts for CMR sampling. Moreover, the time spent to get similar population estimate, such as through CMR sampling, was significantly lower and thus more cost effective. The size of fish detected ranged between 100-300 cm, which fit well with the CMR data but might be problematic for species that don't grow larger or juveniles of some species.*

# 7 Sampling of captures

In the following section, different methods to collect morphological information of sampled sturgeon as well as different methods to tag fish are provided. When handling fish, the recommendations in the Animal Welfare chapter are to be implemented and personnel must be experienced. The majority of the methods described here are covered in more detail in the Technical Guideline for EX SITU Conservation Measures in Sturgeons. (Gessner et al., 2024).

## 7.1 Anaesthesia

The application of anaesthesia depends upon multiple factors, including the time and degree of invasiveness of the procedure, regulations, safety for the user and the environment (waste disposal), and weighing the risk and stress posed by using an anaesthetic versus not using an anaesthetic, which may potentially result in increased stress or injury. Some countries and agencies will require the use of anaesthetics for any invasive procedure to meet animal care protocols and address animal welfare issues (Zahl et al., 2012). For wild sturgeon, the use of anaesthetics is usually regulated in the permit for carrying out monitoring activities.

Guidelines and policies regarding the use of chemical anaesthetics vary between manufacturers and between countries. Local and regional agencies should be consulted for the latest regulations and currently approved-use chemicals. The primary goal of using an anaesthetic is to immobilize the animal while blocking nerve impulses when conducting invasive procedures. The stage of anaesthesia (Summerfelt & Smith, 1990) used depends on the degree of invasiveness and length of time the procedure will take. Procedures like ultrasonography and milt collection do not typically require anaesthesia. Procedures like fin clipping, blood sampling, PIT tagging or external tagging might require sedation, depending on national law. Endoscopy, celiotomy/biopsy, and collection of ovulated eggs as well as the insertion of a telemetry tag by surgical procedures require anaesthesia to stage III (partial loss of equilibrium with increased opercular rate and reactivity only to strong tactile stimuli) or stage IV (total loss of equilibrium, slow but regular opercular movements, loss of spinal reflexes).

### 7.1.1 Chemical

Three of the more widely used fish anaesthetics are tricaine methanesulfonate (MS-222), clove oil, and 2-phenoxyethanol (Neiffer & Stamper, 2009; Priborsky & Velisek, 2018). Both MS-222 and 2-phenoxyethanol are reported to be hazardous to human health (potential carcinogen) and latter is forbidden in France (Marie-Laure Acolas, pers. comm.).

Therefore, personal protective equipment must be used when handling the chemicals and solutions and regional standards and law requirements must be checked. Also, the disposal of the solution after use may require special treatment. The induction and maintenance of anaesthesia is temperature dependent. Concentrations need to be adapted to the water temperature. Supplemental oxygen should be added to the anaesthesia tank and monitored throughout the process.

Immersion is the most common method for fish anaesthesia, as the agents dissolved in solution enter the bloodstream through the gills and skin. Induction and recovery times vary, primarily based on the dosage level, the duration of time the fish is under anaesthesia, and the water temperature. The guideline for dosage is to induce the desired state of anaesthesia within 5-10 minutes and then have a similar recovery time (Neiffer, 2021). For smaller fish (<50 cm), the desired state of anaesthesia should be reached faster (within 2 minutes) to prevent excessive stress. After the invasive manipulation is finished, the fish should be transferred to fresh water to allow for fast recovery. Exact dosages for the lowest induction and recovery time will vary with species, body size, age and life-cycle stage, stage of anaesthesia targeted, and water temperature and quality (Summerfelt & Smith, 1990).

Tricaine methanesulphonate (MS-222), sold under a number of trade names (e.g., Tricaine-S™ and Finquel™, USA; Aqualife TMS™, Canada), is a derivative of benzocaine. Many users prepare stock solutions (10 g/L) to reduce human exposure to the powder. Fresh stock solutions should be made every 30 days, and both the drug and stock solutions should be protected from light (Neiffer, 2021). MS-222 reduces water pH so a buffer, typically sodium bicarbonate, is added initially at a 1:2 ratio (MS-222: sodium bicarbonate) followed by pH measurements and adjustments made as needed to maintain pH at approximately 7.0. Typical doses for induction range from 100-250 mg/L, and maintenance doses reported have ranged from 70-100 mg/L (Divers et al., 2009; Hernandez-Divers et al., 2004; Kahn & Mohead, 2010; Matsche, 2011).

The active ingredients of clove oil are eugenol (approximately 84 %), iso-eugenol (5-10 %), and methyleugenol. Clove oil is not completely soluble in water. Therefore, 95 % ethanol is used as a solvent in a 1:9 ratio, yielding a 100 mg/ml stock solution. Typical induction doses are 20-80 mg/L (Kübra, 2022; Neiffer, 2021). Dosages need to be adjusted, based on the content of eugenol, and on the time required to reach the desired stage of anaesthesia. Water-soluble alternatives to natural clove oil such as synthetic isoeugenol (Aqui-S) exist. Aqui-S contains 50 % active isoeugenol, and dosages are 75-150 mg/L. Aqui-S 20E contains 10 % active isoeugenol, and dosages are approximately 375-750 mg/L (Adel et al., 2016; Feng et al., 2011; Gomulka et al., 2008; Hurvitz et al., 2007; Kahn & Mohead, 2010; Webb et al., 2019).

Ethylene glycol monophenyl ether (2-Phenoxyethanol, 2-PE) has been used on numerous aquaculture species (Priborsky & Velisek, 2018), including sturgeon (Adel et al., 2016; Kübra, 2022; Shaluei et al., 2012). The effective concentration ranges from 0.06-1.20 ml/L which has a wide margin of safety and range of effects from light sedation to surgical anaesthesia (Priborsky & Velisek, 2018). Shaluei et al. (2012) reported concentration of 0.7-0.9 ml/L on beluga sturgeon resulted in deep anaesthesia within 3 minutes of exposure.

In France, Benzocaïne 10 % (benzocaïne 100 mg/ml) is used for the captive stock of European sturgeon. The dosage for short term transport lies between 0.10-0.25 ml/L, and for anaesthesia for short manipulation between 0.5-1 ml/L, whereby the lowest dosage is generally enough. The dosage for euthanasia lies at 2.5 ml/L (MIGADO V. Lauronce, pers. comm.).

There are several other chemicals less frequently used. Some are used as immersion anaesthetics, and include alfaxalone, propofol, and metomidate hydrochloride (Neiffer, 2021). Some, although less commonly used, are administered as injections, such as ketamine hydrochloride, xylazine, medetomidine, midazolam, and diazepam (Neiffer, 2021).

### 7.1.2 Physical

There has also been an increasing interest in using electroimmobilization to anesthetize fish, including sturgeon (Balazik et al., 2013; Balazik & Musick, 2015), and a recent review summarized that it is a useful tool for fish handling that equals or surpasses the capabilities of chemical sedatives (Reid et al., 2019). Direct current power supply is used to limit issues with tetany responses. Electroimmobilization causes a blockage of brain messages to the spinal motor nerves. Some of the benefits listed include no expiration/degradation of chemicals, no disposal protocols, significant shorter induction and recovery times, easier to adjust "dosage", a single device is reusable as opposed to chemicals that must be purchased regularly. Challenges include maintaining equipment in a proper and safe working order, proper application of electricity (too little or too much can be harmful), fish must be positioned properly within the apparatus, and certain devices will have a higher start-up cost compared to chemical sedatives.

## 7.2 Morphological measurements

The standard procedure after fish were captured is the collection of morphological measurements. For robustness, it is suggested to assess total length (TL, from the tip of the snout to the end of the caudal fin), fork length (FL, from the tip of the snout to the fork between upper and lower lobe of the caudal fin) and girth length (GL, the perimeter immediately behind the pectoral fins) of the captured fish using a meter band (Figure 20). Alternately, a measuring board can be used for all lengths except the GL, with the benefit of reducing the error of the body curvature. Measuring the GL will help to increase the goodness of fit of length-weight relationships. In addition, the standard length (SL) from the tip of the snout to the basis of the caudal fin can be taken as well. To take the measurements, the fish is moved to a stretcher, which is placed upon racks for stability. After the fish has calmed down, the measurements are taken and noted along with sample number, date, time, and species (see Annex 14.1).

