Strasbourg, 21 October 2024 TPVS(2024)18

CONVENTION ON THE CONSERVATION OF EUROPEAN WILDLIFE
AND NATURAL HABITATS

Standing Committee

44th meeting
Strasbourg, 2 - 6 December 2024

Technical Guideline for *ex situ* Conservation Measures in Sturgeons

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

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The following Guideline is being produced under service contract No 09.0201/2022/885601/SER/D.3 'Supporting conservation and protection actions to implement the Pan-European Action Plan for Sturgeons' signed between the European Commission and a consortium led by Umweltorganisation WWF Central and Eastern Europe, under a public tender procedure.

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This Guideline represents an update of the 2011 Ex situ guideline and Hatchery Manual by Chebanov et al. (FAO Tech Ser. 558 and 570).
The Guideline was developed in partial fulfilment of the EC Service Contract (09.0201/2022/885601/SER/D.3) Supporting Conservation and Protection Actions to implement the Pan-European Action Plan for Sturgeons

How to cite this document:
Gessner, J. et al 2024. Technical Guideline for EX SITU Conservation Measures in Sturgeons. EC Service Contract (09.0201/2022/885601/SER/D.3) Supporting Conservation and Protection Actions to implement the Pan-European Action Plan for Sturgeons. Publications Office of the European Union, Luxemburg, 308pp.

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Abbreviations and Acronyms

BW	Body weight
CCP	Common carp pituitary
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
cm	Centimeter
d	Day
dph	Days post hatch
ELS	Early life stages
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FFSBC	Freshwater Fisheries Society of British Columbia
FL	Fork length
G	Gram
GI	Gastro-intestinal
GnRHa	Synthetic analogue of mammalian gonadotropin-releasing hormone
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
h	Hour
ICES	International Council for the Exploration of the Sea
ID	Internal diameter
IUCN	International Union for Conservation of Nature
IUU	Illegal, unreported and unregulated (fishing)
KHz	Kilohertz
kg	Kilogram
km	Kilometer
L	Liter
lux	Luminous flux per unit area (unit of illuminance)
m	Meter
MHz	Megahertz
min	Minute
mg	Milligram
mJ	Millijoule
mL	Milliliter
mm	Millimeter
NADEG	Expert Group on Nature Directives
nm	Nanometer
NTU	Nephelometric turbidity unit
PANEUAP	Pan-European Action Plan for Sturgeons
PI	Polarization index
PIT	Passive integrated transponder (tag)
RAS	Recirculating aquaculture system
SP	Sturgeon pituitary
s	Second
TL	Total length
USD	United States dollar
USFWS	United States Fisheries and Wildlife Service
UV	Ultraviolet (light)
YOY	Young of the year
µm	Micrometer (0.001 mm)
µg	Microgram (0.001 mg)

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 were listed by the International Union for Conservation of Nature (IUCN) as threatened with extinction in 2022 and are reported as being in "unfavourable" conservation status within the frame of the reporting under Article 17 of the Habitats Directive (Council Directive 92/43/EEC).
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 "favorable" 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."*

As sturgeons in Europe have declined to a level where self-recovery is considered unlikely or even impossible depending on the specific river basin, the recovery of populations to self-sustaining levels will require active support measures. Therefore, the purpose of this technical guideline is to specifically support the implementation of the Action Plan's Objective 2 "Population Structure is actively supported" namely to "establish *ex situ* brood stocks to secure genetic diversity of sturgeons" and to "put in place and implement reproduction and release programmes" where needed.

Sturgeons although threatened by extinction, are key indicators for the ecological integrity of rivers, as habitat for completing the life-cycle may cover entire catchments (Schiemer 2000). Likewise, viable sturgeon populations depend upon availability of ecologically functional habitats. The underlying reasons for the decline of a population must be well understood in order to start a qualified recovery program. Besides a thorough scientific assessment, any decision for a recovery program must be based on a number of important prerequisites. These also include the decision on the "hosting" organization that is to lead the measures for a long-term period and is responsible for the coordination with partners and stakeholders. It is vital that necessary permissions from authorities are obtained as early as possible. If infrastructure needs to be provided, cost coverage must be ensured not only for its establishment but also for the operation of the facility. The acquisition of broodstock must be based on genetic prerequisites and is to secure the genetic variability of the founder population from the geographic origin.

If controlled reproduction is to be applied, best practice methods with minimal adverse impact upon the broodstock and its offspring are to be applied both for reproduction and rearing of fish for release. The releases should be implemented in accordance with the target to be reached, be it supplementation of an existing population or its reintroduction and must take into consideration the carrying capacity of the system to be stocked. The evaluation of impacts of the releases must follow a scientific approach, with the results being utilized to adapt and optimize both the rearing and release as well as addressing the threats in the given water system that impact the success of the recovery measures.

It is clear that the mitigation or removal of adverse impacts in situ, is the most important prerequisite to ensure population recovery is possible and sustainable in the long run. Monitoring of habitat and population status is instrumental to provide the necessary data for any decision on recovery measures*. Ex situ* measures come into play to overcome recruitment failure or the loss of a population. The ultimate goal of *ex situ* measures is to restore viable self-sustaining populations allowing the subsequent termination of all *ex situ* measures, which can happen only, if *in situ* sturgeon habitat is functional and sufficiently protected from adverse impact and if direct threats to wild populations (i.e. bycatch, poaching, etc.) are reduced to biologically safe levels.

Ex situ measures are a means to safeguard the genetic heterogeneity and diversity of the populations that still exist following detrimental anthropogenic impacts. The measures comprise the safeguarding of remaining individuals under controlled condition (in captivity) to allow their utilization in future reproduction and providing the basis for population support through releases of fish being reared under controlled conditions. This guideline summarizes the currently available knowledge and developments of *ex situ* methods and their application under different population scenarios. It needs to be ensured that future projects follow the best practice approaches outlined in this document in the sense that the research questions are clearly articulated and the methods applied are appropriately chosen to answer these questions. It is of utmost importance to set recovery priorities, which should be summarized in a national sturgeon action plan or conservation strategy, following the framework of the PANEUAP.

Due to the longevity of sturgeons, adult animals may still be around for a long time after natural reproduction ceased, providing ample opportunity to secure animals for *ex situ* conservation. Once a population is functionally extinct, the measures for re-introduction require decades to become effective. Since the *ex situ* approach has to be maintained over extended time periods, due to the long generation time of the species and the fact that available broodstock being the genetic basis of the population in question is rare or even irreplaceable, the *ex situ* measures are complex and cost coverage is challenging. *Ex situ* measures can require the construction or utilization of specialized facilities to safeguard biodiversity which result in long term, costly investments. The later during the decline of a population they are applied, the more intensive and complex they become. Therefore, it is of utmost importance to start these measures timely

enough, e.g. when populations start to struggle and to accompany them with effective *in situ* protection. In order to assess the right point-in-time for the onset of effective *ex situ* measures, monitoring data on population status are essential, yet in a European context these were not available and the onset of *ex situ* measures started too late in most regions.

In the European context because the populations to be restored are shared between countries, a close collaboration among range countries of the species is mandatory to manage the resource jointly which may also include sharing of costs. As a prerequisite, it needs to be ensured that results of *ex situ* actions are shared transparently with the public, relevant stakeholders involved in sturgeon conservation, as well as neighboring countries sharing the same populations in a river or sea basin. As for example the information on timing and locations of releases in an upstream country affect management decisions (for example on fisheries) of downstream countries or vice versa.

In order to seek for political support, it is essential to include the conceptual planning of *ex situ* as well as *in situ* measures in relevant strategic documents. In the long term, this procedure will support national governments and international organizations alike to ensure necessary funding to support the implementation of effective conservation actions. The combination of national sources with regional funding instruments may provide good opportunities to initiate the implementation of *ex situ* measures but, in the longer term, such costs should be integrated into national or even better joint regional budgets since the conservation of migratory species must be considered a truly range wide objective.

Chapter 1: Introduction

Joern Gessner, James A. Crossman, Thomas Friedrich, Beate Striebel-Greiter

Sturgeons are endemic to the Northern hemisphere. They are of significant ecological, commercial and recreational value and importance. The threats adversely affecting sturgeon populations are closely linked to their biological and ecological requirements (see Figure 1.1). All sturgeons share specific traits, like late maturation, longevity, low specific fecundity and expressed homing behavior, that render them extremely susceptible to anthropogenic impacts and complicate recovery actions. Among these anthropogenic impacts, fisheries, obstruction of migration routes and destruction of physical habitats have been associated directly with population declines (see Chapter 2). While combinations of threats may differ regionally, as well as change with time and population status, their devastating impacts remain. As a result, sturgeons are among the most threatened group of species according to the IUCN Red List Reassessment of 2022 which summarized the ongoing decline of sturgeon populations globally. Today, 80% of sturgeon species are classified as endangered or critically endangered with one species having gone extinct in the last decade. Within the EU, all sturgeon species are protected under the Habitats Directive (43/92/EWG), and their conservation is a legal obligation for its Member States. *Acipenser naccarii*, *A. oxyrinchus* and *A. sturio* are listed on Annex II and IV and are considered species of special concern to the EU, requiring member states to "apply a strict protection regime across their entire natural range, within and outside Natura 2000 sites" (art 16). The other five species are listed on Annex V which obliges Member States to ensure that their exploitation and removal from the wild is compatible with maintaining them in a favorable conservation status. Yet, their return from a currently unfavorable status requires urgent and active protection measures. The Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) also lists sturgeons and its Contracting Parties commit themselves specifically (Article 11) to encourage the reintroduction of native species of wild flora and fauna when this contributes to the conservation of an endangered species, provided that a study is first made in the light of the experiences of other Contracting Parties to establish that such reintroduction would be effective and acceptable.

Reflecting the existing legal frameworks and the high risk of extinction for this species group, a Pan-European Action Plan for Sturgeons (PANEUAP)[1] was adopted in November 2018 as a recommendation[2] 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 Expert Group on the Nature Directives (NADEG) also recommended the implementation of the

[1] https://rm.coe.int/pan-european-action-plan-for-sturgeons/16808e84f3
[2] https://rm.coe.int/recommendation-199-2018-action-plan-sturgeon/1680a01895

Action Plan 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 "favorable" conservation 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 recommendation mandated the Secretariat of the Bern Convention to closely monitor the implementation of the Action Plan and to coordinate the implementation of regular reporting on the implementation of the Action Plan at a national level.

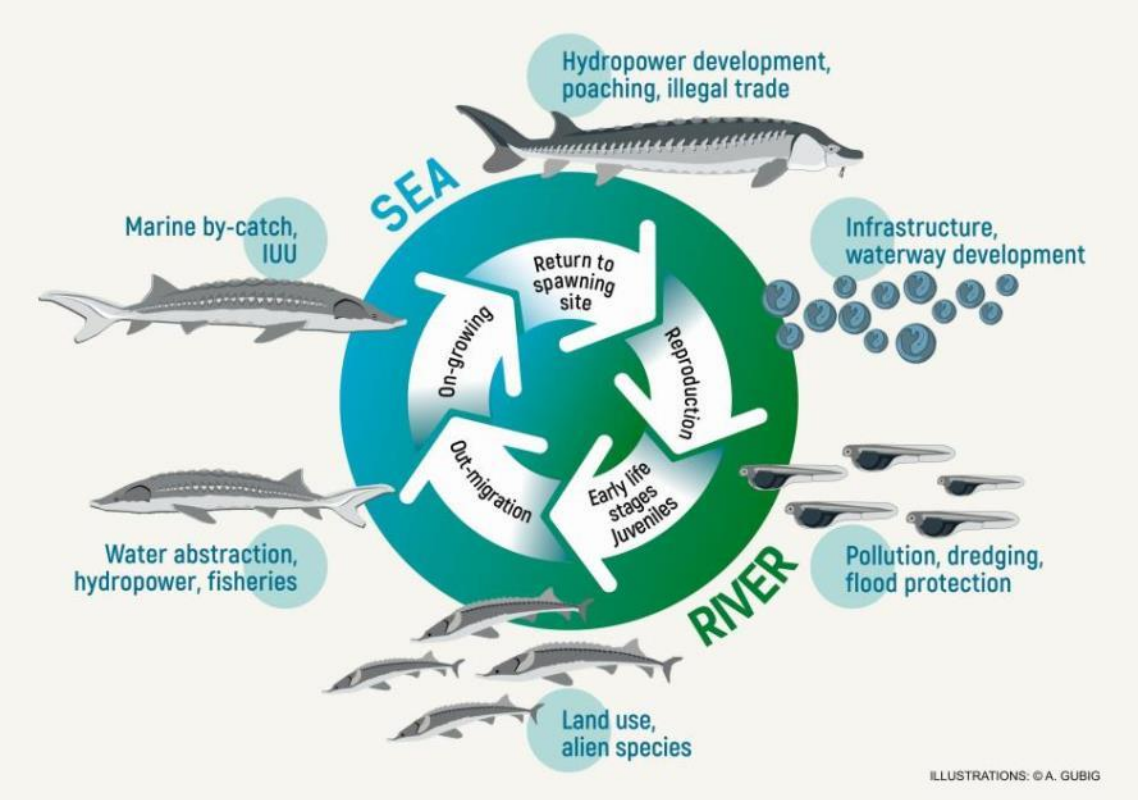

Figure 1.1: *Schematic life cycle and main threats predominantly impacting the various life stages*

Since its adoption, the EU Commission has closely followed the implementation of the PANEUAP and in 2022 issued a service contract (ENV/2022/OP/0019) to support its implementation. The scope of the contract 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 the Danube, Rioni, Po, Vistula, Oder, Nemunas, Gauja, Narva, Elbe, Rhine and Gironde, have been collected and displayed in maps. Further, the contract

encompasses (1) a study about sturgeon by-catch and possible measures to avoid or mitigate it, (2) a technical guideline for sturgeon population monitoring, (3) a technical guideline for habitat monitoring as well as (4) a technical guideline for best practice *ex situ* breeding and release programs.

This document presents the technical guideline for *ex situ* measures, contributing explicitly to reaching the PANEUAP **Objective 2 POPULATION STRUCTURE IS ACTIVELY SUPPORTED TO REVERSE THE DECLINE** focusing specifically on the utilization of *ex situ* measures to bring the populations back to levels that allow sustaining themselves.

Viable sturgeon populations depend upon an ecologically functional habitat for completing their complex life cycle, including the availability of sufficient breeding, feeding and wintering habitats, habitat accessibility and connectivity as well as good water quality. The spread of these habitats may cover entire catchments with adjacent marine areas, which is the reason why sturgeons are considered key indicators for the ecological integrity of rivers.

To endure the success of recovery measures, it is important that the identification of the underlying reasons for the decline of a population are well understood when starting a qualified recovery program. In order to make informed decisions a sound knowledge base on the species habitat as well as on the status and development of the population in question is needed. In this respect the application of the other two support documents, produced under this EC service contract, namely the "Technical Guideline for Habitat Assessment" and the "Technical Guideline for Population Monitoring" need to be considered. Minimizing existing threats, active measures to restore populations, their habitats or their migration routes require substantial political will and resources and should be based on best available knowledge.

The knowledge of the population status, its reproductive performance and the impacting factors on population development are key for the success of the recovery of populations. The knowledge gained from population monitoring can serve multiple uses and would provide the best available knowledge to make informed management decisions on priority settings for the implementation of measures required to sustain a population in question. In cases when natural reproduction and recruitment is decreasing to insufficient levels to maintain the population in question, reproduction has ceased, or in cases when extinct populations are to be restored, *ex situ* measures come into play.

Ex situ measures are applied at different intensities to overcome recruitment failure or the loss of population components as a result of the adverse impacts. The ultimate goal of *ex situ* measures are viable self-sustaining populations and the subsequent termination of all *ex situ* measures, which can happen only if *in situ* sturgeon habitat is functional and sufficiently protected from adverse impact.

Ex situ measures might be targeting a) the safeguarding of the genetic diversity in cases of ongoing attempts to mitigate habitat deficits, b) the support of wild

populations to help reach the desired population size and self-sustaining status faster, or c) the allowance of a certain intensity of harvest. Naturally, the different targets require a different set of approaches or different intensities of the measures to ensure success. Due to the current status of sturgeon populations in Europe, the goal needs to be recovery of populations first, while harvest would be an aim for the distant future.

Unfortunately, in many cases for instance in the Baltic Sea or most parts of Western Europe, sturgeon populations are beyond the point of self-recovery. In these cases *ex situ* measures remain indispensable to recover the populations.

The guideline at hand is to support national authorities and institutions implementing ex situ measures in identifying best practice approaches to design and evaluate *situ* programs and to take informed decisions on funding priorities. Descriptions of methodologies and technologies, their purpose, and advantages and disadvantages provide orientation and guidance for practitioners to develop their own individual solutions to implement targeted methodological approaches to address specific research questions and to close existing knowledge gaps. Technical chapters are complemented with a compilation of required resources, 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.

While the underlying principles can be adapted also to other endangered fish species to a certain extent, the information provided in these guidelines is based on the general similarities of species within the sturgeon family of Acipenseridae. However, variations exist between species and populations of different catchments. Furthermore, there are still knowledge gaps about rearing conditions and the underlying requirements for fish produced using *ex situ* methods. Whenever available, specific information is provided, but it remains the responsibility of the document user to adapt the given information to the conditions in which they are applied.

Chapter 2: Sturgeon biology and life history

James A. Crossman, Molly A.H. Webb, Thomas Friedrich, and Joern Gessner

2.1 Sturgeon systematics, range and characteristics

Sturgeons belong to the family *Acipenseridae* within the order *Acipenseriformes*, a group of primitive ray-finned fishes that have persisted relatively unchanged for millions of years (Peng *et al.* 2007; Brownstein *et al.* 2024). The family comprises 25 species, distributed across freshwater and marine habitats within the Northern Hemisphere. Sturgeons exhibit distinctive morphological characteristics common to all species that include elongated bodies covered in five rows of bony plates called scutes, a heterocercal tail fin, a distinctive elongated rostrum equipped with sensory barbels, and a notochord instead of a spinal column. Their mouths are positioned ventrally, adapted for bottom-feeding habits. Sizes vary greatly among species, with some reaching lengths of over 7 m and weights exceeding 1,000 kg (e.g. *Huso huso*), which presents logistical challenges for *ex situ* programs where adults are required to be held in captivity.

2.2 Global conservation status of sturgeons

Sturgeons are the most imperiled group of species globally (IUCN, 2022), with all species suffering from substantial declines in abundance in the last century. These declines are attributed to anthropogenic impacts associated with habitat loss, ecosystem changes, pollution, overfishing, and restricted migrations due to the regulation of many rivers used by sturgeons (Haxton & Cano, 2016). These impacts substantially change the physical properties and biological functions of the ecosystems and alter productivity throughout the food web. The longer the lifespan and generation interval of a species, the more challenging recovery will become as many anthropogenic impacts have occurred within a single generation for most sturgeon species. Despite implementation of protection and recovery measures for most sturgeon species, populations continue to decline, and many still require substantial intervention beyond the current efforts to ensure their persistence for future generations.

2.3 Life history characteristics and considerations for *ex situ*

2.3.1 Longevity and generation time

Sturgeons are exceptionally long lived with individuals exceeding 150 years of age (Trautman, 1954). Life span depends upon species and latitude, with the northern ranges having overall longer lifespans. Their slow rate of growth contributes to this longevity and is also associated with late sexual maturation. Compounded with

this life history characteristic is the sensitivity of early life stage (ELS) survival to environmental conditions, a factor that is even more pronounced in perturbed systems. All this contributes to altered recruitment patterns that can be difficult to address in recovery programs due to generation times that require long-term data collection to understand their effectiveness.

2.3.2 Migration types and their implications for *ex situ* decisions

Sturgeons are diadromous migrants that return to their natal rivers to spawn. Two different groups can be distinguished in sturgeons: fully anadromous species that spawn in freshwater and spend much of their life in marine environments as well as potamodromous species that migrate within river systems or between connected lakes and rivers for reproduction. Migration distance varies between species and sub-populations and the timing of their migration is influenced by environmental conditions and underlying genetics. Following incubation, ELS and juveniles then tend to move downstream from the spawning sites into feeding and rearing habitats. The presence of different ecological groups varies between river systems with larger rivers generally revealing more differentiated groups that utilize specific reaches (e.g. races *in sensu* Berg, 1959; Gerbilski, 1954). However, most species spawn over a protracted period during which several distinct runs of fish migrate to the spawning grounds.

Generally, individuals that ascend rivers earlier in the year tend to migrate further upstream, later migrations take the fish to spawning habitat in the lower reaches of the river. It has been shown that their migration and its timing is repeatable at the individual level (Forsythe *et al*. 2012) demonstrating the importance of treating different spawning groups or sub-populations as distinct management units which can be affected by different impacts during their life cycle. With a perspective on the *ex situ* measures to be applied, it is of utmost importance to keep these different spawning groups or sub-populations separated if they are used to produce progeny. Controlling for these factors allows for negative effects on fitness and local adaptation to be mitigated to the extent possible before *ex situ* methods are applied (see Chapter 6).

Table 2.1: *Overview of nomenclature of early life stages (ELS). In this guideline, the ELS terminology includes: embryo, yolk sac larvae, and feeding larvae.*

Description	**Conte *et al*. 1986**	**Detlaff *et al*. 1993**	**Balon, 1999**	**Guideline, 2024**
Oocyte	Egg	Egg	Oocyte	Oocyte
Oocyte following insemination and fertilization	Fertilized egg	Fertilized egg	Embryo	Embryo (ELS)

Embryo following hatch	Yolk sac larvae	Embryo	Eleutheroembryo	Yolk sac larvae (ELS)
Late embryo after onset of feeding	Feeding fry	Feeding larvae	Larvae	Feeding larvae (ELS)
Fish after completion of development after stage 45 (Detlaff *et al*. 2019)	Fingerling	Juvenile	Juvenile	Young of Year (YOY), juvenile

2.3.3 Reproduction

Sturgeons are gonochoristic (i.e. eggs and sperm present in separate individuals) and gymnoovarian (i.e. gonads are open to the body cavity). They are late-maturing, and reproduction is controlled by endogenous and environmental factors (Dettlaff *et al*. 1993; Doroshov *et al*. 1997; Webb & Doroshov, 2011). The timing of first maturation depends on age and body size, accumulated energy (e.g. lipids), and environmental cues. The first reproductive cycle is activated by neuroendocrine signals, with endogenous factors acting as the gates to first maturation (see Webb & Doroshov, 2011). Most sturgeons reach first maturity after 3-6 years in small species (e.g. *Acipenser ruthenus*) or 15-30 years in large or northern species (Caron & Temblay, 1999; Caron *et al*. 2002; Cox *et al*. 2022). In general, spawning periodicity (i.e. number of years between spawning events) is 2–11 years for females and 1-6 years for males (Billard & Lecointre, 2001; Forsythe *et al*. 2012; Haxton *et al.* 2016; Cox *et al*. 2022). It has been found that naturally vernalized water temperature is important for successful oocyte maturation and ovulation in sturgeons (Webb *et al.* 1999 and 2001). Most species spawn in the spring to early summer over a wide range of temperatures (6 to 25 °C; Billard & Lecointre, 2001), though there are a few species that have populations that spawn in the fall (*A. gueldenstaedtii*, *A. oxyrinchus*, *A. sinesis*, *A. stellatus, H. huso;* Berg, 1946; Balazik *et al.* 2012; Wu *et al.* 2015). Sturgeons are highly fecund (100,000-1,000,000+ eggs per spawn depending on the species), and fecundity is dependent on body size and egg size. However, over their reproductive life span, sturgeons have low specific fecundity due to the late age at first maturity and long spawning periodicity. Multiple males may spawn with a single female and males may spawn several times within a season (e.g. Duong *et al*. 2013). Spawning substrate selectivity may differ across species but generally includes gravel of different sizes, cobble, and bedrock (e.g. Hochleithner & Gessner 2012). The relative importance of the environmental factors controlling reproduction and the magnitude of change of these factors required to initiate a

spawning event have not yet been well defined for many chondrostean species. It will be important to understand the specific role of environmental factors in driving gametogenesis and spawning to address the mismatch that may occur with global climate change, and understanding these factors is important for successful reproduction in captivity.

2.3.4 Spawning

Sturgeon eggs are broadcast over coarse substrates where they can incubate and develop in fast water either adhered to the surface of rocks (e.g. gravel, cobble boulders) or within the interstitial spaces. Following fertilization, sturgeon embryos progress through a series of developmental stages before hatch, the rate of which is influenced significantly by temperature (e.g. Wang *et al.* 1985). Susceptibility to mortality is higher during earlier development stages (e.g. cleavage and gastrulation) and handling for *ex situ* activities should be limited prior to neurulation. Total incubation time is correlated to water temperatures, with warmer temperatures accelerating development and cooler temperatures reduce development. Optimal temperature ranges vary among sturgeon species but typically fall between 10 °C to 18 °C for spring spawners and exceed 20°C for summer and fall spawning species (e.g. *A. oxyrinchus*). Temperatures exceeding optimal ranges are known to increase rates of mortality as do reduced dissolved oxygen levels. During incubation in the wild, eggs are offered some protection from predation associated with fast water velocity barriers (>1.0 m/s) and the substrates they are incubating within, however, mortality during this life stage is naturally high and predicted to exceed 99% (*A. oxyrinchus desotoi*; Pine *et al.* 2001). Pathways of mortality are not limited to predation from other species but can include microbial infections (Fujimoto *et al.* 2013; Walquist *et al.* 2022), the rate of which accelerates with increasing water temperatures and nutrient load. Infestations can become an ongoing challenge for many *ex situ* programs during captive incubation. In addition to microbial infections, physical properties of spawning habitats such as shear stress and reduced substrate complexity can influence survival, especially in systems with altered flow regimes as a result of regulation.

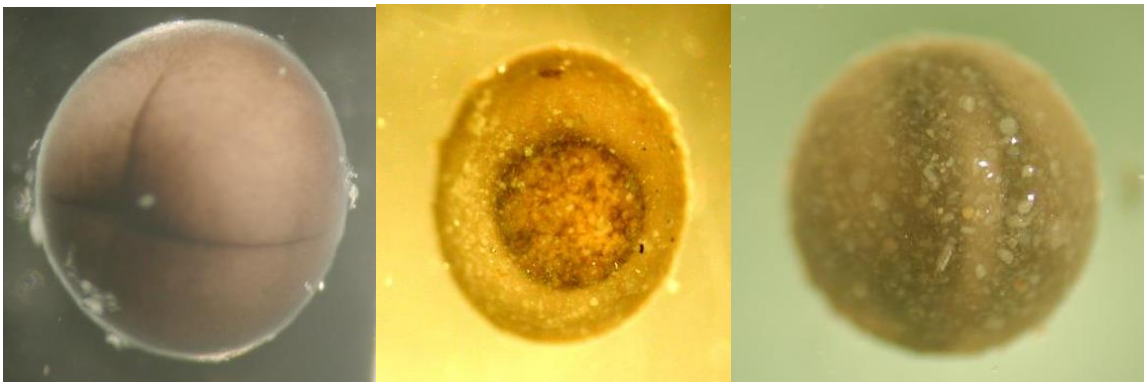

Figure 2.1: *Embryonic development of Acipenser oxyrinchus (4-cell, large yolk plug, and neurulation stages, from left to right) (Photo credit: C.M. Kamerichs).*

2.3.5 Early life stages

For the purpose of this document, the ELS comprise the embryo, yolk sac larvae after hatch, the transition to exogenous feeding and conclude once the individual resembles the adult phenotype and becomes a young of the year (YOY) or juvenile (Dettlaff, et al. 1993). Generally, sturgeon embryonic development commences after hatch with the absorption of the yolk sac and associated differentiation of the embryo. At this point in time, the embryo is measuring approximately a centimeter in length, though size at hatch will vary according to species and environmental conditions during incubation (e.g. Beer, 1981; Wang *et al.* 1985; Wang *et al.* 1987; Dettlaff *et al.* 1993; Hardy & Litvak, 2004; Jay *et al.* 2020).

All sturgeon larvae have a prominent yolk sac at hatch, which provides endogenous resources for initial development either prior to or during dispersal downstream of spawning sites. Many species utilize these endogenous yolk-sac reserves while hiding within interstitial spaces at spawning sites, emphasizing the importance of spawning substrate restoration as a recovery tool (Crossman & Hildebrand, 2014; McAdam *et al.* 2018) in combination with *ex situ* measures. While many species have a dispersal period once endogenous reserves are exhausted (e.g*. A. fulvescens*, Hastings *et al.* 2013; *A. transmontanus*; Kynard *et al.* 2014), some species disperse long distances immediately following hatch (e.g. *S. albus*, Braaten *et al.* 2006; Braaten *et al.* 2008; Guy *et al.* 2015; Marotz & Lorang, 2018; Chojnacki *et al.* 2023) or disperse continuously from hatch until they reach river mouths and the sea (e.g*. H. huso*; Pavlov, 2002). Transition through larval developmental stages is influenced primarily by temperature (Jay *et al.* 2020) but is likely influenced by species-specific variability and habitat conditions (e.g. Nguyen & Crocker, 2006; Boucher *et al.* 2014; Boucher *et al.* 2018).

Larval development and physiology are optimal for most species between 12-18 ^{o} C, with potential reduced survival beyond those limits. Larvae of fall spawning species will encounter much warmer temperatures during development, but this generally remains understudied for this life history characteristic. Like the larval species of many fishes, sturgeon larvae tend to disperse during darkness to reduce the risk of predation, which is high for this life stage (Waraniak *et al.* 2018). In many river systems, natural turbidity associated with spring freshet flows during spawning may also provide adequate cover for dispersal during the day, though natural levels of turbidity have been greatly diminished due to dam regulation (Northcote et al. 2005). Sturgeon larvae begin feeding in areas of fine sediments (e.g. silt) where food of the appropriate size and type (largely macroinvertebrates; Braaten *et al.* 2007) is available.

Figure 2.2: *Free embryos of A. oxyrinchus upon hatch, immediately after hatch, and close to absorption of the yolk sac (Photo credit: C.M. Kamerichs).*

The total duration of the larval stage varies among species but typically lasts a few weeks during which time developmental and physiological changes allow for transition to the juvenile life stage.

2.3.6 Juveniles

The juvenile life stage comprises the period of fastest somatic growth for sturgeons as they transition to reproductive adults. Initially, juveniles are highly susceptible to predation and offset this risk through the development of five rows of bony scutes to provide protection and rapidly grow in length (Beamish *et al.* 1996; Ireland *et al.* 2002; Crossman *et al.* 2023) to escape the gape size of other fish

predators (Gadomski & Parsley, 2005; French *et al.* 2014; Crossman *et al.* 2018; Baird *et al,.* 2020). This allometric growth tends to be faster in length than weight initially to avoid predation followed by an increase in the rate of growth in weight. In favorable environmental conditions, growth in weight will be allocated between somatic growth and reproductive development, the latter taking either a few years (e.g. *A. ruthenus*) or a few decades (*A. transmontanus*).

Juvenile sturgeons tend to be benthically orientated as prey consist primarily of invertebrates and insects within fine sediments or in shallow river margins (Nilo *et al.* 2006; Bock *et al.* 2011; Barth *et al.* 2013). As juveniles grow, diversity in the diet increases, and while prey selection generally reflects availability (Crossman *et al.* 2016), opportunistic feeding or selectivity for specific prey items of high value is common. The diet of juveniles for some species remains focused on invertebrates and insects (*S. platorynchus*, Gerrity *et al.* 2006), while most others transition to a diet that incorporates other fish species (Gerrity *et al.* 2006; Wanner *et al.* 2007; Margaritova *et al.* 2021).

Habitat use varies according to species with many using entirely freshwater during early (age-0) juvenile stages (e.g. *A. fulvescens; A. transmontanus; A. ruthenus*) followed by partial (*A. brevirostrum; A. oxyrinchus*) or even full estuarine habitat use for juveniles of certain species (*A. sturio*). Salinity tolerance is generally influenced by body size (Amiri *et al.* 2009; Allen *et al.* 2011), and most sturgeon species have considerable tolerance for using brackish or marine waters. While *ex situ* rearing could adapt juveniles to rearing in saltwater for many species, sensitivity varies considerably among species with some being less tolerable of salinity regardless of life stage (e.g. *A. naccarii*; Cataldi *et al.* 1999). As juveniles gradually approach adulthood, they may display increased space use and expand their feeding habits. They may also mimic or follow reproductive adults to spawning areas, important feeding areas, or overwintering habitats though this behavior is not well documented or studied. The duration of the juvenile stage varies greatly among sturgeon species, with some completing this stage rapidly (e.g. *A. ruthenus* 3-5 years) and some taking longer (*A. transmontanus* and *H. huso* >16 years).

Therefore, the time required to understand the dynamics of recruitment to, and survival through, the juvenile stage can be a significant undertaking for many recovery programs.

Figure 2.3: *Juvenile (YOY) Acipenser oxyrinchus at an age of 4 months (Photo credit: J. Gessner).*

2.3.7 Adults

Sturgeons are defined as adults once they reach sexual maturity. Depending upon species, this may take up to 22 years at sizes of around two meters (e.g. *H. huso, A. oxyrinchus, A. sinensis*). Smaller species (e.g. *Pseudoscaphirhynchus sp. Scaphirhynchus sp. A. ruthenus*) mature after four to six years. Upon reaching maturation, females are physiologically capable of spawning every two years, but the spawning interval may be quite variable and occur every 2+ years for females. It has been documented in a few, small individuals of smaller species, such as *A. ruthenus*, that females may produce eggs within a year (T. Friedrich, personal observation). In general, males mature earlier than females and tend to stay smaller. Males are physiologically capable of spawning annually, but many males have a longer interval between spawning (e.g. *S. albus*; Cox *et al.* 2022).

Depending upon species and/or population segregation, mature adult sturgeons are found in marine, brackish, riverine and lacustrine systems and continue to forage until the next reproduction cycle. Adult sturgeons feed opportunistically on a variety of prey organisms, including fish, crustaceans and benthic invertebrates. Feeding migrations within and between these systems may span large distances based upon seasonal variations of prey availability. Within riverine systems, winter months are normally spent in deeper sections of the river with reduced movement and feeding (Kynard *et al.* 2002). These migrations and aggregations often lead to mixed stocks of several sub-populations to be present in the same area having direct implications on the collection and assignment of broodstock for sound *ex situ* practices.

Figure 2.4: *Adult Acipenser oxyrinchus showing a shorter, more blunt snout and less dominant scutes as a result of allometric growth altering the morphometic characteristics over time. Photo credit: S. Zankel.*

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Chapter 3: Means of sturgeon conservation and the role of *ex situ* measures

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3.1 Introduction

The guideline presented in this manual has been developed specifically for use by sturgeon hatchery managers, fisheries managers, fisheries policymakers and other authorities responsible for fisheries and aquaculture development. It aims to increase awareness, help make sound decisions in conservation planning and support capacity building on best practices currently available in sturgeon hatchery management.

Several documents provide guidance to preserve and manage resources utilized by humans to reach a level of sustainability that allows long-term utilization along with thriving populations. Approaches have been outlined in the FAO Code of Conduct for Responsible Fisheries (FAO 1995) for example. The prerequisites for sturgeon conservation were outlined in detail in the Ramsar Declaration on Global Sturgeon Conservation (2006) and updated in the Vienna Sturgeon Conservation Declaration (2017). Nevertheless, the sustainability approach has utterly failed in sturgeons due to management approaches that did not sufficiently consider the life history until all of the global populations were heavily affected by the anthropogenic impacts as demonstrated in the current outcome of the IUCN Red List Assessment (IUCN, 2023; Congiu *et al.* 2023).

Conservation attempts in cases when populations are beyond the point of being self-sustaining have to overcome recruitment deficits (Gessner, 2000). In these cases, *ex situ* measures help to protect the last remaining individuals and provide the option for supportive releases. It is therefore of utmost priority to help facilitate the decision as to when *ex situ* measures are mandatory to save a species or population from extinction and to provide a rationale for the inclusion of *ex situ* conservation into a wider sturgeon conservation framework. At this point, population and subsequent habitat monitoring comes into play (Haxton et al. 2023, Neuburg et al. 2024, Reinartz, 2024).

3.2 Challenges in sturgeon conservation

Challenges and threats, which have to be considered in sturgeon conservation and recovery, are inherent to this group of fishes and stem from the specifics of Acipenserid biology, ecology and life cycle. The resulting management approaches are apparent on different hierarchical levels of organization and are furthermore impacted by the target that the conservation approach aims to achieve. All of this

makes sturgeon conservation a complicated endeavor involving a variety of fields of expertise and stakeholders and calling for detailed planning, coordination and synchronization for any integrated approach to be successful.

Major examples for challenges are listed in Table 3.1. Most challenges are related to the late maturity of the species resulting in impacts of even low fisheries related mortality. Other challenges include the large sizes attained, the adaptation to given river systems and the resulting presence of subpopulations that are segregated by the time and distance of reproduction sites from the sea. Furthermore, the long-distance migrations between feeding and spawning habitats and the concentration during spawning runs require adapted management strategies (Boreman 1997, Jaric & Gessner 2013).

3.3 Aims and targets of recovery

The **overall final objective** for rehabilitation measures may be defined as re-establishing/securing self-sustaining viable wild populations. According to the Pan-European Action Plan for Sturgeons this means: "To restore all existing sturgeon populations to 'least concern' (IUCN classification) or 'favorable' (Habitats Directive) status and re-establish self-sustaining sturgeon populations as well as habitat critically required for each stage of their life-cycle habitat in their historic range to an extent that ensures species survival and representation of the subpopulations where possible. "

Other targets might be a) the safeguarding of the genetic diversity in cases of ongoing attempts to mitigate habitat deficits, b) the support of wild populations to help reach the desired populations size and self-sustaining status faster, or c) the allowance of a certain intensity of harvest.

These different targets require a different set of approaches or different intensities of these measures to ensure success. In all cases, determination of the population status is the first input to be provided when considering the need for different recovery measures. This needs thorough analysis to verify if recovery of a population can be feasible. It must be considered if habitat availability and quality allow a population to sustain itself in the long term. It is of further importance to verify if the causes or increased mortalities have been identified and remediated, largely depending upon the size of the remaining population. Based upon the current state of the population in question, different approaches can be considered as outlined in Figure 3.1. Before an *ex situ* conservation program is established, it is important to consider the characteristics and dimensions required, the timeframe and the financial effort it requires.

The decision to include e*x situ* management in the conservation strategy for a species should be determined by weighing the potential conservation benefit for the species against the likelihood of success and overall costs and risks of not only the proposed *ex situ* program, considering also alternative conservation actions or inaction. As is the case for conservation planning in general, these evaluations are made ideally by a multi-stakeholder group, including both *in situ* and *ex situ* expertise and experience.

Table 3.1: *Life history traits of sturgeons and associated threats resulting from anthropogenic pressures with an outline of the approaches in management for mitigation.*

Characteristics	**Resulting management challenges**	**Conservation approaches**
Show a high plasticity of morphological traits	Making it difficult for the unexperienced observer to identify individuals on the species level, hampering the involvement of lay people in conservation work and the evaluation of anecdotal information	Requires availability of reliable determination techniques (through experts or application of AI) to verify reports and prevent illegal harvest
Long lifespans and late maturity	Very sensitive to harvest and prone to overexploitation Conservation effects become assessable only after long periods	Effective fisheries management to limit harvest at tolerable levels; allow for removal only at max reproductive potential; prevention of bycatch Long-term commitment for restoration and funding to ensure targeted conservation outcome
Aggregation during spawning runs	Obscures the impacts of mortality/recruitment failure on adult population level due to large numbers that return annually	Detailed knowledge of population structure and natural mortality to set targets for harvest required; assessment of YOY abundance as an indicator for population recruitment
Individuals do not spawn annually	Different year classes are mixed in spawning runs which does prevent inbreeding	Requiring larger groups of captive breeders being collected over longer periods of time for *ex situ* measures
	Only a fraction of the whole population is represented in the annual reproduction migrations	Breeding protocols need to involve families to preserve and/or maximize the representation of certain genotypes in a population

Multi-aged population structure	Depletion of spawners results in deficits in annual spawning runs affecting reproduction success for decades	Long-term recovery measures to allow rehabilitation of populations
Populations are ecologically adapted to their specific catchment and respective conditions	Limits the transferability of fish between river systems	Selection of hydrologically and genetically similar sources
	Limits the transfer of research results between subpopulations from different catchments	Careful verification of prerequisites and boundary conditions required
	Availability of populations may be seriously affected by human-made changes to the hydrological regimes	Trial and error approach in restoration with clear objectives to be addressed with regard to the population segment to be recovered/replaced
	Renders fisheries management difficult since impacts might be separated temporarily or spatially	Regionally harmonized fisheries management required to minimize adverse impacts of gear types
Populations may display different forms with both vernal and hiemal migration patterns	Bearing the risk of losing such distinct patterns and forms through a) hatchery practices in *ex situ* activities and b) climate change	Proper characterization and mindful utilization of reproductive groups that display differences in maturation timing
	Past overexploitation, habitat destruction, modification and subsequent loss of such substructures result in complications defining a reference population status and environmental (habitat) reference conditions	Needs caution when interpreting population data
Sturgeon populations and their substructures (e.g. subpopulations, different spawning runs and locations) may be stretched out over thousands of kilometers at one point in time (i.e. in different habitat types in a	Rendering the meta-population difficult to assess	Multiple timepoints and sampling sites for assessments annually
	Selective harvest of single population segments may reveal different status	May result in specific segment requiring protection, and for support
	Susceptibility to obstacles of migration routes may be increased	Address migration impacts on a transnational or basin-wide scale

marine-estuarine-riverine catchment system)		
Spawn in freshwater at different distances from the sea	Exposes them selectively to multiple impacts and threats associated utilization of riverine environments (landscape sinks with multiple stressors and excessive impacts by various users, see above)	Knowledge of population structure, differentiated assessment of threats and different requirements for migration facilitation
Inhabit mainly deep stretches in the main channel in the lower and middle stretches of large rivers (potamal)	Being exposed to a multitude of anthropogenic impacts (settlements, water abstraction, navigation in combination with hydropower)	Integrated management and spatial development targets to be elaborated, monitoring effort needs to be high
	Results in overlap with navigation use of the river (dredging, ship strikes), loss of feeding habitat	Integrated management and development targets must be elaborated
	Habitat requirements do interfere with targets for navigation channels (pool, bank structures) bearing the risk of detrimental impact on habitat use in general and reproduction in particular	Key habitats require effective protection, maintenance work must consider species protection as a critical factor
Utilize shallow marine areas on the continental shelf	Results in overlap with intensive fisheries leading to marine bycatch with an important impact upon recovery targets	Adapted fisheries techniques, closed seasons, closed areas and surveillance systems to be implemented; align with protection measures for other species groups for the development of a uniform measure to be applied
Large sizes already as subadult individuals in comparison with other species in a respective system	Subadult individuals (as well as large and potentially reproductively active fishes) are prone for bycatch considering the selectivity of the common gear used for demersal fish species may be harvested without ever having taken part in reproduction	Information campaigns, adapted fisheries techniques, closed seasons, closed areas and surveillance systems must be implemented

Large size animals	Adapted to open spaces and wide rivers; risk of injuries in man-made infrastructures (fish passes)	Appropriately dimensioned passing solutions at migration barriers/obstacles (compared to other fish groups)
Absent or extremely rare in many former distribution areas	Makes population monitoring impossible or extremely difficult	Select targeted methods and life stages to allow data acquisition
Capable of intra- and inter-species hybridization	Renders preservation of genetic variability for establishment of *ex situ* populations difficult (low encounter probability)	Establish long term collection programs based upon eDNA identification of aggregation sites
	Bearing the risk of introgression for populations	Apply restrictions in the transfer and use of exotics in aquaculture and ornamental fish trade (ICES Code of Practice); reverse habitat shortage (spawning sites); intensify the safeguarding against escapement; monitor hybridization

Box 3.1: Recovery targets may vary depending upon the status of the population in question. In cases where a functional population exists and removal of individuals is permissible, optimizing the conditions for reproduction and recruitment might be sufficient to increase the harvestable population size over a longer period of time, requiring a moratorium for the fishery or strict catch limits that the allow the recovery of the population still.

In cases where natural recruitment is limited through adverse environmental conditions (i.e. regulation and damming), the supplementation of the population through stocking has been carried out over decades, resulting in an increased harvestable population at the cost of impaired genetic integrity.

If striving for the (re-)establishment of a self-sustaining population, the condition of the remaining population and the persistence of the adverse impacts that have reduced the population in the first place and that have not allowed recovery after the initial pressure has been removed will determine the strategy to be applied. This largely depends upon the outcome of the assessment if the recovery efforts will be in time to allow the remaining population to recover before it becomes too small to ensure sufficient recruitment. If the assessment is positive, *in situ* measures might suffice to bring the population back to historic levels. If the assessment is negative, remaining populations are to be safeguarded in *ex situ* measures through releases of offspring to avoid loss of genetic diversity and heritable adaptations for the recovery of the population following habitat restoration.

Temporal recruitment failure due to adverse environmental conditions, low population size or temporal overharvest may not require *ex situ* measures if the impacting causes can be addressed within a generation time. On the other end of the spectrum, reintroductions following the extinction of populations are a classical case where *ex situ* measures are required. It is therefore important to verify the status of a population and the aims that underly the recovery program.

The aim to reestablish historic population levels in most cases might not be realistic considering the level to which the environment, including the riverine habitats have been altered over the last 150 years. As such, substantial proportions of the habitat might be missing in turn limiting carrying capacity. This can be evaluated when monitoring the populations under recovery. If historic populations sizes do not qualify as a criterion for recovery success, alternative criteria would comprise the population trend and the reproductive efficiency of the population.

If a self-sustaining population is the final target of the recovery, it is essential to ensure that monitoring the population over at least 2 generations for successful and sufficiently sized recruitment to cope with the overall mortality encountered by the population is implemented. The monitoring results must be the basis for the management actions to address any shortcomings during this period. Only if recruitment and natural reproduction exceed the mortality encountered for a multi-generation population over the entire period, it can be considered a relevant quantifier for the persistence of the population.

3.4 Strategies for recovery

Viable sturgeon populations depend upon an ecologically functional habitat. The identification of the underlying reasons for the decline of a population must be well understood to start a qualified recovery program (Lioubimtseva 2023, Reinartz et al. 2016, Ralls & Ballou 2013, Primack. 1998, Pavlov et al. 1985). As such, mitigation or removal of adverse impacts is instrumental to ensure population recovery is possible (IUCN, 2014). To obtain the information, assessment of habitat and monitoring of population status are instrumental to provide the background data that allows for decisions on the measures to be taken (Luk'yanenko et al. 1999). *Ex situ* measures come into play to overcome recruitment failure or to prevent the loss of population components as a result of the adverse impacts. The ultimate goal of *ex situ* measures are viable self-sustaining populations and the subsequent termination of all *ex situ* measures (see Box 1), which can happen only if *in situ* sturgeon habitat is functional and sufficiently protected from adverse impact (WWF 2023, Rosenthal et al. 2017, Seddon et al. 2014, Rosenthal & Pourkazemi 2006, Rochard et al. 1997). The habitat of sturgeons as a basis for the sturgeon life cycle of wild and reintroduced populations comprises the marine, estuarine and riverine water bodies of the ecological corridor. Therefore, their connectivity is of paramount importance, as well as the functionality of the associated habitats. The habitat mosaic represents a functional unit into which any *ex situ* measures feed (Reinartz 2024).

Wild populations need to be protected *in situ* in order to be able to thrive. One major aspect of this protection addresses fisheries to prevent overexploitation either directed through Illegal, Unregulated, Unreported (IUU) fishing as well as the removal of individuals through bycatch associated mortalities. Targeted fisheries, to be considered sustainable, must be based upon the annual population growth and the recruitment of the population in question. A special case is represented by scientific fisheries for monitoring purposes or for *ex situ* measures, which are essential for the understanding of the response of the population towards management, but removal of individuals needs thorough justification and must be in accordance with the conservation targets. The options and approaches for conservation and recovery of a population are summarized in Figure 1.

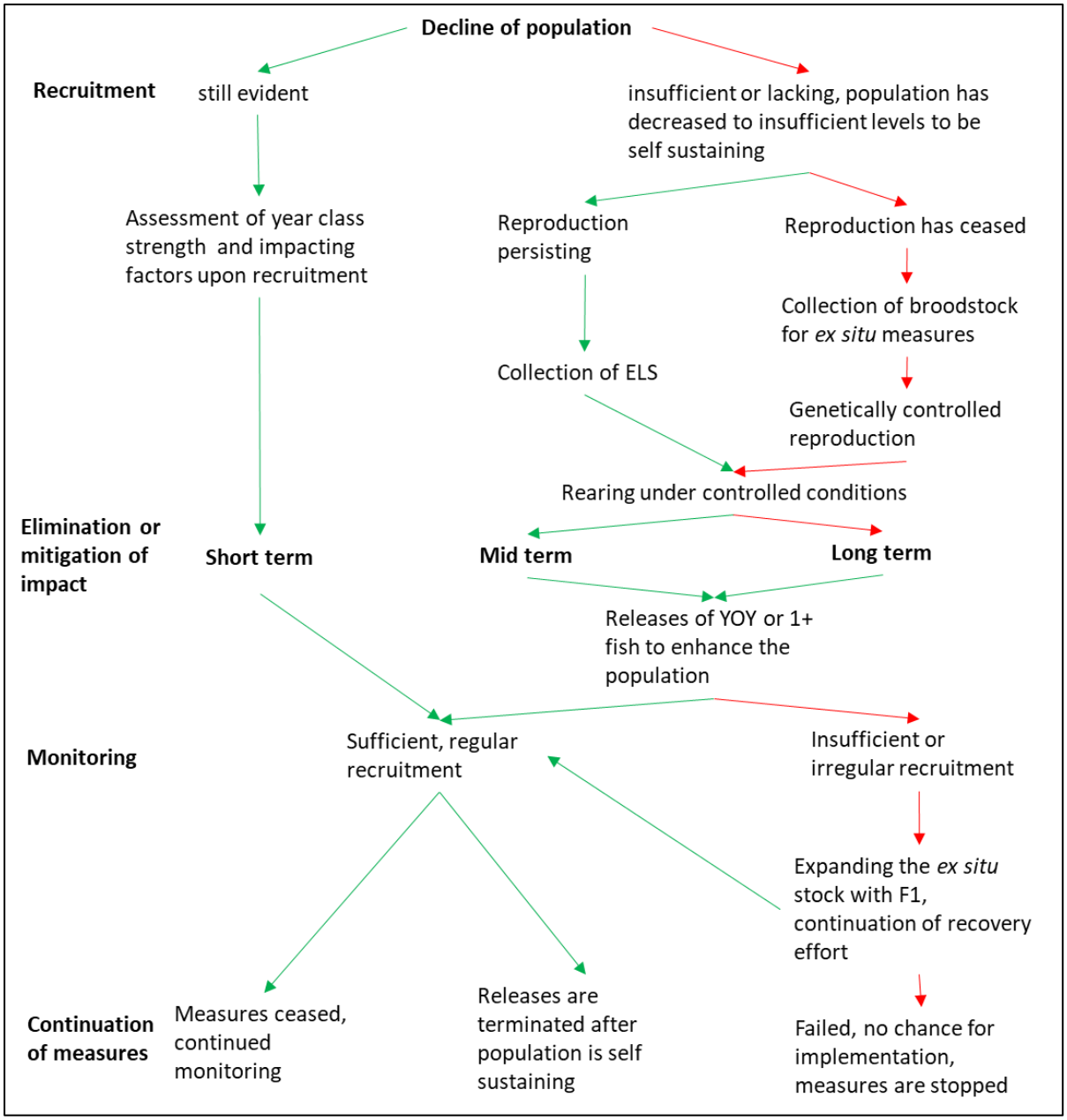

Figure 3.1: *Decision tree for population recovery strategy implementation where "elimination of impact addresses physical, managemental and socio-economic impacts upon the population in question.*

Local communities, stakeholders and the general public need to be informed about the importance of sturgeon conservation in general, the options for their implementation and the benefits of sustainable fishing practices. Furthermore, the public as well as the stakeholders must be involved in the decision-making processes throughout the conservation activities to ensure long term support (Arlinghaus et al. 2019). *Ex situ* measures (e.g. rearing of fish under controlled conditions and subsequent releases) provide plenty of opportunities for public involvement which can also be used to help support *in situ* measures.
Finally, international cooperation and exchange on methodology and results obtained for both *in situ* and *ex situ* measures is essential to minimize repetition

of mislead approaches and to actively promote sturgeon conservation across borders to ensure the long-term survival of sturgeon populations (Bern Convention 2018). As a result, an integrated, coordinated and synchronized approach is necessary to combine the different fields of expertise in a harmonized regional strategy (Gessner et al. 2019).
The following main steps should be taken into consideration and coordinated within an integrated approach for the recovery of a population:

- Pressures need to be identified and their effects upon populations have to be assessed
- Detrimental impacts have to be eliminated or mitigated
- Population responses to elimination or mitigation of impacts have to be monitored
- In case of an insufficient or lacking response of the population and an ongoing decline, *ex situ* populations have to be established and *in situ* measures need to be intensified
- Aims and measures of *ex situ* programs need to be clearly defined on a population level. For this purpose, the prerequisites, recovery targets and milestones as well as the exit strategy have to be outlined.

3.5 What is *ex situ*?

When a population is becoming rare and decline is progressing, the decision - regardless of the effectiveness of the habitat and fisheries associated measures taken - must be made if a representative part of the population is to be secured under controlled conditions to prevent the loss of the inherited diversity or even the species in total (Boscari & Congiu 2014, see also Chapter 6). Here, the *ex situ* conservation comes into play as one element of the recovery strategy which aims at supportive measures from the increase of recruitment to securing the genetic heterogeneity by maintaining selected population segments under controlled conditions to produce offspring for supportive stocking.

In line with the IUCN Guideline, "*ex situ*" is defined as "conditions under which individuals are spatially restricted with respect to their natural spatial patterns or those of their progeny, are removed from many of their natural ecological processes, and are managed by humans. In essence, the individuals are maintained in artificial conditions under different selection pressures than those in natural conditions in a natural habitat" (IUCN, 2014).

The first objective to deal with is the question to which degree the previously defined conservation target can be reached through minimizing the mortality of the natural population and most importantly if the population is viable under the current conditions. In cases when the population in question is not sufficiently large to recover even when the anthropogenic pressures have been dealt with or if longer time periods are required to fully mitigate these pressures, the population

support through increased recruitment becomes mandatory to prevent extinction (c.f. Figure 3.1).

3.6 Why *ex situ* conservation?

There are two main purposes for *ex situ* measures in sturgeon conservation: Firstly, the conservation of residual sturgeon biodiversity in captivity, and secondly, the provision of genetically appropriate individuals for the support and restoration of populations (Dugo et al. 2004). If the population in question is reproducing naturally still, this can be achieved through the collection of F1 for rearing to overcome the high natural mortality from the embryo to YOY stages to be released at larger individual sizes. If the population does reproduce only sporadically or reproduction has ceased, broodstock development in captivity as a basis for subsequent releases would be the option to prevent deterioration of the remaining genetic heterogeneity (Boscari et al. 2022, Fisch et al. 2015). In this case, timing is a decisive factor, and as such, it needs ample consideration to assess the time at which to interfere with the system to start *ex situ* measures. Here criteria such as the lack of or insufficient natural reproduction, the genetic diversity in the remaining population when compared to the initial population to be recovered, the knowledge about the extent and seasonality of the migration as well as access to the different population segments and evidence for the population trend need to be considered. The extreme case would be an extinct population that is reestablished through translocations utilizing a captive rearing program of genetically and behaviorally similar fish of a sister population to facilitate release to reestablish an extirpated population (Williot et al. 2011, Gessner et al. 2011).

Small populations are particularly vulnerable to primary threats and stochastic processes (Allee Effect). As such, they require more rapid and comprehensive management measures to avoid extinction. But even in larger populations revealing a substantial decline, the establishment of a diverse and sustainable *ex situ* population may be critical to prevent species extinction. Especially if a sufficiently rapid reduction of primary threats is uncertain or has been unsuccessful for the respective population, timely action is required while the population in question still inherits sufficient diversity and heterogeneity. The potential to revert the population trend by addressing the main adverse impacts must be critically evaluated and an assessment of the factors that might impede or likely contribute to conservation success must be carried out (Caron et al. 2002, Auer 1996).

Table 3.2: *Causes for population dysfunction and proposed countermeasures.*

Symptoms	**Cause**	***In situ* measures to address the impacts**	***Ex situ* measures to be taken besides remediation of causes**
Lack of reproduction	Subcritical number of spawners	Reduction of mortality	Establishment of an *ex situ* broodstock and controlled reproduction
	Unsuitable flow or temperature conditions	Improved river basin management	Utilization of wild spawners for controlled reproduction
	Lack of suitable spawning habitat	Facilitation of lateral erosion, artificial spawning habitat	Utilization of wild spawners for controlled reproduction
Recruitment failure in juveniles	Excessive embryo mortality under natural conditions	Habitat improvement (water quality, substrate quality)	Collection of embryos in the wild and rearing under controlled conditions
	Predation	Improved substrate quality, habitat enrichment	Collection of embryos, larvae and juveniles in the wild and rearing under controlled conditions
	Habitat deficiency	Habitat restoration	Utilization of wild or establishment of captive broodstock
Recruitment failure in broodstock	Excessive mortality in fisheries	Effective protection, enforcement, increased compliance	Utilization of wild breeders or establishment of captive broodstock
	Predation	Habitat restoration, reduction of introduced predators, fisheries management	Utilization of wild breeders or establishment of captive broodstock

	Mortality through hydro constructions	Migration facilitation, habitat improvement	Utilization of wild breeders or establishment of captive broodstock
	Migration obstacles	Migration facilitation, habitat improvement	Utilization of wild breeders or establishment of captive broodstock
Continuous decline of wild population of all age classes	Unknown or combination of reasons	Research	Establishment of an *ex situ* broodstock and controlled reproduction to obtain sufficient time to solve the underlying reasons of the decline
Releases to account for insufficient sturgeon resources to support a regional fishery	Insufficient regulation of harvest	Increased natural recruitment, fisheries management	Utilization of wild breeders or establishment of captive broodstock
Releases to maintain the species in natural environments	Lack of recruitment, deficit in carrying capacity, overambitious project aims	*In situ* measures to improve habitat quality, recovery of functional populations	Establishment of an *ex situ* broodstock and controlled reproduction as a basis for release
Sturgeon rivers and seas void of sturgeon	Extinction in the wild	Reintroduction and *in situ* measures for habitat provision	Establishment of an *ex situ* broodstock and controlled reproduction, controlled translocation and re-colonization based upon releases

3.7 How to apply *ex situ* measures?

Ex situ management has been applied in several different approaches in support of threatened or endangered sturgeon species. Sturgeon species extinctions have largely been prevented this way so far. At the same time, conservation measures

or re-introductions that were carried out following periods of *ex situ* management were rarely successful if such measures were focusing solely on supporting populations through releases, while at the same time the associated habitat deficits were left unconsidered. Therefore, the need for and suitability of an *ex situ* program must be carefully evaluated as part of an integrated conservation strategy (IUCN, 2014). This strategy should comprise:

- a status review of the species and its habitat,
- a threat analysis,
- the identification of the role of the *ex situ* measures in the conservation of the species,
- an analysis of the population segments and sizes that need to be represented in the *ex situ* measures,
- an assessment of the resources required,
- a feasibility study that the targets will be achieved,
- a risk analysis for the adverse impacts upon the program,
- a transparent decision on the fate of the planned measures incorporating all stakeholders involved.

The process of establishing and implementing *ex situ* measures for sturgeons requires long-term planning (Friedrich et al. 2019, Chebanov et al. 2011). Therefore, the conservation plan should assess the overall duration of the *ex situ* process until reaching the conservation target. This assessment would be subject to recurring evaluations based upon the recovery of the population. Once the primary threats have been addressed and the impacts mitigated, *ex situ* populations may be used for population restoration or the re-introduction in an assisted colonization. As such, these guidelines are complementary to the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN, 2014). For proper *ex situ* planning, it is essential to describe the conditions and the timepoints at which to intervene with the system to start *ex situ* measures.

Table 3.3: *Prerequisites associated with different objectives of ex situ applications to ensure its effectiveness.*

Target	Purpose(s)	Method	Prerequisites
Protect remaining genetic heterogeneity of species/population	Prevent extirpation through a natural catastrophe, natural mortality and lack of recruitment, fisheries; temporarily overcome adverse conditions that potentially affect the viability of the population	Catch of representative segments of an available population and transfer into *ex situ* facilities	Ensure genetic integrity, proper identification of substructures of a population and that they are represented in the *ex situ* population, safeguard rearing through qualified personnel, fail-safe rearing technology and procedures

Support natural recruitment	Enhance recruitment by reducing mortality of early life stages	Collect embryos/larvae of natural reproductions for hatchery rearing to YOY or 0+ size or establish *ex situ* population	Minimize selection of hatchery environment on offspring and ensure behavioral plasticity
Replenish stocks	Support recreational/commercial harvest	Catch and reproduction of natural spawners	Prevent adverse impact of releases upon natural reproducing population segments
Reestablish extirpated population	Release fish to initiate a self-sustaining population	Establish and expand *ex situ* population, reproduce and rear offspring fit for release	Utilize a suitable (genetics, habitat quality) source population, identify the bottlenecks and reasons for initial population decline, ensure that threats have been terminated

Recommendations for the establishment of conservation hatcheries and conservation stocking so far have focused on broodstock to be established (DSTF-WSCS Recommendation, 2021). This provides the essential prerequisites for the development of a captive bred population segment, such as the utilization of the widest possible range of diversity at all biological levels to ensure the adaptive potential of populations in unpredictable changes of natural environments (i.e. climate change). The establishment can be carried out by catching mature fish in the wild (when accessible) or through the collection of juveniles. Alternatively, animals can be acquired from fish farms, as long as the origin from the population to be protected and reestablished/supported can be ensured.

A general principle for the establishment of *ex situ* stocks is the prerequisite that the collection of animals from the wild should not endanger the wild population any further (IUCN 2013 and 2014). Any removal of animals from the wild needs to be justified, ensuring the safeguarding of the animals in captivity over a prolonged period of time and must guarantee access of public conservation bodies to the resource. It must be emphasized that the establishment of *ex situ* stocks is a last resort to prevent the natural population from becoming extinct. Therefore, it can be justified if their survival in the wild is at stake to transfer the entire remaining population to captive conditions (Williot *et al*. 2011). An alternative that applies to populations with successful reproduction is the collection of fertilized eggs or wild juveniles to reduce the initial mortality during early life stages (Anderson et al. 2021). This approach benefits from natural mate choice and excludes the common drawbacks of controlled reproduction. The interplay of the

different impacts upon fitness and the wild population are summarized in Figure 3.2.

In any case, the acquisition, collection, transportation and documentation of origin of animals must be conducted on the basis of legality and traceability. Furthermore, all animals to be used in the establishment and reproduction of the broodstock need to be assessed genetically to ensure the use of native strains, the differentiation into different recognizable conservation units and families for pedigree documentation and subsequent propagation (see Chapter 6, e.g. Boscari et al. 2014). A comparison with the genetic diversity of the wild population is mandatory to represent the diversity of the natural population in the conservation program (Dugo et al. 2004). In an attempt to effectively safeguard the rare animals incorporated in the broodstock and the resulting F1 (or later) of the conservation units, it is suggested to distribute the animals to two or more *ex situ* facilities for risk split and to avoid the total loss due to local adverse events.

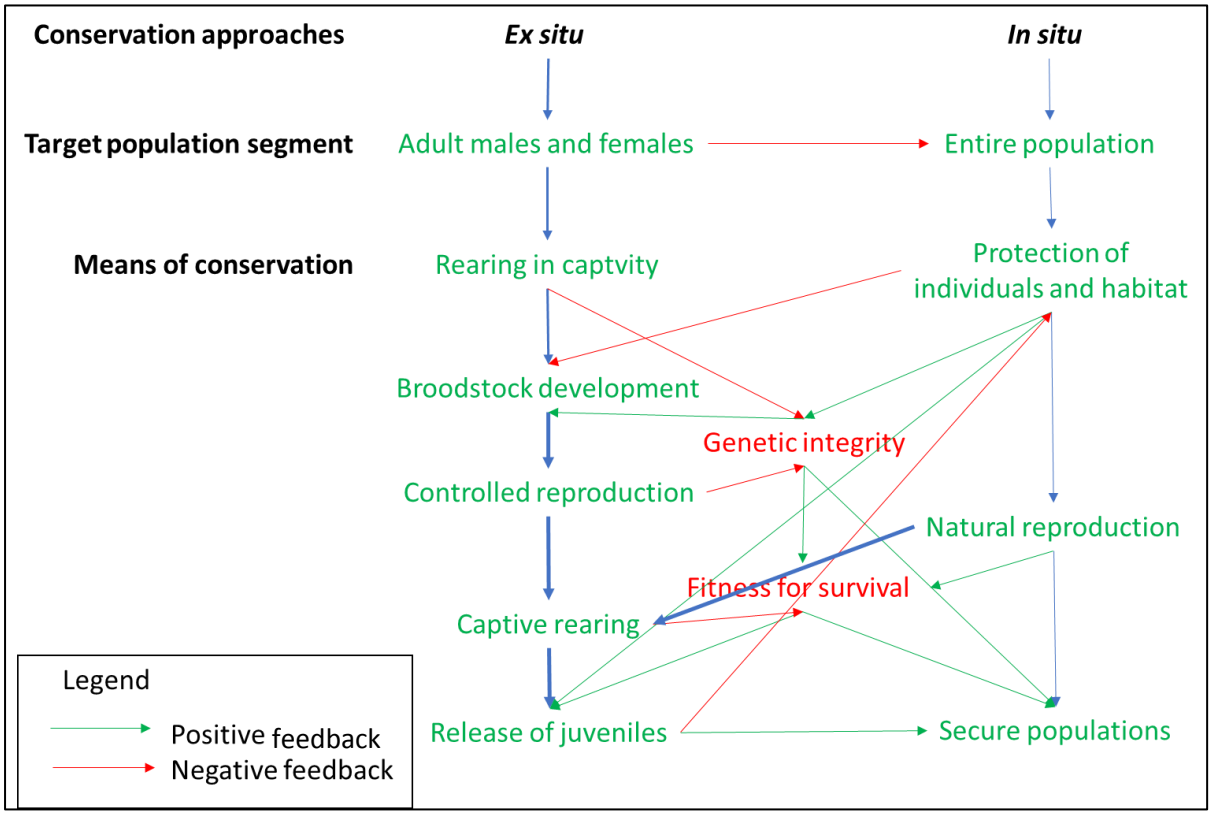

Figure 3.2: *Potential interaction of conservation approaches and measures upon genetic integrity and fitness for survival.*

In cases where natural reproduction has ceased and collection of naturally reproduced offspring is infeasible, controlled reproduction of broodstock is used to sustain both the long-term operation of the living gene bank and the support of the population through releases of animals in the wild. Maturation of broodstock should preferably be initiated under near natural rearing conditions to match the biological cycle of the animal. Hormonal induction is required to induce ovulation

in most cases. Still, it would be preferable that the animal's physiological response could be triggered by the modification of environmental stimuli (see Chapter 8).

Countermeasures might be necessary in the rearing facilities to address effects of climate change on the maturation of the fish (Sobieraj & Metelski 2023, Lassalle et al. 2010, Ruban et al. 2019), such as temperature regulation during the maturation process. To ensure the safeguarding of the broodstock, minimal invasive techniques are to be applied to collect eggs from the females (see Chapter 9).

3.8 Resource requirements

The development of species- and/or population-specific *ex situ* programs must consider the status of the respective population and take its biological and ecological requirements and peculiarities (time of spawning, migration patterns, etc.) into account when designing the rearing facilities (Chebanov & Galich 2010). To meet the aims of genetic purity of the population in question, the identification of potential sources for broodstock development or for progeny that represent the genetic variety and identity of the population in question must be implmented (see Chapter 6). To prevent the loss of animals that represent the entire heritage of a population segment, a suitable facility to maintain them is essential (see Chapter 5). This aim might be facilitated by using an existing (commercial) infrastructure or through the development of facilities to safeguard the animals and to produce offspring adapted to be released into natural conditions. It is inevitable, however, to ensure that the experience/expertise to handle, maintain and raise the animals in question is available ensuring also that animal welfare is guaranteed in the operation of the facility. This also includes the presence of failsafe plans, backup devices and a long-term strategy for the development of the captive population. In a second step, establishing and implementing propagation plans based on molecular genetics to ensure diversity is maximized and to avoid the risks usually associated with controlled propagation (e.g. inbreeding, outbreeding, domestication; see Chapter 6). Both the genetic basis and the application of releases have the potential to adversely affect the supported population in the wild (Ruban et al. 2019, Ralls & Ballou 2013). This can be the case through introgression of fish that originate from different genetic subgroups or simply by releasing a huge quantity of genetically identical fish or fish that are large enough to outcompete the wild fish and dominate subsequent reproduction. As a result, the recovery of population demographics that resemble the natural population should be a long-term goal.

Because of the very large size of these *ex situ* facilities and the required financial investment, it can be difficult for single countries to implement such measures, especially when more than one species is to be addressed. These restrictions further emphasize the need for basin-wide collaboration, allowing coordinated programs to be developed between several facilities not only to split the costs and

risks but also to preserve genetic diversity in captivity over many decades (Rosentahl et al. 2017).

3.9 Timeframe for recovery

Being species that reach maturity only after a decade or more, sturgeon recovery requires an extended timeframe. The verification of the target to reach a sufficiently large population size to ensure self-sustaining status over time without further intervention will have to be monitored over prolonged periods to be able to exclude unforeseen drawbacks. Therefore, releases might require to be continued until the population reveals continuous and regular reproduction and recruitment. Even if the pressures on the population have been addressed properly and the released individuals survive, it is essential to monitor the performance during this period to verify if recruitment is consistent and sufficient to maintain the population at a stable level (Haxton et al. 2023, Neuburg et al. 2024).

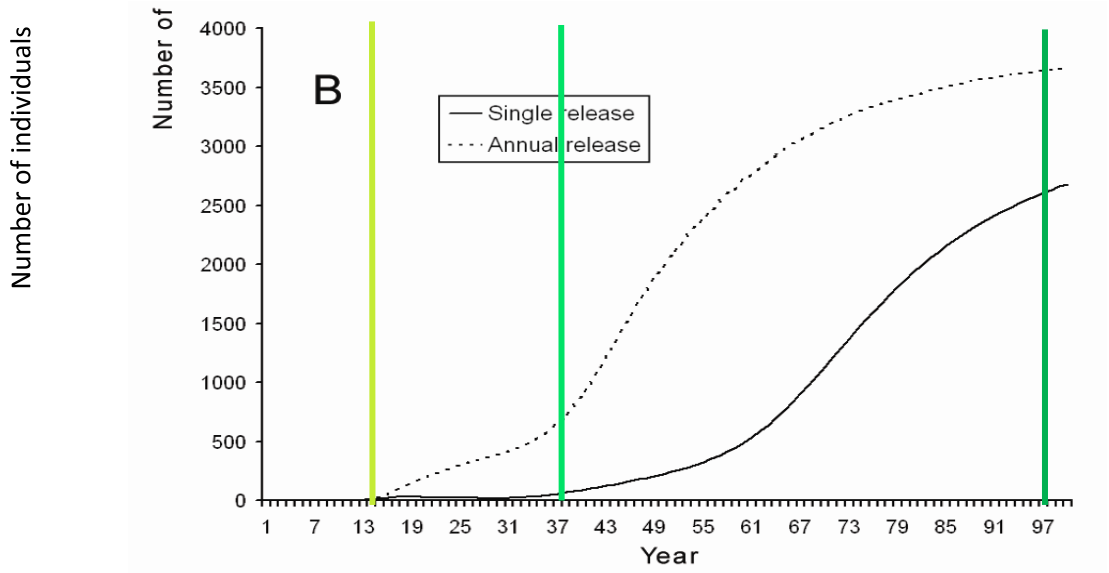

Figure 3.3: *Timeframe for recovery of sturgeon populations under continued release (broken line) and based upon single release; yellow line indicates onset of reproduction; green line indicates the start of exponential growth of the population; dark green line indicates the endpoint reaching plateau phase in supported recovery (after Jaric & Gessner 2013).*

If it is considered that the *ex situ* population at this stage might not be needed for supportive releases anymore, it is highly recommended to establish and maintain it for another generation period to be able to respond in cases when the recruitment suffers from adverse or temporally fluctuating impacts and the population begins to decline again. The initial safeguarded population segment is the most precious part of the *ex situ* process since it represents the entire genetic heterogeneity of the population in question (Boscari *et al*. 2014).

In most cases, it has taken a century or more to deplete the populations of sturgeon, and it seems rather optimistic to expect that the recovery would be a process that can be achieved in a much shorter timeframe by simply "filling" population deficits with individuals from controlled propagation over a short period of time.

3.10 Adaptive management

In order to be successful, *ex situ* programs need to be carefully planned and implemented since it has been shown in a variety of fish species that the rearing of individuals under controlled conditions alters their behavior and their cognitive abilities exemplified in salmon (Jonsson & Jonsson 2009), trout (Bradshaw 1996), and sturgeon (Carmara Ruiz *et al.* 2019). Therefore, the need to further develop scientifically based and innovative approaches of *ex situ* conservation is evident and requires constant updating to employ best practice methods and make them widely available.

When applied properly, *ex situ* conservation can be a potent tool for species conservation that supports *in situ* conservation and *vice versa*. Potential *ex situ* goals, objectives and actions should therefore be evaluated alongside the *in situ* activities in the process of conservation planning to ensure that they secure the most benefit for the overall program.

In order to allow the recovery measures to be effective, the monitoring of the effects of the measures is paramount. For this purpose, the definition of the aims of the monitoring becomes essential. For *ex situ* measures, two main approaches have to be followed:

a) The performance of the *ex situ* facility in rearing the fish comprises the growth and mortality of the fish as a summary parameter for the appropriateness of the rearing conditions, their genetic integrity, their maturation and reproductive effectiveness. Fitness of fish to be released is to be assessed prior to release (Chebanov & Galich 2011, see Chapter 12). It is noted that behavior assessments in broodstock are difficult to be carried out but with time improved rearing designs might ease this assessment in the years to come.

b) The effectiveness of the releases in contributing to the population establishment or enhancement must be assessed. For the evaluation of the performance of the released fish the following indicators are of key importance: survival, population genetics, migration and habitat use, reproduction and recruitment.

In both cases, it is essential to note that the provision of funds for monitoring is a prerequisite for successful *ex situ* measures as well as for conservation management in general. Without assessment of the measures undertaken, their effectiveness remains unknown preventing any means to be improved by adaptive

management. Conceptual mistakes in such cases are perpetuated and contribute to the wasting of resources that could be used sensibly if monitoring data would be at hand (Haxton et al. 2023). Population monitoring and habitat monitoring practices are described in detail in the Technical Guideline for Sturgeon Habitat Monitoring (Reinartz, 2024) and the Technical Guideline for Population Monitoring (Neuburg *et al.* 2024) developed under the same EC contract.

In this context, the monitoring of fish released into the wild and their performance in the natural environment, including the growth, mortality, migration and effectiveness of reproduction and recruitment are key issues to be determined (ASMFC 2024). Only sound data on the response of the released fish and eventually the fish that still reproduce naturally in addition to the impact of the releases can help to support adaptive management of the recovery measures, an adaptation of the time plan or to conclude on necessary alterations to the program and develop alternative means of population support.

The effectiveness of conservation actions will become visible through the population monitoring, namely the survival, mortalities, and recruitment or a reintroduced population. Nevertheless, habitat plays a decisive role in the performance of the released fish. As such habitat monitoring and monitoring of habitat use, including tagging and tracking to assess migration and dispersal, are essential parts of the assessment to evaluate the effects of ongoing habitat alterations through climate change or anthropogenic pressures (e.g. navigation facilitation or damming). Furthermore, population genetics in both the released individuals and the *ex situ* population will provide indications of adaptation of the offspring to the conditions of the habitat into which they are released.

In order to be able to assess the mortalities observed in the population under recovery, monitoring of fisheries and their impact through bycatch, poaching or habitat disturbance needs to be incorporated in the data acquisition.