Weighing on a moving ship can be extremely demanding and imprecise. Nevertheless, it is suggested to collect length-weight data on every occasion possible to be able to establish a length-weight relationship for the different seasons of the survey as well as for each species and sex. Taking standardized pictures is recommended to be able to document morphological specifics of individuals (injuries, scute patterns, etc.) or to carry out further meristic measurements. To identify hybridization between different species, the lateral scutes should be counted on both sides and documented (Margaritova et al., 2021).

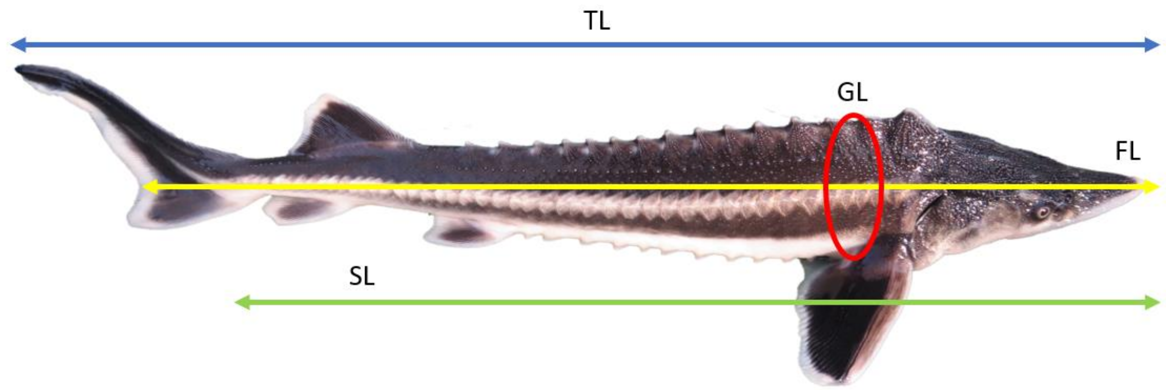


***Figure 20:*** *Total length (TL, blue arrow), fork length (FL, yellow arrow), standard length (SL, green arrow), and girth length (GL, red circle) measurements of a sturgeon (© BOKU, T. Friedrich).*

## 7.3 Tagging

In order to follow individual or batch growth and survival, to distinguish between fish that were stocked and those born in the wild, or to allow a monitoring of a tagged population, tagging fish might become necessary. Tags can be applied at the hatchery before release or in the field during monitoring of the population. Tagging methods should be adapted to the species and to fish size. Depending on the purpose of the tagging (individual identification, mass marking, temporary marking, etc.), one needs to choose and adapt the best method. Tagging or marking fish requires careful handling (see chapter 5). From a general point of view, the difficulty of the tagging method (qualified trained technician), retention rate, country regulation concerning animal welfare, and cost should be considered when implementing the tagging strategy.

There is abundant literature which deals with methods, limits, and advantages of tagging fish (e.g., Bégout et al., 2016; Bridger & Booth, 2003; Hastein et al., 2001; Macaulay et al., 2021).

Ideally, the behavior, growth, and survival of tagged and untagged fish should be similar. When a tagging strategy is designed, corresponding literature should be reviewed, and the pros and cons of specific methods should be considered.

External tags and marks can be used for visual identification, whereas internal tags or marks usually require specialised equipment for detection and identification. The solutions have been detailed hereafter which can be applied to sturgeon species.

### 7.3.1 External Tags

External tags are visible structures which are usually attached to the fish by piercing tissues (McFarlane et al., 1990). Such tags, which may carry an individual code, batch code or visible instructions, can be easily detectable without specialised equipment. External tags include a variety of tagging material such as ribbons, threads, wires, plates, discs, dangling tags, straps, etc. (McFarlane et al., 1990). However, most commonly used in research targeting sturgeon are either T-bar tags (Hamel et al., 2012), Floy Fingerling tags (FFT; Mihov et al., 2022; Paraschiv et al., 2006), or Wire On Tags (WOT, Marie-Laure Acolas, pers. comm.).

By permanently penetrating the skin, the tag may provide an access route for infection. In the hatchery, the use of external tags should be limited due to the risk of abrasion in the tanks, but in a natural environment it can be useful to easily detect recaptures. External labels are easily visible, allowing observation during handling and facilitating qualified reporting of bycatch. Before inserting the external tag, skin disinfection is required, diluted (1:10) $H_2O_2$ or vetedine is recommended, but these substances should not come into contact with fish gills. To apply such external tags in sturgeon, the tag is usually applied at the basis of the dorsal fin to maximize retention rates. T-Bar tags or Floy tags are usually applied through the base of the dorsal fin (Figure 21). Hamel et al. (2012) observed 100 % retention rates of T-bar tags in shovelnose sturgeon (421-720 mm FL) over a timeframe of 98 days. However, the fish were kept in a hatchery.

WOT are attached using a hollow needle through which the wire in inserted. After removing the needle, the two wires are twisted to secure the fixation (Figure 21).

The FFT are used to monitor YOY sturgeon in the Lower Danube (Mihov et al., 2022; Paraschiv et al., 2006). These are usually small plastic tags which contain individual numbers which are sewn through the base of the dorsal fin.

In general, the optimal location for above-mentioned tags is through the base of the dorsal fin, interlocking the tag with the bony structure. However, external tags can be lost in a short time and subsequent recaptures may go unnoticed. Regarding the tagging procedure, the same basics as for PIT tagging should be applied. Heavy biofouling can be observed especially in freshwater, making tags unreadable.

### 7.3.2 Internal Tags

The need to identify fish, individually or by group, with minimal influence on behaviour, health or survival has led to the development of internal tags. Even though a variety of internal marks is available, only the most commonly used options are described here. A more detailed description of different tagging techniques is given in the Technical Guideline for EX SITU Conservation Measures in Sturgeons. (Gessner et al., 2024).


***Figure 21:*** *T-Bar (upper left, © BOKU, T. Friedrich), FFT (lower left, © DDNI, M. Paraschiv), and WOT (right, © INRAE, M. L. Acolas), all applied through the base of the dorsal fin.*

#### *7.3.2.1 PIT*

RFID (Radio Frequency Identification) tags, often called passive integrated transponder tags (PIT tags), can be read by an external antenna. Once implanted, they provide a non-invasive and non-destructive means of individual identification (Gibbons & Andrews, 2004) by hand-held readers and in-stream antennas located on the bottom of rivers or in fish passes (Downing et al., 2001). PIT tags are the most commonly used tag for fish biological research and they outperform external tags in tag retention (Moser et al., 2000). Each PIT tag has an individual code and tag retention is variable, depending on the implant site and species, but is usually high (Briggs et al., 2019; Hamel et al., 2012; Liss et al., 2022).

Even though PIT tags are widely used, it must be ensured that each institution involved in sturgeon monitoring has access to functional readers to avoid missing detections of tagged fish. Furthermore, each institution in a catchment should ensure that the tags they are using are compatible with the readers of other institutions and vice versa. Another consideration are the costs of using PIT tags. Currently, they cost between 2-4 Euro apiece, which is quite expensive. Cheaper alternatives are available but come with the disadvantage of higher levels of malfunction. Whenever PIT tags will be used, the number of malfunctioning tags should be identified.

For sturgeon, an optimal location for PIT tagging is under one of the anterior-most dorsal scutes (second, third, scute) (Briggs et al., 2019). To insert the PIT tag, the use of sterile/disinfected needles is required. The needle should be inserted no more than the opening face and the PIT tag should be gently pushed underneath the skin via the syringe without moving the needle (Figure 22). The needle can be restricted from entering too deep by using the index finger. After removing the needle, it is recommended to check the presence of the PIT tag with a hand-held reader to avoid releasing a fish with unread or malfunctional tags. To avoid the use of malfunctional tags, each tag should be checked prior to the tagging procedure. The regulation and legal requirements for the respective project or country determines if anesthetization of the fish is necessary. Usually, the procedure of PIT tagging only takes a few seconds, hence, the use of anaesthetics should be evaluated based on legal requirements and the expected stress to the fish. During the procedure of tagging, the fish has to be gently secured to safely deploy the tag. This usually requires two people, one to hold the fish and another to tag it, though it might change with the size of the fish.

PIT tags are available in various sizes, and the larger the tag, the higher the detection range. Individuals above 25 cm can be equipped below the anterior-most dorsal scutes with 12 mm long PIT tag (2 mm in diameter, weight 0.1 g) (current practice in sturgeon hatcheries in Austria, France, Germany, etc.). Moser et al. (2000) reported retention rates of only 50 % for *A. oxyrinchus* below 20 cm due to the lack of musculature at this size; the PIT tag was placed posterior to the dorsal fin, where tissue growth is least (Moser et al., 2000). Similar observations were made for *A. ruthenus* (Thomas Friedrich, pers. comm.). A study highlighted that 8.4 mm PIT tags can be safely used for shovelnose sturgeon from 8 cm (Schumann et al., 2017) when inserted into the abdominal cavity. Fish between 80-120 mm had tag retention rates to 49 days of 97% and fish between 40-70 mm 60%, respectively. Very small microtags are also available and could be useful in the hatchery to individually identify very small fish. A study on *A. baerii* highlighted a retention rate of 77% of said microtags in the abdominal cavity for fish of 14 cm, however, the study was not focused on microtags, (Carrera-García et al., 2017) and this can be improved for sturgeon as it is successful on other species (Cousin et al., 2012). The distance of detection in microtags is very small at present and they may not be detectable once the fish has grown, especially by fixed antennas within streams; therefore, they are considered temporary tagging.

Different locations to set the PIT tag have been tested. In pallid sturgeon, Hamel et al. (2013) tested insertion into the operculum and along the base of the dorsal fin of age-1 individuals. After 189 days, retention rate was 83 % for tags inserted into the operculum (mainly for fish around 26 cm and during 60 days after tagging) and 85 % for tags inserted near the dorsal fin (mainly for larger individuals 30 cm and continually during the experiment). The position below a front dorsal scute seems to be the one with the highest retention rate (99 %) in Briggs et al. (2019) for *A. fulvescens* but the smallest fish tagged were 60 cm. Liss et al. (2022) observed PIT tag (12 mm) retention rates of 96 % after 101 days in white sturgeon with a mean length of 385 mm. The tags were inserted into the dorsal musculature next to the dorsal fin with a needle and syringe. Hamel et al. (2012) tagged shovelnose sturgeon (421-720 mm FL) with 12 mm tags in the same location next to the dorsal fin and in the operculum. Tag retention after 98 days was 73 % next to the dorsal fin and 77 % in the same area when sealed with cyanoacrylate. Tag retention in the operculum was 92 %. The region below the pectoral fin is also used for PIT tagging of broodstock as well (Chebanov and Galich, 2010).

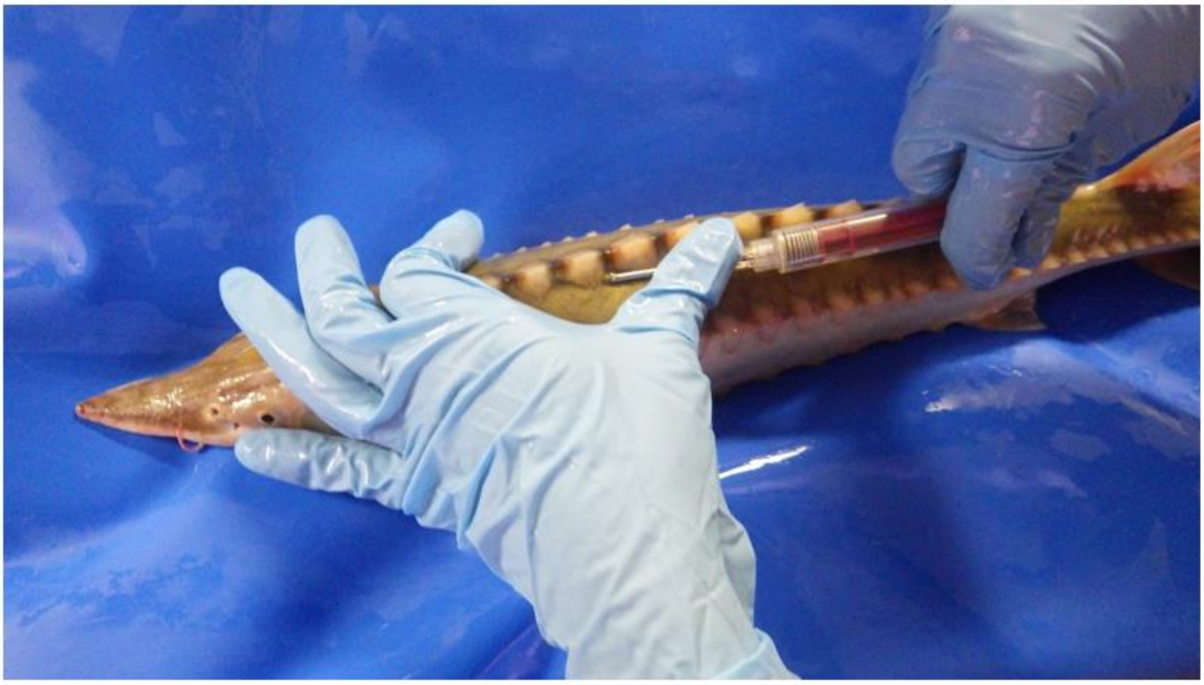

***Figure 22:*** *PIT tagging of a sturgeon below a dorsal scute (© INRAE, R. Le Barh).*

#### *7.3.2.2 Coded Wire Tags*

Some internal tags include plastic or glass tubes, metal plates and small pieces (size 0.5-2 mm × 0.25 mm) of magnetised stainless steel that may have a binary code of Arabic numbers engraved or laser etched on their surface. The latter, known as coded wire tags (CWT) are extensively used for identifying large numbers of fish and, due to their small size, can be used on fish of a large range of sizes.

In sturgeon, they can be inserted into the rostrum, under the scutes (USFWS, 2019), or into the first pectoral fin ray (Paraschiv et al., 2006). For tag detection, a hand-held metal detector is required and to obtain the code, the tag has to be removed from the fish. USFWS (2019) recommended tagging pallid sturgeon between 50-70 mm with CWT. CWT are also used for several species of sturgeon in the Caspian Sea from 3 g (Fadaee et al., 2006). In Romania, more than 600,000 released sturgeon are tagged with CWT, which helped in identifying returning hatchery released Russian sturgeon (Marian Paraschiv, pers. comm.).

#### *7.3.2.3 Visible Implant Elastomer*

An alternative which can be used for batch tagging comes with the visible implant elastomer tags (VIE). These tags consist of a biocompatible two-part fluorescent silicone elastomer material that is mixed and injected into tissue as a liquid with a hypodermic syringe. After 24 h at room temperature, it cures into a pliable solid, providing an externally visible internal mark. The fluorescent elastomer is available in several colours. Recognition of individuals is possible through the use of different body locations and colours as is the marking of very small fish (<30 mm, Frederick, 1997; Olsen & Vøllestad, 2001). Even though VIE can be used to tag a large number of fish with limited resources, identification of a large number of individuals is not possible in the same extent as compared to PIT or other external tags.

Depending on the number of batches, the tagging plan should be prepared in advance. By combining colours and site of injection, several batches can be distinguished (USFWS, 2019). It is recommended to use a two-mark combination per batch in case one mark disappears.

The elastomer should be prepared just before tagging and only the amount necessary for the tagging session as there is a limited time to inject it before it begins to harden. For injection, the needle should be inserted in a flat angle for a few millimetres below the skin and the elastomer injected at the same time as the needle is removed with application of constant pressure on the syringe. To prevent the elastomer from escaping, a stop with the needle a few millimetres before removal should be made.

The rostrum is the most common place for VIE injection (Figure 24, current practice in Austrian sterlet hatchery) but injection under the eye, in the opercula or within the scutes for small fish are promising (Figure 23, current practice in French *A. sturio* hatchery). In *A. oxyrinchus*, Kapusta et al. (2015) reported a survival above 90 % and a retention rate of the VIE of 100 % in the rostrum and of 93.5 % at the base of the pectoral fin 8 weeks after tagging; tagged fish were between 10 and 17 cm. In pallid sturgeon, the minimal size recommended is 7 cm (USFWS, 2019). Kozłowski et al. (2017) tagged fish as small as 5 cm with a 90 % retention rate in the rostrum after 70 days; the survival rate was similar with controls but it was generally low (40 %). Moreover, VIE marks can eventually be covered by overgrowing tissue and hence may become invisible over time (Olsen & Vøllestad, 2001).