3.11 Potential drawbacks of *ex situ* measures

While the safeguarding of wild fish (and their offspring) under controlled conditions must be considered a last resort to "buy time" to overcome the obstacles for sufficient natural reproduction and recruitment, supported recruitment can be helpful under conditions of low natural reproduction and recruitment or for reestablishing populations that have been lost. In these attempts, sturgeon hatcheries play an important role. The release of hatchery produced offspring requires thorough planning and practice to prevent adverse effects. Due care must be applied to minimize the impact of hatchery fish upon naturally reproducing conspecifics to prevent adverse effects upon an already stressed resource (Arlinghaus *et al.* 2019).

When implemented on a small but functional population, supportive stocking can have the goal to increase the speed of population recovery or focus on a different target like supporting fisheries. While the first option has been applied on several

occasions, for instance, in *A. sinensis* in the Yangtze River (Wei *et al.* 2010), in the Lower Danube between 2005 and 2009 for A*. gueldenstaedtii, A. stellatus and H. huso* (Holostenco *et al.* 2019) as well as currently in *A. ruthenus* in the Upper Danube (Friedrich *et al.* 2022), these measures require cautious implementation in order to avoid dominance of released fish over potential natural recruitment through the release of older and more robust fish and pathogen transmission. It has been recognized that stocking of hatchery-produced progeny may include the risk of altering the genetic structure of the natural populations while at the same time producing juveniles not fit for survival in nature (Sulak et al. 2014).

Usually, fish of hatchery-origin have a low genetic diversity, originating from few parental specimens, due to limited holding capacities of conventional hatcheries (see Chapter 6). In contrast, long term collection of broodstock has resulted in almost complete coverage of natural genetic diversity in the *ex situ* stocks (e.g. *S. albus*, Saltzgiver *et al.* 2012). Releases for re-introduction are currently applied for instance in the European sturgeon *A. sturio* (Germany) and the Atlantic sturgeon *A. oxyrinchus* in the Baltic Sea, *A. fulvescens* (Gessner et al. 2019; Bruch et al. 2016; Gessner et al. 2014; Kirschbaum et al. 2011).

As a result of the above considerations and based on well-established knowledge for conventional hatchery operation for any fish species, the current hatchery practices have to be optimized in terms of their appropriateness for future sturgeon rehabilitation programs (see Chapter 10, e.g. Anderson et al. 2021, Jonsson & Jonsson, 2009). Therefore, the entire husbandry and breeding process, as well as the strategy for conservation culture, must be critically assessed to adjust rehabilitation programs and subsequently the design of hatcheries and their operation mode to serve the targeted release purposes. In order to assist in understanding the requirements for conservation aquaculture for sturgeon, one has to emphasize clearly the fundamental differences in hatchery operations for commercial aquaculture and for culture for release into natural waters.

3.12 *Ex situ* rearing and aquaculture production

Identifying the commonalities and differences of conservation and production-oriented culture techniques is one of the main and pivotal goals of this document. Aquaculture production focuses on the performance of the cultured specimens of any species in the aquaculture system and meeting consumer preferences. Therefore, the entire operation aims at ensuring maximum survival (producing high numbers) and fast growth (producing a large biomass in the shortest time possible) under farm conditions. In this context the production of disease-free specimens and/or specimens resistant to disease is paramount to avoid any losses while at the same time achieving high feed conversion efficiency to reduce production costs, which leaves the fish without need to train to hunt for food. On top of the previous points, the production process requires appropriate meat composition/quality; adaptation to all-year-round spawning and need to be hardy when handled, allowing easy management for sorting and live transportation.

Practices applied in commercial hatcheries conflict with ecological, environmental and socio-economic objectives of the recovery programs (Bradshaw 1996, Braithwaite & Salvanes, 2012, Johnsson et al. 2014). They are a result of economic pressures rather than ecology and oriented towards maximizing a short-term profit. As a consequence, none of the target objectives mentioned above for commercial aquaculture of sturgeons or any other aquatic species applies for conservation cultivation for release.

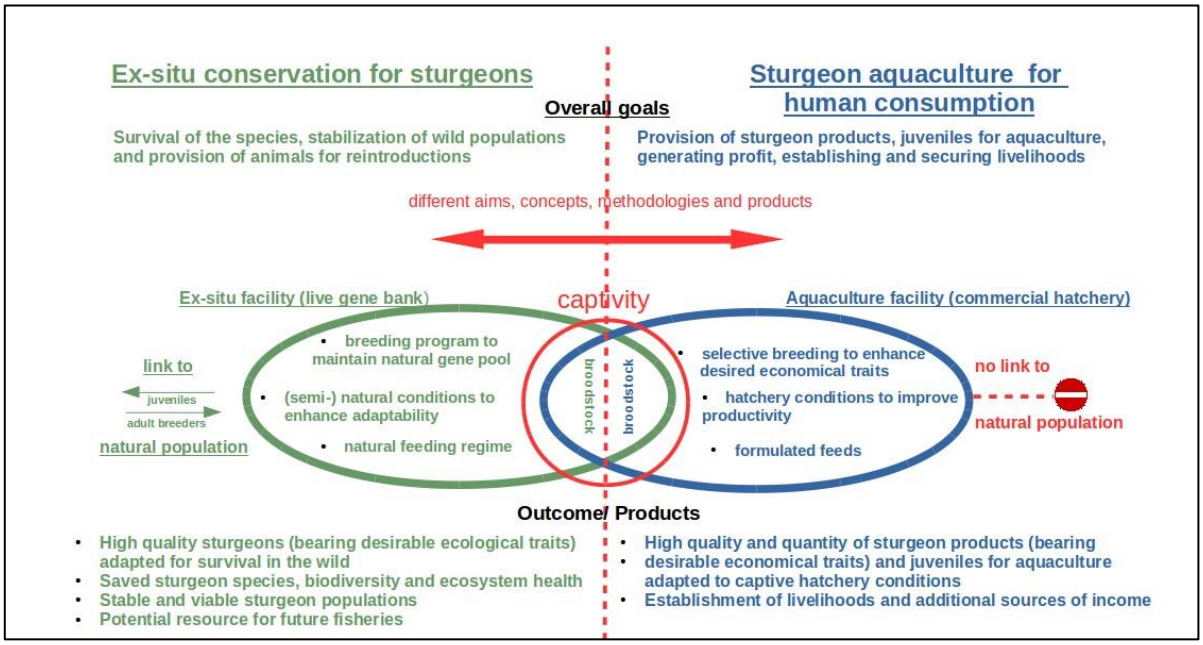

Figure 3.4: *Comparison of rearing for release and commercial production for consumption of sturgeons providing an overview of main differences (after Reinartz, 2015).*

As a result, numerous projects and activities in recent years have aimed to increase the release of sturgeon to rehabilitate the populations in the wild, but many have failed to achieve their expected goals due to an insufficient preparation and duration of the measures planned (see Chapter 12).

In general, the rearing for release aims at the provision of heterogenous rearing conditions with regard to water quality, light, feeding regime and responsiveness to predator presence. The approaches vary from incubating and rearing early life stages in river water, with substrate in semi natural river-like structures to application of rather controlled conditions that are followed by training schools during which the fish are challenged with the location and uptake of natural diets, coping with temperature fluctuations, exposition to increased current velocity and heterogeneity and predator exposure. In all cases, the aim is to increase the responsiveness and behavioral flexibility to increase survival after release.

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Chapter 4: Capture, Handling, Sampling and Fish Welfare

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4.1 Introduction

Conservation programs usually rely on the capture of wild fish (see chapter 7) for the establishment of *ex situ* programs where progeny is either reared for an indefinite period (e.g. larvae, age-0, etc.) or released into the wild at a specified age, size or time (Anderson *et al.* 2022). This chapter will briefly cover the legal requirements for collection, and possession, animal welfare and review the techniques to capture, handle, collect samples, and anesthetize sturgeon. In addition, recommended data collection and potential sampling techniques are reviewed.

4.2 Legal Framework and Permits

Animal protection laws are a result of changing attitudes and social norms that have been developing over many years, and every country has its own established regulations for collection in the wild and for use of animals in conservation programs, research, teaching, and farming. For example, the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) concerns the use of animals in research, and its provisions cover areas such as care and accommodation, conduct of experiments, and humane euthanasia. In North America, the Endangered Species Act (US) and Species At Risk Act (Canada) govern access to the resource either in the wild or captivity.

The legal framework is typically provided at the national level, addressing not only animal use and welfare, but for almost all sturgeon populations worldwide, there are species-specific regulations in place due to their threatened and endangered status. Since the laws, restrictions, and required permits vary from country to country, they will not be presented here, but it is emphasized that it is of utmost importance that the personnel involved in capture, handling, sampling, and retaining sturgeon at *ex situ* facilities implement the conditions of the relevant legal framework for the species in question.

4.3 Fish Welfare

Fish welfare remains a controversial issue (Diggles *et al.* 2024) since it is an anthropogenic reflection upon the response of an animal to the environment it is exposed. As such, the underlying notion is based upon similarities between humans and its conspecifics which might or might not be fully justified and reflect a cautionary approach. We use the term welfare to describe the condition under which the behavior and physiological state of an animal is optimized. Reference for this state is the behavior and response observed in the natural environment and is challenging to measure accurately in captivity. In this respect, deviating conditions imposed upon the animal is interpreted as stress resulting in a compromised physiological and behavioral state of the animal.

Since stress in animals is a critical aspect of welfare, which is triggered through changing or suboptimal conditions of rearing, unsuitable handling and feeding, precise planning, selection of methods and careful handling and rearing should be self-evident principles. Below are some highlighted aspects of sturgeon welfare when capturing, handling, and sampling.

4.4 Collection and Handling

4.4.1 General Considerations

Since most sturgeon species are endangered or threatened, special attention must be devoted to ensuring safe and careful fish handling to avoid adverse impacts upon the fish and the population as a whole. The collection, processing, and sampling of fish must only apply safe techniques and must consider fish welfare during all stages of the operation. Applying the precautionary principle, especially when dealing with critically endangered species, should be self-understood. When large fish are captured, the fish can do considerably damage to themselves, the gear, and the crew if not handled appropriately and quickly. The amount of handling and sampling procedure after capture will vary by the aims the program has and thus between different conservation programs. For example, if only mature fish are being targeted for transport to *ex situ* facilities, then maybe only limited assessments (e.g. length data and tagging) will be done in the field, and the rest of the data collection and sampling may be conducted in stable conditions following transport to reduce total stress. Stress in mature individuals may lead to reduced reproduction and recruitment success which can further endanger populations. It is known that stress can impede effectiveness of reproduction through reduction of gamete and subsequently progeny quality (e.g. Campbell *et al*. 1994; Pankhurst & Van der Kraak 1997; Semenkova *et al*. 1999; Schreck *et al*. 2001; Bayunova *et al.* 2002), increase incidence of gonadal atresia (e.g. Clearwater & Pankhurst, 1997; Pankhurst & Van der Kraak, 1997; Cleary *et al*. 2000), and potentially compromise immune function (e.g. Georgiadis *et al*. 2001). Immature fish are typically more robust prior to being reproductively active and may be more thoroughly sampled (see below), tagged, and then released back into the wild. Since stress is cumulative, stress associated with handling events

should be reduced under all circumstances. Reducing stress during each handling event should be attempted by assessing the relevance of the procedure (e.g. is there a less invasive technique that will provide the data needed with acceptable accuracy), the duration of the procedure, and the conditions under which the animal is handled (e.g. temperature, air exposure, dissolved oxygen, water quality, direct sunlight, etc.). Stressors experienced in one developmental stage can have an effect during later developmental stages (see Schreck, 2010), and as such, minimizing stress is essential to ensure minimal impact upon the fish.

4.5 Capture methods

Eggs, embryos, juvenile, subadult and adult sturgeon can be captured by active (e.g. drift, gill and trammel netting, trawling, seining, electrofishing) or passive (gill and trammel nets, fyke, stow and hoop nets, D-frame nets, baited setlines, egg mats) methods. The methods of choice vary between life cycle stages, locations, and purpose (e.g. Wanner *et al.* 2007; Doyle *et al.* 2008; Kahn & Mohead 2010; Hupfeld *et al.* 2022; Haxton *et al.* 2024). They are presented in detail in the Technical Guideline for Sturgeon Population Monitoring (Neuburg *et al.* 2024) and are only summarized briefly.

4.5.1 Active Fishing Methods

4.5.1.1 Drift Gill Netting

The operation of drifting gill or trammel nets is from a boat or between two boats, which allows coverage of a large area of water. The net is typically deployed from a boat, tied to buoys at both ends, and maintained in position while drifting, rowing, or motoring the boat to match the speed of the drift. The net is fished while dragging on the bottom while it is maintained perpendicular to the flow.

4.5.1.2 Trawling

Trawling utilizes a motored vessel that drags a net bag of multifilament equipped with a bottom chain and floats on top between two otter boards that keep the net open. For sturgeon, the net is usually fished with continuous bottom contact. Due to the constant motion and the tendency to collect debris, fish in the trawl can suffer trauma, but short trawl sets (30 minutes) can help to minimize the impact.

4.5.1.3 Seining

Seining refers to the utilization of a net to encircle a target either being operated from shore in rivers or coastal waters or from two boats when operated at sea (purse seining). The net is generally made of polyfilament nylon netting and is equipped with a leaded bottom and float line.

4.1.5.4 Electrofishing

Electrofishing has proven to be an effective method for collecting sturgeon mainly in shallower water (e.g. Harris *et al.* 2017; Hupfeld *et al.* 2022; Haxton *et al.* 2023). Fishing with a direct current field between a cathode at the boat and the

anode that typically is the dip net operated in front of the boat, leads to electronarcosis, and tends to drag fish to the anode where they can be captured. The major drawback of electrofishing is the limited range of the field reducing its effectiveness on fish in deep water.
An alternative option for deep, mid-river application is the use of an electrified bottom trawl with either cathode and anode being placed on bottom chain and top line or the field being generated on a static frame that comprises the opening of the net. The length of the net has to be sufficient to keep the captured fish out of the electric field. The method is generally only efficient for smaller-sized individuals, such as young of year.

4.5.2 Passive Fishing Methods

4.5.2.1 Gill and Trammel Nets

Stationary gill and trammel nets (Figure 4.1) are equipped with a lead line and a float line and are set between two anchors that keep it fixed in position. As such the net collects fish that are moving perpendicular to the net when they are either entangled or are captured by running their heads into the mesh. In some cases, mortalities have been documented and were associated with elevated water temperature, extended soak times, and net interactions (clamped opercula) that prevented normal respiration. To decrease mortality risk, collection methodology should be modified (e.g. shorter soak times) as needed based on the environmental conditions.

Figure 4.1: *Sturgeon (Scaphirhynchus albus) captured in a trammel net on the Missouri River, USA (Photo credit: D. Ritter).*

4.5.2.2 Fyke, Stow and Hoop Nets

These generally similar nets consist of netting in the form of a cylindrical or cone-shape (fyke), cone or pyramid shape (stow), or nylon mesh hung on round frames (hoop), all with a funnel shape end to retain the fish. These nets are fixed at the bottom with stakes or anchors. These large passive gear types have the advantage of low mechanical injury and stress on the fish, and as a result the survival and fitness are high (Gessner *et al.* 2011).

4.5.2.3 D-Frame Drift Nets

Anchored D-frame drift nets are set below known spawning sites and have been used for sampling embryos, larvae and early juveniles (e.g. Wei *et al.* 2009; Usvyatsov *et al.* 2013; Poytress *et al.* 2015; Haxton *et al.* 2023). The nets are a steel frame in a D- or rectangular- shape that is maintained in position by an anchor or by steel piles driven into the sediment. The frame is equipped with a conical net and the cod end is equipped with a detachable mesh sampling container. Depending on the current velocity and amount of debris accumulation, the soak times must be adjusted. In strong currents or high debris drift, a retrieval might become necessary every 10-20 min. Mortalities associated with this sampling technique can be high, due to the mechanical pressure and stress on larvae.

4.5.2.4 Baited Setlines (Trotlines)

The use of baited setlines (Figure 4.2), also known as trotlines, for capturing sturgeon has been successful for many species (e.g. Thomas & Haas, 1999; Holtgren & Auer, 2004; Wanner *et al.* 2007; Steffensen *et al.* 2013; Patton *et al.* 2020; Haxton *et al.* 2023). Setlines will vary in length, and hook sizes (commonly 2-0 to 20-0) and type will vary among target species and body size. They are often set in mid-river with anchors and buoys at each end. Hooks should be spaced at a sufficient distance to reduce the chance that captured fish become entwined. Set times (4-24 h) will vary depending on the rate of fish capture and/or bait loss. Setlines have versatility as they can be set deep and in currents where other capture techniques may not be as effective (Haxton *et al.* 2023).

Figure 4.2: *Single baited setline used to catch Acipenser transmontanus on the Columbia River, USA. A setline consisted of 125 ft of ¼ inch three strand nylon rope and a separate 25 ft of rope both attached to a 30 lb Columbia River style four-prong anchor. The other*

end of the 125 ft rope was attached to a buoy, while the other end of the 25 ft rope had a 20/0 mustad circle hook with a stainless-steel halibut snap (8/0 swivel) baited with one third of the body of an adult Alosa sapidissima (Photo credit: S. Bettin).

4.5.2.5 Egg Mats

Due to sturgeon egg adhesiveness, egg mats can be used to verify spawning locations and collect embryos to be hatched at *ex situ* facilities. This technique requires knowledge of potential spawning regions in a river system and has been successfully utilized (e.g. McCabe & Tracy, 1994; Sulak & Clugston, 1998; Duncan *et al.* 2004; Poytress *et al.* 2015; Gillespie *et al.* 2020; Haxton *et al.* 2023). Egg mats can range in design from simply wrapping concrete cinder blocks with air filter material (Haxton *et al.* 2023) to use of two rectangular pieces of air filter material secured back-to-back within a welded steel frame (Brown, 2007) held in position using various types of anchors, with a marker float or buoy attached. Egg mat retrieval and sampling can range from 1-3 days. Fertilization dates can be back calculated using known rates of development by recording the observed development stage and the average daily water temperature at the time of collection (Dettlaff *et al*. 1993).

4.6 Handling Procedures

Removal of fish from the capture gear has priority when bringing the gear into the boat. Depending upon the conditions and gear type used, the length of it might need to be fully retrieved before removal of fish can be started. In this case, water supply and cover from direct sunshine are important means to reduce stress in captured fish. Nets must be treated as consumables as the value of a live sturgeon is higher than the cost of the nets. It is advisable to have knives, ideally with a sickle blade for worker safety, at hand to cut fish out of nets if removal exceeds 1-2 min. If a sturgeon removed from fishing gear appears to be non-responsive, it is often possible to resuscitate these fish by flushing water, preferably enriched in oxygen, over the gills or into the mouth until recovery is obvious. Dragging the fish back and pushing it forward requires careful operation to prevent injuries so that the gill filaments are not damaged.

If it is necessary to hold sturgeon for short periods while fishing continues, there are various methods depending on fish size and number caught. When water quality is acceptable, portable net pens are a good option for holding fish. Mesh sizes should be large enough to allow for the free exchange of water but small enough to prevent entanglement of all sizes of fish being sampled. It is preferable to use nets with knotless webbing and have a means of covering the net pen to prevent escape as well as sunburn. It is not recommended that fish be held for extended periods of time when water temperatures are high. Thermal maximums will differ between species and populations based on their acclimation to local water temperature regimes and the rate of temperature change the fish are

exposed to during collection and holding. If air temperatures are lower than 0° C, handling sturgeon should be avoided to prevent damage to skin and tissues and potential death. If freezing temperatures cannot be avoided, the sturgeon must always remain in water while collecting morphometric data and sampling.

Holding tanks should be designed to accommodate the size and number of fish that might be held before being handled. When fish are held on board a research vessel, they should be placed in tanks that accommodate the size of the fish (Figure 4.3). The tanks are filled by a water supply that allows for total replacement of the water volume every 15-20 min. If maintained in a static bath, aeration or oxygenation the water should be exchanged every hour to maintain water temperature and quality. A sump pump equipped with a long hose allows collection from deeper water levels to minimize the temperature fluctuations between catch and handling on board. Water level in the tank should be sufficient to entirely cover the fish. Temperature and oxygen levels should be frequently monitored and controlled. Oxygen saturation should not exceed 110%. This can result in decreased respiration and thereby cause accumulation of CO_2 (hypercapnia), which can be lethal. If pure oxygen is used to augment ambient oxygen levels, it is to be measured frequently. In static water, osmotic stress can be relieved by the addition of 0.25-0.50% uniodized salt depending upon body size and/or the use of commercial electrolytic solutions like Stress Coat™ can be beneficial.

Figure 4.3: *Holding tank on board of a sampling vessel with Scaphirhynchus albus (Photo credit: C. Guy).*

A hooded stretcher of appropriate size (see Figure 4.4), with a drain at the base of the hooded region, is commonly employed to hold fish. The stretcher, which should be the size of the fish and allow to circumvent its entire girth, is made from a coated textile with a soft surface, equipped with drains to let the excess water runoff, a hood to cover the head of the fish and two handles. For the ease of

measurement, a meter band can be sewn into the stretcher or a soft, flexible measuring tape may be used to collect fish length. Individuals are captured from either the holding tank or net pen by carefully guiding an individual (by pectoral fin and snout) directly into the stretcher, which is then moved to the stretcher holder/sampling area. For very large fish, the stretcher can be tethered to the side of the boat for collection of samples. Once in the stretcher, the fish is rolled ventral side up, and upon reaching the sampling area, a hose of oxygenated fresh water is placed directly above the mouth or in the mouth. Flow is adjusted to have a consistent outflow from both ventilating opercula. The stretcher holder should be designed with sufficient pitch to allow water to drain toward the hood. Mature sturgeon under this condition typically exhibit tonic immobility and begin ventilating their opercula due to the water flow (Wagner & Cooke, 2005; Chapman *et al.* 2019; Marbury *et al.* 2020; Colborne *et al.* 2022). One of the most useful physiological benefits of tonic immobility is analgesia or the decreased sensation of pain, and Páez *et al*. (2023) and Kessel & Hussey (2015) proposed that tonic immobility be recognized as an acceptable anesthetic technique for some surgical procedures. However, the smaller, immature sturgeon tend to be more active and may require some level of sedation (see section 4.6). Fish should not be held for extended periods of time in the stretcher. Captured wild sturgeon often have extremely sharp scutes and denticles which can easily slice skin. Thus, it is recommended that gloves be used when handling sturgeon and have back-up stretchers available.

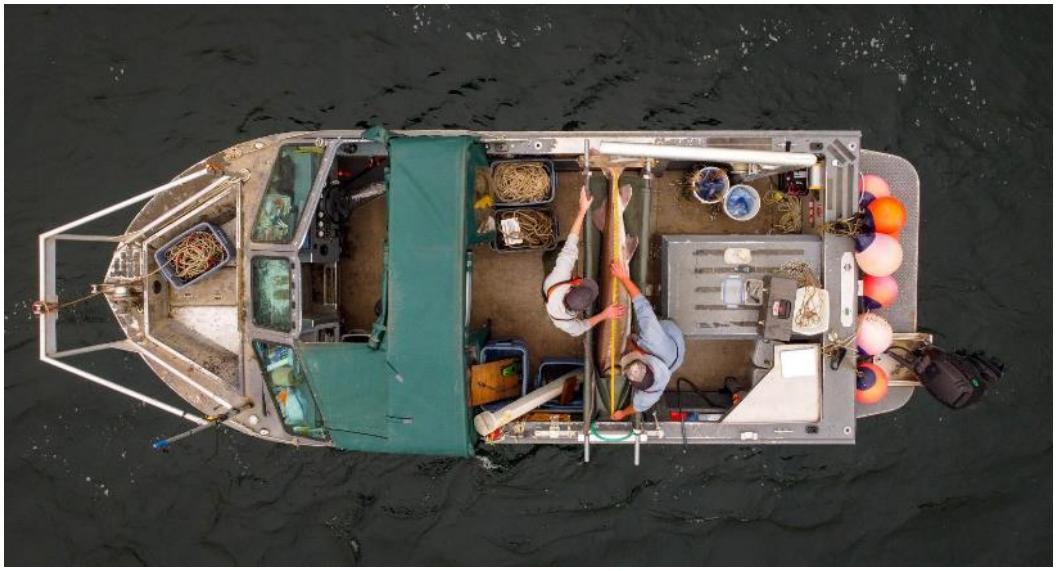

Figure 4.4: *Hooded stretcher for safe handling and transport of sturgeons following catch shown extending across the gunwales of the boat (Acipenser transmontanus) while sampling (Photo credit: J. Crossman).*

When sampling sturgeon downstream of dams, be aware that while the turbines are operating, total gas pressure (TGP) of the water may be elevated. This condition can cause stress and in extreme cases gas bubble disease. When fish are retrieved from the depths in these conditions, even mortality may result.

Therefore, TGP should be tested in such locations and sampling rescheduled when TGP is too high.

Release may be the final step in field sampling of some sturgeon while some may remain in a holding tank, transported to shore and then to an *ex situ* hatchery. Sturgeon are physostomes in which the swim bladder is connected to the gut through a pneumatic duct. They can overinflate their swim bladder when stressed during capture and handling by engulfing air and not releasing the excess air sufficiently. Also, when collections are made from extreme depths, the fish should be brought to the surface very slowly, allowing the fish to release gas from the swim bladder as they are brought to the surface. This is evidenced by "bubbles" when slowly retrieving a net from depth. If the fish has excess air in its swim bladder and it seems unable to release the gas, return the fish to neutral buoyancy prior to or during release. Slight pressure on the anterior part of the belly can be applied in a posterior to anterior motion to assist with gas release or keeping the fish upright upon release and slowly moving it back and forth in the water helps to release gas from its swim bladder. In any case, the behavior of the fish must be monitored after collection and sampling until a full recovery and normal swimming is reestablished to ensure high survival.

4.7 Anesthesia

A key component of sturgeon welfare when sampling after capture is the use of anesthesia which is based on multiple factors, including the time and degree of invasiveness of the procedure, legal restrictions, cost-effectiveness, user safety and environmental impact (waste disposal). In general, it is recommended to weigh the risk and stress imposed by using an anesthetic against the expected impact. Some countries and agencies will require the use of anesthetics for any invasive procedure to meet animal use and care protocols and address animal welfare issues (Zahl *et al.* 2012; Williot *et al.* 2018).

Guidelines and policies regarding the use of chemical anesthetics vary between manufacturers and between countries. Hatchery personnel should consult local and regional agencies for the latest regulations and currently approved chemicals. The primary goal of using an anesthetic is to immobilize the animal while blocking nerve impulses when conducting invasive procedures. The stage of anesthesia used (Table 4.1) will depend on the degree of invasiveness and length of time the procedure will take. Procedures like ultrasonography, fin clipping, blood sampling, tagging for identification purposes do not typically require anesthesia. While endoscopy, celiotomy/biopsy, pectoral fin ray sampling, as well as the insertion of a telemetry tag by surgical procedures will require anesthesia to stage III or stage IV (Table 4.1.).

Table 4.1: *Stages of anesthesia (modified from Summerfelt & Smith, 1990; Burka et al. 1997; Sneddon, 2012).*

Stage	Anesthesia level	Criteria
I	Light sedation	Disorientated, reduced activity, slight loss of reactivity to stimuli
II	Excitation	Agitated, increased activity and gill ventilation rate, difficulty maintaining equilibrium
III	Light anesthesia	Partial loss of equilibrium, decreased gill ventilation and muscle tone, reaction to strong stimuli only
IV	Surgical anesthesia	Total loss of equilibrium, occasional and shallow gill ventilation, relaxed muscle tone, no reaction to stimuli, reduced heart rate
V	Deep anesthesia	Rare gill ventilation and heart contractions, no muscle tone
VI	Overdose	No gill ventilation, cardiac arrest and death (euthanasia)

4.7.1 Chemical

Three of the more widely used fish anesthetics 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 (potentially carcinogenic), therefore personal protective equipment must be used when handling the chemicals and solutions. Also, the disposal of the solution after use may require special treatment.

The induction and maintenance of anesthesia is temperature dependent. Concentrations must be applied with care and should be adapted to the water temperature. Supplemental oxygen should be added to the anesthesia tank and monitored throughout the process.

Immersion is the most common method for fish anesthesia, as the agents dissolved in solution enter the bloodstream through the gills and skin. An individual may be maintained at the induction dose after reaching the desired stage of anesthesia if the procedure(s) last no more than 10-15 min. After this, the induction dose will likely result in excessive anesthetic depth (Neiffer, 2021), so a maintenance dose (approximately half the induction dose) is used to maintain the desired level of anesthesia until the procedure has been completed. Induction and recovery times vary, primarily based on the dosage level, the duration of time

the fish is under anesthesia, and the water temperature. The guideline for dosage is to induce the desired state of anesthesia within 5-10 min and then have a similar recovery time (Neiffer, 2021). Exact dosages for the lowest induction and recovery time will vary with species, body size, age and life stage, stage of anesthesia targeted, and water temperature and quality (Summerfelt & Smith, 1990).

Tricaine methanesulphonate (MS-222), sold under several trade names (i.e. 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 d, and both the drug and stock solutions should be protected from light (Neiffer, 2021). The 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 60-250 mg/L, and maintenance doses reported have ranged from 75-90 mg/L (Conte *et al.* 1988; Hernandez-Divers *et al.* 2004; Divers *et al.* 2009; Kahn & Mohead, 2010; Matsche, 2011, 2013; Johnson *et al.* 2016; Webb *et al.* 2019).

The active ingredients of clove oil are eugenol (approximately 84%), iso-eugenol (5-10%), and methyl eugenol. Clove oil is incompletely water soluble, so using 95% ethanol as a solvent with a mixture of one-part clove oil to nine parts ethanol yielding a 100 mg/mL stock solution is often used. Typical induction doses are 20-70 mg/L (Neiffer, 2021; Kübra, 2022). Dosages need to be adjusted based on the percent content of eugenol and how fast or slow induction takes to reach the desired stage of anesthesia. Water-soluble alternatives to natural clove oil like 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 (Hurvitz *et al.* 2007; Gomulka *et al.* 2008; Kahn & Mohead, 2010; Feng *et al.* 2011; Adel *et al.* 2016; Johnson *et al.* 2016; Webb *et al.* 2019).

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

The anesthetic properties of carbon dioxide (CO_2) on fish have been documented, including sturgeon (Holcomb *et al*. 2004; Gomułka *et al*. 2015; Baaberoo *et al*. 2016), and although it can decrease stress and immobilize fish for a quick procedure, like ultrasonography, its analgesia properties may not be appropriate for invasive procedures (Mylniczenko *et al*. 2014). The blood gas and acid-base balance of a fish are altered when using CO_2 (Gomułka *et al*. 2015), thus sodium bicarbonate should be added to buffer the bath to neutral pH (maintain pH 6.5-

7.5; Mylniczenko *et al*. 2014). Typically, CO_2 compressed gas is introduced into a large water bath with ceramic plate diffusers, and fish are immobilized within 5-10 mins. The use of CO_2 is routinely used on sturgeon farms in the USA when sexing large numbers of fish with ultrasonography. However, exposure of fish over a longer period should be avoided since accumulation of CO_2 in the blood reduces blood pH and renders the fish susceptible to elevated water pH values during recovery, especially if ammonia levels are elevated (Gessner *et al*. 2009).

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

Euthanization of fish is commonly carried out by chemical anesthesia overdose (usually for larvae and fish < 5 kg) or in larger fish by concussion/percussive blow to the head and subsequent pithing or severing of the notochord just behind the head (Neiffer 2021).

4.7.2 Physical

There has also been an increasing interest in using electroimmobilization (review by Reid et al. 2019) to anesthetize fish, including sturgeon (Henyey *et al* 2002; Hudson *et al.* 2011; Balazik, 2015; Johnson *et al.* 2016). 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 (Gosset & Garaïcoechea 1974; Gosset & Rives 2004), however, its effect upon receptors for pain/stress perception is not shown. Some of the benefits listed include no expiration/degradation of chemicals, no disposal protocols, significant shorter induction and recovery times, easier to adjust "dosage", and a single device is reusable as opposed to chemicals that must be purchased regularly. Challenges include maintaining equipment in 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.

4.8 Sampling

In order to safely handle large sturgeon, experienced individuals and a crew with predefined duties are recommended to minimize risk. Because of inherent risks to both fish and humans, all staff who wish to conduct collection efforts are strongly encouraged to initially participate in collection efforts with experienced personnel and to undergo appropriate training prior to participating or conducting independent efforts.

Each conservation program that utilizes wild-caught sturgeon will develop their sampling procedures and own set of data to be collected. One set of data typically covers the details of the gear utilized (e.g. gill net: location, habitat, depth, date, time, length of set, length and depth of net, mesh size, etc.). A second set of data comprises each fish captured, listing the sampling procedures conducted and samples collected, tag type and identification number, and whether the fish is either released or held for transport to an *ex situ* facility. Below are some of the common sampling procedures.

4.8.1 Physical Examination

Depending on the location and ongoing sturgeon conservation programs, collected sturgeon should first be examined for evidence of previous capture events. This could include the presence of internal and/or external tags and/or external markings (e.g. removed scutes, fin clips) (see Chapter 13). Each captured fish should also be examined for injuries, scars, deformities or other physical abnormalities, and photographs of each fish collected and/or sampled are recommended.

4.8.1.1 Length, Girth and Weight

Sturgeon should be measured for total length, fork length and girth (circumference of the body at the widest section of the body; Figure 4.5). Weighing, especially on a moving or swaying vessel can be difficult and imprecise but should be conducted if it is possible. Precision of the measurement depends upon the size of fish and the error associated to the technique applied. Fish that will be transported to an *ex situ* facility can be accurately weighed when unloading from the transport vehicle.

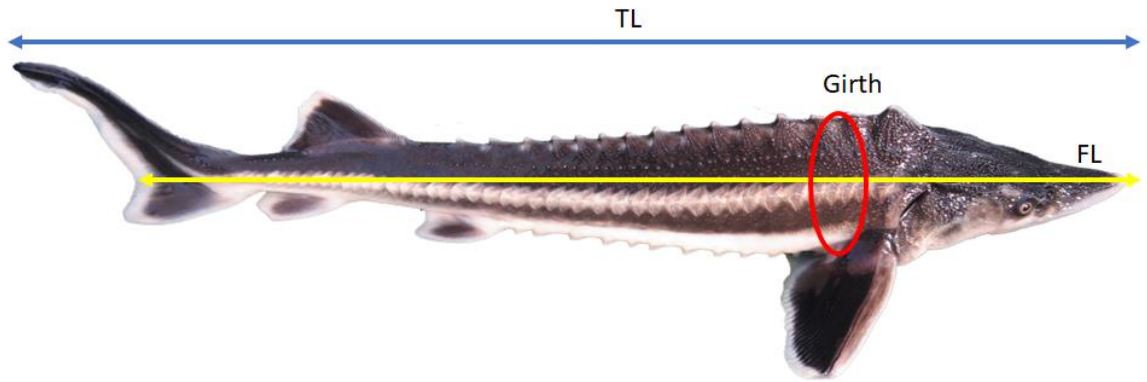

Figure 4.5: *Measurement of Total Length (TL), Fork Length (FL) and girth (Photo credit: T. Friedrich).*

4.8.1.2 Sex Determination and Maturation Assessment

To obtain a comprehensive picture on the state of the population, the identification of sex and stage of maturity of a given fish is a vital element and is most important for selecting mature individuals to retain and transport to an *ex situ* facility for

spawning induction. Sexing of wild fish can be difficult because sexual differentiation occurs only after age 3-4 years, and examination requires either invasive surgical methods or non-invasive ultrasonography (reviewed in Chapter 8). Newly developed sex markers using genetic analysis (Kuhl *et al.* 2021) are proving proficient for several sturgeon species and may be conducted along with other genetic analyses, although this analysis will only provide sex, not the stage of maturity, and the analysis must be conducted in a specialized laboratory.

4.8.1.3 Genetic Sampling

Applying genetic techniques allows us to understand the presence of a species in each area (i.e. native, non-native, hybrid, or aquaculture origin) and the movements of sturgeon on different spatial scales. Also, for some species, multiple spawning populations may exist within a single river system leading to different lineages utilizing different spawning sites. Genetic technologies may thus also be used to assign individuals to certain natal rivers in marine catchments, where sturgeon from different spawning populations may aggregate. For mature fish to be used for spawning in *ex situ* facilities, it is important to establish their genetic relatedness for breeding plans (see Chapter 6).

Genetic sampling involves collecting a small piece of fin (0.5 – 1.0 cm^2) which is placed in an ethanol resistant labeled screw cap or snap-cap tube filled with 95% non-denatured ethanol using surgical scissors and forceps. To prevent cross-contamination between fin samples, the surgical tools should be thoroughly cleaned and disinfected between samples. Samples can be stored at room temperature.

Figure 4.5: *Taking a fin clip for genetic analysis with an automatic sampler (Acipenser ruthenus) (Photo credit: T. Friedrich).*

4.8.1.4 Gastric Lavage

The identification of food organisms for different life stages of sturgeon may lead to identification of certain habitat types where such organisms are abundant and such favorable conditions could help to identify locations where the fish are aggregating. Gastric lavage is a reasonably safe and effective technique for flushing food items from the stomach of live sturgeon (Haley, 1998; Barth *et al.* 2013; Brosse *et al.* 2002).

The use of light anesthesia (Stage III, Table 4.1) is recommended to relax the fish and minimize the risk of injury during the procedure. Intramedic semi-rigid polyethylene tubing, with a blunted leading edge is used, and the outer (i.e. 2.1-7.0 mm) and inner diameter (i.e. 1.6-5.8 mm) will increase depending on the size of the fish. Larger diameter tubes have also been utilized to act as a sleeve to assist in getting the smaller diameter tube down the esophagus. The tubing is carefully and slowly inserted down the pharynx and esophagus, and into the forestomach (Figure 4.6; proventricle; Vajhi *et al.* 2013; Crossman *et al.* 2016).

Various sized syringes, garden sprayers, 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 very limited to protect the fragile internal organs.

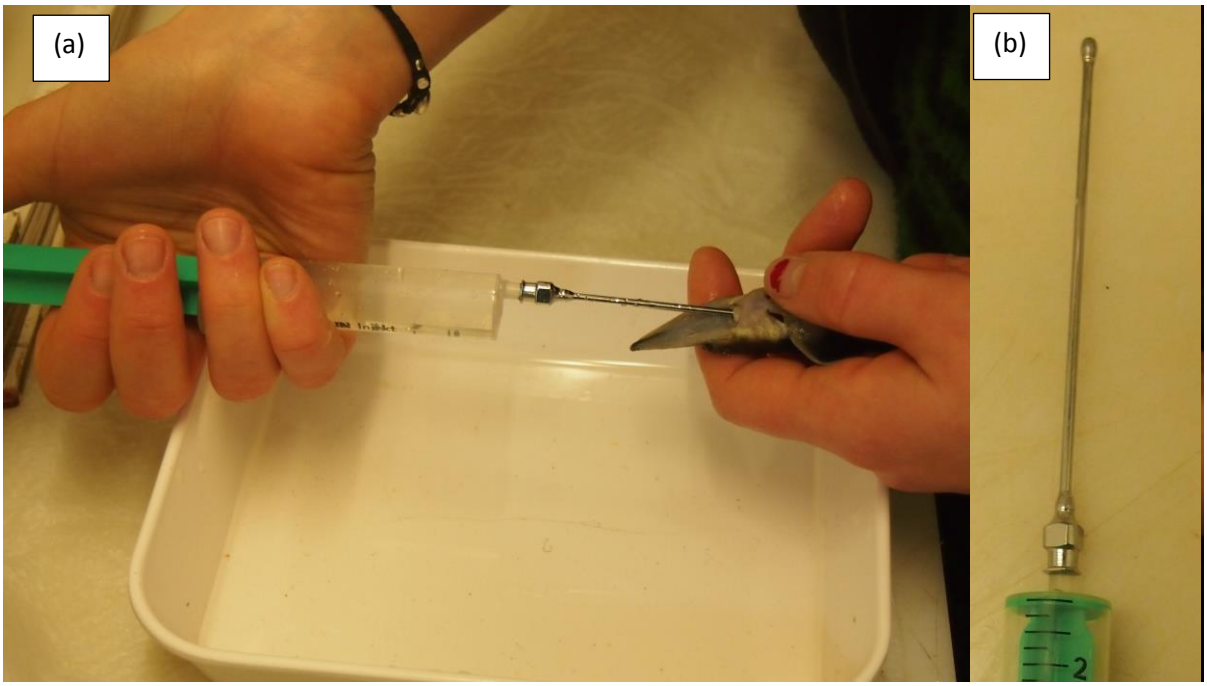

Figure 4.6: *Gastric lavage in young of year sturgeon using a syringe (a) with a blunt needle (b) (Photo credit: J. Gessner).*

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. Gently moving the tube in and out while pumping seems to enhance the effectiveness of regurgitation.

4.8.1.5 Age Determination

In sturgeon, age estimation is commonly applied using a 1-2 cm section of the first pectoral fin ray that is removed with a fine (diamond) saw in the field. After air-dried, the fin ray is sectioned into thin slices and sanded smooth with increasing grades of sandpaper, examined under a dissecting scope until it's at the right thickness to see the growth rings (annuli), and the annuli are counted from the center and working outward (Figure 4.7). Alternatively, otoliths or bony scutes can be used for aging, but because otolith removal is a terminal procedure, this method is only for the evaluation of carcasses, occasional mortalities, or fish that must be euthanized.

Figure 4.7: *Location of collection on the first pectoral fin ray to age sturgeon. Growth rings (annuli) are counted from the center and working outward (Acipenser transmontanus) (Photo credit: J. Crossman).*

Analysis of non-lethally collected fin rays can provide an estimated age but caution must be applied since there are several factors affecting the aging procedure. Feed deprived years, reduced growth during reproduction years, and the compaction of slow growth increments for older individuals increases the difficulty of accurate readings. A number of studies have reported the unreliable nature of determining age estimates from older sturgeon pectoral fin spines (e.g. Rien & Beamesderfer 1994; Hurley *et al.* 2004; Whiteman *et al.* 2004; Ghere *et al*. 2024). However, Bruch et al. (2009) provided a verification model for older lake sturgeon using

bomb radiocarbon assays allowing for a mathematical correction for reading errors in mature fish.

4.9 Tagging, Release or Transport

Sturgeon captured and sampled may be released back into the wild (Figure 4.8) or transported (see Chapter 14) to an *ex situ* facility (see Chapter 5), and some or all sturgeon will be tagged (see Chapter 13) with an internal tag and in some cases an additional external tag.

Figure 4.8: *Fish released after sampling (Acipenser transmontanus). Photo credit: M. Marrello.*

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Chapter 5: *Ex situ* facility concept and design

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5.1 Purpose of *ex situ* rearing facilities

As outlined in Chapter 3, the main purpose of an *ex situ* program is to secure the genetic heterogeneity of a species population under the risk of extirpation while supporting it through limiting the major bottleneck of ELS mortality. There are three different strategies to reach this aim which have been discussed in Chapter 3:

1) Capture wild-caught embryos and larvae to be reared *ex situ* until a target release size is reached to minimize natural mortality,

2) Annual capture of pre-spawn wild broodstock, induced reproduction, and rearing of progeny for release. Post-spawned broodstock are released back at the locations of capture or

3) The establishment of a captive broodstock to secure the genetic variability of the natural population and to reproduce them in captivity. Post-spawned wild caught broodstock, and immature fish in some cases, remain captive as part of a multi-generation broodstock development and maintenance program. This facility is especially important for species at risk of extinction. Fish are conditioned for controlled reproduction, and subsequent progenies are reared for both future broodstock and juvenile release.

The choice of which strategy depends upon the status of the natural population and its risk of extinction. In the following sections, a variety of prerequisites and potential options are illustrated. These can serve as examples but depending upon the national policy regarding water and wastewater rights and regulations, availability of land, etc. the design of the facility as well as its technical components would have to be adapted to fit the regulatory framework and the intended scope of that the program.

5.2 Basic considerations for facility design

Depending upon the strategy applied, the complexity of the facility required varies with the life-cycle stages involved and the duration of the rearing process. Based upon the above descriptions, three *ex situ* facilities are envisioned which are listed in the order of increasing size and complexity in Table 5.1. The status of the given population and the goals of the conservation program will define the approach to

be taken, and as such, not all rearing steps must be integrated in the facility design.

Table 5.1: *Methods associated with three different approaches to ex situ facility programs rearing YOY juveniles for release: 1) collect wild ELS, 2) wild caught broodstock are collected annually, spawned and then released, 3) wild caught fish are collected, spawned and retained, to begin a captive broodstock program. Not applicable (NA).*

	Approaches		
Methodologies	Collection of ELS	Collection and release of wild broodstock	Retention of wild fish for captive broodstock
Broodstock collection	NA	Overwintering of fall collected broodfish and/or spring assessment for suitable spawners.	
Controlled reproduction	NA	Final maturation assessment, spawning induction, collection of gametes and fertilization.	
Egg incubation and rearing of ELS	Assessment of fertilization rates, incubation to hatch, adaptive rearing conditions for free embryos.		
Rearing YOY	Transition to exogenous feeding, using live feed and commercial diets. Constant grading and enrichment of rearing conditions. Training schools.		
Release of YOY or age 1+ fish	Testing for fitness, tagging, preparation for transport, transport, and release.		
Integration into future captive broodstock	NA	NA	Retain post-spawn wild fish and some YOY for captive rearing.
Long-term rearing	NA	NA	Rearing of multiple age classes, development of a breeding plan.

5.2.1 Location of site

When choosing a suitable site for locating a hatchery facility the following critical components should be considered (modified from Chebanov *et al*. 2011):

- The location must be in accordance with national and local government regulations to obtain the required permits for construction and operation.
- The location must be protected from floods or located in areas of low flood risk.
- The type of facility (utilizing ponds, tanks, flow-through, recirculating or a combination) will determine the amount of land required.
- The facility should be located at minimal distances from infrastructure (e.g. roads, canals, electricity, gas).
- A properly designed facility (e.g. for power requirements, pumping and discharging water, tank/pond design and layout, recirculating systems), will require a qualified aquaculture engineer.

- Reliable water source(s) for both quantity and quality must be available, whether river or surface water and/or groundwater.
- Water discharge capabilities and requirements must be considered (e.g. required water treatment or not, permits for release).
- Protection devices to prevent escapement of fish into the environment (especially if fish are maintained outside of their natural range)
- The facility should be a short distance (e.g. < 30 km) from the source of wild broodstock (if applicable), and short distances (e.g. < 20 km) from locations of release, to minimize transport stress.
- Access to water of the river of release is instrumental for imprinting and acclimation of juveniles prior to release.

5.2.2 Water supply

For any *ex situ* facility the decision to use river water, ground water, stored river or ground water in a reservoir (e.g. elevated head pond), or a combination of these, comes first and is mainly driven by the condition on site as well as the approach taken. Preferably, river water of the recipient water body where the fish are to be stocked is used to allow for early adaptation to the natural fluctuations in water quality parameters to increase the survival potential of the released fish. However, in some cases this may not be practical (e.g. no construction in a potential flood zone, water use and discharge restrictions).

Water supply needs are calculated based upon the size and type (pond, flow through or RAS) of the facility in question to determine the water demand of the facility. Many aspects need to be considered: quantities for flushing of water supply and discharge systems, capacities of water treatment facilities, and the maximum water flow to be used in each pond and tank system. The climate of the construction location (precipitation, evaporation and filtration related losses) and hydrogeological data (e.g. seasonal variations in level and temperature of ground water) must be incorporated.

5.2.2.1 River water

River water can either be pumped from a river or gravity fed if the facility is located below a dam. Since river water often contains debris, suspended solids, organic matter and small fish and invertebrates the use of a screened inlet before the pump is important. Further smaller particle removal can then be done with mechanical filters, such as a drum filter. To reduce the chance of disease in the hatchery disinfection of the water is crucial. The most common system is UV and it is also possible to use ozone, but extra care must be taken as ozone is toxic to humans and fish. Cooling or heating systems may be required depending on the water source as well as the goals of the *ex situ* facility, and the water should be oxygenated to reach >70% saturation.

5.2.2.2 Ground (well) water

Pumping of well or groundwater typically does not require extensive filtration before use but will require nitrogen removal and oxygen enrichment through aerators/degassing columns, trickle filters, oxygenation systems. Depending on the water quality analysis of your water source, the removal of certain chemicals, like iron and manganese may be required.

5.2.2.3 Surface water

Modified water sources for an *ex situ* facility could be surface water, originating from either river or ground water and pumped to a large elevated reservoir. The reservoir water is then either gravity fed or used with an in-line pump system to increase flow rates. Some facilities use this design to degas and oxygenate ground water as it is continuously pumped into the reservoir several meters above the water surface, into a screened box, to agitate and aerate the water. The elevated reservoir also acts as a partial back-up system in case of a pump failure as the head pressure alone will allow continued water flow into the facility.

5.2.3 Water quality

The water used in sturgeon hatcheries should be of high quality. It is important to ensure that the levels of harmful substances and impurities are below the threshold values or within the range of values given (Table 5.2).

Table 5.2: *Optimal water quality parameters for rearing and breeding of sturgeons (adapted from Chebanov & Galich, 2011; Hochleithner & Gessner, 2012; Svobodová et al. 1993; Conte et al. 1988).*

Water Quality Parameter	Range
Oxygen Saturation	70 – 100 %
Total Gas Pressure, for N_2	< 110 % <102 %
Carbon Dioxide (CO2)	< 10 mg/L
Nitrogen (N2)	< 103 %
pH	6.5- 8.0
Ammonia (NH3-N)	< 0.01 mg/L
Ammonium (NH4-N)	< 0.10 mg/L
Nitrite (NO2-N)	< 0.01 mg/L
Nitrate (NO3-N)	< 100 mg/L
Total Hardness (CaCO3)	50-400 mg/L
Turbidity	20-30 NTU
Total Suspended Solids (settleable and colloidal)	< 80.0 mg/L
Total Alkalinity (carbonate + bicarbonate + hydroxide)	40-300 mg/L
Biological Oxygen Demand (BOD_5)	< 7 mg/L
Chemical Oxygen Demand (COD)	< 10 mg/L
Hydrogen Sulfide (H2S)	< 0.002 mg/L

Iron (Fe)	< 0.01 mg/L
Magnesium (Mg)	< 40 mg/L
Manganese (Mn)	< 0.10 mg/L
Chloride (Cl)	< 30 mg/L
Chlorine (Cl2)	< 0.01 mg/L
Phosphate (PO4)	< 0.3 mg/L
Sulfate (SO4)	< 50 mg/L
Calcium (Ca)	< 180 mg/L
Cadmium (Cd)	< 0.003 mg/L
Lead (Pb)	< 0.003 mg/L
Zinc (Zn)	< 0.03 mg/L

5.3 Facility Type 1 – Rearing of wild-caught embryos and larvae under near-natural conditions

For some populations of sturgeon there are known regions of river systems where the collection of embryos and larvae is possible (e.g. *Acipenser transmontanus* (Idaho Power Company 2021), *A. medirostris* (Poytress *et al.* 2015), *A. fulvescens* (Hunter *et al.* 2020), *A. sinensis* (Wei *et al.* 2009)). The advantages of rearing these wild caught individuals under more controlled near-natural conditions include, reducing natural mortality that would have occurred if left in the river, while maintaining naturally produced genotypes, and at the same time maximizing fitness of juveniles at release. Capture and transport of these early life stages into the facility and to the release sites are covered in Chapters 4, 7, and 14.

The facility design for early life stages utilized for release has adopted new approaches targeting at increased fitness of the fish produced, and aiming at improved survival through faster adaptation to post release conditions (see Chapter 10, Crossman *et al.* 2011, 2014; Friedrich *et al.* 2019; Gessner *et al*. 2023). Key elements of this type of facility include: 1) incubation of eggs and rearing of fry in river water to mimic natural conditions and to ensure homing behavior, and 2) rearing of juveniles in river water to expose them to natural fluctuations in temperature, turbidity, water chemistry, and bacterial fauna, improving adaptability and survival to conditions in the wild when released.

Feeding of natural live food and irregular feeding patterns minimize domestication effects and require suitable feed storage or feed production facilities to be incorporated in the design. Additional stimuli like different flow patterns, substrate, changing light regime, smell of predators etc. can further improve performance of released juveniles in the wild. In cases when rearing conditions reveal deficits in fitness, the implementation of training schools to increase the adaptation and uptake of natural feed, to temperature fluctuations, to swimming performance or to improve predator recognition, can all enhance survival upon release (Cámara-Ruiz *et al*. 2018, 2019, see Chapter 10). As such the hatchery

design needs to address these needs by providing extra capacities for the measures to be implemented.

5.3.1 Incubation and rearing facility design

Systems utilized for early life stages can be permanent or mobile, buildings or containers, indoors or outdoors or a combination of both, with water treatment and holding systems for incubating embryos and rearing larvae until they reach juvenile release size. The specific design will be dependent upon the site, the goals of the stocking program (number and size of juveniles to be released), and whether it will be a flow through and/or semi-recirculating system(s).

The main advantage of a mobile container system is the fact that it can be moved between sites or rivers. Holtgren *et al*. (2007) provide a very detailed design for their successful lake sturgeon portable streamside rearing facility used for lake sturgeon. Briefly, the facility used a portable generator, and later a local power line as the power source. Screened river water connected to intake pipes and a pump system, with a sediment removal system, since too much silt and fine sediment can be detrimental to sturgeon fry, clogging their gills and increasing the mucus production. Water is then supplied by two separate pipes, one to the incubation system and one to the rearing tanks system.

The most common sturgeon egg incubation systems typically utilized for large numbers of eggs (Yuschenko, Osetr, Weiss and McDonald jars, Eagar Inc. upwelling incubator) could be too large, and so smaller versions may need to be specially constructed or acquired from aquaculture supply companies. The number of incubators needs to be sufficient to handle the estimated multiple batches of embryos to be collected. Illumination in the incubation area should be controlled as direct light can negatively affect the embryonic development of sturgeon. If overhead skylights are used to maintain a natural photoperiod in the facility, a shaded area for incubation is needed, and any incandescent lighting should be on dimmer switches. Post hatch larvae are reared, away or protected from direct light, in troughs or circular tanks that have adjustable (directional and quantity) water inflow, and aeration near the screened outlets to help keep them clean.

An example of a streamside container is given in Figure 5.1. The largest portion of the container is set up for the multiple hatching containers and troughs for rearing post hatch larvae. It also has a separated laboratory and water treatment compartment that serves for rearing of Artemia for use at the onset of feeding, a sink and a small lab bench for a microscope, feed storage/preparation area, a fridge/freezer for supplies and feed as well as the water and aeration system. The incoming water is filtered mechanically by a drum filter (60-100 µm) and treated with UV (20-120 mJ/cm^2 @254nm) to reduce the load of pathogens in the supply water.

Regarding feed storage, the space available should plan for weekly or biweekly deliveries to avoid using too much space in the container just for storage. For example, a 5 kg bulk pack of Artemia could be stored in the container, along with

25-40 kg of brine. For frozen natural feed the capacity of a freezer to hold 7 days' worth, and any formulated diets (semi-moist and dry) could be stored in appropriately sized ice chests in the container.

Another example for ELS rearing could be a two-container facility with one container as described above and then a second container for longer term juvenile grow out to larger sizes. The standard 12.2 m container offers room for 8 troughs of 400 x 65 x 30 cm or 4 circular tanks 200 x 40 cm if stacked. Some hatcheries prefer troughs for rearing, although they typically require more time for cleaning. The circular tanks can make fish care more difficult due to restriction of working and handling space, but for larger fish they have very low maintenance due to the self-cleaning aspects of having a circular flow and center standpipe. Keeping the screen clean can be challenging but aeration near the drain will help keep it from clogging.

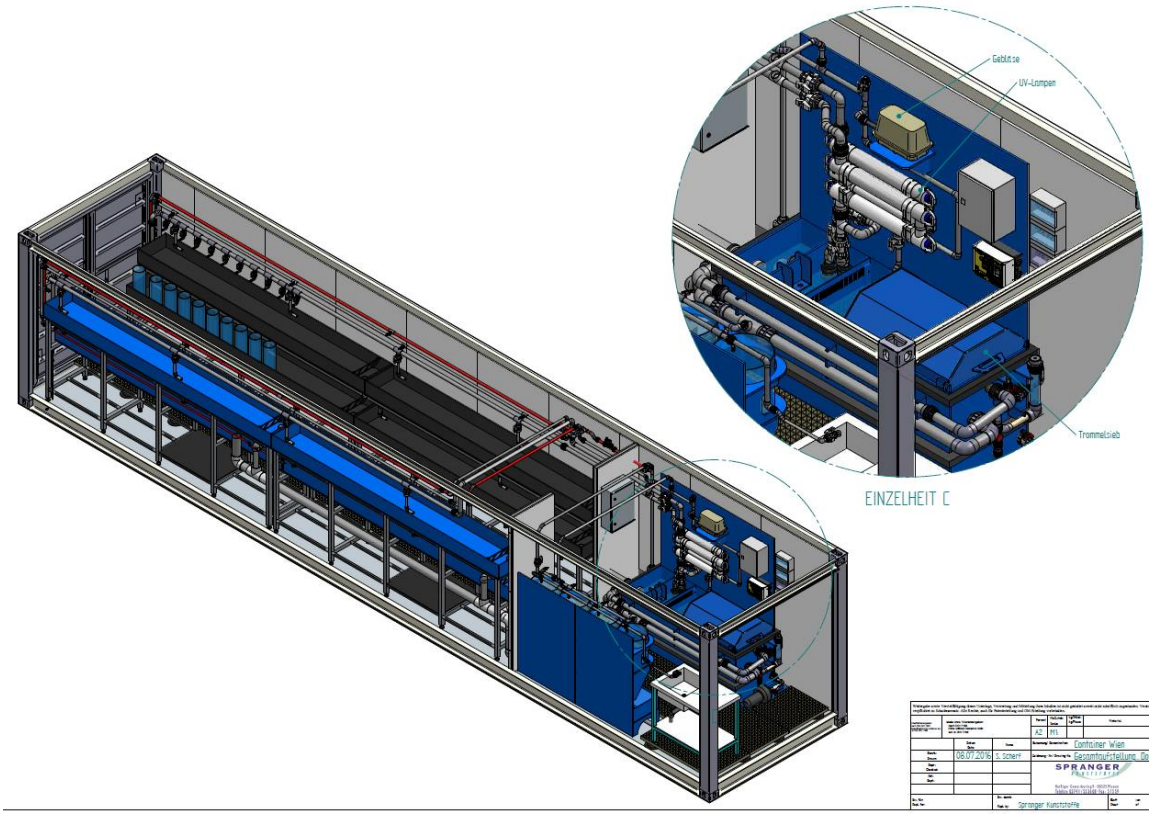

Figure 5.1: *Streamside rearing facility container (bottom) with an inside schematic showing both a large rearing compartment (top left) and a close up of the smaller compartment for the water management, includeing mechanical filtration, supply pumps, UV unit and blower for compressed air, as well as Artemia systems (top right) (Photo credit: Spranger Kunststoffe GmbH, Plauen, Germany).*

A recently constructed permanent hatchery for *A. transmontanus* ELS (Idaho Power Company, 2021) comprises a 325 m^2 facility designed for the annual production of up to 2,500 juveniles at approximately 200 g (30 cm FL). The hatchery building houses an embryo receiving room, an incubation area with 2-L

upwelling hatching cones, rearing area with various diameter circular fiberglass tanks (16-0.6 m, 8-1.2 m, and 6-2.4 m), mechanical room, wet lab, office and an attached separate dormitory (Figure 5.2). This facility utilizes pathogen free gravity fed spring water (14 °C) and two electric water heaters to have the ability to increase rearing temperatures to 16-18 °C.

Incubation and rearing tank units for any ELS facility could be designed as both flow-through and recirculating. Recirculating would allow for thermoregulation of very cold river water to facilitate growth, if desired, for larger juveniles at release and/or release at different times of the year. To ensure the continued operation, replacement pumps and aerators, and an automated emergency electrical supply system must be available.

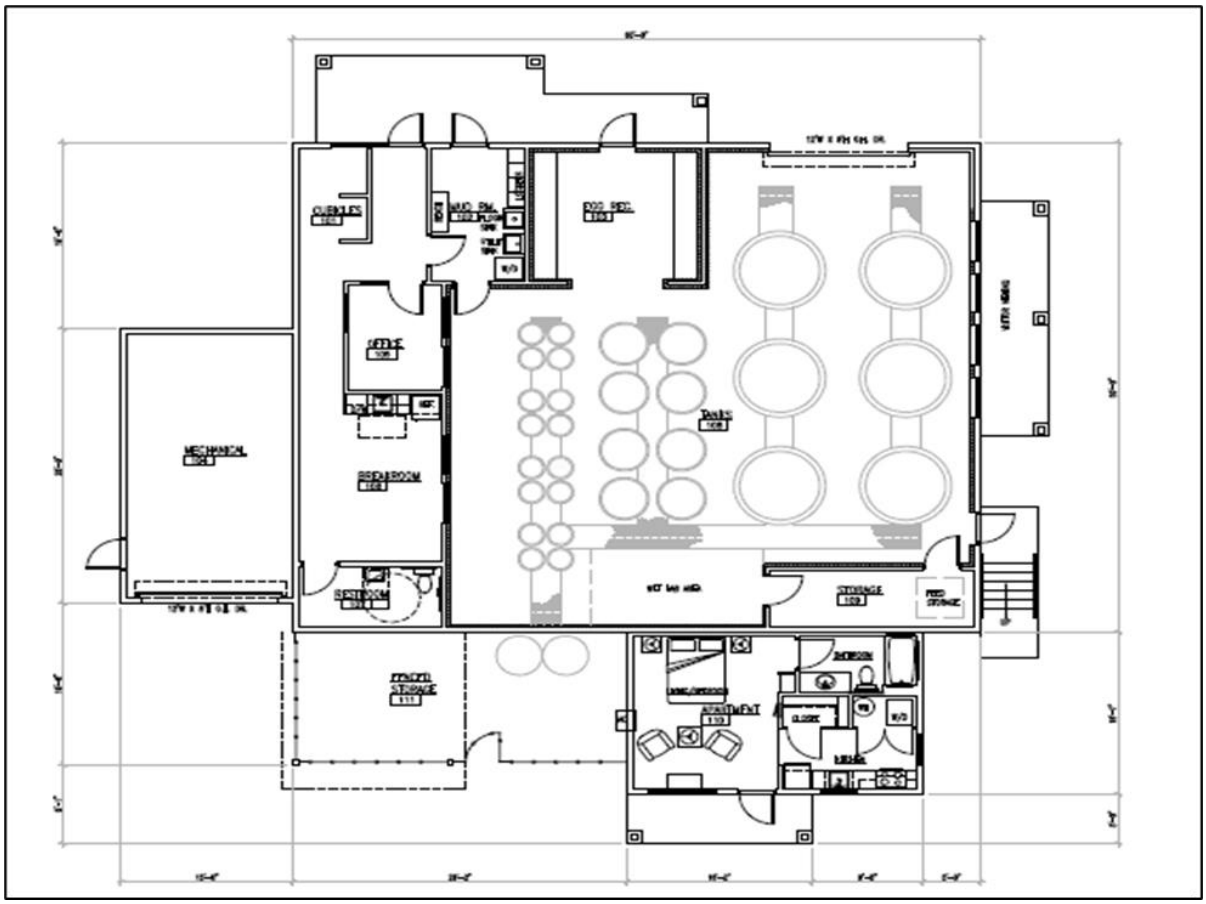

Figure 5.2: *Floor plan of the Niagara Springs Sturgeon Hatchery (Idaho Power Company, 2021). The largest footprint of the facility is for the various diameter circular fiberglass tanks (16-0.6 m, 8-1.2 m, and 6-2.4 m) (Photo credit: J.P. van Eenennaam).*

5.4 Facility Type 2 – Wild-caught broodstock spawned and released

In cases where natural reproduction is insufficient or the reproduction sites are unknown or not accessible to collect ELS from natural spawns, while at the same time sufficiently sized spawning migrations are observed and are accessible, the collection of broodstock and their transfer to a controlled reproduction facility is a common *ex situ* hatchery alternative, with the subsequent release of the broodfish after gamete collections. Although, due to the manipulation of both the broodfish and the offspring, the potential to reduce fitness of the fish under captive conditions should be considered. The reproduction of wild breeders to maximize genetic heterogeneity (see Chapter 6), use of appropriate rearing techniques (see Chapter 10) and feedback from fitness assessments (see Chapter 12) will improve the overall fitness of released juveniles. Some of these facilities may also start to incorporate a captive broodstock program, from the rearing of some of the non-released progenies, to act as a gene-pool back-up if wild broodstock become unavailable during spawning migrations (e.g. *Scaphirhynchus albus* propagation program; Webb, *et al*. 2016).

5.4.1 General design considerations

The critical components of the facility location were presented above, and the main purpose of these *ex situ* facilities is to conduct procedures for long-term (decades) holding and propagating broodstock under controlled conditions. As mature fish are the most valuable and irretrievable genetic resource for conservation, utmost care and fail-safe procedures must be used during capture and handling (see Chapter 4) and transport (see Chapter 7) to reduce stress and to maximize the success of artificial reproduction (see Chapter 9) The size of your overall facility will be based on the projected number of broodstock to be brought to the facility and their maximum size, and the program's production goal for juveniles to be released. If available and practical, the visitation of nearby existing *ex situ* sturgeon facilities and/or a sturgeon aquaculture facility would be extremely valuable to help design a proper facility. Discussions with the facility staff should cover what rearing systems work well and what they would design differently if they had had the choice.

Basic infrastructures include the water supply, electricity, and accessibility of the facility by truck, and boat for some facilities. The facility must be equipped with an alarm system for water supply, power supply and have an emergency generator to provide power to maintain essential operations in case of power outages. A control room where the essential functions and data are collected and displayed, will ease the supervision of the facility. This room ideally has visual contact with the main rearing facility. Furthermore, it is essential to strive for failure safe operation. Replacement pumps, aeration or oxygenation equipment, and back-up water sources are essential to prevent losses when equipment failure occurs. It is highly recommended that a water quality monitoring/alarm system also be

installed for parameters such as, water flow (pump operation), water temperature, dissolved oxygen, and total dissolved gas. The human error factors in operations, handling and servicing must be considered, and the "four eyes principle" should be applied to all critical decisions, to prevent any unintentional mistakes that could lead to drastic consequences for the well-being of the animals in question. Surveillance cameras, fences, and alarm systems for human or animal intruders are usually a necessity due to the high value of the animals.

In general, there are two main criteria that will guide the design of these hatcheries, the short-term holding and spawning of ripe broodstock caught during the spring spawning run, or the longer-term overwintering of pre-spawning broodstock collected during the fall-winter season. Several different broodstock holding systems should be considered: 1) holding systems for monitoring final maturation for spring caught broodstock and/or for over-wintering of fall caught broodstock and then assessment of final maturation during spring, 2) spawning tanks for injected males and females, 3) recovery systems for holding post spawned fish until transported for release. These holding systems can be outdoors or indoors or a combination of both.

5.4.1.1 Broodstock overwintering and/or pre-spawning holding system

After wild broodstock have been captured (see Chapter 4) and transported to the *ex situ* facility, they are placed into some type of holding system until selected for spawning induction (see Chapter 8). Holding densities, in general, should range from 10-30 kg/m^3 with tolerance levels being species specific (Chebanov & Galich, 2011). Females and males should be held together to take advantage of any possible behavioral and pheromone cues that may occur during maturation.

5.4.1.1.1 Ponds/Raceways (earthen; Figure 5.3 or concrete; Figure 5.4)

Historically, sturgeon hatcheries have used a variety of facilities for fish holding, rearing and maturation (reviewed in Chebanov & Galich, 2011). For example, Chebanov & Galich (2011) describe the "Kazansky" type facility as an earthen-pond system for holding broodstock. The earthen pond has a length of 130 m and comprises two parts, a wider (100 m) section of 2.5 m depth and a narrower (30 m) shallower (0.5–1 m deep) section that simulates conditions of sturgeon passage to the spawning reach of a river (i.e. high current velocity, gravel bottom). The water flow through the pond at the onset of the holding period is 30–40 L/s and at the end of prespawn holding, it can be elevated to 300 L/s. The disadvantage of this system is the large water demand.

The ponds for rearing sturgeon are generally rectangular in shape with a side ratio of 1:2 or 1:3, with a maximum depth of 2.5 m. A constant slope from inlet to outlet helps in draining the ponds effectively. The system supplying water to the ponds consists of a main canal or pipe with supply lines (regulated flow) to each pond with the capability to ensure rapid filling (1-2 d) or flushing. The water supply system should ensure a full water exchange in the ponds every 1–2 d based on water quality parameters. The design of the ponds should allow easy access of

personnel and the dikes between ponds must be sufficiently wide and stable to allow access by trucks and equipment for pond maintenance and fish transportation.

Figure 5.3: *Earthen raceways used for sturgeon culture (A. transmontanus) with Oncorhynchus mykiss as a natural food source (left) (Photo credit: J.P. Van Eenennaam).*

Figure 5.4: *A concrete pond used for sturgeon culture (Photo credit: J.P. Van Eenennaam).*

5.4.1.1.2 Tanks (concrete, fiberglass, plastic, metal with plastic liner)

Tanks for broodstock are typically circular or rectangular with rounded corners, usually made of concrete or fiberglass (Figure 5.5), with a diameter >3 times the

body length of the species, and water flows to create a current (>0.2m/s). Depending on the clarity of the water, tanks are either deep enough (~2.5m) or are covered/shaded (>60% shade cloth) to minimize stress caused by intense sunlight or if indoor incandescent lighting is not dimmable.

Figure 5.5: *Concrete (left) 15 m diameter, and fiberglass (right) 6 m diameter circular tanks used for A. transmontanus broodstock rearing (Photo credit: J.P. Van Eenennaam).*

5.4.1.1.3 Cages (stainless steel, fine-mesh netting)

Cages 20-100 m^2 and depth 3-3.5 m can also be used for broodstock, typically placed in ponds or reservoirs (Figure 5.6), at a maximum density of 10-25 kg/m^2 (Chebanov & Galich, 2011). These are also covered to keep low light conditions and to prevent any escape.

5.4.1.1.4 Controlled RAS

Due to the increasing concern of limited water resources (river, surface, and ground), and increasing regulations for use and discharge, and compounded with climate change, the most rapidly developing option is to hold wild-caught broodstock under controlled conditions to thermoregulate (heating and/or cooling) the holding systems using RAS. Many of these RAS systems are indoor facilities (Figure 5.7), however some are also used outdoors.

The ability to maintain overwintering water temperatures, for example, will allow for holding selected pre-spawning broodstock to reach final maturation during later months of the spawning season. And with heated water some females could be acclimated to spawn earlier in the season. This could allow a facility to produce multiple progenies spread over a longer time frame and thus allow for maximum production of juveniles.

The basic layout of an indoor facility is an area of circular tanks for holding pre-spawning or overwintering broodfish. The facility design should allow easy access to these tanks by broodstock transport vehicles and allow sufficient space to handle the fish in stretchers or for the larger species, space enough to use a small

crane or bobcat with a lift net. Smaller sized circular spawning tanks can be used for post-injected fish, and then at least one of the larger sized tanks for holding post-spawned fish until release back into the river.

A separate area is for collection of gametes, usually right next to the egg incubation unit, and then an area for rearing tanks of increasing diameter, to hold the larval sizes through the target release size of the juveniles (see Chapter 10). All these systems should be RAS to maintain temperature control during final maturation, spawning induction, and embryo incubation. The most critical area(s) of the facility is the operational area(s) of the RAS(s) as there is usually more than one (e.g. the egg incubation system usually stands alone as it only requires a recirculating system with a small chiller to maintain optimal water temperature). Hatched larvae typically have a more tolerant thermal range, and, in some programs, it is desirable to add warmer water for faster growth and thus shorter time to release size.

There has been a rapid development over the last two decades in the technologies involved in RAS and it is beyond the scope of this manual to go into details of designing an e*x situ* RAS sturgeon facility. There are numerous publications and books on the subject (e.g. Timmons *et al.* 2002; Aich *et al.* 2020; Ahmed & Turchini, 2021; Bregnballe, 2022) and we highly recommend that a qualified engineer help design the more complex systems for any *ex situ* facility. Generally, and briefly, the basic RAS design is to continuously treat circulating water by removing waste products (solid and chemical) excreted by the sturgeon and add oxygen to maintain optimal levels. As water drains from the fish tanks it passes through a mechanical filter, and then a biological filter, and then it is aerated and stripped of carbon dioxide. Further steps can include oxygen enrichment, UV disinfection, automatic pH regulation, and heating or chilling.

5.4.1.2 Broodstock spawning system

Broodstock ready for hormonal stimulation (see Chapter 8) are moved into a system of tanks with thermoregulation to maintain ideal spawning temperature of the fish. The tank size should allow the fish to move freely, the diameter being 2-3 times the length of the fish, and the depth should just be 3-4 times the width of the fish for easy capture and removal for gamete collection. The rearing density is recommended not to exceed 10-15 kg/m^3 (Conte *et al.* 1988; Chebanov & Galich, 2011). To reduce stress and prevent escape, the tank is covered. To ease the determination of onset of ovulation by spotting eggs, the tank bottom should have a light blue to gray coloration and the water, if needed, should be filtered for sufficient transparency. Optimal water quality must be maintained either in RAS or in flow through. Some facilities prefer having only one female per tank although some will place more than one female per tank which can make it more challenging to determine which female started releasing eggs first. These tanks can be multi-purpose and subsequently be used for juvenile grow-out after the spawning season has ended.

5.4.1.3 Gamete collection and fertilization area

For gamete collection, fish are removed from the spawning tank system, and typically the collection of gametes requires a suitable sized tank for narcosis of the fish (see Chapter 4), sufficient space to move and handle the fish by forklift or in the stretcher, a stable table or suitable support device (e.g. adjustable height sawhorses) for the stretcher, access to water to flush the gills with fresh oxygenated rearing water and capacity to place the required materials (e.g. towels, syringes, milt flasks, egg bowls, beakers for fertilization water, etc.) on two different portable-folding tables. A freely accessible space of 4 x 4 m is sufficient for small and medium sized sturgeon, and for large fish of more than 2.5 x 2.5 m additional space might be required.

5.4.1.4 Post-spawning recovery system

After gamete collection (see Chapter 9) the wild caught broodstock can be moved back into one of the empty overwintering and/or pre-spawning holding systems or a similar sized recovery tank, until transported back for release at their capture site.

5.4.1.5 Embryo incubation system

Various types and sizes of incubators are used to hold sturgeon embryos from post-fertilization through hatching. The most common systems (see Figure 5.8) utilize either horizontal (Yuschenko system, Osetr system) or vertical (Weiss jar, McDonald jar, Eagar upwelling incubator) flows of water and are described and pictured in detail by Chebanov & Galich (2011). Ideally, UV sterilized ground water should be used that is semi-recirculated with a chiller if needed to maintain optimal incubation temperature. Oxygen levels should be at greater than 70% saturation. In general, the incubation systems should be in low light sections of the hatchery (<100 lux) and never in direct sunlight, which can lead to developmental abnormalities (Chebanov & Galich, 2011). It should be noted that some species of eggs may survive better in specific incubation systems. For example, it was found that the McDonald jars, typically used for *A. transmontanus* embryos, were too vigorously tumbling the thin-chorion *A. medirostris* embryos resulting in high mortalities compared to the gentler upwelling Eagar incubators (Van Eenennaam *et al.* 2008).

5.4.1.6 Number of incubators

The number of incubators will depend on the number of females induced to spawn at one time and their estimated fecundity. The volume or number of eggs placed into an incubation system will depend on the size of the section/box/jar, and species egg size (Chebanov & Galich, 2011) and in general a Yushchenko section can have 150-260 thousand embryos, an Osetr box 100-250 thousand, a 6-L McDonald or Eagar upwelling incubator 20-65 thousand, and an 8-L Weiss jar 20-35 thousand.

Figure 5.6: *Incubators frequently used for sturgeon embroys a) Osetr incubator (Photo credit: Techni-Prom-Mash UA), b) McDonald jar with S. albus embryos (Photo credit: Cory Hagemeister), c) Zuger/Weiss jar with A. oxyrinchus embryos (Photo credit: Gerd-Michael Arndt), d) Eagar upwelling incubator (below) with A. medirostris embryos (Photo credit: Joel Van Eenennaam).*

5.4.1.7 Larval and Juvenile rearing systems

Hatched larvae are often stocked in tanks (fiberglass, concrete or plastic), that are circular, rectangular or square, with rounded corners. Additionally, net cages and small ponds have been used but juveniles should be at least 100 g in size before stocking into ponds (Chebanov & Galich 2011).