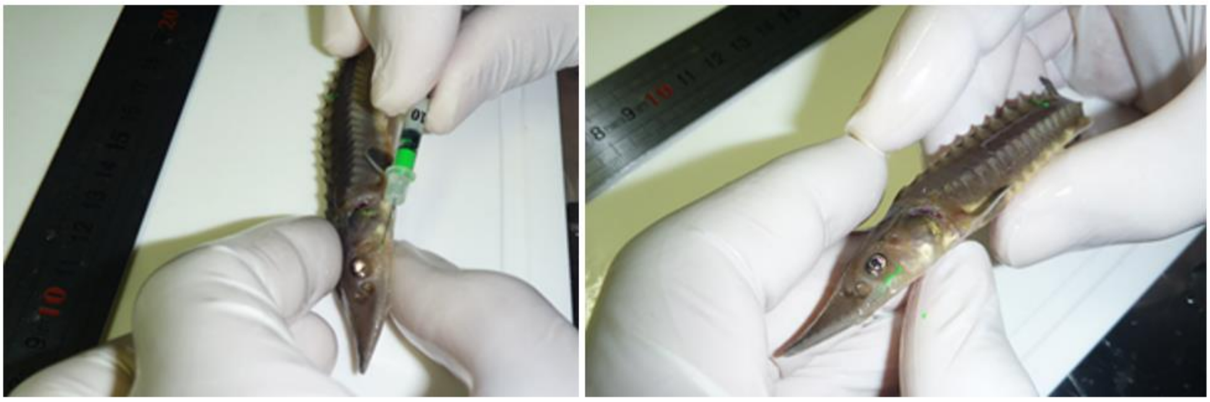

***Figure 23:*** *Injection of VIE below the eye (© INRAE, M. L. Acolas).*

***Figure 24:*** *Injection of VIE in the rostrum (© INRAE, L. Jacob).*

To separate different cohorts, different colours can be used for each year class. One example as it is applied in the LIFE Boat 4 Sturgeon project for stocked and wild fish is as follows:

***Table 4:*** *Example of a colour scheme.*

| **Year** | **Colour** |
|---|---|
| 2023 | Red |
| 2024 | Blue |
| 2025 | Orange |
| 2026 | Green |
| 2027 | Red |
| 2028 | Blue |
| 2029 | Orange |
| 2030 | Green |
| | |
| Wild fish | Pink |

As with external tags, marked fish can be recognized without the necessity of additional equipment such as PIT readers. It may therefore be useful if the population of interest is exposed to commercial fishing. However, the visibility of VIE highly improves under UV light, which is why the use of a UV lamp is recommended.

## 7.4 Age determination

Age determination is required to determine growth, population structure and demographics, size at age, and interannual mortality. It is recommended to determine the age of individuals on a regular basis as a supplement to length-frequency-diagrams to allow the establishment of a reliable representation of a population structure.

To facilitate age estimation in sturgeon, a 2 mm slice of the first pectoral fin ray is removed after anaesthesia, approximately 5 mm from the articulation (Figure 26), using a 24/1 tooth hacksaw (Figure 25). The material is stored in paper sample pouches in a cooler for further processing (species, sample ID and protocol no as well as date are to be noted). The fin ray regenerates within a year (Figure 27).

***Figure 25:*** *Removal of a small part of the first pectoral fin ray (© INRAE, M. L. Acolas).*

***Figure 26:*** *A small (~2 mm) piece removed from the first pectoral fin ray for age determination (© INRAE, M. Lamour).*

Side cutters can be used on smaller sturgeon; however, this will cause the pectoral ray to crack on large sturgeon, thereby making it difficult to section and age. It is important to obtain sections from as close to the articulation as possible, without causing haemorrhaging, to ensure annuli are not lost. A knife is used to cut the posterior end of the leading pectoral ray from the fin along the section to be removed. Diamond saws are used to cut >1 mm thick slices, which are then sanded/polished/burnt for microscope analysis. The preparation of the sample and the age determination process are explained in detail in (Izzo et al., 2021; Wilson, 1987).

***Figure 27:*** *Partial removal of first pectoral fin ray (right) and regenerated first pectoral fin ray within one year after removal for aging. Practice has shown that smaller pieces are sufficient for analysis (© T. Haxton).*

## 7.5 Sex determination

To obtain a comprehensive picture on the state of the population, the identification of sex and eventually the stage of maturity of a given fish is a vital element. Sexing in wild fish can be difficult since the sexual differentiation to a degree allowing macroscopic sex determination occurs only late in life, usually after 3-4 years. Secondly, the determination requires invasive surgical methods to be performed. An alternative approach using ultrasound diagnostics is well established in aquaculture (Chebanov & Galich, 2011; Webb et al., 2019) but is challenging under field conditions due to the technical prerequisites that are essential, among which include the costly equipment, the necessity for waterproof positioning on board and the protection of the screen against reflections and sunshine are the most important. Furthermore, extensive training is required to reliably determine sex in early stages of development up to stage 3.

Newly developed sex markers using genetic analysis are proving proficient for sturgeon and are an easy and cost-effective way to determine the sex-ratio as they can be conducted along with other genetic analyses (Kanefsky et al., 2022; Kuhl et al., 2021; Scribner & Kanefsky, 2021).

A visual determination of the sex based on morphological traits was done successfully on >80 % of sterlet (n = 29, Neuburg & Friedrich, 2023) but a double-check with other methods is recommended. Kahn et al. (2021) identified the sex for Atlantic sturgeon during a surgery when implanting telemetry tags or when the fish expressed gametes when pressure was applied to the ventral surface of the body. This kind of sex determination is only possible during spawning season, however.

### 7.5.1 Ultrasonography

Ultrasonography, as the least invasive tool to assign sex and phase of sexual maturity, has been the fastest growing technique, and has been used on most sturgeon species and their hybrids (e.g., Gessner et al., 2024; Chebanov & Galich, 2011). The accuracy of this technique (68-100 %; Webb et al., 2019) depends on the quality of the ultrasound equipment, the species, the age or body size, phase of maturity, and the degree of sturgeon expertise of the ultrasound technician. Ultrasound can provide immediate identification of sex and phase of maturity and images can be saved for further analysis.

### 7.5.2 Celiotomy/Biopsy

Celiotomy is a surgical incision of the abdomen that allows for direct observation of the gonad by eye or by endoscopy and when appropriate, collection of a gonadal biopsy for histological processing and analysis. Like endoscopy, this technique can provide immediate determination of sex and the exact phase of gonadal maturity, which can then be verified with histological analysis to determine stage of gonadal maturity (e.g., Chapman & Van Eenennaam, 2012; Doroshov et al., 1997; Falahatkar et al., 2013; Webb et al., 2019). The accuracy of this technique (100 % if the gonadal tissue is sampled; Webb et al., 2019) depends on the individual's

knowledge of sturgeon phases of gonadal maturity, expertise in biopsy collection of gonadal tissue, and the availability of a histology processing lab. If the fish is a spawning capable female, ovarian follicles may be collected.
The procedure takes approximately 5 minutes per fish to make the incision, to view the gonad by eye or through endoscopy, to biopsy the gonad if desired, and suture the incision closed. Details of the procedure, surgical instruments required, and suturing techniques are given in Chapman & Van Eenennaam (2012) and Webb et al. (2019).

Briefly, an individual fish, whether anesthetized or not, is placed in a holding device, such as a hooded-stretcher, ventral side up, with fresh oxygenated water irrigating the gills. An incision (1-3 cm) is made approximately 1-3 cm off the ventral mid-line, opposite 3-5 ventral scutes anterior from the pelvic fin, and the exact position depends on species and body size. The goal is to make the incision directly above the gonad. The incision is opened using Adson-Brown tissue forceps and a pair of Allis forceps to view the gonad by eye or with an endoscope. The Allis forceps or Miltex cup jaw biopsy forceps can be used to collect a gonadal tissue sample. This incision is then sutured closed with a Cruciate (cross-mattress, interrupted-X, or multiple single stitches).

## 7.6 Genetic Sampling

In order to assess the genetic diversity of a population or other population specific characteristics, to genetically sex a fish, or to obtain estimates of the number of breeders contributing to the offspring, it is necessary to take genetic samples for analysis (e.g., Roques et al., 2018). It should be clear which type of analysis should be done prior to taking the samples. If several analyses are planned, the sample taken needs to be larger. It is recommended to coordinate with the geneticists in charge of the analysis to make sure what amount of tissue is necessary. Genetic sampling can be carried out by collecting tissue samples such as a piece of fin or mucus swabs. To prevent contamination of samples, clean sampling is essential. Sterilized equipment (cotton swab, scissors, forceps) can be required, depending on the planned analysis.

### 7.6.1 Fin clips

Sampling a fin has the advantage of being less susceptible to contamination than a mucus swab, but it results in a wound on the fish, which may be negligible in the case of larger animals but significant in the case of smaller ones. For this reason, especially with small animals, it is necessary to take some precautions. It is vital though, to not remove the fin entirely but to only obtain a piece of fin; preferably the pelvic or anal fin to avoid major impacts on the mobility of the individual following release. In the case of large animals, it is possible to remove a strip of approximately a few centimeters in length and two to three millimeters thick from the margin of the fin, so as to not affect highly vascularized tissues.

The rim or tip of the fin is held with forceps and a piece of tissue is removed with scissors or a scalpel. The tissue section is transferred immediately to a prepared Eppendorf tube with >90 % pure ethanol. The vial is sealed and the sample number is marked on the vial with an ethanol resistant label. Replacement of the ethanol with fresh ethanol after a few days will improve the shelf-life and therefore quality of the sample during long-term storage (Steven Weiss, pers. comm.). In the absence of ethanol-resistant labels, it is possible to insert a strip of paper into the ethanol together with the sample after writing the code with a pencil; pencil markings are not removed by the ethanol. If the sample is not stored in 90 % ethanol, it can be frozen at -20 °C (stored in a cooler in an Eppendorf tray during processing of the catch). It is recommended to clean scissors and tweezers between each sample of different fish in order to avoid cross-contamination of the sample.

As during the tagging procedure, each fish should be gently secured while taking the genetic samples to avoid injuries of either the fish or the executing person. Moreover, depending on the locality where the research is conducted, permits may be necessary to transport the samples and legal requirements should always be checked before conducting any research activities.

### 7.6.2 Mucus swab

An alternative for fin clipping is the use of sterile cotton swabs to scrape mucus off the skin of the fish (Ignatavičienė et al., 2023). While this method is not invasive and sampling permits may not be required as compared to fin clipping, it has several major disadvantages.

Fish mucus, a complex glycoprotein that is produced continuously in specialized cells of the skin, is a protective device that reduces pressure on the fish from ectoparasites, bacteria and fungus. It furthermore reduces friction during swimming and adds to the barrier function of the skin against the osmotic effects of water on the ion contents of the body. Since it is produced by the skin, it contains cells and fragments of skin tissue that allows the extraction of DNA. While at first glance the sampling of mucus may look like an easy and relatively harmless option to collect DNA from the animal, the method comes with some drawbacks.

The sampling of mucus swaps requires clean cotton swabs and a sealable sterile tube. The mucus swab is usually taken from the ventral side of the fish or along the fin base. Essentially, the mucus barrier is damaged in order to ensure that the sample contains cellular elements. As such, the sections where samples have been taken have a higher risk to be invaded by pathogens.

The swab is transferred immediately to a prepared Eppendorf tube with >90 % pure ethanol. The swab is cut off the supporting stick with robust scissors to fit into the tube. The vial needs to be sealed and the sample stored in a freezer at -20 °C.

The main drawback is the fact that the Glycoproteins camouflage the DNA and as such the isolation of DNA is more complicated and requires additional purification steps. The sample is prone to decay and as such, immediate cold storage is required as storage in ethanol is not feasible. Furthermore, the intensive contact of the fish while sampling, for instance in trawls, increases the potential of cross contamination with the risk to increase the amount of non-target DNA in the sample. Additionally, it is likely that the mucus will contain a significant amount of exogenous DNA that can be detected by genetic analyses which do not employ strategies to target analysis solely on sturgeon DNA (RAPD, AFLP, RAD-seq methods, etc.). Finally, this approach yields less genetic material as compared to the fin clip in terms of collected genetic material.

It is for the variety of reasons given above that a small piece of fin clip is the much better option for genetic analysis, minimizing risk for the fish, providing reference material and improved storage. The shelf-life of fin clip samples is also much higher than mucus samples.

### 7.6.3 Analysis of genetic samples

There are several methods which can be used to analyse genetic sample, always based on the research question of a specific study. The choice of the genetic approach to be used depends on the evaluation of various parameters, with the primary one being the biological question one aims to address. However, economic feasibility, the laboratory's expertise in conducting the analyses, the urgency of obtaining results, the need for reproducible data, the number of samples to be analysed, the requirement to compare the analyses with existing data, and many other variables also play a significant role in the decision-making process. The choice of the approach to be used should be made in collaboration between those responsible for sample collection and those who will conduct the genetic analyses; indeed, as previously mentioned, the choice of the approach to be used depends on the type of sample that needs to be collected. Conversely, the choice of technique to be used depends on the available samples.

Special consideration should be made for studies aimed at identifying diagnostic markers for a specific condition. This is the case, for example, in species identification, geographic allocation of samples, or gender identification. In these cases, the analyses are based on comparing samples belonging to different categories to search for diagnostic markers that can be applied to animals for which the category of membership is not yet known. In these and similar cases, it is essential that the animals used as "standards", for which the condition is considered known, are absolutely reliable. Animals for which the condition cannot be certified a priori should not be included in the analysis, as they could undermine the study.

Some examples of genetic analyses done at the population level have aimed to discriminate different populations (Holostenco et al., 2022; Kohlmann et al., 2017, 2018), to describe the population structure and conduct parentage and kinship assignments (Roques et al., 2019), to reveal the origin of individuals as well as hybridization (Dudu et al., 2011, 2022; Friedrich et al., 2022; Ludwig et al., 2009) and to estimate the population size based on pedigree analyses (Friedrich et al., 2022).

## 7.7 Gastric lavage

The identification of main food organisms for different life phases of sturgeon may lead to certain habitat types where such organisms are abundant and encounter favourable conditions to build large and dense populations.

A variety of techniques for nonlethal sampling of stomach contents have been developed for sturgeon, but gastric lavage is recommended. Gastric lavage is relatively cost effective, not too labour intensive, and reasonably safe and effective. Although this automatically extends handling time, use of anaesthesia is encouraged to minimize the risk of injury during the procedure. Relatively flexible small diameter tubing is an essential part of this procedure. Tubing with 2 mm inside diameter were used to successfully sample stomach contents from sturgeon between 18-58 cm TL (Margaritova et al., 2021). High flexibility of the tubing is mandatory to prevent injuries or ruptures of the walls of the alimentary canal. Aquarium tubing and the like should be used due to their rigidity and stiffness. Intramedic type tubing is most suitable due to its ductile nature and small diameter. The leading edge of the tubing should be blunted. While the flexibility of intramedic tubing seems to protect sturgeon from injury, it takes some practice to get the tubing into the esophagus. Forcing water out of the tubing while inserting the tubing into the alimentary canal may help entry and prevent puncturing the walls of the canal by the tube. Researchers must take extreme care to prevent forcing the tubing into the fish and thus causing damage. Gently moving the tube in and out while pumping seems to enhance the effectiveness of regurgitation. Large diameter tubes have been utilized by researchers to aid in inserting the flexible small diameter tube down the esophagus; they have been used as a sleeve to assist in getting the highly flexible small diameter tube past the oral cavity (Brosse et al., 2002). This technique works well for some researchers and better for some species. Sampled fish should be kept separately after the procedure because they may regurgitate some stomach contents after the flushing procedure itself (Borislava Margaritova, pers. comm.).

Syringes, garden sprayers (approx 9.5 l) as well as hand operated and electric pumps have been used to provide the "flushing water". Regardless of the water delivery device used, the amount of applied water pressure should be limited to protect the fragile internal organs. Therefore, if high volume or pressure pumps are used, a flow/pressure restricting device is imperative. Positive results have been noted for both continuous water flow and pulsed or interrupted flow. Conducting lavage under freezing weather conditions may present unique dangers to the fish.

General guidelines discourage exposure of fish to air temperatures below freezing for more than a couple of minutes, rendering lavage ineffective, as it would be difficult to collect stomach contents while keeping a sturgeon submerged in water.

Highly stressed individuals (from temperature change, capture stress, or other means) should not be subjected to lavage techniques.

The stomach content can be identified via taxonomic traits under the microscope (Strelnikova, 2012) or through metabarcoding techniques similar to eDNA sampling (Rebecca Tibbetts, unpublished data).

# 8 Data Analysis

The techniques used for data analysis depend on the objectives and research questions and depend upon the methods chosen to sample the respective life-cycle stages. A sampling design that is developed to meet the objectives of the study is essential to ensure that collected data allow to conduct proper analyses and conclusions. The data obtained must reflect the state of the sampled population. Since the goal of a monitoring program is the establishment or evaluation of management practices, the measures cannot be better than the underlying data that inform them.

In this chapter, several techniques and methods to analyse monitoring data are exemplified. Techniques and methods described here focus on sturgeon literature and the respective analyses that are suggested. Several books, book chapters and articles (e.g., McComb et al., 2018; Powell & Gale, 2015; Sanderlin et al., 2019; Williams et al., 2002) are focusing on the analysis of monitoring data in detail.