For circular tanks, initial water flow is low and almost vertical into the tank to prevent any vortex that will impinge larvae onto the center standpipe or cause forced clumping of larvae. Screens are initially ~500 µm mesh and subsequently increased to 4 mm+ dependent upon juvenile size, and aeration near the screened center standpipes or outlets will help to keep them from fouling and clogging. Water inflow is angled more and more as the juveniles grow and which will also make the tank more self-cleaning. In general, the initial post-hatch larval tanks

should have 3-5 complete changes of water every hour with aeration to maintain dissolved oxygen >70% saturation (see Chapter 10). Water depth from 0.2-0.5m with blueish to gray colored preferred, as white is too bright and black too dark (difficult to see the larvae).

5.4.1.8 Stocking densities

Initial stocking densities range from 1,000-5,000 larvae per square meter for initiation of exogenous feeding (see Chapter 10), and to help design a facility layout, Table 5.3 provides guidelines for stocking densities for the different sized juveniles and rearing systems.

Table 5.3: *Size and stocking density for systems used to rear different size sturgeon juveniles in tanks, net cages, and small ponds (modified from Chebanov & Galich, 2011). Not stocked at that size (NS).*

Mean Body Weight (g)	**Tank Sizes Small Ponds**	**Net Cages**	
	5-20 m² 750 m²	**4-25 m²**	**250-**
	Stocking Density (kg/m²)		
1	2.5 – 5	NS	NS
5	5 – 10	NS	NS
30	7 – 14	6	NS
200	9 – 18	7	3
400	10 – 20	8	4
800	12 – 24	9	5

5.4.1.9 Storage, food preparation and laboratory unit

Backup equipment of essential, life-saving systems should be maintained on site in a dry storage area for equipment, cold or deep freezer storage is needed for fish feed, and an area for wet equipment, such as nets, raingear, boots and waders. Nearby should be a changing area with lockers for personnel to store personal items/shoes while changing into wet gear. A small workshop/repair area is also needed.

The feed preparation area needs to incorporate a sink, a workbench (stainless steel), storage racks, scales, bowls, etc. The provision of live feed makes it necessary to designate a room for the tanks for rearing live food such as Artemia

and for the enrichment of live feed items. A rearing area is also required for additional live feed (e.g. polychaetes, insect larvae) if they will be utilized.

The laboratory unit should be one room with two workstations; one for a microscope, dissecting scope and analytical equipment for water quality, and one to act as a wet lab for fish examination.

5.4.2 Office space

To operate the facility in daily business, a small office with 2-3 workstations, as well as a bathroom with toilet, sink and shower and a kitchen and meeting area are necessary. If presence of personnel overnight is required, or if research infrastructure is included in the facility, bedrooms are useful.

5.4.3 Outdoor storage-backup power-parking area

For larger equipment and vehicles, fish transport trailers and boats, an outdoor storage area must be planned into the design. An emergency power system must be installed to provide electricity in case of a power loss, which is also located outdoors. And an appropriate number of parking spaces should be included for the facility personnel.

5.4.4 Planning of workflow and facilities layout

Overall workflow and routines must be envisioned to be able to plan for the arrangement of the different sections of a facility within the overall layout, especially proper distance in between tanks. In principle, quick and easy access for both material transport as well as movement of fish is essential. Deliveries of materials (e.g. feed, chemicals, equipment, fish) requires various sized transport equipment (e.g. forklift, crane, bobcat) to the storage areas, and different holding facilities. Accessibility of infrastructures (e.g. pumps, filters, pipes) needs to be easy and provide sufficient space for maintenance and replacement.

5.5 Facility Type 3 - Wild caught sturgeon become a captive stock

In cases when *ex situ* facilities are covering the entire life cycle, to preserve rare species in living gene banks, the facility will be much larger and it is a multi-decade approach (Figure 5.9). These *ex situ* facilities must house and protect extremely valuable genetic heterogeneity and biodiversity components. For this reason, it is essential to minimize losses in the process of maintaining them under captive conditions. This requires the efficient control and manipulation of rearing conditions as needed, which means that the rearing facility should provide different options for environmental conditions either through e.g. the water sources utilized, aeration, oxygenation, and temperature control.

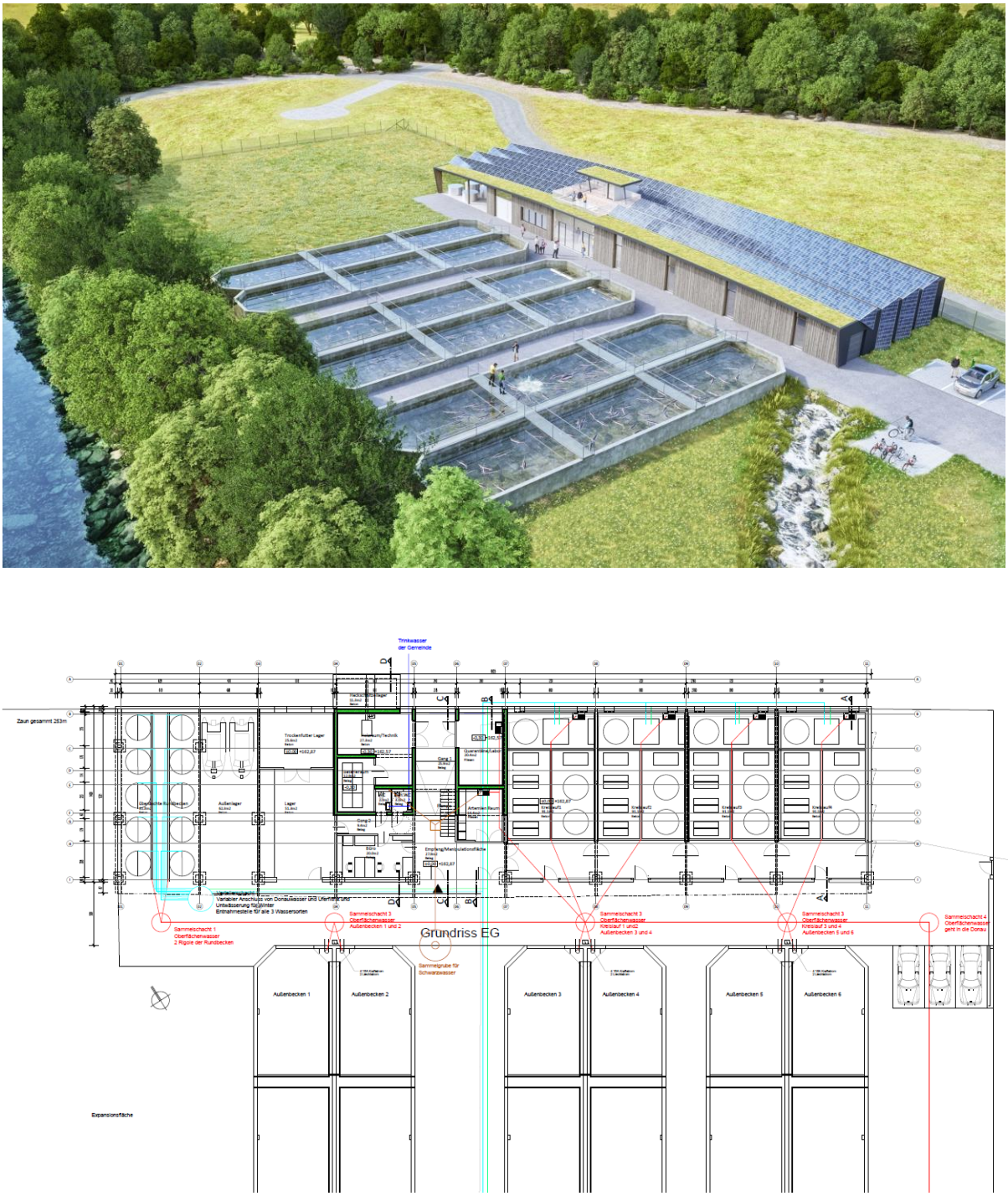

Figure 5.7: *Hatchery design for an integrated facility with river water, comprising six raceways for future broodstock, four incubation and fingerling systems, grow-out tank systems, lab and office facilities (Photo credit: T. Friedrich).*

5.5.1 General design considerations

For this type of *ex situ* facility, the number and sizes of different rearing facilities must be estimated based on the total biomass of fish that is considered necessary for goals of the program. This can be based on the average weight of the fish to be reared from age 1 to age of maturity, the number of different year classes to

be reared concurrently from hatch to maturity, and the optimal stocking densities. Different year classes have to be identifiable during their whole life-cycle requiring a reliable marking technique or separation in the rearing facility. This procedure could further increase the number of rearing units required.

In general, the decision is to be made if the facility will solely comprise a tank-based facility or if ponds will also be utilized for juvenile and subadult or even adult fish to be reared until maturation. In the first case the design of the facility largely depends upon the water source and water treatment (i.e. flow through or RAS), and this decision is largely influenced by environmental legislation and water supply. In general, ground water flow through and RAS provide better control than surface/river water systems. The manipulation of the rearing water makes the use of RAS more sensible for heating and cooling, even though RAS are more energy intensive than flow through systems, they reduce water use.

The basic facility design is the same as Facility Type-2, but the systems used for the rearing of juveniles for release need to be kept separate for biosecurity reasons, and because the requirements for the rearing environments will be different between fish for release and the fish being reared for future broodstock. The development of captive broodstocks require the same rearing systems, as described in Type-2 facilities, but systems are needed to transition wild-caught fish into long-term captive rearing conditions, which primarily involves weaning them onto artificial diets (see Chapter 10) and similar systems but with increasing numbers and sizes of tanks and/or ponds, to grow out and close the reproductive cycle of each subsequent year class to be reared as future captive broodstock. Because of the high initial capital cost to construct a large facility all at once, the facility could be designed for anticipated expansion of tanks and/or ponds. While the initial year class and wild fish are being reared, more and larger tanks/ponds can be added to the layout as the fish grow and more subsequent year classes are added.

5.5.2 Ponds, tanks, net cages, RAS considerations

Beyond the general description of these systems in the above Facility Type 2 sections, the development of captive broodstock program will require a much larger capacity, and likely even larger sized tanks/ponds to maintain the increasing number of captive adults from different age classes as they age.

5.5.3 Stocking densities

To help design a facility layout, Table 5.4 provides guidelines for stocking densities for the different sized fish and the different rearing systems.

Table 5.4: *Size and stocking density guidelines for systems used to rear different size sturgeon for future broodstock in tanks, net cages, and small ponds (modified from Chebanov & Galich, 2011).*

Mean Body Weight (kg)	**Tank Size Ponds** **20-40 m² 1,500 m²**	**Net Cages** **10-50 m²**	**Small** **250-**
	Stocking Density (kg/m²)		
1.5-3.0	15 – 30	10	6
3-4	18 – 36	12	7
4-6	25 – 50	15	8
6-10	30 – 60	25	9
>10	40 – 80	30	10

5.5.4 Guidelines and considerations for size of a facility

The size of the captive broodstock to be maintained should be based on the genetic diversity of the natural populations and the breeding plan underlying the program (see Chapter 6). This information will help calculate the overall size of the facility.

Considering the age at maturation of each species and using the estimated survival rates of 75% from yearlings to five-year old animals and 90% survival thereafter, until maturation, the total number of fish to be kept from each year's reproduction can be estimated. As controlled reproduction can be stressful for the fish, mortalities must also be considered when planning for broodstock size.

The estimated size of a tank facility rearing 18 age classes of sturgeon, and one tank of spawning fish, and rearing 130,000 juveniles for release at 5-gram size, and some at age 1 and 2 years, is provided as an example (Table 5.5). The total biomass reared in each system is based on the hypothetical mean body weight at each age and the number surviving, from an initial 20 fish at age 2, is given in Table 5.6. These high numbers of large animals demand large facilities, the size of which is ever increasing with increasing numbers of older and larger individuals to be maintained. The required size of this tank facility can be reduced on the other hand by rearing subadults age 3-15 years, in pond facilities.

Table 5.5: *Dimensions of the rearing systems calculated for 18 age classes of future broodstock of A. sturio sturgeon (including one spawning brood tank), and systems to rear 130,000 five-gram, age 0 juveniles and larger age 1–2-year-old fish for release.*

Tank Size (m)	**Tank Height (m)**	**Tank Area (m^2)**	**Rearing Density (kg/m^2)**	**# of Tanks**	**Total Biomass (kg)**	**Age Classes Reared**	**Rearing Area (m^2)**	**Water makeup Systems (m^2)**	**Gross Area (m^2)**
Future									
Brood									
4x4	0.8	12.5	10	2	242	2,3,4	25	7.3	48
6x6	1.2	28.3	10	3	856	5,6,7,8	85	44	264
10x10	1.4	78.5	10	5	3900	9-18	393	236	960
10x10	1.4	78.5	7	1	550	Brood	79	45	188
For release									
4.5x0.6	0.4	2.7	5	50	650	0	135	75	315
2x2	0.8	3.2	5	12	16	1	38	12	76
4x4	0.8	12.6	5	5	64	2	63	14.5	120

Tanks are circular except the troughs used for age 0 fish. Total space requirement (gross area) for all the tanks, water handling systems/treatments, and working space in between tanks, is approximately 2000 m^2. Note that *A. sturio* is very sensitive to rearing density, which is the reason for the low broodstock stocking densities suggested.

Table 5.6: *The mean body weight at each age for A. sturio and the estimated number of survivors, from an initial 20 fish at age 2.*

Age (year)	**Mean Body Weight (kg)**	**# of Fish (N)**	**Total Biomass (kg)**	**If stocked at 10 kg/m^2; tank area required (m^2)**
2	2	20	40	4
3	4	19	76	7.6
4	7	18	126	12.6
5	10	17	170	17.0
6	13	16	208	20.8
7	15	15	225	22.5
8	18	14	252	25.2
9	21	14	294	29.4
10	24	13	312	31.2
11	29	12	348	34.8
12	35	12	420	42.0
13	35	11	385	38.5
14	38	11	418	41.8
15	41	10	410	41.0
16	44	10	440	44.0
17	47	9	423	42.3
18	50	9	450	45.0

5.6 Biosecurity (Prevention of Escapement)

In cases where *ex situ* facilities are placed outside of the range /catchment of the (sub-) population to be maintained, it is vital to ensure that the escapement of fish into the environment is prevented by the establishment of security measures. For the different *ex situ* facilities, security levels can be described as follows:

5.6.1 ELS rearing facility

If rearing takes place in RAS, there are three successive security measures (Figure 5.10):

1. The water supply flows into a reservoir where it is aerated and passes through the mechanical (sand or mesh) biofilter system.

2. A grid with a mesh size of 0.3-2 cm (depending upon fish size) is placed at the outlet of each rearing tank.

3. Two grids with mesh size below 1 cm are placed at the overflow of the effluent- drainage channel. The water is then pumped back into (1) above to close the system.

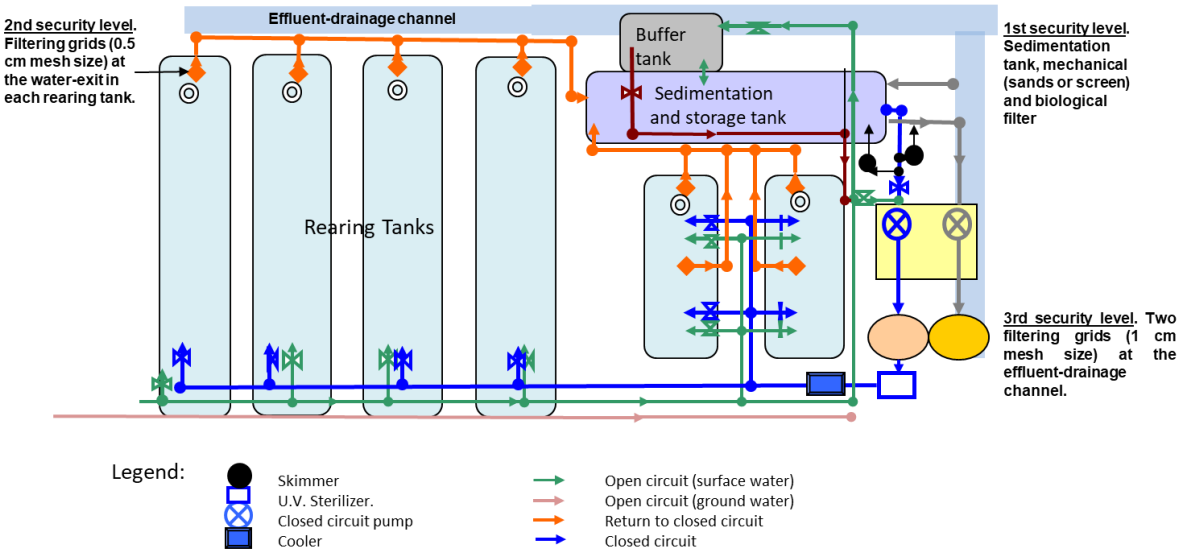

Figure 5.8: *Hatchery design utilizing surface and ground water sources and three security levels against fish escapement (Photo credit: M. Chebanov).*

The same approach holds true for any RAS that is used to grow sturgeon broodstock or temporarily maintaining spawners for their reproduction and then subsequently releasing them back to the wild. In cases where flow-through facilities are utilized, the principal design can follow the approach for pond rearing.

5.6.2 Sturgeon grow-out in earthen ponds

An example of semi-intensive earthen ponds for rearing of sturgeon is shown in Figure 5.11. The ponds are aligned in a cascade to allow water flow by gravity or by using pumps from pond to pond. A cross section from an irrigation channel to a water-drainage channel is illustrated in Figure 5.12, to show the locations of fish screening to prevent movement between ponds or escapement from the facility.

Figure 5.9: *Semi-intensive pond/raceway system for sturgeon rearing with surface water supply (top) (Photo credit: L. Congiu).*

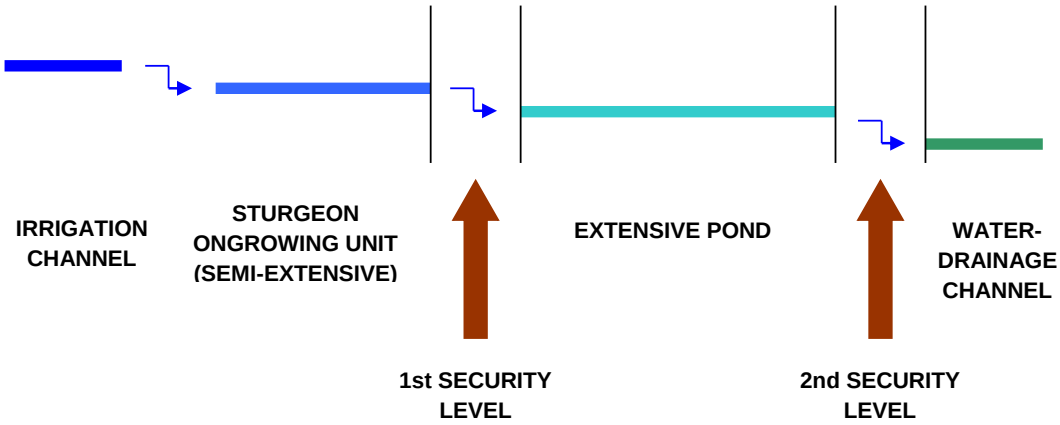

Figure 5.10: *Water level scheme with screening devices between subsequent pond facilities to prevent fish escapement (Photo credit: M. Chebanov).*

The outlet weirs have filtering mesh grids, with mesh size adapted to the size of the fish in the ponds. Such grids prevent fish escape from the semi-extensive culture system into the extensive ponds, and from the extensive pond, into the drainage channel (Figure 5.11).

Figure 5.11: *Inflow of a semi-extensive pond equipped with a spherical filtering screen (red circle) mounted (left) and detached (right). (Photo credit: M. Chebanov).*

A third security level would be a filtering grid placed in between the end of the water-drainage channel and the recipient water body, further minimizing the possibility of any accidental escape from the facility into the river. All of the screening devices should be inspected on a daily basis for any required cleaning and repair.

5.7 Transplants out of range or out of country

In cases when fish are transferred into bioregions outside of the native range of the species, it must be ensured that the establishment of the broodstock follows the ICES code of practice for introductions and transfers (ICES, 2005) to prevent the co-transfer of pathogens along with the fish. In these cases, the transfer of fish originating from a captive broodstock should be certified disease free. Any transplants between countries will require CITES permitting.

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Chapter 6: Genetic considerations in *ex situ* measures

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6.1 Introduction

Many *ex situ* conservation plans include the establishment of a captive breeding population. Whether the goal is to support recovery of natural populations or reintroduce a population to an area where it has locally become extinct, the establishment of this breeding population should represent, as much as possible, the genetic composition of the target population in order to minimize the inevitable impact after release. Managing an *ex situ* conservation program for sturgeons involves addressing a range of genetic issues. Decisions such as which source population to use, how many individuals to maintain in captivity and how to select breeding pairs all necessitate a scientifically informed approach. Therefore, it is crucial for operators to understand the potential genetic consequences of their activities and carefully consider them before implementing any actions.

In this chapter, the most common issues that can arise in the management of *ex situ* sturgeon populations and their potential effects on genetic diversity will be briefly discussed, while also providing guidelines for informed management. First and foremost, it is important to outline briefly some unique characteristics of sturgeons that are significant for their genetic conservation. These characteristics not only impact the evolutionary consequences of management decisions but also pose constraints on researchers conducting genetic analyses on these organisms.

6.2 Main sturgeon features with genetic relevance

In sturgeons, genetic analyzes must deal with complex genomes and different levels of ploidy, resulting from different whole genome duplication events. The first duplication event occurred in a common ancestor which possessed 60 chromosomes, leading to a genome with approximately 120 (110-130) chromosomes (Peng *et al.* 2007). The species belonging to this group include *Acipenser nudiventris, A. oxyrinchus, A. ruthenus, A. stellatus, A. sturio, Huso dauricus, H. huso, Scaphirhynchus albus, S. platorynchus, S. usii, Psephurus gladius* (recently declared extinct, see Congiu *et al.* 2023a), and *Polyodon spathula*. A second whole genome duplication event occurred in both the Pacific and Atlantic clades, leading to a total of approximately 240 chromosomes (220-276). The species in this group include *A. baerii, A. fulvescens, A. gueldenstaedtii, A. medirostris, A. naccarii, A. persicus, A. schrenckii,* and *A. transmontanus*. Lastly, a third event led to the unique karyotype of 360 chromosomes observable in *A. brevirostrum* (Kim *et al.* 2005). The determination of ploidy associated with each chromosome number has been the subject of extensive debate between two

principal theories. The first argues that species with 120 chromosomes resulting from a duplication event in the common ancestor should be considered tetraploid and those with 240 chromosomes should be considered octoploid. The second position focuses more on functional aspects, attributing diploidy and tetraploidy conditions to species with 120 and 240 chromosomes, respectively (Fontana *et al.* 2007). Both positions can be considered correct since a whole genome duplication event is generally followed by a ploidy reduction process. For example, a nominally tetraploid genome can be largely diploidized (Fontana *et al.* 2008; Stift *et al.* 2008; Birstein, 2002). However, the process of functional ploidy reduction is gradual, making it likely that different regions of the genome are characterized by different ploidy levels (Dalle Palle *et al.* 2022). This considerably complicates genetic analyses.

6.3 Genetic identification of conservation units

Maintaining genetic diversity within and among populations is a crucial priority for recovery and rehabilitation programs (Palsbøll *et al.* 2007). The differentiation between natural populations can be adaptively neutral, resulting from reproductive isolation and random divergence of gene pools. However, the genetic differences among populations may also hold adaptive value, particularly if the ecological conditions in their respective environments differ. In such cases, the forced mixing of populations adapted to different conditions can lead to a decrease in fitness in subsequent generations, known as "outbreeding depression" (Frankham *et al.* 2011). Therefore, understanding the natural geographic patterns of genetic variability is of paramount importance in informing the different phases of an *ex situ* conservation program. All activities, ranging from the establishment of broodstocks to the release of captive bred animals, should be designed to preserve the natural differentiations between populations. The breeding stocks must consist of individuals from the same conservation unit, and their offspring should be exclusively used for restocking the corresponding natural populations. Transferring animals between different populations or, even worse, crossing breeders with different evolutionary histories must be avoided.

In many instances, establishing breeding populations for controlled reproduction cannot be carried out based on animals from wild populations. Instead, integration of captive-bred individuals becomes necessary, which in many cases obscures the geographic origin of the individual. Therefore, it is crucial to develop genetic tests for each species that enable rapid and reliable determination of geographical origin or source population. In some cases, population differentiation is pronounced enough to be easily detected, facilitating straightforward geographic allocation. For example, the *A. naccarii* exhibits distinct populations from Italian rivers and the eastern coast of the Adriatic Sea, which can be distinguished even through simple analysis of mitochondrial DNA (Ludwig *et al.* 2003). However, in other cases, detecting genetic structure among populations is more challenging and may require labor-intensive investigations, as seen with *H. huso*. Only recently, a

whole-genome RAD approach allowed differentiation between Black and Azov Seas populations and the Caspian Sea population. This differentiation was impossible using more conventional markers (Boscari *et al.* 2021).

Considerable efforts are being made by researchers to reconstruct the pattern of geographic differentiation and identify the conservation units that require separate management. For certain species like, for example the above cited *A. naccarii* and *H. huso*, the information is already accessible and should be incorporated into the planning of *ex situ* conservation programs. This is intended to lead towards the establishment of specific broodstocks for each conservation unit.

For other species, conservation units have not yet been identified, and a concerted effort involving geneticists, aquaculture facilities, and conservationists is needed to elucidate the natural patterns of genetic differentiation. Unfortunately, for several species, decades of translocations and releases across basins have massively impacted the different gene pools. As a result, it has become strenuous to identify animals with certain geographical origin and reduces the chances of collecting reliable reference samples with known geographic origins. In these cases, an adequate representation of the genetic diversity of the source population is almost impossible to identify. Aquaculture facilities can be of great importance if they possess very old animals captured in well-known geographic areas. This greatly enhances the chances of accurate geographic allocation based on the history of individual specimens and represents one of the advantages of working with such long-lived animals.

6.4 Analyses of relatedness

Once the population to be used as a source of individuals for establishment of a broodstock as a basis for the planned release into the natural environment has been identified, another priority is to represent a significant portion of the natural genetic diversity. Genetic diversity can be regarded as the adaptive potential of a population. The greater the heterogeneity of a population's gene pool, the higher the chances that some individuals will possess genetic traits that enable them to respond effectively to the various selective pressures of the natural environment. Therefore, it is important to strive for maximum genetic diversity in a population while respecting the differentiation between populations discussed in the previous paragraph.

The ideal scenario is one where wild mature individuals are available in a natural population which can be kept in a controlled environment temporarily or permanently to facilitate their reproduction or where offspring of such parental stocks can be collected in the wild to increase their survival in captive rearing. In such cases, the likelihood of dealing with related individuals can be disregarded.

However, if wild individuals are unavailable in sufficient numbers or protected and cannot be captured for conservation purposes, reliance on aquaculture stocks

becomes necessary for selecting breeders. This presents several potential problems, primarily due to the fact that the rearing conditions in facilities intended for caviar production and the criteria for acquiring, selecting, and breeding animals are not aligned with the conservation of natural populations and their genetic diversity. Hence, it is crucial to be aware of the potential genetic issues and take appropriate precautions to prevent or at least mitigate their impact.

The first significant issue with aquaculture stocks that renders them unsuitable for conservation purposes is the prevalence of closely related individuals. Typically, fish farmers personally carry out breeding within the facility or acquire animals in the form of embryos. In both cases, due to the large number of individuals produced by each female, the number of crosses performed is limited in general, resulting in a population of closely related individuals. As a result, over time, each farm typically ends up with a small number of closely related groups of individuals. It is crucial, therefore, to determine the kinship relationships among the different animals in order to identify family groups and prevent mating between relatives. Mating between relatives has two major disadvantages. First, it exposes offspring to potential inbreeding depression, which refers to the reduced fitness or health of individuals resulting from reproduction between parents with shared genetic ancestry. When parents share their genetic ancestry, alleles with adverse effects on traits related to survival, growth, fertility, or disease resistance are more likely to be co-inherited and expressed in the offspring. In sturgeons, a certain role of ploidy in modulating the effects of inbreeding was never been proven but it can be hypothesized with some reason. In fact, the probability that a deleterious mutation is in complete homozygosity in a tetraploid is lower than in a diploid and it is therefore conceivable that the effects of inbreeding are somewhat reduced by a polyploid chromosome set.

The second negative consequence of mating between relatives is the reduced contribution of their offspring to the genetic diversity of the natural population, again limiting genetic diversity and as such adaptability compared to mating between unrelated individuals.

Therefore, it is strongly recommended to implement breeding plans that consider both the level of relatedness among different breeders and the overall genetic diversity within a given facility. The objective should be to release the highest degree of diversity into the natural environment, and to achieve this, it is important to maximize the diversification of genetic lines in future generations. These breeding plans should be developed over several years, gradually involving all available genetic lines. If the diversity within a single facility is limited and insufficient to develop appropriate breeding plans, it is advisable to transfer animals or gametes between different facilities as long as they still belong to the target population.

Another measure that should be adopted in crossbreeding planning is to delay the crossing of new generations and continue crossing older generations as much as

possible. This maximizes the portion of genetic diversity from the older generations that will be successfully transmitted, thereby reducing the rate of genetic erosion.

In the management of breeding plans, it is important to consider that not all animals are ready to reproduce every year, especially females, who tend to reproduce intermittently.

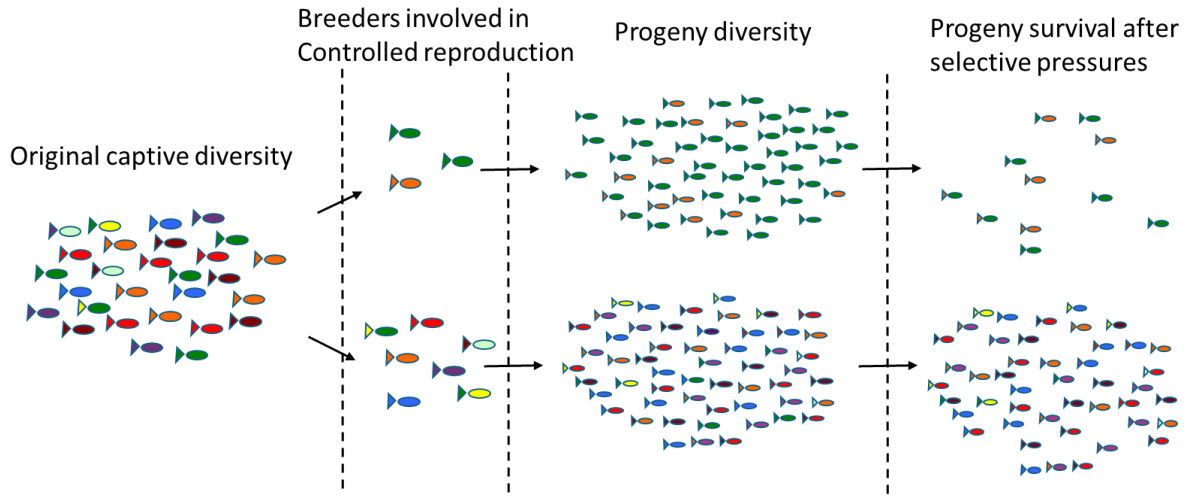

Figure 6.1: *Effect of genetic diversity erosion on adaptability (Photo credit: L. Congiu).*

This fact imposes strong constraints on the selection of animals for reproduction and makes long-term planning impossible. One way to overcome this limitation is to consider families as reproductive units rather than individual animals. This increases the likelihood of having some females available from a specific family group when needed. Of course, this assumes that a sufficient number of individuals from each family group are retained over time, accounting for intrinsic mortality, to ensure at least ten adult breeders per family. An alternative approach, focusing on the individual basis of reproduction would be the implementation of a gene bank for cryopreserved sperm, allowing to select suitable partners for each female according to the genetic breeding plan.

Relatedness analyses for diploid species are well-established procedures in genetics, and there are many software tools available that facilitate the inference of the degree of relatedness based on genetic data (e.g. ML-relate, etc.). However, for tetraploid species, the available approaches are more limited and generally less automated. Nevertheless, methods do exist to identify related individuals in tetraploid species, both through parental allocation analyses (if genotypes of the parental generation are available) and through estimates of genetic distance between tetraploid profiles (e.g. Congiu *et al.* 2011; Boscari *et al*. 2021).

Info-Box: Risks of captive breeding as associated with controlled propagation in aquaculture

Genetic drift is the change in the frequency of an existing gene variant (allele) in a population due to random sampling of organisms. The alleles in the offspring are a sample of those in the parents, and chance has a role in determining whether a given individual survives and reproduces. Genetic drift may cause gene variants to disappear completely and thereby reduce genetic variation. It can also cause initially rare alleles to become much more frequent and even fixed. If only a few copies of an allele are present within a population, the effect of genetic drift is larger, especially in small populations.

Inbreeding is defined as the production of offspring from the mating of individuals related by ancestry. Inbred offspring are more likely to inherit recent copies of the same allele from both parents, that is, alleles that are identical by descent (derived from a common ancestor of both the parents).

Natural populations contain low frequencies of deleterious recessive mutations (due to the balance between their occurrence by mutation and removal by selection) are normally found as heterozygotes and are thus not expressed. Inbreeding increases the probability of individuals inheriting these recessive alleles as homozygotes, thus enabling the expression of their deleterious effects. Consequently, in most populations of animals and plants, inbreeding results in a decline in reproduction and survival (reproductive fitness), also known as **inbreeding depression**. Another cause of inbreeding depression is overdominance. Different genotypes have different effects on fitness. In some traits or loci, even though alleles are not recessive, the homozygous genotypes are, on average, less fit than the heterozygous genotype. These traits or loci are referred to as over dominant or showing heterozygote advantage. As inbreeding decreases, the proportion of the fitter heterozygotes in a population, the average fitness (e.g. reproduction and survival) of the population declines, resulting in inbreeding depression. Studies show that most inbreeding depression is due to the accumulation of deleterious recessive mutations, rather than overdominance.

Mating between distantly related individuals, such as individuals from different populations or subspecies, is called **outbreeding**. Crossing populations may increase reproductive fitness by increasing heterozygosity and thus preventing the expression of deleterious recessive alleles (aka "hybrid vigor") or may decrease fitness because of various kinds of genetic incompatibilities between the genes from the different populations (**outbreeding depression).** Outbreeding depression is a reduction in reproductive fitness, reduced ability to mate or pollinate, fertilize, produce offspring, survive, or reproduce, in the first or later generations following the crossing of populations (Ralls *et al.* 2013).

6.5 The threat of hybridization

Another important characteristic of sturgeons is their genetic plasticity, to the extent that they can readily form interspecific hybrids with almost all existing sturgeon species (Birstein *et al.* 1997; Havelka *et al.* 2011; Zhang *et al.* 2013; Rachek *et al.* 2022; Vasil'ev *et al.* 2021). This has led to the generation of hybrid types in nature and aquaculture, which can pose a significant challenge for conservation, as will be discussed later. When hybrids are formed between species with the same ploidy level they are often fertile, like the widely cultured bester (*H. huso* x *A. ruthenus*) for meat and caviar on aquaculture farms, and some hybrids between species with different ploidy levels are also fertile (Vasil'ev *et al.*

2021; Rachek *et al.* 2022). Sturgeon aquaculture has continued to experiment with various hybrids, combining species that would rarely encounter each other in nature, such as species from the Pacific clade with species from the Atlantic clade. This experimentation, conducted with the aim of finding hybrids with desirable productive characteristics, has extended beyond first-generation hybrids, resulting in backcrosses or multiple hybrids with the contribution of more than two species. This process has generated a multitude of variants that are often no longer identifiable based on morphology (Congiu *et al.* 2023b).

These genetically impure animals present in aquaculture facilities are often mixed knowingly or unknowingly with individuals of pure species. As a result, in stocks intended for conservation purposes, hybrid individuals with different species can occur accidentally (e.g. the example of *A. naccarii* hybridization in aquaculture). It becomes crucial, therefore, to be able to identify these animals in order to remove them. Several genetic methods are available for the identification of species and interspecific hybrids, summarized in Congiu *et al.* (2023b) and Ludwig (2008). However, in some cases, identification based on the established and widely used genetic methods is still impossible, requiring further development of diagnostic genetic markers. The issue of reference markers is also central in this case. To identify species-specific markers, it is necessary to compare an adequate number of unrelated individuals of the species is to be characterized with representatives of all other species. It is crucial that the reference samples for these analyses are pure; otherwise, it may be impossible to identify diagnostic markers. An emblematic example is the hybrid between *A. schrenckii* and *H. dauricus* which is widely produced and used in Chinese aquaculture (Boscari *et al.* 2017). The two parental species are very different morphologically and belong to different genera. Therefore, it is expected that the identification of distinctive markers between the two species would be straightforward. However, probably due to the presence of hybrids in the reference samples used, this has not been possible (Boscari *et al.* 2017). It is absolutely crucial that breeding stocks intended for conservation programs are established using breeders of which the purity has been verified genetically.

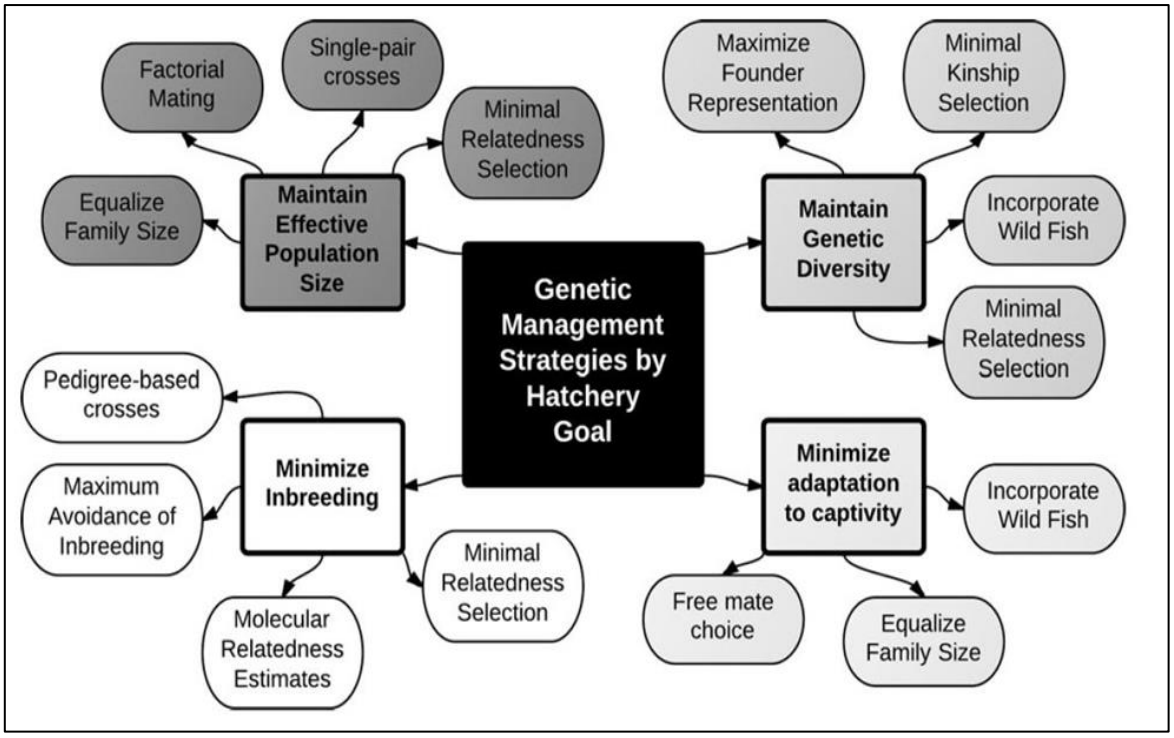

Figure 6.2: *Main objectives and related actions to consider when designing ex situ propagation facilities/ conservation hatcheries (after Fisch et al. 2015)*

6.6 Genetic effects of long-term rearing

When an organism is held in captivity, it is exposed to a different set of stimuli compared to its natural environment. These stimuli effectively act as selective pressures that impact both the physiology and the behavior of the animal. The environmental conditions in aquaculture facilities, where sturgeons are typically kept, can vary significantly in terms of structural characteristics, climatic regions, and water quality. Each facility's conditions exert selective pressures that favor individuals (genomes) capable of surviving, growing, and reproducing more effectively under those specific conditions. This phenomenon is known as captive selection (Frankham *et al.* 1986)) and is often reinforced by operators selecting individuals that demonstrate better performance, thereby increasing the frequency of their genetic traits over generations.

Furthermore, another evolutionary process observed in aquaculture, as opposed to the natural environment, is the relaxation of natural selective pressures. In controlled conditions, where abundant food is provided and parasites and predators are controlled, genetic traits that would be swiftly counter-selected in nature can increase in frequency, especially if they are randomly associated with the aforementioned characteristics that promote superior performance in captivity.

*Table **6.1:** Overview of the safety measures to be applied depending upon the source of the fish used in an ex situ program*.

Preliminary analyses suggested for broodstock establishment	
Breeders from the wild	**Farmed breeders**
• Geographical allocation • Check for purity	• Geographical allocation • Check for purity • Analyses of relatedness
Suggestions for rearing and propagation	
• Split the stock to different facilities • Do not prioritize animals • Involve all breeders over the long term • Breed wild generations as longer as possible • Cryopreserve sperm • Retain part of the offspring from all crosses • Translocate non-ovulating adult females to different conditions	• Use families as breeding units • Avoid mating related animals • Delay the reproduction of new generations • Split the stock to different facilities • Do not prioritize animals • Involve all families over the long term • Breed older generations as long as possible • Cryopreserve sperm • Retain part of the offspring from all crosses • Translocate non-ovulating adult females to different conditions

To mitigate or at least slow down this process of genetic adaptation resulting in a loss of fitness, certain management strategies are recommended (see Figure 6.2). The first strategy is to utilize wild-origin animals for as long as possible, as their genetic heritage has been naturally selected. For sturgeon, which have a life cycle spanning several decades, this can significantly delay the process of genetic erosion. In this sense, also the cryopreservation of sperm can prolong the genetic contributions of males. The second approach is to implement breeding plans that aim to utilize all available breeders (or all family groups in the case of a family-based breeding plan), giving lower priority to animals that have already been bred in the past. A third approach is to distribute animals across different facilities with diverse environmental conditions, thereby diversifying the selective pressures and, consequently, the genetic variants inadvertently selected. It should be noted that there are documented cases of animals that did not reach sexual maturity for 40 years in facilities where other conspecifics were reproducing regularly until, immediately after being transferred to a new facility with river water instead of groundwater, they ovulated for the first time (Storione Ticino S.r.l. pers. comm.). This demonstrates the crucial role of the facility environment in influencing the performance of different animals.

6.7 Effects of Aquaculture Practices on Genetics

The most recently discovered genetic risk for *ex situ* hatcheries to consider is the occurrence of spontaneous autopolyploidy which has been documented for multiple species (Schreier et al. 2021). Although the rate of spontaneous autopolyploidy is higher in hatcheries and is primarily due to post-ovulatory aging of oocytes and mechanical shock during egg de-adhesion (Van Eenennaam et al. 2020) there is also spontaneous autopolyploidy documented in wild stocks, including some recovery programs (Schreier et al. 2021). Since spontaneous autopolyploids are fertile any mating with normal ploidy individuals will produce viable intermediate ploidy females that are either sterile or have delayed maturation and that will pass the new ploidy level to their offspring. For captive *A. transmontanus* autopolyploids, instead of maturing at ages 7-9 years, maturation was delayed until ages 12-16 years for some females (Van Eenennaam, unpubl. data; Fiske et al. 2023). Of course, for any released spontaneous autopolyploid sturgeon into the wild, they first need to survive to maturity and then spawn with a normal ploidy individual, and those intermediate ploidy progenies then need to survive to adulthood, thus it could be decades before the potential impact on a conservation program could be identified. Therefore, the risk of release of spontaneous autopolyploids needs to be assessed on a program-by-program basis. For example, the Snake River *A. transmontanus* conservation program (Idaho, USA) began checking for spontaneous autopolyploid individuals in 2015-2016. A total of twelve families were made with wild-caught broodstock and three of the families had 6%, 13% and 27% incidence of spontaneous autopolyploids (Idaho Power Company, 2021). In 2019 they shifted to a repatriation program and began to screen every fish using coulter counter analysis of RBC nuclei volume (Fiske *et al.* 2019) to ensure only normal ploidy fish were released. They identified and culled 98 spontaneous autopolyploids (1.5%) during 2019-2022 (Brown, 2023), considering the large size of the individuals that would have been released (200-400g) and probability of survival to maturation and spawning (Ireland *et al.* 2002; Justice *et al.* 2009), their implementation of verifying the normal ploidy of all released individuals has been a critical component of their *ex situ* hatchery program.

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Chapter 7: Approaches for incorporating wild sturgeon populations into *ex situ* programs

James A. Crossman, Molly A.H. Webb, Thomas Friedrich, and Joern Gessner

7.1 Introduction

Ex situ facilities for sturgeon commonly rely on the collection of gametes from wild caught or captive bred broodstock native to the river system where recovery efforts are required (Anderson *et al.* 2022). While gametes from broodstock of the same species but from adjacent catchments have been used (e.g. *Acipenser oxyrinchus and A. sturio*), this practice is now much less common and only considered if extirpation of the native population is imminent or populations are reintroduced after their extinction. For the latter case, a structured approach to sourcing suitable broodstock to ensure genetic and evolutionary consequences are mitigated is discussed in Chapter 6. A key emphasis of *ex situ* is to limit selective rearing pressures to the extent possible and this has included adapting more traditional hatchery methods to incorporate more species-specific biology through the use of wild breeders or their progeny. Reflecting more natural conditions during both spawning and rearing of early life stages has been a priority for *ex situ* programs utilizing wild breeders. Much success has been attributed to transitioning from a focus centered on large numbers of juveniles for release and rather optimizing hatchery practices for producing smaller numbers of robust progeny for release across multiple life stages or ages. This chapter expands on the review by Anderson *et al.* (2022) and discusses the rationale for incorporation of wild fish into conservation aquaculture activities and outlines specific methodology associated with different approaches for directly spawning wild adults or collecting naturally produced progeny from the wild.

7.2 Rationale for use of wild fish

As covered in Chapter 3, objectives for each specific program will drive the rationale for how progeny are produced or collected. Briefly, prevention of extinction while promoting measures to retain genetic diversity of the wild population are generally the overarching goals governing how *ex situ* programs are developed. The use of wild breeders to ensure genetic diversity benefits from natural selection while adopting strategies outlined in Chapter 6 allows some mitigation of genetic selections by environment during hatchery rearing. Progeny are ideally sourced from the wild population that is of focus, provided sufficient numbers of annual breeders can be captured and directly spawned. Alternatively, collecting naturally produced gametes or larvae at or near spawning grounds in

the wild allows for circumvention of controlled reproduction in the hatchery altogether.

Ideally, concurrent population monitoring would confirm effectiveness of these approaches through monitoring spawning activity, larval dispersal, and recruitment to the juvenile stage. In many cases, *ex situ* programs are occurring in parallel to other recovery activities where the goals (e.g. relationship between wild recruitment and environmental conditions) could be compromised if *ex situ* programs are not designed comprehensively within the larger framework of recovery.

7.3 Approaches for incorporating wild populations

There are three common strategies that can be employed to incorporate individuals from the wild population into conservation aquaculture programs. These include, from least invasive to most: 1. collection of embryos or larvae from spawning events that occur naturally in the wild, 2. capture of mature adults for short-term holding and direct spawning in the hatchery, and 3. long-term holding of mature adults for direct spawning in the hatchery. Each approach is described in detail below, with a direct comparison provided in Table 7.1.

7.3.1 Direct collection of naturally produced progeny

Embryos or larvae produced through natural spawning events can be collected and incorporated into conservation aquaculture programs (Figure 7.1). Because wild spawning allows for natural mate selection and spawning location, embryos and larvae collected experience natural selective pressures at early developmental stages. This approach has been shown to improve genetic diversity compared to the more traditional method of spawning adults under controlled conditions (Crossman *et al.* 2011; Jay *et al.* 2014; Thorstensen *et al.* 2017). Success requires detailed knowledge of spawning and egg deposition locations and larval dispersal behavior, which can be species-specific. Embryos or larvae are collected from the river using several techniques but most commonly include egg mats or drift nets (Haxton *et al*. 2024). The design of egg mats traditionally consists of filter material (synthetic or latex coated animal hair) fastened to a square steel frame, the size of which will be specific to hydraulic conditions in the river. Egg collection mats are deployed to rest on the river substrate at or immediately downstream of spawning sites and work by entrapping drifting or deposited embryos in the filter material. Egg mats are especially beneficial in situations where frequent monitoring is not possible and can be left to passively sample for multiple days assuming the frequency of assessing them is less than the time required for embryo incubation and hatch.

Standard drift nets consist of a rolled stainless-steel frame in a "D" shape with a tapered plankton net (0.16 cm delta mesh size) attached ending with a collection cup device. Drift nets are deployed to stand perpendicular to the river bottom and

collect drifting embryos and larvae in the tapered plankton net (Figure 7.1). They are more labor intensive compared to egg mats as they can quickly become filled with organic material in the water column which will reduce survival of sturgeon progeny if nets are not inspected and cleaned frequently. Drift nets are used successfully to capture passively dispersing yolk-sac or feeding larvae for many sturgeon species including *A. transmontanus* (Crossman & Hildebrand, 2014), *A. fulvescens* (Auer & Baker, 2002), and *A. brevirostrum* (Moser *et al.* 2000). For programs that intend to use naturally produced progeny, a combination of egg mats and drift nets is recommended as the mats allow for detection of spawning and a better idea of where spawning occurs, while drift nets can then be placed downstream of these sites. In shallow streams with small substrates, kick nets or other tools that can collect and separate substrate materials to allow embryos to be removed have also been used with some success (Crossman *et al.* 2011), but this method is not practical for the majority of sturgeon species that spawn in deeper rivers.

Figure 7.1: *Standard egg mat (left) and drift net (right) used to collect embryos and larvae. The tapered plankton net ends with a collection cup device (not shown in the photo) (Photo credits: Egg mat (J. Crossman) and drift net (M. Webb)).*

When possible, collection should capture the entire spawning distribution to maximize genetic diversity (Jay et al. 2014; Anderson et al. 2022) as individual spawning time appears to be repeatable for some species (Forsythe et al. 2012), and most species spawn over a protracted period of 4-8 weeks. During collection in the field, embryos and larvae should be developmentally staged to identify spawning timing and allow for rearing groups to be identified (Figure 7.2). Fertilization date for collected embryos and larvae can be estimated by back-calculation from the capture date and time based on developmental stage and mean incubation water temperature (Dettlaff et al. 1993).

Separate rearing by distinct periods (e.g. early vs late spawning runs in *A. fulvescens*) or spawning events to control for disproportionate survival ensures all groups are better reflected in the final stocking numbers. While it is expected that

this approach will increase genetic diversity compared to direct spawning of adults, genetic monitoring is recommended during rearing or before release to confirm effectiveness.

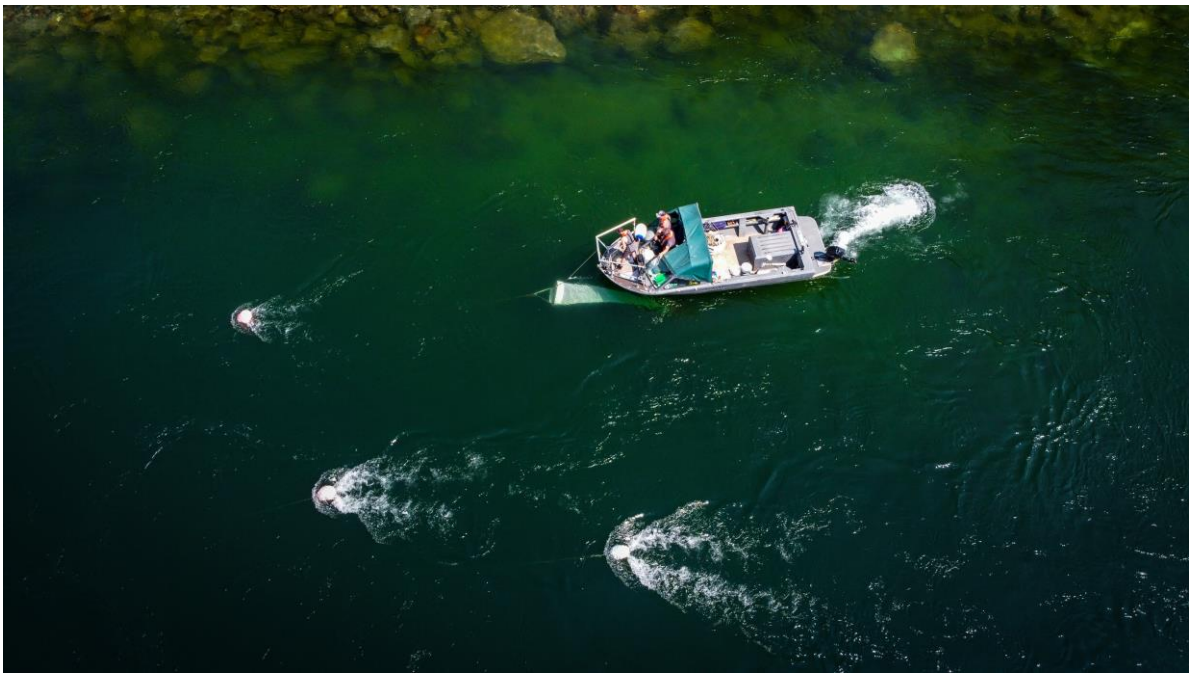

Figure 7.2: *Example of a drift net being deployed off the bow of a boat immediately downstream of spawning grounds for Acipenser transmontanus (Photo credit: J. Crossman).*

Figure 7.3: *A): Embryos of Acipenser transmontanus at different developmental stages collected from wild spawning events for transfer to a streamside hatchery for incubation. B): Wild caught larvae of Acipenser transmontanus for transfer to a streamside hatchery (Photo credits: Laura Henderson).*

Natural reproduction or population persistence would not likely be impacted by collection of embryos or larvae from wild spawning events given that natural rates of mortality at these life stages are extremely high for sturgeons (Jaric & Gessner, 2013).

To improve survival during early life stages, streamside incubation can be used to allow for less transportation and handling effects and can ensure early life stages are exposed to natural environmental profiles and chemical signatures. There may be benefits associated with imprinting, though further study is required to identify mechanisms and life stages. Several species have transitioned to streamside rearing including *A. fulvescens* (Holtgren *et al.* 2007; Crossman *et al.* 2011, 2014), *A. transmontanus* (Snake River, Thorstensen *et al.* 2017; Upper Columbia River, Anderson *et al.* 2022), *A. oxyrinchus* (Gessner *et al.* 2023) and *A. ruthenus* (Friedrich *et al.* 2019). Streamside rearing can involve temporary, semi-permanent, or permanent facilities that rear certain life stages or are used for the entire rearing process up to release. In some cases, embryos are incubated streamside (Figure 7.3), and larvae transferred to conservation facilities for rearing.

Figure 7.4: *Example streamside trailer used for Acipenser transmontanus on the Columbia River, Canada for incubation of embryos. After hatch, larvae are transported to a hatchery to be reared for 9 months before being released back into the river (Photo credit: J. Crossman).*

Transportation of embryos and larvae to the hatchery would generally follow methods described in Chapter 14 but would have additional considerations. Embryos should experience natural deadhesion, but if they are collected close to the time of fertilization, they may require treatment with diatomaceous earth or other deadhesion techniques as described in Chapter 9. This should occur as close to collection as possible, either prior to transport or during transfer into a streamside facility. Handling in the field should be limited, especially for embryos that have not reached neurulation. Lastly, a possible concern for some facilities associated with bringing in embryos and larvae from the wild is that initial incubation periods in the river allow for microbial community development to occur

naturally on embryo surfaces (Fujimoto *et al.* 2013; Fujimoto *et al.* 2021; Walquist *et al.* 2022). This could complicate their use at certain facilities if quarantine is not possible or if there are disease concerns (Chapter 11).

7.3.2 Capture, short-term holding and direct spawning

7.3.2.1 Streamside spawning of wild adults

Streamside spawning can be the method of choice if access to adults in the act of spawning is possible. Gametes in this case can be collected directly on the spawning grounds (Crossman *et al.* 2011; Bruch *et al.* 2009) and either be fertilized on site or transported to a controlled hatchery setting. If an adult is collected that is expressing gametes, gentle hand stripping can be used to collect eggs or milt directly, similar to the approach described in Chapter 9. The amount of time adults are handled should be limited to ensure they return and continue spawning. Holding tanks on the river's edge could be considered while adults are collected, but eggs and milt can also be collected and stored until sufficient numbers have been collected to meet breeding objectives. By preserving eggs in ovarian fluid in plastic bags floating in ambient river water and milt in bags filled with oxygen kept at ambient river temperatures, a wider array of breeding combinations may be possible. This is likely worth the minor compromise to egg quality or sperm motility. Milt can be held for longer durations in a refrigerator if oxygen is replenished every few days. Eggs likely require fertilization within 12 hours of collection while being mindful of other potential effects, like influencing rates of spontaneous autopolyploidy by fertilizing overripe eggs (Schreier *et al.* 2021). If adult capture and gamete collection is occurring later in the spawning period when temperatures are elevated (e.g. >16 °C), fertilization of eggs may need to occur more quickly following collection as egg quality declines with increasing temperatures for most species.

There are risks associated to streamside spawning of wild adults as even though small amounts of gametes are collected (<1 L), fish may abandon spawning in the wild. This risk should be weighed against allowing fish to spawn naturally and collecting embryos or larvae after spawning is over (as described above), especially in populations where some evidence of natural recruitment has been documented.

7.3.2.2 Short-term captive holding for direct spawning

Wild adults that are typically captured close to (within 6-8 weeks) or during the natural spawning time, either at staging grounds or directly on the spawning grounds, are subsequently spawned under captive conditions. Successful examples include *A. guedenstaedtii* (Ruban *et al.* 2019), *A. ruthenus, A. stellatus, A. transmontanus, Huso huso,* and *Scaphirhynchus albus*. Adults captured prior to the spawning period and assessed as spawning ready are transported to a conservation facility and held in tanks with water temperature matching ambient river temperatures with natural photoperiod. Fish should be closely monitored at hourly intervals immediately after arrival. Segregation by sex should be performed initially to ensure spawning does not occur naturally. The water temperature in

female holding tanks should be increased slowly (< 2 °C/day) until it reaches known spawning temperature for the population to mimic natural environmental conditions to the extent possible. If more precise temperature control is possible, following the natural temperature profile of the river of origin is recommended. Wild males can be kept at the initial ambient river temperature and then increased to the spawning target after being induced to spawn. Typically, wild adults being held for short duration (~1 month) are offered either live feed or are not fed while in captivity. In most cases, hormonal induction is still used for adults that are transported to a facility for short-term holding.

Following spawning, adults in facilities are held for a period of observation of recovery before release near their location of capture. While slightly less invasive compared to holding wild adults for longer periods in captivity, many of the same risks as detailed below need to be managed. In the Upper Columbia River White Sturgeon Recovery Initiative, 21% of the adults brought to the hatchery (n=220) for short-term holding over a 14-year period failed to spawn, with 2 adult moralities occurring in captivity (FFSBC, 2020). Further, as described above, cumulative stress and physiological impacts may last well beyond captivity following release back into the wild, and this aspect remains understudied. Facility requirements are similar to long-term holding but may allow for additional wild adults to be used following initial rounds of direct spawning when fish have been returned to the river.

Natural tank spawning could be an option for adults that are very close to spawning but has not been rigorously assessed for effectiveness with wild fish brought in for short duration. It has been successfully applied for a few captive species including, where adults in spawning condition are allowed to mate semi-naturally with (*A. medirostris*, Van Eenennaam *et al.* 2012) or without *(A. oxyrinchus oxyrinchus*, Henne unpublished; *A. brevirostrum*, Kynard *et al.* 2011) hormonal induction. This approach can be conducted in traditional circular holding tanks where gametes are collected every few hours with a fine mesh dip net scraped along the tank bottom (Van Eenennaam, *et al*. 2012) or by using mesh netting incorporated into the outflow but is potentially limited to a single female with one or more males. The approach used by Kynard *et al.* (2011) and proposed by Chebanov (2008) incorporates a larger artificial stream structure with natural water velocities and spawning substrates allowing for embryos to incubate under more natural conditions. This approach has been shown to improve survival, growth, and development in some species (e.g. *A. transmontanus*, Boucher *et al.* 2014; McAdam, 2011; Gessner *et al.* 2004). In the more complex artificial stream, Kynard *et al.* (2011) was able to incorporate multiple females and males to allow for more natural mate choice. However, this approach may be less feasible to incorporate for many programs as it requires considerable infrastructure.

7.3.2.3 Long-term holding for direct spawning

Utilization of wild adults after a prolonged lead time in captivity typically applies when spawning or staging sites prior to spawning are unknown. Further, this strategy may be applied to small populations when encountering fish is difficult or in systems where sampling is less practical during the spawning period (e.g. during spring freshet in large rivers). They can be selected a year to 9 months in advance during the spawning migration (potamodromous species, hiemal migrants) of natural spawning. This may also include adults that have been reconditioned to reach spawning readiness in the hatchery. Adults are captured and assessed for sex and stage of maturity as described in Chapter 8 and in Webb *et al.* (2019). Individuals that are expected to spawn in the coming year are transferred to a facility where they are held (see Chapter 5) and spawned in a similar manner to the methods described in Chapter 9 for hormonal induction.

To adapt the wild-caught fish to captive conditions, the fish should be maintained in an acclimation unit for 2-3 weeks at low stocking density and matching ambient river temperatures with natural photoperiod. Dissolved oxygen should be high (~ 8 ppm). Stress factors should be minimized during this period, and handling, if necessary, may be postponed to the hours of darkness and carried out under red light with a wavelength of 680 nm that is invisible for sturgeon.

Feeding of transferred fish should be initiated with natural feeds (e.g. crustaceans, mollusks, worms, and fish), and a gradual transition to prepared compounds, composed of natural feeds, should occur. During the period of acclimating fish to feed, they should be held at optimal temperature for the species, as at higher temperatures, non-feeding individuals quickly lose weight and their physiological condition decreases, which can lead to irreversible dystrophy. Fish that do not consume formulated feeds after more than 90 d of acclimation should be released into natural water bodies if live feed cannot be continued indefinitely.

Weaning of wild-caught fish to feeding with formulated dry diets, if necessary, begins with carefully selected suitable (healthy, well-conditioned) fish that are successfully feeding in captivity on natural diets. It should be considered that different sturgeon species in natural conditions consume different food organisms, and the composition of the feed mixture during acclimation to formulated feeds should contain different feed sources. The process of adding formulated diets is started with less than 5% of dry diet that is introduced into paste-like natural feeds (minced fish, mussels, shrimp, etc.). Feed additives (omega-3 fatty acids) can help to increase acceptance of the paste-like diets, and only when the fish begin to consume this mixture, the content of artificial feeds in the mixture is gradually raised, increasing the size of feed pellets.

The effectiveness of acclimation of wild sturgeon to a captive environment, as with pre-spawning holding, can be increased through injections of vitamins. For this purpose, vitamin "C" (ascorbic acid) and "E" (α-tocopherol) are used, which are affecting the fat, protein and mineral metabolism. Effective daily doses of vitamins in the course of acclimation are lower than at pre-spawning administration, and

amounts of 5 mg/kg of ascorbic acid and 10 mg/kg of α-tocopherol may be given. The duration of injections is 5-7 d. When feeding begins, vitamin C can be added to the feeds at the rate of 2 g/kg of feed.

For the next successive maturation of the ovaries, domesticated females need not only to restore energy and compensate losses incurred during the period of wintering, spawning, holding, curing of injuries and the period of acclimation to feeding, but also to accumulate a sufficient amount of reserves for the formation of a new generation of oocytes.

Breeding plans will follow population-specific guidelines that will depend on the numbers of breeders available within a year and the genetic objectives for the population (see Chapter 6). During the period of captivity, rearing methods vary by species, but feeding typically occurs until fish are spawning ready (Chapter 8). Following spawning, adults are held for a short period to ensure they recover before being released back into the wild near their location of original capture. It is recommended that adults are individually tracked using PIT tags to ensure their genetic contributions to the program can be controlled, as some programs have elected to not reuse adults once they have contributed to a single year class (Anderson *et al.* 2022). Wild adults are recommended to have a genetic assessment to ensure spontaneous autopolyploidy is not a concern (see Chapter 6) and to develop a database of genotypes to allow for genetic measures of the program to be determined. The conservation aquaculture program for *S. albus* for example requires that genetic relatedness (see Chapter 6) is estimated for all available spawners within a year to optimize breeding pairs that minimize relatedness while maximizing effective population size.

This approach is considered the most invasive of those covered in this chapter as removing reproductive adults from the wild comes with elevated risks associated with capture, transport, and captive rearing. Cumulative stress associated with these activities could result in direct mortality or have sublethal effects that persist following release back into the wild. While these effects can be mitigated to some extent following best practices outlined in Chapter 4, not all adults that are brought into facilities for spawning the following year respond well to the controlled conditions, and all precautions should be taken to maximize individual welfare, even if that means returning the individual to the wild. Further, this approach has the most facility requirements and may conflict with optimal breeding designs if facility limitations preclude sufficient numbers of adults from being held in captivity.

Table 7.1: *Comparison of approaches that can be used to incorporate wild adults into ex situ programs.*

Approach	Benefits	Challenges
Direct collection of naturally produced progeny	• Least invasive • Increased number of wild adults represented • Increased genetic diversity • Natural mate selection • Natural spawn timing • Less facility infrastructure	• Knowledge of spawning locations • Sampling logistics for capturing early life stages • Treatment of microbial infections • Increased handling of sensitive life stages • Facility requirements for streamside
Streamside spawning of wild adults:	• Less invasive than holding adults • Includes more adults in the breeding design • Natural spawn timing • Less transportation infrastructure • Less facility infrastructure	• Limited number of systems where this is possible • Controlling conditions for gamete holding and fertilization • Unequal volumes of gametes from different individuals • Capturing sufficient numbers of adults in the act of spawning • Access to spawning sites
Short-term captive holding for direct spawning of wild adults	• Less risk to wild adults through reduced holding • Final maturation stages occur in natural environmental conditions • Adults are closer to spawning, less risk of failed spawn	• Removal of adults that could contribute to natural spawning events • Increased stress that may result in individuals failing to spawn (e.g. atresia in females) • Increased risk of unintended mortality

Approach	Benefits	Challenges
Long-term holding for direct spawning of wild adults	• Ensures availability of adults for small populations • Spawning time can be controlled through hormonal induction • Field sampling can be protracted in a year in advance of spawning	• Space for adequate numbers of wild adults, especially for large-bodied species • Appropriate feed for adults while captivity • Increased stress that may result in individuals failing to spawn (e.g. atresia in females) • Increased risk of unintended mortality • Removal of adults that could contribute to natural spawning events

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Chapter 8: Broodstock Management and Selection

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8.1 Introduction

Broodstock management requires identification of the different stages of maturity in both females and males (see 8.2). Subsequently, selection of mature broodstock for breeding can be divided into holding regimes to optimize the maturation process throughout the last stages of gonadal development:

- Initial Selection Assessment (see 8.3)
- Autumn-Winter Holding Regimes (see 8.4)
- Maturation Assessment (see 8.5)
- Pre-spawning Holding Regimes (see 8.6)

8.2 Gonadal Stages of Maturity

Gonads of sturgeons are gymnoovarian (open to the body cavity), paired, and consist of both fatty (adipose) tissue and germinal tissue where the ovarian follicles or spermatozoa develop. Within a reproductive cycle, females develop all oocytes synchronously and release eggs over a short period of time. There are multiple books and guides (e.g. Dettlaff *et al.* 1993; Bruch *et al.* 2001; Chebanov & Galich, 2011) and papers (e.g. Doroshov *et al.* 1997; Van Eenennaam & Doroshov, 1998; Webb *et al.* 2002; Flynn & Benfey, 2007; Webb & Erickson, 2007; Wildhaber *et al.* 2007; Chebanov & Galich, 2009; Webb & Doroshov, 2011; Du *et al.* 2017; Kazemi *et al.* 2019; Webb *et al.* 2019) describing gonadal maturation. However, the gonadal classification schemes in each guide or paper can be slightly different based on the study objectives, the actual phases encountered, and the selected detail in describing broader or more specific aspects of gametogenesis (Webb *et al.* 2019) resulting in limited comparability of the stages described. Standardized terminology for the stages in the reproductive cycle for sturgeons and the use of the stage name (e.g. for females: differentiation, pre-vitellogenic, early to mid-vitellogenic, late vitellogenic, post-vitellogenic, oocyte maturation and ovulation, post-ovulatory, mass atresia) rather than a number or combination of numbers and letters is recommended for comparison purposes. Fish that have reached sexual maturity and spawned may have spermatogonia or primary oocytes with no other visible indicators of previous spawning (i.e. residual spermatozoa or post-ovulatory follicles or mass atresia). These animals will never return to the immature stage/phase but may be classified as regenerating with spermatogonia or pre-vitellogenic oocytes (Table 8.1).

8.2.1 Classification of Maturation Stages

The stages used in this publication to describe decisive progress in the maturation process are based on Webb *et al.* (2019), which provides one of the most recent macroscopic and histological descriptions for sturgeon females (Table 8.1) and males (Table 8.2), and the classification scheme of Bruch *et al*. (2001) has been summarized for comparison. The classifications by Trusov (1972) for both females and males have been described in detail using ultrasonography diagnostics by Chebanov & Galich (2009, 2011).

Table 8.1: *Phases and stages of female gonadal development. Descriptions (macroscopic and microscopic) with notes on ultrasonography observations modified from Webb et al. (2019) and compared with classifications by Bruch et al. (2001) and Chebanov & Galich (2011). Because oocyte diameter differs by species, a range is given that incorporates all species (sf=semi-fatty; f=fatty; i=incomplete; c=complete).*

Phase	Stage	Webb *et al.* 2019	Bruch *et al.* 2001	Chebanov & Galich, 2011
Immature	Differentiation: formation of ovigerous lamellae embedded in the adipose tissue along the gonad length; oogonia along periphery of lamella with perinucleolar oocytes developing that are not visible macroscopically; not clearly discernible by ultrasonography	1	Fv	F1
Developing	Pre-vitellogenic: obvious well-developed ovigerous folds with small translucent oocytes in the early endogenous growth phase; oocyte diameter 0.1 - 0.4 mm; ovigerous folds can be identified by ultrasonography	2	F1	F2, F2sf, F2f
Developing	Early to mid-vitellogenic: ovigerous folds contain small white to larger yellowish oocytes with differentiation of 1-2 layers of the zona radiata and yolk platelets in the cytoplasm; nucleus (germinal vesicle) is centrally located; oocyte diameter 0.3 - 1.5 mm; ovarian follicles can be identified by ultrasonography	3	F2	F2-3
Spawning Capable	Late vitellogenic: ovarian follicles begin to darken in color as melanin pigment is deposited under the oolemma; follicular layers and three layers of the chorion are fully differentiated; nucleus begins to move off-center toward the animal pole; oocyte diameter 1.0 – 3.0 mm; ovarian follicles can be identified by ultrasonography	4	F3	F3
Spawning Capable	Post-vitellogenic: fully grown ovarian follicles that have a polarized structure with the animal hemisphere containing small, round yolk platelets and lipid inclusions, while the vegetal hemisphere contains	5	F4	F4i, F4c

	large, oval-shaped yolk platelets and large lipid inclusions; nucleus displaced to the animal pole; oocyte diameter > 2.0 mm; ovarian follicles can be identified by ultrasonography			
Spawning	Oocyte maturation and ovulation: oocytes have undergone germinal vesicle breakdown and are ovulated; eggs are freely flowing from the vent when captured in the wild or from a hormonally induced fish	6	F5	F5
Regressing	Post-ovulatory: ovaries contain post-ovulatory follicles and the next generation of oocytes (pre-vitellogenic or early vitellogenic); some recent atretic follicles from non-ovulated eggs may be seen	7	F6	F6
Regressing	Mass atresia: ovarian follicles are soft and break easily with deformed cell shape and melanin pigment in the yolk; have a marbled appearance (i.e. black with white swirls); advanced follicular atresia can be identified by ultrasonography	8		
Regenerating	Pre-vitellogenic with histological indications of past spawning event: small translucent oocytes in the early endogenous growth phase; small atretic bodies or fully collapsed post-ovulatory follicles that are almost fully resorbed; oocyte diameter 0.1 - 0.4 mm; ovigerous folds can be identified by ultrasonography	9		

Table 8.2: *Phases and stages of male gonadal development. Descriptions (macroscopic and microscopic) with notes on ultrasonography observations modified from Webb et al. (2019) and compared with classifications by Bruch et al. (2001) and Chebanov & Galich (2011).*

Phase	**Stage**	**Webb *et al.* 2019**	**Bruch *et al.* 2001**	**Chebanov & Galich, 2011**
Immature	Differentiation: testes appear as a smooth; thin, white thread on top of adipose tissue; clusters of primary spermatogonia in less-defined testicular cysts; not clearly discernible by ultrasonography	1	Mv	M1
Immature	Spermatogonia proliferation: testes appear as a thicker white thread (≈0.5 cm) on top of adipose tissue (≈ 0.5-1.5 cm); spermatogonia undergoing mitosis in differentiated testicular	2	M1	M2f

	cysts; testes may not be discernible by ultrasonography			
Developing	Onset of meiosis: testes are whitish with a turgid texture (≈0.5-2.0 cm thick) on top of variable amounts of adipose tissue (≈1.0-3.0 cm thick); approximately 50% testicular cysts contain spermatogonia and the remaining contain primary and secondary spermatocytes and spermatids; a few may contain spermatozoa; testes can be identified by ultrasonography and have a distinct white boundary (tunica), while the adipose tissue is dark (hypoechoic)	3	M1	M2sf, M2
Potentially Spawning Capable	Late spermatogenic: gonad is primarily testicular tissue, with little adipose tissue; testes are turgid, lobular and white (2.0 - 4.0 cm thick); most testicular cysts contain spermatocytes, spermatids, and spermatozoa, with less than 10% of cysts containing spermatogonia; testes can be identified by ultrasonography, and areas of each testis starts to appear lobular	4		M3
Spawning Capable	Ripe: completion of spermatogenesis; the testes are large (≈ 3.0 – 8.0 cm), white and lobular; differentiated spermatozoa in > 90% of the testicular cysts; animal is not actively spermiating; ultrasonography reveals a bright white, fine-grained homogeneous testis that fills the majority of the screen	5		M4
Spawning	Spermiation: spermiating males release thick, white milt (peak spermiation) to thin "watery" milt (either at the beginning or end of spermiation); spermatozoa in all testicular cysts; ultrasonography reveals a white, fine-grained homogeneous testis that fills the majority of the screen with no obvious boundaries (tunica)	6	M2	M5
Regressing	Post-spermiation: residual spermatozoa in regressed testicular cysts, and clear seminal fluid can sometimes be collected from the	7	M3	

	urogenital pore; this fluid when placed in a clear glass beaker reveals white flocculants of residual clumped non-viable spermatozoa; ultrasonography of post-spermiation males have not been well documented			
Regenerating	Spermatogonia proliferation with histological indications of past spawning event: spermatogonia undergoing mitosis in differentiated testicular cysts; lumens of cysts may be open; testes should be discernible by ultrasonography	8		

8.2.1 Methods for Assessment of Phases of Gonadal Maturity

Because external sexual dimorphism is not apparent in sturgeon before sexual maturity, different techniques are used to determine the sex and the phase of sexual maturity. The most widely used techniques are ultrasonography, endoscopy, celiotomy/biopsy and determination of plasma sex steroid concentrations. Technique selection will depend on the *ex situ* program objectives and limitations. Limitations may be financial (limited funds), time sensitive (need immediate results), regulatory (biopsies or bleeding of threatened or endangered species may require permits), results based (high accuracy required), or a combination of these factors (Webb *et al.* 2019).

It is important that new personnel obtain training from an experienced individual for any of the methods described. Training can come from another *ex situ* hatchery, a research facility using one or more of these techniques, or if available an established sturgeon farm (e.g. many farms use ultrasonography for broodstock management).