## 8.1 Describing a population

When working with a population, it is important to know the species occurring, their size and age distribution, the associated sex ratio, and the genetic characteristics of the population etc. Examples for the genetic assessment of populations are given above (chapter 7.6.3). Here, some examples describing a population morphologically will be provided.

The most basic procedures upon capturing sturgeon include measuring and weighing the fish, providing valuable information about occurring length classes which – at least for the first two to three years – can be assigned to the age of the fish (length-frequency (L/F) distribution, Figure 28), as well as on the condition of the sampled population with regard to missing year classes or condition factors of single individuals (Figure 29). Analysing length distribution informs about occurring or missing year classes (Neuburg & Friedrich, 2023) or might show habitat preferences of specific life-cycle stages (Haxton et al., 2018).

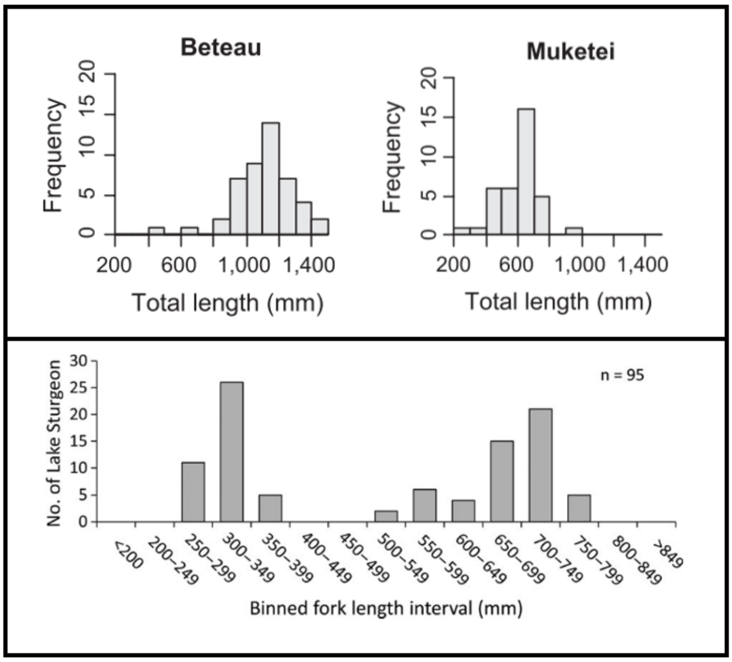


***Figure 28:*** *Examples for length-frequency diagrams. Haxton et al. (2018) showed site-specific L/F distributions indicating differing habitat preferences of juvenile and adult lake sturgeon (upper L/F diagrams) and the L/F diagram produced by McDougall, Pisiak, et al. (2014) shows clear size differences between age-0 and age-1 lake sturgeon (lower L/F diagram).*

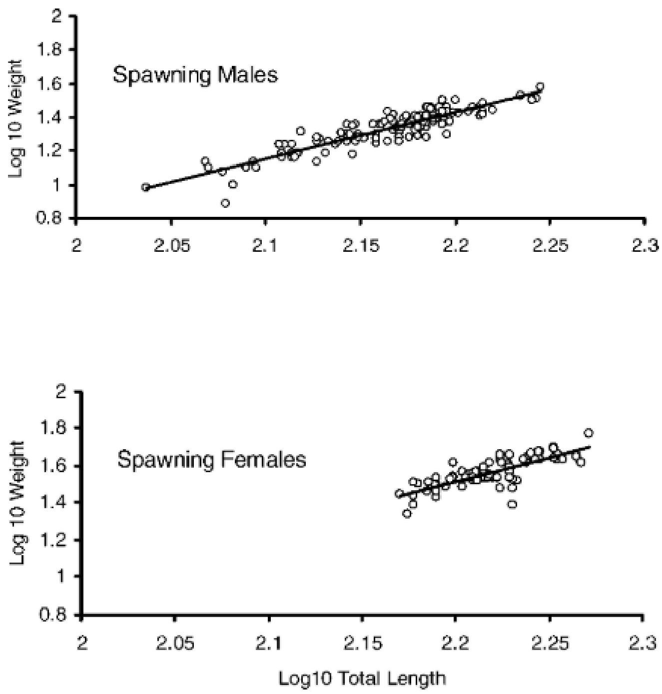


***Figure 29:*** *Differences between spawning males and females based on their L/W relationships (Smith & Baker, 2005).*

Additional incorporation of age analyses can yield information about demographic differences among populations or changes in a population over time (Haxton,

2006) or yield growth rates for young fish (Auer & Baker, 2002; Paraschiv & Suciu, 2005). Even though Haxton (2006) interprets the differences of the relationship between age and length between the samples taken 50 years apart (Figure 30) with caution due to possible aging mistakes, some significant differences in the population could be observed. Kennedy et al. (2007) obtained mortality rates for specific age classes using the information from aging sampled fish.

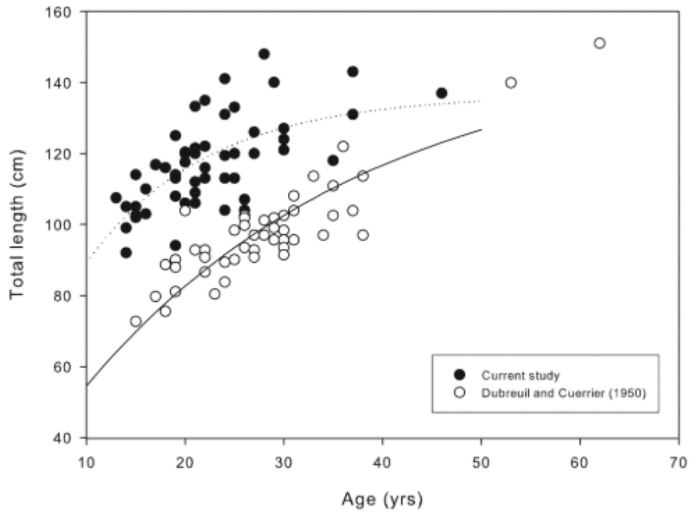


***Figure 30:*** *Differences between age and length plots of a lake sturgeon population that was sampled in 1950 and between 2001-2004 (Haxton, 2006).*

When, in addition, sexing of the fish is carried out (see chapter 7.5), the description of the population increases in detail and estimates of spawning populations can be conducted (Kahn et al., 2021). Cox et al. (2022) showed mass ovarian follicular atresia occurring in pallid sturgeon, which affects the actual size of any spawning run as atretic females may join a specific spawning run but cannot contribute to that year's reproduction. Follicular atresia generally occurs when spawning conditions are not conducive (Webb et al., 1999).

Morphological measurements and results from genetic analyses provide a detailed picture of the status of the population. In addition, the data serve as the basis of a population description and as input data for any further modelling or can increase the detail of any models.

## 8.2 Modelling population parameters

To obtain population parameters that inform about recruitment or survival rates in the population, it is necessary to utilize more complex models. Those models always have prerequisites that need to be met in order to obtain reliable results.

Moreover, they require standardization to make samples comparable since some parameters can only be obtained from multiannual sampling (survival, recruitment, movement, capture probability, etc.).

Obtaining the above-mentioned parameters usually requires repeated sampling over the course of years and therefore, the underlying assumption is that sampling targets an open population with occurring recruitment, deaths, immigration, and emigration, which resultingly affect the population size and composition.

Estimating survival rates yields important information about the stability of the population or specific life-cycle stages. This parameter can be obtained from Cormack-Jolly-Seber (CJS) models (Cormack, 1964; Jolly, 1965; Seber, 1965) and require uniquely marked individuals (Powell & Gale, 2015). Next to estimates for survival, estimates for capture probability can be obtained through these models as well. Kahn et al. (2023) used this type of model to estimate both survival and capture probability of the York River population of Atlantic sturgeon with data obtained from an acoustic telemetry array over seven years. Survival was very high, which can be expected from an adult sturgeon population. However, the authors were able to show a clear difference between female and male capture probability and an effect of the mean length of the fish, providing important information about the ecology of this species. Since capture probability is an important parameter to estimate population size, it should always be accounted for (Kahn et al., 2021). Also, since varying capture probabilities could result in biased population estimates (Hightower & Gilbert, 1984; Nichols et al., 1981), the quality of the data should be maximized to provide reliable input data.