8.2.1.1 Fish Handling

Fish handling for each method of assessment is similar. Individuals are captured from either inside the rearing tank/pond/cage or river (see Chapter 4) by carefully guiding an individual (by pectoral fin and snout) directly into a hooded stretcher, sling or cradle, or from outside the rearing system by using some type of dip net or tube-net made of knot-less netting material. The animal is then moved to the stretcher/sampling area which is set-up close so the fish is out of water for only a short time. Depending upon the size of the female, sufficient personnel or technical support measures (forklift, bobcat, crane) might be required. Once in the stretcher and at the sampling area, the fish is rolled ventral side up, and a hose of oxygenated fresh water is placed directly above the mouth or in the mouth. Mature sturgeon under this condition typically exhibit tonic immobility (see Chapter 4).

8.2.2 Diagnostic methods

8.2.2.1 Ultrasonography

Ultrasonography, as the least invasive tool to assign sex and phase of maturity, has been the fastest growing technique and has been used on most sturgeon species and their hybrids (Annex 8.1). 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 expertise of the ultrasound technician. Ultrasonography can provide immediate identification of sex and phase of maturity and images can be saved for further analysis.

Depending on the make and model, an ultrasound unit and transducer will cost $12,000-35,000+ USD, with refurbished units at a discount. There are less expensive units, but the sonograms tend to have poor resolution making the phases of gonadal maturation more difficult to discern. More recent systems that are being successfully used are hand-held computer tablets, with software to view the sonograms on the tablet screen, and a transducer. These systems basically are the cost of the transducer ($6,000-8,000 USD). Most companies will allow on-site testing of units, and this is highly recommended prior to purchase.

The settings used on each ultrasound/transducer unit will need to be determined by the user for their species and body size. In general, a 2D mode is used, with a small parts exam, and depth and gain are adjusted based on the fish body size. What can confuse the user in the interpretation of a sonogram is the overall gain and the far and near gain controls. These controls brighten or dim the sonogram and can potentially make the easily distinguished light-dark ovigerous folds to be too bright and indistinguishable or make the image too dark. Either extreme can make the homogeneity or heterogeneity of gonadal tissue and the hyperechoic and hypoechoic echo signals difficult to interpret (Webb *et al.* 2019).

Regardless of the make/model of ultrasound used, the proper transducer is most critical and will depend on the species, body size, body wall thickness, and gonad size. Higher MHz provides greater resolution but lower penetration and vice versa. A convex 2.0-3.5 MHz transducer allows for imaging at > 20 cm depth, which is important for large fish with a thick abdominal wall (> 50 kg; Chebanov & Galich, 2008) like the *Huso huso*. Linear transducers from 5-10 MHz were used in most sturgeon studies (Novelo & Tiersch, 2012), and currently a 6-15 MHz linear transducer is being used to sex and determine phase of maturity in *Acipenser medirostris*, *A. transmontanus*, *A. fulvescens*, *A. oxyrinchus*, *Scaphirhynchus albus*, *S. platorynchus* (Annex 8.1). The use of ultrasound gel is not usually required when pressing the transducer down onto the body, as in the water environment keeping the head of the transducer wet is adequate. However, it is recommended for most species to use a protective plastic sheath to cover the transducer, with gel on the inside of the sheath, to help protect the transducer head from getting small cuts from sharp dermal scutes. Although in some cases, the scutes are so sharp they can cut the protective sheath.

General guidelines for ultrasound use include: 1) position the unit and fish so that the user working with the transducer can easily observe the ultrasound screen at the same time, 2) protect the unit from splashing water, and 3) angle and shade the unit screen from direct and indirect light if working outdoors or in brightly lighted indoor facility. Good resolution and contrast of the screen is essential. A monitor hood/sunshade can be very useful.

There are three common body positions for sexing and assessing phase of sexual maturity: the ventral side up (provides frontal or longitudinal view of the gonad), the half-lateral recumbent position (fish is half-way between being on its side and ventral side up), and the lateral recumbent position (fish is on its left or right side). For ventral side up fish, the transducer is placed lengthwise onto the abdomen (longitudinal or frontal scan; Figure 8.1A), 3-5 ventral scutes anterior from the pelvic fin, directly above the gonad, and is the preferred position for differentiating late vitellogenic and post-vitellogenic females based on ovarian follicle size (Table 8.1) and male testes size (Table 8.2) during the autumn and spring assessment.

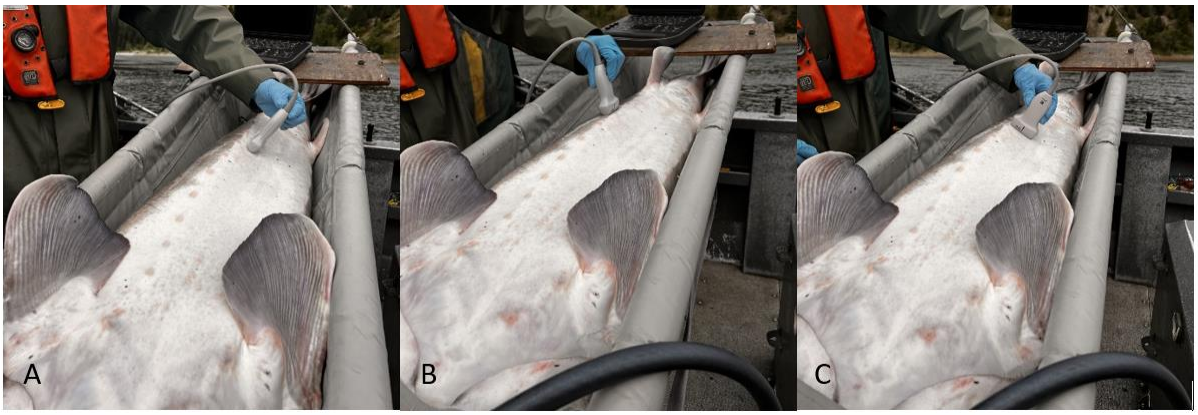

Figure 8.1: *From left to right, ultrasound transducer location on Acipenser transmontanus to obtain A) longitudinal or frontal sonogram, B) longitudinal or frontal sonogram from under the ventral scute line, and C) transverse sonogram (Photo credit: J. Crossman).*

Longitudinal views are especially good for sexing sturgeon as it more easily allows you to position the transducer just above or below (Figure 8.1A and B) the ventral scute line (3-5 scutes anterior to the pelvic fin) at just the right location and angle so the germinal tissue of the developing gonad is directly below. In all body positions, the transducer can be turned ninety degrees to obtain a transverse section of the gonad (except in cases of species with large ventral scutes; Figure 8.1C). Each view can be used to examine the entire length and degree of thickness of ovarian tissue or the thickness and lobes of testes, and the amount of adipose tissue. Details on the use of ultrasonography for determining sex and phases of maturity, with representative sonograms of the different phases is presented in Annex 8.2 and Chebanov & Galich (2009).

8.2.2.2 Endoscopy

Endoscopy is a procedure in which an instrument is introduced into the body for visual examination. In sturgeons, one may use an endoscope, laparoscope, borescope, or otoscope inserted primarily through the abdomen (celiotomy; see 8.2.3.3) and occasionally through the urogenital opening. The success of endoscopy has been reported for numerous sturgeon species (e.g. Wildhaber *et al.* 2005; Hernandez-Divers *et al.* 2004; Bryan *et al.* 2007; Hurvitz *et al.* 2007; Divers *et al.* 2009; Trested *et al.* 2010; Falahatkar *et al.* 2011; Matsche, 2013; Kazemi *et al.* 2019; Maskill *et al.* 2022). Endoscopy can provide immediate identification of sex and most phases of maturity, and images can be saved for further analysis. The accuracy of this technique (85-100%; Webb *et al.* 2019) depends on the individual's knowledge of the phases of sturgeon gonadal maturity, expertise in locating the germinal tissue, especially of immature fish, and the degree of clear visualization of gonads. Although most endoscopy systems currently used are just for visual inspections of sturgeon gonads and image archiving, specialized instruments are available that can be inserted into the probe to collect small biopsies.

Endoscopy systems utilize similar components: a slender and tubular probe that is flexible or rigid with a miniature video camera fitted with a lens that permits viewing in a variety of directions, a halogen or LED adjustable light source with a fiber optic transmission cable that allows brightness, contrast, and resolution to be controlled, a camera control unit, and a color monitor or computer screen. Images can be saved and, in some systems, further edited to improve contrast and clarity. This is useful to later confirm sex and phase of maturity, if there is uncertainty in the field, and provides material for training purposes.

Examinations are typically made though a small (~0.5-2.0 cm) abdominal incision 3-4 scutes anterior to the pelvic fins and 1-3 cm off the midline (Figure 8.2).

Figure 8.2: *Abdominal incision made for insertion of an endoscope and collection of a gonadal biopsy, 3-4 scutes anterior to the pelvic fins and 1-3 cm off the midline in Acipenser transmontanus (Photo credit: S. Bettin).*

A few studies have made examinations through the Mullerian duct by inserting the instrument into the urogenital opening. However, the view of the gonads is not as clear as through an abdominal incision due to the opaqueness and the presence of blood vessels in the Mullerian duct wall (Wildhaber *et al.* 2007), and the degree of haziness can vary by species and body size (Wildhaber *et al*. 2005). Using a boroscope inserted into the urogenital opening of *A. brevirostrum*, Kynard & Kieffer (2002) could only identify females with vitellogenic ovarian follicles (39%) and could not distinguish between male and an immature female (61% sex undetermined).

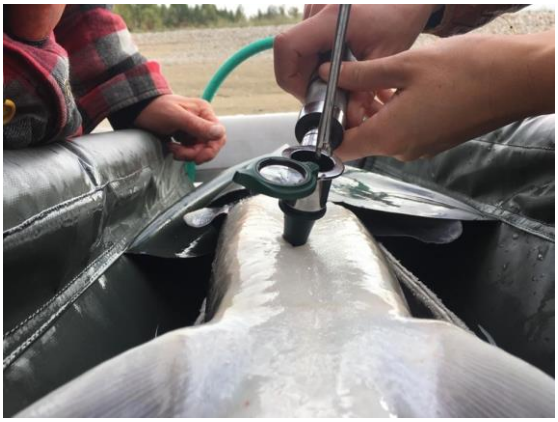

Figure 8.3: *Use of an otoscope for visualization of gonadal tissue in Acipenser transmontanus. A biopsy can be taken through the specula with a Miltex biopsy cup (Photo credit: S. Bettin and J. Crossman).*

Using an otoscope to assign sex in prepubertal and post-pubertal *A. transmontanus* was 98-100% accurate (Maskill *et al.* 2022) and is the most portable and easiest instrument to use for endoscopy. The exam is made through an abdominal incision (1-2 cm) in a similar position as described previously.

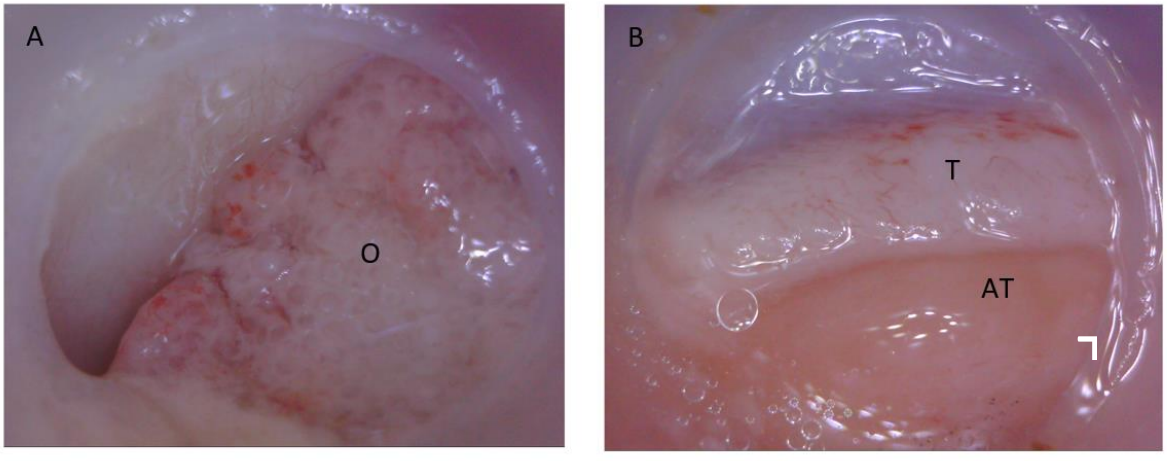

Figure 8.4: *A) Previtellogenic ovarian tissue (O) and B) testicular tissue (T) with adipose tissue (AT) seen through the otoscope specula in Acipenser transmontanus (Photo credit: J. Crossman).*

The otoscope contains a light source and a simple magnifying lens (3x) with a removable front speculum that can be exchanged for different lengths and diameters (Figure 8.2) depending on the size of gonad being observed. In most otoscopes, the lens can be removed which allows other instruments to be inserted through the specula into the body cavity. Once the gonadal tissue is identified in the body cavity (Figure 8.4), a gonadal biopsy can then be collected through the specula using an instrument, like a Miltex cup jaw biopsy forceps (Figure 8.3) and the sample histologically processed to verify sex and phase of maturity (Maskill *et al.* 2022).

8.2.2.3 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 assignment of sex and phase of gonadal maturity, which can then be verified with histological analysis to determine the exact stage of gonadal maturity (e.g. Amiri *et al.* 1996a, 1996b; Doroshov *et al.* 1997; Webb *et al.* 2002; Chapman & Park, 2005; Petochi *et al.* 2011; Chapman & Van Eenennaam, 2012; Falahatkar *et al.* 2013; Kazemi *et al.* 2019; Webb *et al.* 2019; Maskill *et al.* 2022). 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 (see Section 8.5.2.2).

The procedure takes approximately 5 minutes per fish to make the incision, view the gonad by eye or through endoscopy, biopsy the gonad if desired, and suture the incision closed. The exact time will depend on the individual's experience in surgery, biopsy sampling, and suturing. 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 is placed in a holding device, such as a hooded-stretcher, ventral side up with fresh sufficiently 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. For collection of ovarian follicles for assessment of oocyte polarization index, a trocar, extraction system, or shoup (Candrl *et al.* 2010; Chebanov & Galich, 2011) may be inserted through the abdomen (Figure 8.5).

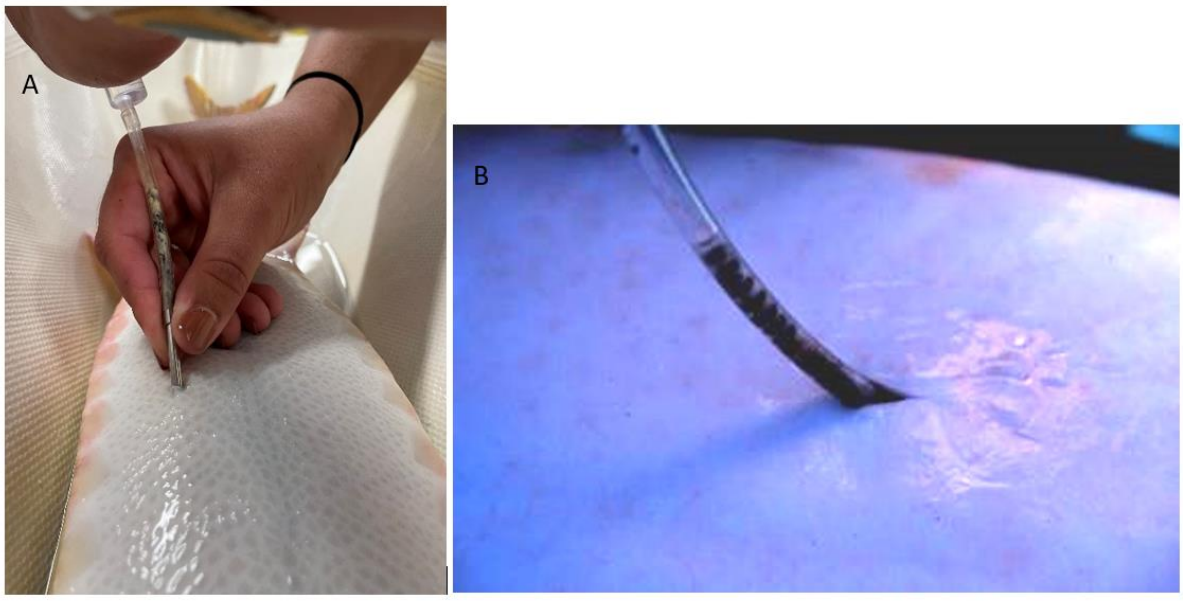

Figure 8.5: *Collection of ovarian follicles through the abdominal wall 3-4 scutes anterior to the pelvic fins and 1-3 cm off the midline using A) an extraction system described in Candrl et al. (2010) in Scaphirhynchus platorynchus and B) a catheter made from tygon tubing made rigid by soaking in 100% ethanol for 48 hours in Acipenser transmontanus. Ovarian follicles shown in this image are undergoing follicular atresia (Photo credit: M. Webb (A) and J.P. Van Eenennaam (B)).*

The goal is to make the incision or insertion point directly above the gonad but away from organs such as the liver and intestine. After making an incision, the incision is opened using tissue forceps and a pair of Allis forceps to view the gonad by eye or with an endoscope. The Allis forceps or a Miltex cup jaw biopsy forceps (Figure 8.3) can be used to collect a gonadal tissue sample. The incision is then sutured closed with a Cruciate (cross-mattress), interrupted-X, or multiple single stitches.

8.2.2.4 Plasma Sex Steroids

The pattern of sex steroid production in sturgeon allows for discrimination of sex and stage of maturity (Webb & Doroshov, 2011). Steroid concentrations are undetectable or low prior to initiation of puberty and during the non-reproductive phase of gametogenesis once puberty has been reached (Webb *et al.* 2002; Webb & Doroshov, 2011; Du *et al.* 2017; Webb *et al.* 2019). When sturgeon have reached puberty and are in the advanced phases of gametogenesis, using testosterone (T) and estradiol-17β (E2) to assign sex and stage of maturity has a high accuracy of 72-100% (e.g. Webb *et al.* 2002; Malekzadeh Viayeh *et al.* 2006; Webb & Doroshov, 2011; Maskill *et al.* 2022). The collection of the blood sample from the caudal vasculature (Figure 8.6) requires some training, but much less than ultrasonography, endoscopy and celiotomy. However, the disadvantages to this technique are the lack of immediate assignment of sex and phase of maturity, and the required specialized laboratory to conduct the analysis by

radioimmunoassay (Webb *et al.* 2002) or liquid chromatography tandem mass spectrometry (LC-MS/MS) (Nouri *et al.* 2020).

Figure 8.6: *Collection of blood from the caudal vasculature immediately posterior to the anal fin using a syringe in Scaphirhynchus albus (Photo credit: C. Guy).*

8.2.2.5 Other Methods

There are several other techniques to potentially sex and determine the phase of maturity in sturgeon, but they have not been verified with multiple species, have variable accuracy, or are still undergoing or require further research and development. These include morphological characteristics such as the shape of the urogenital opening (Vecsei *et al.* 2003; Wheeler *et al.* 2019), shape of the pectoral fins (Podushka, 2008), scutes (Barulin, 2018), and morphometrics of the head (Mal'tsev & Merkulov, 2006; Balazadeh & Litvak, 2018).

The use of biomarkers like vitellogenin (Craig *et al.* 2009) as well as the determination of calcium levels in the blood, which serves as a proxy for the vitellogenin concentration since the plasma concentrations are correlated with vitellogenin (Doroshov *et al.* 1997; Linares-Casenave *et al.* 2003; Stahl *et al.* 2009), have been successful. As well, using Fourier transform infrared (FT-IR) spectroscopy (Lu *et al.* 2011) and near infrared spectroscopy (Servid *et al.* 2011) have been used with limited success to predict the onset of follicular atresia in females.

Recently, the discovery of a genetic sex marker has provided a new opportunity for the determination of sex in very early stages of development (Kuhl *et al.* 2021).

The marker has been tested to be effective in *A. sturio, A. oxyrinchus, A. ruthenus, A. gueldenstaedtii, A. baerii, A. fulvenscens,* and *H. huso* (Kuhl *et al.* 2021; Scribner & Kanefsky, 2021), while the verification of its applicability in *Scaphyhrinchus* and *Polyodon* and the Pacific clade of sturgeons (*A. transmontanus* and *A. medirostris*) is still pending. Other recent studies have examined whole-genome inter-sex variation in *A. gueldenstaedtii* (Degani *et al.* 2022), real-time PCR for sex determination in *A. fulvescens* (Kanefsky *et al.* 2022), and the search for genetic sex markers in *A. baerii* and *A. oxyrinchus* (Panagiotopoulou *et al.* 2023).

8.3 Initial Selection Assessment

Regardless of how captive broodstock were established (see Chapters 4 and 7), an initial maturation assessment during autumn is done to select the late vitellogenic and post-vitellogenic females and the late spermatogenic and mature males (Tables 8.1 and 8.2). These fish are held together in facilities under controlled temperature regimes through autumn-winter and the spring spawning season. Males and females that are not spawning capable are returned to the grow-out facility, or if the hatchery has enough empty tanks/cages/raceways/ponds, further segregation of different phases of maturity could be done after each fish is assessed. If a hatchery works with both captive and wild-caught broodstock, captive and wild fish must be kept in separate broodstock holding systems for biosecurity reasons. Some hatchery programs may not have any autumn assessments if they only work with wild-caught, spring spawning fish that are returned to the wild.

8.3.1 Methodology

The fastest, easiest, and least invasive method for assessment is using ultrasonography. The large, white lobular testis of a mature male (Stage 4) and the large late or post-vitellogenic ovarian follicles of a potential spawnable female in the spring (Stage 4) are clearly distinguishable. At this assessment, depending on the species, population, and objectives of the hatchery, ovarian follicles could be sampled for size and phase of maturity (see section 8.6.2). For example, in the captive *A. medirostris* population in California, USA, autumn spawning has been identified at the fall assessment (Van Eenennaam, unpubl. data). *S. platorynchus* (Tripp *et al.* 2009), *A. oxyrinchus* (Balazik *et al.* 2017; Smith *et al.* 2015; Hager *et al.* 2014), and *A. oxyrinchus desotoi* (Randall & Sulak, 2012) sturgeon populations have also had evidence of autumn spawning. Initially, the existence of up to four distinct spawning subgroups was described for the Volga River and the tributaries to the Caspian and Azov seas (Gerbilskii & Petrunkevitch, 1955; Pickford, 1955; Berg, 1959). The separation of these subgroups increases the complexity of the *ex situ* management since the "autumn" assessment in this case has to take place in spring, and holding temperature regimes will be different. For this reason and for biosecurity reasons, the separation of the different subpopulations is important to avoid intermingling between these groups.

8.3.2 Data Collection

Individuals selected for potential spring spawning should be, at a minimum, weighed, measured (fork length and total length), and if not already tagged upon integration into the broodstock or at sexing, they should be injected with a passive integrated transponder (PIT tag) (see Chapter 13). The tag will be maintained for the fish's life, and all subsequent assessments/spawning inductions of the individual can be tracked. Mature wild-caught individuals are often weighed, measured and PIT tagged on the vessel prior to arrival at the hatchery holding facilities. Some of these individuals may be released back into the wild after spawning, while some may be held in captivity for subsequent reproductive cycles.

8.4 Autumn-Winter Holding Regimes

There are many different types of holding regimes, including circular tanks (fiberglass, concrete, plastic), cages, earthen or concrete raceways or ponds (see Chapter 5). The required dimensions and number of holding systems needed, will depend on the maximum spawning size of the species and number of broodstock to be maintained.

8.4.1 Rearing conditions

Broodstock holding systems are usually outdoors, subject to natural photoperiod, although indoor systems can also be used (see Chapter 5). The overall goal is to have an optimal environment with low stress during final maturation. Ideal water quality parameters (see Chapter 5) are critical for broodstock. Optimal water temperature profiles are essential, which will vary with species and sub-populations, and any substantial temperature fluctuations during the day must be avoided. Dissolved oxygen levels (>70% saturation) should be monitored continuously or, if monitored daily by a person, should be monitored at least twice daily in the morning and afternoon. Densities should be lower than in commercial grow-out facilities (which can vary between 50-100 kg/m^2; Van Eenennaam *et al.* 2004) and, in general, should range from 2-20 kg/m^2 with tolerances being species specific (Chebanov & Galich, 2011; Webb *et al.* 2018). Selected females and males should be held together to take advantage of any possible behavioral and pheromonal cues that may occur during final maturation.

8.4.2 Feeding

Broodstock of North American species are typically given a grow-out diet of 42-52% protein and 12-18% fat until early vitellogenesis and are then offered reduced fat (8-10%) feed (Gille *et al.* 2017). Fedorovykh *et al.* (2015) reported the optimal fat content in diets for *A. gueldenstaedtii* females to be 12-13%. The actual feed rate (approximately 0.15% – 0.30% of body weight per day) will depend on water temperature. It is important to prevent overfeeding which adversely affects gonad quality and high fat storage in gonadal tissue. High fat storage can also indicate an unbalanced composition of fatty acids in the diet since the triglycerides mainly contain saturated fatty acids (Wirth *et al.* 2000). The feeding rate can be further

reduced (<0.10%) during overwintering based on feeding activity and observations of eaten/uneaten food. Conversely, a period of no feeding is often applied in Eurasian species at temperatures below which feeding occurs or following a finishing diet feed with the highest vitamin and mineral levels and enriched in fatty acids for up to two months prior to maturation and spawning (Chebanov & Galich, 2011). Wild fish that do not transition to formulated feed should be fed natural prey items.

8.4.3 Overwintering

Wild sturgeon populations have a natural cold winter (vernalization), and this should be replicated as close as possible using ambient cold-water sources or artificial chilled water for successful completion of ovarian development (e.g. Kazanskii & Molodtsov, 1973; Williot *et al.* 1991; Chebanov & Savelyeva, 1999; Webb *et al.* 1999). Sturgeon are known to spawn within relatively wide temperature ranges, with most species spawning at 10-20 °C (Dettlaff *et al.* 1993), and the degree of winter temperature drop and length of time will vary between species and river systems. Chebanov & Galich (2011) recommend for Russian species a drop to 2-6 °C for 2-3 months, while Sacramento River *A. transmontanus* of western USA seldom have winters below 9-10 °C, which is the lowest temperature captive stocks have been overwintered, and temperatures are usually closer to 11-12 °C (Webb *et al.* 2001). *A. sturio* are maintained at minimal sea water temperatures of 10-13 °C for the overwintering period in France. In Germany, European sturgeon are maintained at ambient temperatures of below 2 °C, which would reflect the temperatures in the outer estuary in the North Sea. As such, it can be concluded that a substantial drop in winter temperatures seems to help in synchronizing maturation and provides a cue for final maturation, but the amplitude seems to be of lesser importance as long as it resembles the natural conditions of the population in question. General guidelines for grow-out, overwintering and spawning temperatures for different species of sturgeon are given in Table 8.4.

8.5 Maturation Assessment

Wild-caught broodstock during spring are usually assessed on the vessel used to capture the fish, and any found to be post-vitellogenic should be moved to and placed into a separate pre-spawning quarantine tank upon arrival at the hatchery. Any wild-caught fish at other phases of maturity that are brought to the hatchery to be maintained as future broodstock should be placed into a separate culture system to prevent transfer of pathogens.

All autumn-assessed fish held over the winter are reassessed to verify they are post-vitellogenic and spawning capable in the spring. Any fish that are found to have atretic follicles or regressed testes are moved back to their original grow-out systems or released back into the wild.

Spring assessment is conducted at a time based on historical population data for spawning migration of the species or from other spawning data if available from other hatcheries or farms. The goal is to time the spring assessment to occur a few weeks before the earliest females are capable of spawning while being fully matured already. Due to their own internal/genetic time clock, any given population of same-age, mature females will have early, middle, and late season spawners, therefore assessment of some individuals may have to be repeated during the spring.

Some facilities schedule the spring assessment of broodstock while fish are still maintained at the overwintering temperature (ambient or artificially chilled), while some that have the capability to modify temperature will gradually warm (1 °C/every 2-3 d) tank temperatures to 1-2 °C below spawning temperature and then assess their broodstock. Facilities with no artificial chilling capacity will assess their broodstock when ambient temperatures naturally warm to 1-2 °C below spring spawning temperatures.

8.5.1 Selection of Mature Males

Mature males that will most likely respond to exogenous hormone injections are usually selected using ultrasonography, although some hatcheries may prefer endoscopy. With the individual placed ventral side up on a stretcher, the transducer is moved along the entire length of both testes, and the maturity status and readiness to spawn can usually be assessed by the bright white reflection of the echoes due to increased density of the testicular tissue containing primarily spermatozoa (Chebanov & Galich, 2011). The size of the testicular lobes is not as critical as the bright white tissue, as size of testicular lobes does not always confer stage of maturity and large lobes could also be at the post-spermiation stage (Webb *et al.* 2019). Endoscopy will reveal the bright white testes and if sampled, the mature testis tissue is soft and milky (spermatozoa releasing from the broken testicular cysts).

An alternative option to test for spawning readiness of males for hormonal induction is the verification of urine pH using an indicator paper (preferably for a pH range of 5.5-9.0 (e.g. Merck Neutralit or similar). The male is placed ventral side up in a stretcher and slight pressure on the belly results in the fish expelling clear urine. In smaller fish, a slight bend backwards of the fish often increases the success of the pressure treatment. If the fish does not readily expel urine, the moisture developing on the urogenital pore is usually sufficient for the pH test. In unripe males, pH varies between 6.0 and 6.5. In males that have progressed in maturation and are responsive to hormonal induction by successfully spermiating (accuracy 100% if hormonal injection is administered within 2 weeks of the test), the pH varies between 7.5 – 8.0 (J. Gessner, unpublished). This test is quick to apply and provides a reliable result within seconds.

8.5.2 Determination of Oocyte Maturation and Acquisition of Maturational Competence in Females

8.5.2.1 Principle

Ovarian follicle maturation (OFM) in fish includes maturational processes of the nucleus (germinal vesicle; GV) and cytoplasm of the oocyte. The main processes are germinal vesicle migration (GVM), a switch in follicular secretion of C18 to C21 steroids, and acquisition of oocyte maturational competence (OMC; the ability of the oocyte to resume meiosis in response to a progestin) (Patiño & Thomas, 1990; Nagahama *et al.* 1995). Unlike most modern teleosts, OFM in sturgeon is prolonged and occurs over 3–4 months before spawning (Doroshov *et al.* 1997; Webb *et al.* 1999). Due to the heterogeneity of post-vitellogenic females held under the same culture conditions, individual females' ovarian follicles are sampled to determine the stage of oocyte maturation using the oocyte polarization index (PI), which is the distance of the germinal vesicle from the animal pole divided by the maximum oocyte diameter, and acquisition of maturational competence is assessed by the response of ovarian follicles *in vitro* to progesterone with resumption of meiosis, germinal vesicle breakdown (GVBD). These two tools may be used in concert to determine the optimal oocyte PI to hormonally induce maturation and ovulation, and it should be noted that germinal vesicle breakdown and ovulation are two distinct hormonal events.

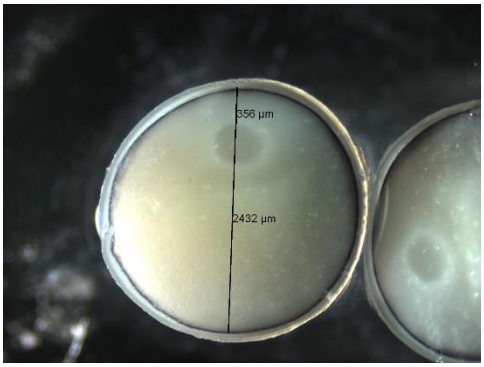

Figure 8.7: *The stage of oocyte maturation is assessed using the oocyte polarization index (calculated in the image as 0.1464) in Scaphirhynchus albus, which is the distance of the germinal vesicle from the animal pole (356 µm) divided by the maximum oocyte diameter (2432 µm; Photo credit: M. Webb).*

8.5.2.2 Sampling and Processing

Ovarian follicles are collected by either a hollow stainless-steel probe that is 3-6 mm internal diameter (ID), depending on the size of the ovarian follicles, inserted through the abdominal wall or through the lateral muscle (Figure 8.5; Candrl *et al.* 2010; Chebanov & Galich, 2011) or by a 4-6 mm ID rigid tubing inserted through a small 6-9 mm abdominal incision (Figure 8.5; Conte *et al.* 1988). It is recommended the incision be closed using a single suture, especially if a female will be hormonally induced to spawn within a few days or the incision be disinfected with iodine/iodine gel. Individual females can be sampled repeatedly in this

manner and can successfully be induced to ovulate, producing eggs with high fertility and hatch rates. It is recommended to refrain from sampling at higher intervals than every 3-4 weeks since stress of repeated sampling can lead to the arrest of oocyte maturation and atresia.

Approximately 20-30 ovarian follicles from each female are collected to calculate oocyte PI. Additional ovarian follicles (Goncharov *et al*. 1999, Chèvre *et al.* 2019) are needed if performing an *in vitro* progesterone assay (see section 8.5.3). For the determination of oocyte PI, ovarian follicles are placed into a container of modified Ringer solution (Dettlaff *et al.* 1993) and kept in a cool environment (14-16 °C). To make one liter of Ringer solution, add 6.5 g of NaCl, 2.0 g NaHCO3, 0.3 g CaCl, and 0.25 g KCL to one liter of distilled water. Each females' ovarian follicles are placed into separate small glass beakers, all with equal amounts (e.g. 15 or 20 mL) of Ringer solution and gently boiled for approximately 3-5 min. Beakers are placed onto wet ice for 15-30 min and then the follicles are placed into containers of 10% buffered formalin overnight. Follicles can be sectioned after chilling, but it is much more difficult to get good cross sections compared to post-formalin fixed oocytes.

For shorter processing times, oocytes can be transferred into a mixture of ethanol, formalin and acetic acid (6:3:1) for 3.5 h before they are ready to be dissected. It is recommended to use protective measures (ventilation) whenever dissecting the follicles after formalin treatment or rinsing the follicles a couple times in water before sectioning due to health concerns.

The ovarian follicles are sectioned along the longest axis (the animal-vegetal axis) usually recognized by the oval shape, and fortunately in most cases, the animal pole can be distinguished by coloration (rings and/or a faint white spot of variable size). For accurate measurements, the follicles need to be bisected so there are two equal-size halves, with equal size GV in both halves. To calculate the oocyte PI, measure the distance from the outer edge of the GV to the oolemma at the animal pole (thin black pigment layer) (Figure 8.6) and the oocyte diameter (thin black pigment layer at the animal pole to the thin black pigment layer at the vegetal pole). Oocyte PI is the distance of the GV to the animal pole divided by the diameter of the diameter of the oocyte. Details of this procedure can be found in Chapman & Van Eenennaam (2007a).

To make measurements for PI calculations, the sectioned ovarian follicles can be simply projected from a USB connected adjustable-zoom magnifier so the half oocyte can be adjusted to fill a large portion of the computer screen, and an accurate mm ruler used to measure the distances on the screen. A more technical system would include a zoom dissecting microscope with a digital camera connected to a computer and monitor and image analysis software used to make the measurements.

8.5.2.3 Segregation of Females for Spawning Induction

Females can be segregated into general groups based on their calculated oocyte PI and can either be induced to spawn immediately or potentially resampled before the PI is at the appropriate value for spawning induction (Table 8.3). Females with

a PI <0.04 maybe overripe and this can be verified by the maturation assay (see section 8.5.3) as overripe oocytes will not respond to the progesterone. If the females have the same high PI value (> 0.16) after a period exceeding 4-5 weeks, they have stopped final maturation and are not spawning candidates. The mechanisms for arrest of oocyte maturation are not well understood, though the effects of non-optimal temperatures and stress have been described (Webb *et al.* 2001). These females should be moved back to their grow-out facilities. The most common reason for arrested development is stress or suboptimal water temperature profiles. In outdoor systems, suboptimal temperature regimes are becoming more common due to global warming. Recommendations and PI grouping will vary depending on species and actual temperature regimes, and data should be collected over several years to establish stock-specific selection criteria.

8.5.3 Oocyte Maturation *In Vitro* Progesterone Assay

The oocyte PI provides a good indication of female spawning readiness (Dettlaff *et al.* 1993; Chapman & Van Eenennaam, 2007b), and many hatcheries use only this criterion for scheduling spawning induction. However, oocyte PI does not actually measure whether the ovarian follicles have acquired maturation competence (i.e. the ability to undergo germinal vesicle breakdown in response to progesterone, the maturation inducing steroid), therefore, an oocyte maturation assay can be performed to determine if the ovarian follicles will respond to hormonal injections.

8.5.3.1 Principle

During the completion of oocyte maturation, the GV reaches the top of the animal pole (PI = 0.00) where it then undergoes GVBD. Once GVBD occurs, the oocyte is released or ovulated from the follicle and is now called an ovum and the genetic material of the female, released from the broken nucleus, can mix with the genetic material of the male carried inside the cytoplasm by the fertilizing sperm.

8.5.3.2 Methodology

Briefly, the *in vitro* oocyte maturation assay, as described in Dettlaff *et al.* (1993) is conducted in Ringer solution. Incubation time can be developed for each species and incubation temperature is usually 16 °C. Ten to fifteen oocytes are incubated with 5 µg/mL progesterone, and the same quantity is incubated without progesterone to serve as a control (Figure 8.8). After incubation, the oocytes are boiled, chilled on wet ice, and stored in 10% phosphate buffered formalin overnight, bisected, and examined for GVBD (Figure 8.9).

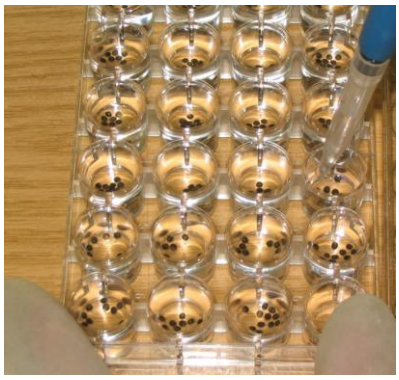

Figure 8.8: *Ovarian follicles in Ringer solution in a 24-well incubation plate to conduct the in vitro oocyte maturation assay. Ovarian follicles are incubated with or without 5 µg/mL progesterone at species specific times and temperature (usually 16 °C) to determine the ability of the fish to respond to hormonal injection with germinal vesicle breakdown (Photo credit: M. Webb).*

A female with oocytes at a PI <0.10 and 100% GVBD in the progesterone assay is a good candidate for hormonal injection and should be induced to spawn as recommended in Table 3. If there is no GVBD in the assay, this female will not ovulate when injected with GnRHa. Injection of pituitary extracts will likely induce ovulation; however, the eggs are often lower quality (see Chapter 9). For females with oocytes at a PI of 0.10 – 0.15 and less than 100% GVBD in the assay, it is recommended to wait a set amount of time depending on temperature before inducing ovulation or resampling prior to injection to ensure the oocytes have matured to an established oocyte PI for the species (Table 8.3). Details of the oocyte maturation protocol and the materials/equipment required are given in Chapman & Van Eenennaam (2007b), and the major limitation to this assay is the necessity for an accurate low temperature laboratory incubator.