An index of cohort strength or of juvenile abundance (Haxton & Friday, 2020) might be helpful to track population changes and assess future population trends. The modelling of recruitment can be carried out applying one of several models. McDougall, Pisiak, et al. (2014) showed differences in the relative recruitment success between age-1 and age-0 lake sturgeon using a simple model based on the relation between captured and stocked individuals. However, they were not able to identify recruitment into the population but rather differences between life-cycle stages which might inform stocking practices. Modelling recruitment into the population was done using different open population models including Pradel (Pradel, 1996) or POPAN (Schwarz et al., 1993; Schwarz & Arnason, 1996) models. Dieterman et al. (2010) used both approaches to estimate recruitment in a lake sturgeon population over a 15-year period, showing that the population has just been maintaining itself. Nevertheless, the authors found a negative development of the population ($\lambda<1$), especially in the latter years of the study, which they explain due to lack of recruitment with possible effects from sand deposition after a dam removal. Recruitment deficiencies in the Lower Fraser River white sturgeon population was shown using a Bayesian model (Nelson et al., 2020) and an integrated spatial and age mark recapture model (ISAMR; Challenger et al. (2017, 2020)). Those estimates result from a high angling sampling effort between 1999-2019 with >140,000 captures analysed. Hence, getting detailed insights into the recruitment process of a sturgeon population requires a long-term monitoring plan as well as a standardized sampling approach. The detailed results from the Lower Fraser River could only be realized through a sampling program that relies on recreational angling for white sturgeon and the voluntary work and data reporting of angling guides, resulting in a very high, continuous effort.

However, working with sturgeon might often result in captures of only a few individuals per year (Counihan et al., 1999), especially in European rivers (Mihov et al., 2022; Neuburg & Friedrich, 2023; Paraschiv & Suciu, 2005; Rochard et al., 2001), where sturgeon populations are considerably small. Hence, obtaining data about specific population parameters may not be easy to acquire but proper design of sampling based on specific research questions can even provide important information about population size (Kahn et al., 2014) or recruitment success (Counihan & Chapman, 2018).

## 8.3 Population size - Capture-Mark-Recapture (CMR) and other methods

The assessment of population dynamics and population trends to understand how populations arrived at a given state and how it might develop in the future, is of high importance for the management of fish populations (Pope et al., 2010). Capture-Mark-Recapture (CMR) studies provide opportunities to estimate recapture rates, population size, to obtain mortality assessments, as well as to track population trends. Incorporating information on fish weights, lengths, sex ratios etc. can increase the information gain (Pope et al., 2010) and the understanding of processes on the population level.

However, in order to generate reliable results, a CMR study requires consistency. For example, if population sizes are to be analysed and the change in size is of interest, it is important to keep the sampling effort similar between years.

A CMR study always includes the marking of animals to some extent. The marking method largely depends upon the aim of the study. If recapture analysis is restricted to scientific personnel or only utilizes dead fish landed in the region, PIT tags are the prime choice. Tag loss in PIT tags, when properly applied, is minimal and the tag remains functional (if not broken) during the entire life of the fish (Gibbons & Andrews, 2004). An alternative tagging method, which allows visibility of the tag and as such does not require additional equipment for the identification of a tag, are external tags. They are especially useful if fishermen are to identify and report the fish captured. Their application has the disadvantage that the tags are subjected to wear and tear. Overgrowth with tissues can limit the readability and recognition of the tag. Often, using both tag types combines their respective benefits and adds flexibility for future studies.

Alternately, individual sturgeon can be genetically identified if the sampling strategy permits. Individuals could be tracked over multiple sampling occurrences temporally (e.g., multiple years).

Close-kin mark-recapture techniques using genetic analyses can be employed to estimate population size if an adequate sample size from multiple generations is obtained (Scribner et al., 2022). This can be an effective means to estimate prior abundance if a collection of tissue samples from past projects is available.

Even though widely used, CPUE is considered unsuitable to assess fish populations. The problem being changing catchability with time, species, area, age class etc. (Maunder et al., 2006). Hence, CPUE might remain high while species abundance declines (Harley et al., 2001) but examples exist where CPUE correlates with species abundance (Steffensen et al., 2017). Small variations in CPUE between years might be sufficient for it to be used for analysing population changes however (Haxton & Friday, 2020), and it was suggested to make monitoring data comparable among countries (Mihov et al., 2022). Standardization in the used methodology may make CPUE comparable but it is paramount to consider catchability and not to ignore or consider it constant. Also, several ways to standardize CPUE to reduce impacts of factors other than abundance on the catch rate exist (Maunder & Punt, 2004). To track the development of sturgeon populations and to obtain robust estimates of population sizes, the use of different metrics and more complicated methods is usually recommended. However, given the scarcity of sturgeon in Europe, the necessary data quality for such models might not always be obtainable and thus the use of standardized CPUE data or zero-inflated negative binomial models (Minami et al., 2007) might be reasonable ways to obtain statistical veracity regarding sturgeon populations. Models based on the latter approach were used to obtain population estimates of sturgeon through N-mixture models (Hughes et al., 2018; Vine et al., 2019). N-mixture models were developed to assess spatiotemporal variation in abundance for small populations or populations with low detection probabilities due to their secretive habits (Royle, 2004) and do not require the marking of individuals. As all models do, N-mixture models come with a suite of assumptions, among them the closure of the population between surveys (Powell & Gale, 2015).

The determination of population size is of special interest if the development of populations is to be tracked. In the past, the assessment of spawning populations was attempted several times. A spawning population estimate can be determined through a CMR study (e.g., Haxton, 2006; Kahn et al., 2019; Neuburg & Friedrich, 2023; Paraschiv & Suciu, 2005; Steffensen et al., 2017). However, multiple years of sampling would need to be conducted to account for spawning periodicity of the species if an estimate of the mature segment of the population was sought (Haxton & Friday, 2019). The maximum spawning periodicity of females for the respective species would be the required duration of such a project. This would require use of an open population estimator as there would be mortality and recruitment through individuals maturing over the multi-year study. The size of spawning runs can be estimated during a single spawning period using closed population models (Kahn et al., 2014; Lallaman et al., 2008). In order to generate population estimates, a variety of methods can be used from simpler closed population models during a single spawning period (Hale et al., 2016; Kahn et al., 2014, 2019; Lallaman et al., 2008), more complex open population models (Caroffino et al., Sutton, & Lindberg, 2009; Paraschiv & Suciu, 2005), combined models (Steffensen et al., 2017), as well as Bayesian models (Nelson et al., 2020).

Alternatively, the effective number of breeders ($N_b$) can be estimated by genetic analyses of a cohort, which is effective for egg, larval and age-0 stages

(Blankenship et al., 2017; Friday & Haxton, 2021; Welsh et al., 2015). Furthermore, the number of spawners contributing to a population can be determined using pedigree accumulation analysis (Friedrich et al., 2022; Sard et al., 2021).

### 8.3.1 Study design and methods

When assessing population sizes or survival rates of sturgeon populations, study design is crucial and has to be adequate for the methods used for data analysis (Lindberg, 2012). In general, models consider the population in question as either open or closed. Closed population models assume that no demographic (no births or deaths) or geographic (no immigration or emigration) changes occur, while open models do allow those changes (Lindberg, 2012). In both cases, several prerequisites exist and must be met to ensure robustness of the estimates. Pollock (1991) summarized them as follows:

- **Closed population models**
    - The population is closed to additions and deletions
    - All animals are equally likely to be captured in each sample
    - Marks are not lost or overlooked
    - Marking does not affect catchability

- **Open population models**
    - Every animal present in the population has the same probability of survival until the next sampling time
    - Every animal present in the population at a particular sampling time has the same probability of capture
    - Marks are not lost and overlooked
    - All samples are instantaneous (short-term) and each release is made immediately after the sample

In general, closed population studies should only cover a relatively short period of time (Pollock, 1980, 1991) and a variety of models exist (Krebs, 2014; Otis et al., 1978; Ricker, 1975). In sturgeon research, they have been successfully used to estimate spawning runs of Atlantic sturgeon (Kahn et al., 2019) among others. Additional telemetry data can be used to confirm if fish leave the sampling area during the survey and, thus, help to verify the model assumptions. However, often the assumption of closure will be violated and respective models may only give uncertain estimates. Therefore, the use of open population models or a combination of open and closed models, for example Robust Design (Kendall & Nichols, 1995; Pollock, 1982), are necessary (Pollock, 1991). More complex models can be powerful and provide meaningful population estimates and trends (Nelson et al., 2020) but require proper planning and data acquisition during monitoring, resulting in an adequate amount and sufficient quality of data, as well as substantiated statistical knowledge.

When using population models, an important consideration when calculating the population size is the sampling intensity defined as the proportion of the population that is sampled per trip. To generate reliable results, capture probabilities should exceed values >0.1 (Hightower & Gilbert, 1984; Nichols et al., 1981) to 0.2 (O'Brien et al., 2005). Nevertheless, since it is impossible to determine capture probabilities prior to conducting a survey, the sampling strategy possibly needs to be adapted according to occurring capture probabilities. In general, the higher the sampling intensity and population size, the more precise the estimates of population size will be (Nichols et al., 1981). However, reliable estimates of the population size can also be made with lower sampling intensity, but only if survival is high and sampling size is large (Hightower & Gilbert, 1984). Sparse data combined with low capture probabilities have adverse effects on the quality of obtained estimates (White & Cooch, 2017). Both issues can easily arise when working with rare species (Lettink & Armstrong, 2003) and have to be considered in sampling design. Most often, the design providing the highest capture probabilities is the preferred design (Lindberg, 2012). As mentioned above, conducting CMR studies on sturgeon populations in Europe might not always be possible due to their scarcity. Therefore, the feasibility of such a study as well as suitable alternatives should always be evaluated in each individual case in order to allocate available resources most effectively and to get the most important information out of the survey in order to increase the knowledge about a given population.

Also, the timing of sampling is important and can influence model estimates due to differing sampling success. Passive methods, like static nets or stow nets, require animals to move in order to be captured. These aspects are critical to be considered in study design (Haxton & Friday, 2019). The chosen sampling area should be selected based on the highest probability of captures and safe access. Depending on the ecology of the species, different areas should be targeted during different seasons (Kahn et al., 2019; Paraschiv & Suciu, 2005; Rochard et al., 2001). If necessary, river sections can be divided into several sampling areas as was done in the Fraser River due to different river morphology and recapture rates (R.L.&L., 1999) in order to improve parameter estimates. It is important, however, to include the entire range of habitats when applying a stratified sampling approach in order to avoid biased estimates when omitting a random sampling approach. Moreover, the use of additional data about morphology, environmental characteristics or sampling gear and effort can improve parameter estimates and, therefore, it should be collected and used wherever possible (Lindberg, 2012).

The reliability of estimates and the resulting success of any monitoring program aiming to describe changes in population size, assess recruitment or the development of a sturgeon population, is dictated by a rigorous implementation of the program. If the size of a population is the target of any monitoring, it should be clear in the beginning which kind of model is applicable given the biology of the species and the available resources of the implementing institution. Since each model comes with specific requirements, they must be considered before designing the sampling. Next to the spatiotemporal sampling, the tagging method is important to be chosen mindful of the type of analysis that will be conducted. Questions like "Is it possible to mark every individual?", or "Is the marking of a cohort sufficient?" strongly depend on the research question and the amount of money available for any monitoring.

# 9 Working plan for sturgeon population monitoring

Any monitoring plan needs to be adapted to the specific circumstances in a given system, as suggested in the five-step plan in chapter 4.1. It might be possible that some things are known about a sturgeon population, yet others are completely unknown. Therefore, the importance of research questions regarding different populations may be different, depending on already available knowledge or prevailing management needs.

Since it is impossible to give a specific working plan for any catchment, no general working plan can be provided. In the following, steps are suggested to establish a proper management plan for any sturgeon population. But one must be aware that any management plan might be target to adaptive changes due to unsuccessful sampling or, in the best case, the ability to enhance the sampling campaign because the initial research question can be sufficiently answered and resources are available to answer additional questions.

**1) What do we know?**

Before beginning any monitoring on a sturgeon population, the status quo must be clear. It is important to get an overview on the available knowledge about a population. This can start with a comprehensive literature review and data mining on historic distributions of species or habitats or about currently implemented projects or bycatch information. Learning about historic habitats of a population ideally provides indications where to start with specific monitoring actions. One could ask "which sites were used and what were their abiotic properties?" and "are there similar sites in the current system?", which provide a direct link to the information provided by the respective habitat assessment. Another possibility is to look for information about a specific species from another system where it might still occur in larger quantities. Some of the information could be used to formulate hypothesis for the system in question.

**2) Definition of research aims/questions**

When some types of information such as historic distributions and properties of used habitats are available, or even information about recent but sporadic captures, it is vital to define a specific research aim or question. If there are sporadic captures of adults, possible research aims would be to describe the population genetically to identify its diversity, to assess the population size, or to collect information about habitat use.

Another example would be an already known estimate of an adult population but with no information about recruitment. Hence, one could be interested to find spawning locations, evidence of spawning, or nursery habitats that are used by YOY sturgeon. Whatever the aim is, it should be reflected through a specific research question. From the examples above, all target life-cycle stages require the application of different methods, different timing of sampling and different sampling sites. If the aims are unspecific, the results of the monitoring will suffer from this deficit. Even though results may be obtained, the quality of results will be higher and the information gained will be more accurate if the research aims are clearly addressed.

**3) Designing the sampling campaign and choosing the method(s) of sampling and analysis**

As soon as a specific research aim or question is decided on, the design phase of a monitoring program begins. The most important part is to know what results one aims to achieve and what are the relevant metrics to obtain because the expected results and prerequisites define the methods that can be used to collect and analyse the data which, in consequence, define the amount and quality of data that are required. Also, the expected results define which life-cycle stage to address. Regarding these aspects, the possible designs of the monitoring approach are already pretty constrained. Furthermore, it must be considered when, where, how often and for how long sampling should be conducted. If the goal is to gain an estimate of a spawning run, it might be necessary to design the sampling in such a way that a closed population model is applicable and, thus, the timing is short enough to achieve this, and the effort is intensive enough to obtain sufficiently high capture probabilities to get reliable estimates. In any case, it is important to choose the right area/site where a sufficient number of sturgeon can be sampled, which already requires some background knowledge on the timing of spawning, migration routes or spawning sites.

Whatever the research aim is, it must be clear which variables need to be collected in order to achieve the research aims and to feed the models with the correct data. Establishing a field protocol before conducting field work is highly recommended because it helps to collect all necessary data and to not forget any (see Annex 14.1).

Lastly, the available resources will dictate the possibilities of sampling. Even though the recruitment into the population might be of highest priority among research interests, sampling and data analysis are very time-consuming and hence cost-intensive. In the context of species recovery, key questions to be answered can be ranked as low, medium and high priority. And, within this ranking, the best cost-benefit method can be chosen (see Table 2).

### 4) Interpretation of results, data storage and planning of further steps

When the initial sampling campaign is completed and the collected data analysed, it is important to interpret them correctly, always mindful of the restrictions of applied methods for sampling and analysis.

After data interpretation of collected monitoring data, one stands at the beginning of the suggested monitoring plan again to target the next objective. The only difference while asking "What do we know?" is that through the monitoring already conducted the knowledge base has changed and the next monitoring steps will increase the details of the knowledge about a given sturgeon population. Hence, the monitoring program ideally is adaptive and allows to build on already collected information while still maintaining the appropriate effort and data quality to answer one's research questions.

Another important aspect is the proper storage of collected research and monitoring data. Since the sampling design dictates the amount and quality of data that is necessary to be collected, the data storage should be realized in a way that all collected data appears in the database (see Annex 14.2). A best-case scenario would be the availability of a region-wide database where monitoring data collected in the whole catchment can be stored in a standardized way. This ensures standardization during data collection and acts as quality control for collected data.

# 10 List of figures

# 11 List of tables

# 12 Glossary

CMR = Capture-Mark-Recapture

CPUE = Catch per Unit Effort

CWT = Coded Wire Tag

eDNA = environmental deoxyribonucleic acid

e.g. = exempli gratia; for example

FFH = Fauna, Flora, Habitat

FFT = Floy-Fingerling tag

FL = Fork length

GL = Girth length

HD = Habitats Directive

IUCN = International Union for Conservation of Nature

IUU = Illegal, unreported, and unregulated fishing

PANEUAP = Pan-European Action Plan for Sturgeons

PIT = Passive Integrated Transponder tag

RBMP = River Basin Management Plan

SL = Standard length

TL = Total length

VIE = Visible Implant Elastomere tag

WFD = Water Framework Directive

WOT = Wire On Tag

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# 14 Annexes

## 14.1 Field Protocol

See separate PDF file: **[LINK here]**

## 14.2 Example of datasheet used in the LIFE Boat 4 Sturgeon project

See separate Excel file: **[LINK here]**

## 14.3 STURIO database development and structure (© IRSTEA, E. Quinton)

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