A female with ovarian follicles at their full size (species specific determined egg diameter), a PI < 0.10 with distinct yolk polarization, and 100% GVBD in the maturation assay should ovulate after injection with exogenous hormone and produce high quality eggs (see Chapter 9).

Once the analysis of oocyte PI and the oocyte maturation assay are completed, and water temperature has been transitioned to spawning temperatures, the identified individuals for first spawning (PI = 0.05-0.10, Table 8.3) are moved out of the overwintering or pre-spawning holding tanks usually at the time of first hormonal injection to minimize handling stress and are placed into spawning tanks (see Chapter 9).

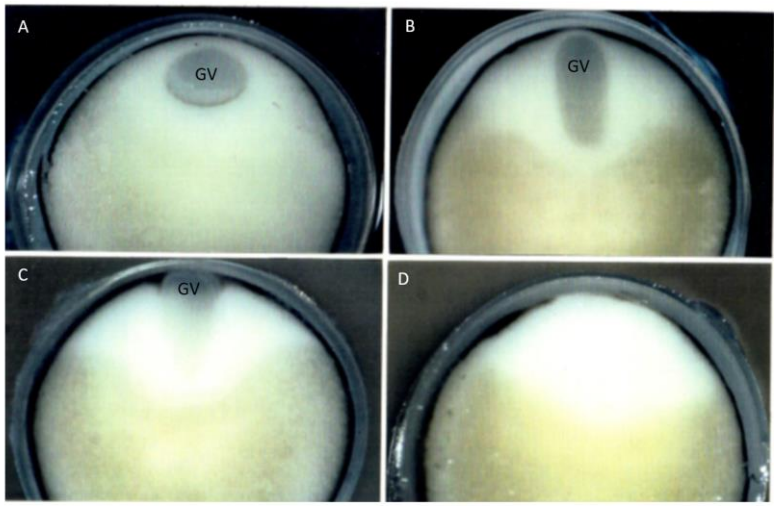

Figure 8.9: *Bisected ovarian follicles following incubation and fixation after the in vitro oocyte maturation assay. Microplatelets of yolk are seen at the animal pole (white), and macroplatelets of yolk are seen in throughout the remainder of the animal hemisphere and vegetal hemisphere of the oocyte (yellowish). A) the germinal vesicle (GV) is at the top of the animal pole where fertilization will occur once ovulation and oviposition occur; B) the GV has elongated as the nuclear membrane prepares to break down; C) the GV is about to undergo germinal vesicle breakdown (GVBD); D) GVBD is complete (Photo credit: J.P. Van Eenennaam).*

8.5.3.3 Duration of Progesterone-induced Oocyte Maturation Time in vitro as a Criterion for Selecting Sturgeon Females for Breeding

Other criteria can be used for the segregation of females to optimize reproductive success (i.e. to select females with high egg fertilization, survival rate and normal development of fry). Oocytes that have reached the post-vitellogenic stage and entered the final stages of oocyte maturation with cytoarchitectural changes (i.e. yolk polarization and low oocyte PI values) should have the ability to mature *in vitro*. However, not all females whose oocytes are able to respond *in vitro* to the hormone are able to respond to hormonal stimulation, and among those who respond, not all produce eggs of high quality. The speed of progesterone-induced oocyte maturation varies greatly (Goncharov, 1976), and females with rapidly maturing *in vitro* oocytes are more capable of responding to hormonal treatment and produce higher quality eggs (Goncharov, 1993). The target of 50% GVBD (T50) has proven to be the most effective criterion of female selection for breeding in *A. baerii* females (incubation medium Ringer solution containing $NaHC0_3$ (2 g/L) plus 17α,20ß-DHP (1 µg /mL) rather than progesterone). When the T50 took longer than 18 hours (incubation at 18.0 ± 0.5 °C), females did not respond to hormonal injection or produced eggs of low quality (Goncharov *et al*. 1999). This method of female selection has been successfully used for many years in France by a company specializing in the production of *A. baerii* larvae (Chevre, pers. comm.), as well as for the breeding of the critically endangered *A. sturio* (Chevre *et al*. 2019).

Table 8.3: *Guidelines for grouping of spring assessment females based on the oocyte polarization index (PI), and general recommendations for spawning induction or resampling (modified from Chebanov & Galich, 2011). These guidelines are based on females being held at overwintering temperatures, initially sampled for oocyte PI, and then acclimated to spawning temperatures (approximately 1 °C/day). There are a few species that are at the extreme ends (Acipenser medirostris are hormonally induced at oocyte PIs of 0.04-0.06 and A. oxyrinchus and A. baerii are hormonally induced at oocyte PIs of 0.10-0.15).*

PI Range	Group Description	Recommendations
PI < 0.04	Potentially Overripe	Attempt to spawn immediately (especially if 100% GVBD in the maturation assay, see 6.6.4) or if your species consistently does not ovulate, then transfer back to the original grow-out facilities.
PI = 0.05 - 0.10	Ripe 1 (should respond to hormonal injections)	Induce spawning after reaching spawning temperature.
PI = 0.10 - 0.12	Ripe 2 (spawning capable in approximately 1-2 weeks)	Acclimate to spawning temperature, hold for 2-7 days, and then induce spawning.
PI = 0.12 – 0.15	Ripe 3 (spawning capable in approximately 2-4 weeks)	Either inject after acclimation and then holding for 2-3 weeks or resample 4 weeks after first assessment (to ensure PI has reached 0.05-0.10).
PI = 0.15 – 0.19	Spawnable	Resample after 4-5 weeks.
PI > 0.19	Potentially Spawnable	Resample after 5-6 weeks.

8.5.3.4 Application of Oocyte PI and Oocyte Maturation Assay

The real need for the oocyte maturation assay occurs if a hatchery is using only the oocyte PI to schedule spawning induction and has an unacceptable low rate of ovulatory success, depending on the goals of the program. The use of both tools in combination to choose females for hormonal injection is more effective than PI alone, and fewer spawners may be required, which promotes animal welfare (reduction and refinement aspects). It is also of great relevance for the

reproduction of threatened and endangered species which have low numbers of spawning adults.

There are some species that do not follow the general guidelines in Table 8.3 and so individual hatcheries should refine this table as they accumulate data for their species. For example, Klamath River *A. medirostris* are found to enter the river at PIs of 0.04, and they are typically hormonally induced at oocyte PIs of 0.04-0.06 (Van Eenennaam *et al.* 2006; Van Eenennaam *et al.* 2012), while *A. oxyrinchus* and *A. baerii* are hormonally induced at oocyte PIs of 0.10-0.14 (Fuest *et al.* 2024).

8.6 Pre-spawning Holding Regimes

The advantage to having a separate pre-spawn holding system is that some individuals can be selected to remain in the overwintering colder temperature tank to slow the process of oocyte maturation, while some females can be moved out into a separate tank (initially at the winter water temperature) and gradually warmed (approximately 1 °C/day) to spawning temperatures. This management regime can provide broodstock for spawning later in the spawning season and is especially important for maintaining high quality males. The duration (days) of holding females with higher oocyte PI values at different water temperatures (also expressed in degree-days) was given by Chebanov & Galich (2011).

Table 8.4: *General guidelines for grow-out, overwintering, and spawning temperatures for different sturgeon species in captivity. Overwintering temperatures are maintained approximately 2-4 months prior to slowly acclimating (1 °C/day) to spawning temperatures. Some species (e.g. Acipenser transmontanus, A. oxyrinchus, A. brevirostrum, Scaphirhynchus albus, S. platorynchus) will have lower ranges of temperatures for more northern latitude populations and higher ranges of temperatures for more southern latitude populations that may not be given in the references cited. Different temperatures ranges will exist for summer or autumn spawning populations.*

Species	Grow-Out Temp (°C)	Over-Wintering Temp (°C)	Spring Spawning Temp (°C)	References
Acipenser baerii	19-22[a]	5-9[b]	9-18[c]	[a]Rad *et al.* 2003; [b]Williot *et al.* 1991; [c]Gisbert & Williot, 2002
Acipenser brevirostrum	18-20[a]	1-6[b]	9-18[c]	[a]Flynn & Benfey, 2007; [b]Fernandes *et al.* 2010; [c]Kynard, 1997
Acipenser fulvescens	17-22[a]	1-2[b]	12-16[c]	[a]Aloisi *et al.* 2019; [b]Brandt *et al.* 2022; [c]Bruch & Binkowski, 2002
Acipenser gueldenstaedtii	18-20[a]	4-5[b]	14-18[b]	[a]Castellano *et al.* 2017; Elhetawy *et al.* 2020; [b]Chebanov & Galich, 2011
Acipenser medirostris	18-19	9-12	13-16	Van Eenennaam *et al.* 2012
Acipenser nudiventris	21-25[a]	4-5[b]	14-18[b]	[a]Feshalami *et al*. 2019; [b]Chebanov & Galich, 2011

Acipenser oxyrinchus	15-19[a]	1-6[b]	13-26[c]	[a]Kelly & Arnold, 1999; [b]Fernandes *et al.* 2010; [c]Hager *et al.* 2020
Acipenser o. desotoi	21-30[a]	5-12[b]	17-20[c]	[a]Lazur *et al.* 2008; [b]Vine *et al.* 2019; [c]Sulak & Clugston, 1999
Acipenser ruthenus	19-24[a]	4-5[b]	10-15[b]	[a]Sion *et al.* 2011; [b]Chebanov & Galich, 2011
Acipenser stellatus	18-23[a]	4-5[b]	17-21[b]	[a]Bubunets *et al.* 2022; [b]Chebanov & Galich, 2011
Acipenser transmontanus	18-20	9-12	14-16	Van Eenennaam *et al.* 2004
Huso huso	21-23[a]	4-5[b]	9-14[b]	[a]Mani & Ebrahimi, 2022; [b]Chebanov & Galich, 2011
Scaphirhynchus albus	17-20	>7[*]	12-20	Webb *et al.* 2018
Scaphirhynchus platorynchus	12-24[a]	>10[a**]	12-24[b]	[a]Kappenman *et al.* 2009; [b]Phelps *et al.* 2016

*To prevent iridovirus outbreaks, *Scaphirhynchus albus* are maintained above 7 °C in captivity.

**To prevent cessastion of growth overwinter, *Scaphirhynchus platorynchus* are maintained above 10 °C.

8.7 References

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Annex 8.1. Wild and Captive Sturgeon Species Assigned Sex and Stage of Maturity Using Ultrasonography.

Use of ultrasonography as a technique to assign sex and stage of maturity in different species of sturgeon is summarized in the table below. Age and body weight ranges for individuals examined with a specific transducer are provided. Accuracy percent is for identifying sex, and a range of percentage values covers the different phases of gametogenesis. Data not reported is given as "na" (not available) and "wild" indicates wild-caught sturgeon with no available age and/or size data. Colombo *et al.* (2004) reported the size of the wild *Scaphirhynchus platorynchus* sampled to be 444-714 mm in fork length.

Species	**Age (yr)/ Weight (kg)**	**Transducer**	**Accuracy (%)**	**Reference**
Huso huso	1.5/4-6 3/3-6 3-5/7-12 9-16/50 - 118	Linear 9-13 MHz Linear 6-13 MHz Linear 5-10 MHz Convex 2-3.5 MHz	81 98 100 100	Esmailnia *et al.* 2018 Masoudifard *et al.* 2011 Chebanov *et al.* 2004 Chebanov *et al*. 2002
Huso dauricus	na/62-115 wild	Convex 2-3.5MHz	100	Galich & Chebanov (unpublished data)
Acipenser persicus	2/1.5-1.8 na/13-27 wild/wild	Linear 5-10 MHz Linear 5-10 MHz	100 100	Chebanov & Galich, 2008 Galich & Chebanov (unpublished data)
Acipenser persicus	13-45/16-48 na/ 8-25	Linear 5-7.5 MHz Linear 5-9 MHz	100 100	Vajhi *et al.* 2013 Chebanov (workshop at ISS5, 2005)
Acipenser stellatus	6-16/5-16 2-3/2-2.5	Linear 5-7.5 MHz Linear 5-10 MHz	76-99 na	Moghim *et al.* 2002 Chebanov & Galich, 2009
Acipenser baerii	3-4/0.8-3 2-3/2-2.5	Linear 3-10 MHz Linear 5-10 MHz	88 na	Munhofen *et al.* 2014 Chebanov & Galich, 2009
Acipenser transmontanus	10-17/6-7.5 wild/32-57	Linear 6-15 MHz Linear 6-15 MHz	49-65 56-81	Maskill *et al.* 2022 Maskill *et al.* 2022
Acipenser medirostris	9-12/20-49	Linear 5-10 MHz	100	Van Eenennaam *et al.* 2012

Acipenser mikadoi	7/5-8 wild/15-27	Linear 5-10 MHz	100	Galich & Chebanov (unpublished data)
Acipenser fulvescens	wild/11-51	Linear 5-10 MHz	88-96	Chiotti *et al.* 2016
Acipenser gueldenstaedtii	10-11/3-13 1+-3/2-2.5 7-15/6-18 wild/wild 3-8/2-14	Linear 7.5 MHz Linear 5-10 MHz Linear 5-9 MHz Linear 5-9 MHz Linear 5-9 MHz	100 na na na na	Memiş *et al.* 2016 Chebanov & Galich, 2009 Chebanov *et al.* 2004 Chebanov *et al*. 2002; Chebanov, 2005 Chebanov & Galich, 2009
Acipenser sinensis	10-17/45-166 12-21/37-215 4-8/9-27	Convex 2-5 MHz Convex 2-3.5	88 100	Du *et al.* 2017 Chebanov & Galich 2008, 2009
Scaphirhynchus platorynchus	16-19/0.7-1.1 wild/wild	Linear 5-10 MHz Linear 5-10 MHz	68 86	Wildhaber *et al.* 2005 Colombo *et al.* 2004
Acipenser ruthenus	2.8/1.3-2.3 1-2/0.3-0.5 3-8/1-4.5	Convex 4.5-7.5 MHz Linear 5-10 MHz Linear 5-9 MHz	64-85 100 100 na	Igna *et al.* 2017 Chebanov & Galich, 2009 Chebanov *et al*. 2002; Chebanov, 2005
Acipenser naccarii x Acipenser baerii	6/9-13	Linear and Convex 3-7.5 MHz	na	Petochi *et al.* 2011
Acipenser gueldenstaedtii x *Acipenser baerii*	1+-2+/2-2.5	Linear 5-10 MHz	na	Chebanov & Galich, 2009
Huso huso x *Acipenser ruthenus*	2-3/2.5-3	Linear 5-10 MHz	na	Chebanov & Galich, 2009

Annex 8.1. References

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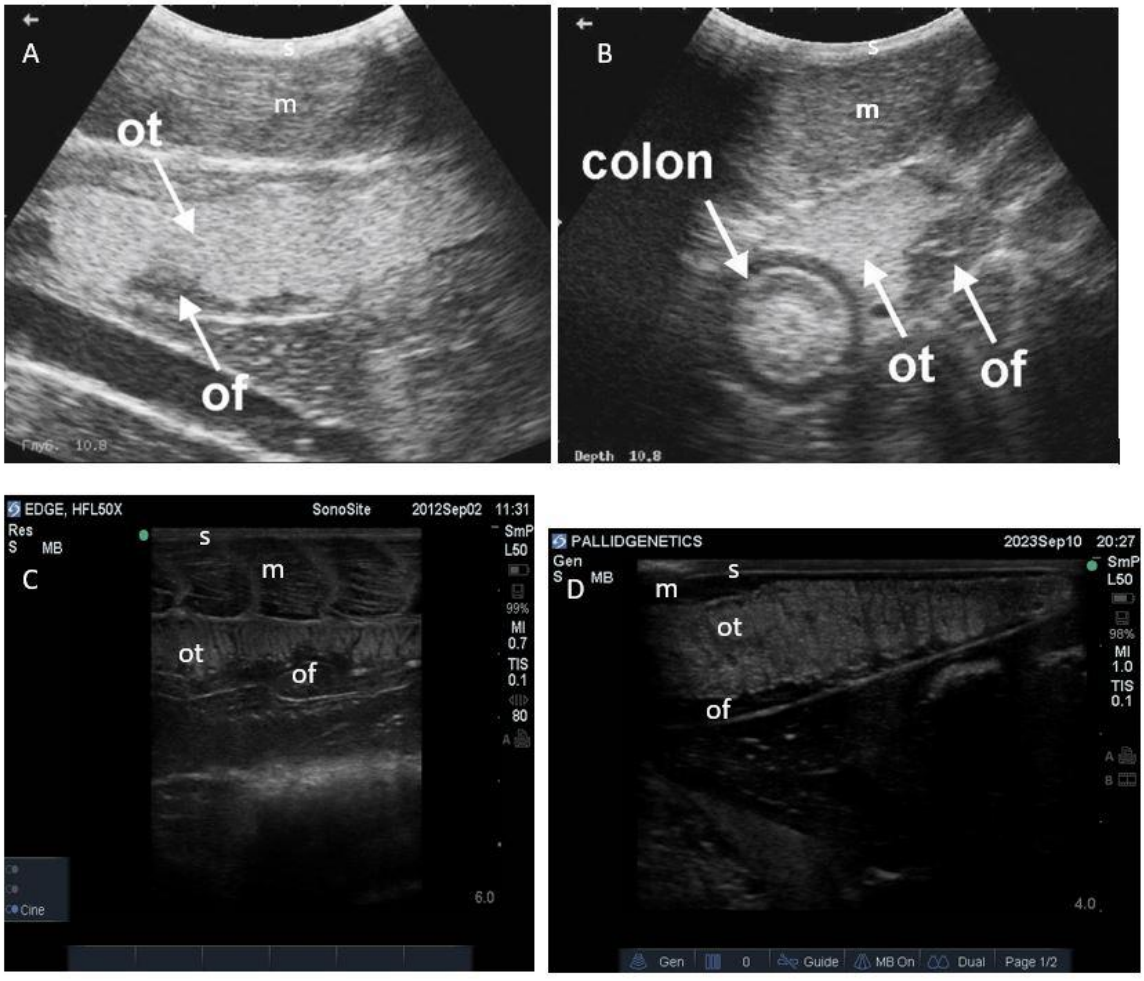
Annex 8.2. Atlas of Sonograms Depicting the Different Reproductive Phases of Sturgeon

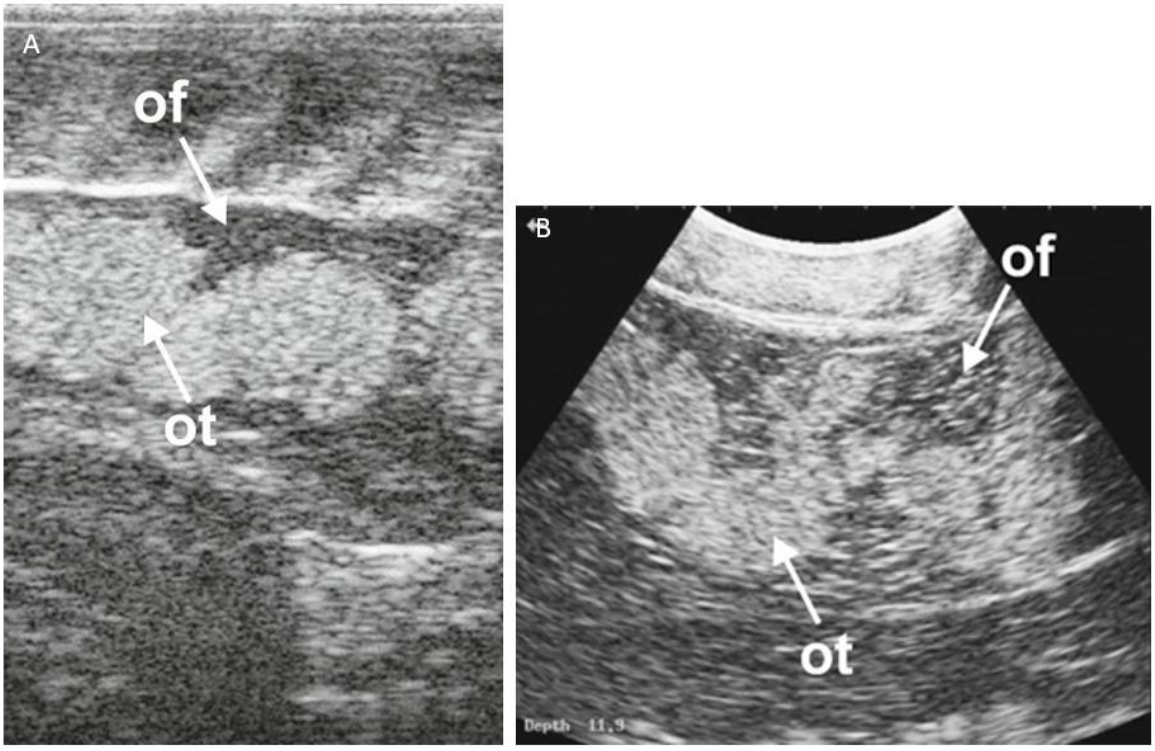
A detailed analysis of sonograms of different species of female and male sturgeon at different stages of gonadal maturity has been described in many publications (e.g. Chebanov & Galich, 2009, 2011, 2018). Therefore, only a brief atlas of the most typical sonograms of females and males are included here. Maturity phases and stages are described in Tables 8.1 and 8.2. Transducer positions are longitudinal (i.e. frontal) or transverse (see Figure 8.1). The muscle and skin in the sonograms are labelled for the first series of longitudinal and transverse images only (Annex Figure 8.2.1.). If arrows are not shown, the letter identifying the organ is placed on top of that organ.

Females

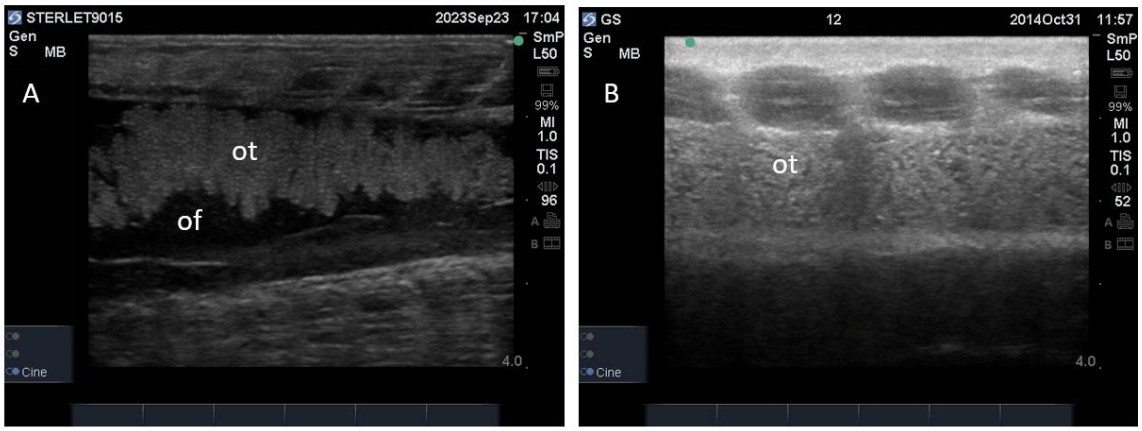


Annex Figure 8.2.1: *A) Longitudinal sonogram of pre-vitellogenic ovary in Huso huso female (ot – ovarian tissue, of – ovarian fat, s – skin, m – muscle) convex transducer), B) transverse sonogram of pre-vitellogenic ovary in Huso huso female (ot – ovarian tissue, of – ovarian fat, s – skin, m – muscle, convex transducer), C) longitudinal sonogram of pre-vitellogenic ovary in Acipenser transmontanus (ot – ovarian tissue, of – ovarian fat, s – skin, m – muscle, linear transducer), and D) transverse sonogram of pre-vitellogenic ovary*

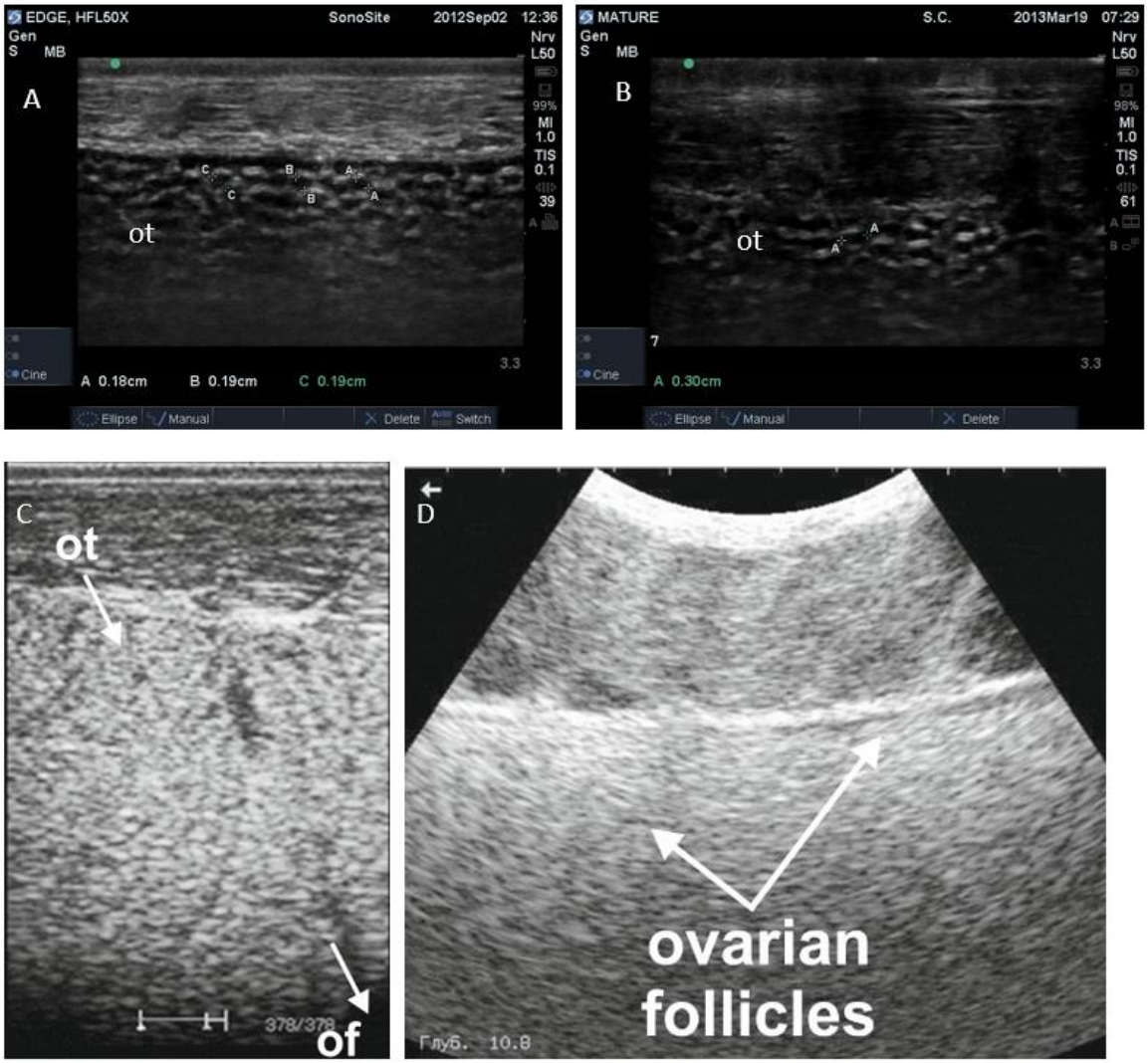
in Scaphirhynchus albus female (ot – ovarian tissue, no apparent ovarian fat, s – skin, m – muscle, linear transducer) (Sonogram credit: M. Chebanov and E. Galich (A and B); J.P. Van Eenennaam (C); and M. Webb (D)).



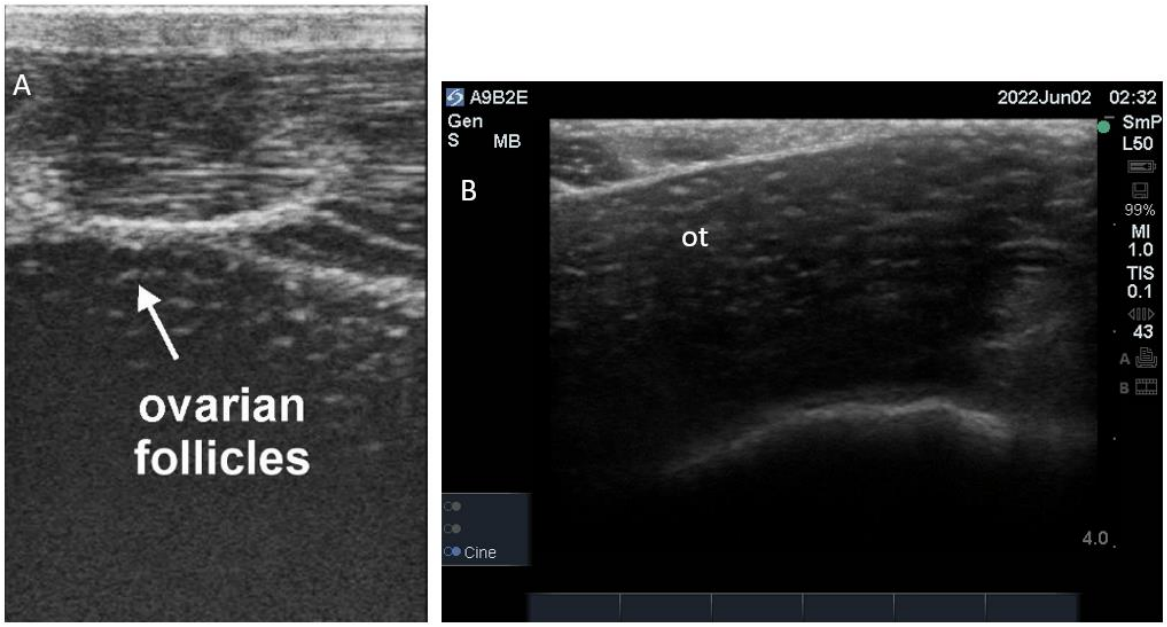
Annex Figure 8.2.2: *A) Longitudinal sonogram of fatty pre-vitellogenic ovary in* Acipenser gueldenstaedtii *female (ot – ovarian tissue, of – ovarian fat, linear transducer) and B) longitudinal sonogram of fatty pre-vitellogenic ovary in Huso huso female (ot – ovarian tissue, of – ovarian fat, convex transducer) (Sonogram credit: M. Chebanov and E. Galich).*



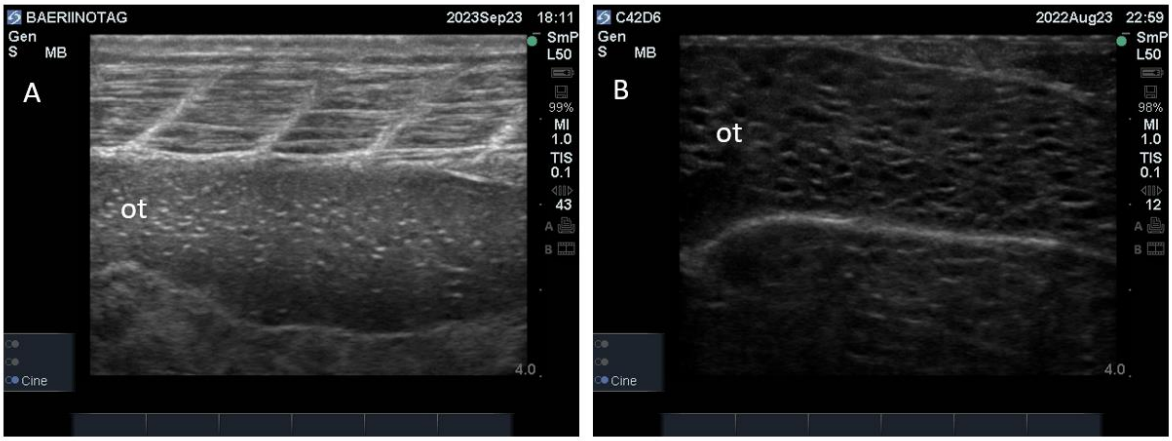
Annex Figure 8.2.3: *A) Longitudinal sonogram of early vitellogenic ovary in Acipenser ruthenus female (ot – ovarian tissue, of – ovarian fat, linear transducer) and B) longitudinal sonogram of early vitellogenic ovary in Acipenser medirostris female (ot – ovarian tissue, no apparent ovarian fat, linear transducer) (Sonogram credit: T. Friedrich (A) and J.P. Van Eenennaam (B)).*



Annex Figure 8.2.4: *A) and B) Longitudinal sonograms of mid to late vitellogenic ovaries in Acipenser transmontanus (ot – ovarian tissue, no apparent ovarian fat, linear transducer; note the ovarian follicle diameters measured between each respective points), C) longitudinal sonogram of late vitellogenic ovaries in Acipenser nudiventris female (ot – ovarian tissue, of – ovarian fat, linear transducer), and D) longitudinal sonogram of late vitellogenic ovaries in Huso huso (convex transducer) (Sonogram credit: J.P. Van Eenennaam (A and B), M. Chebanov and E. Galich (C and D)).*

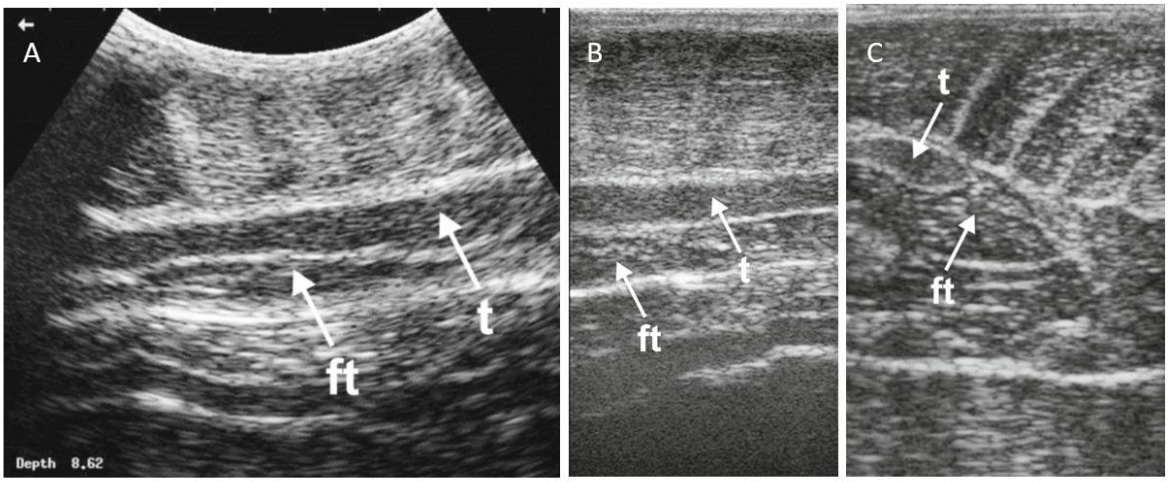


Annex Figure 8.2.5: *A) Longitudinal sonogram of post-vitellogenic ovary in Huso huso female (linear transducer) and B) transverse sonogram of post-vitellogenic ovary in Scaphirhynchus platorynchus female (ot – ovarian tissue, linear transducer) (Sonogram credit: M. Chebanov and E. Galich (A) and M. Webb (B)).*

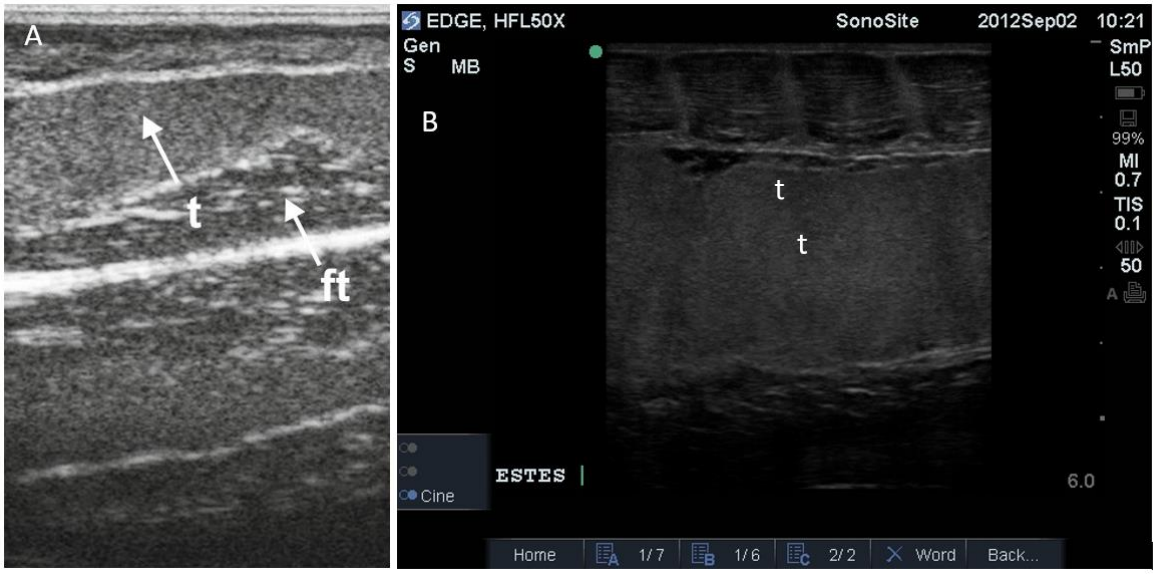


Annex Figure 8.2.6: *A) Longitudinal sonogram of atretic ovarian follicles in ovary of a Acipenser baerii female (ot – ovarian tissue, linear transducer) and B) transverse sonogram of atretic ovarian follicles in ovary of a Scaphirhynchus platorynchus female (ot – ovarian tissue, linear transducer) (Sonogram credit: T. Friedrich (A) and M. Webb (B)).*

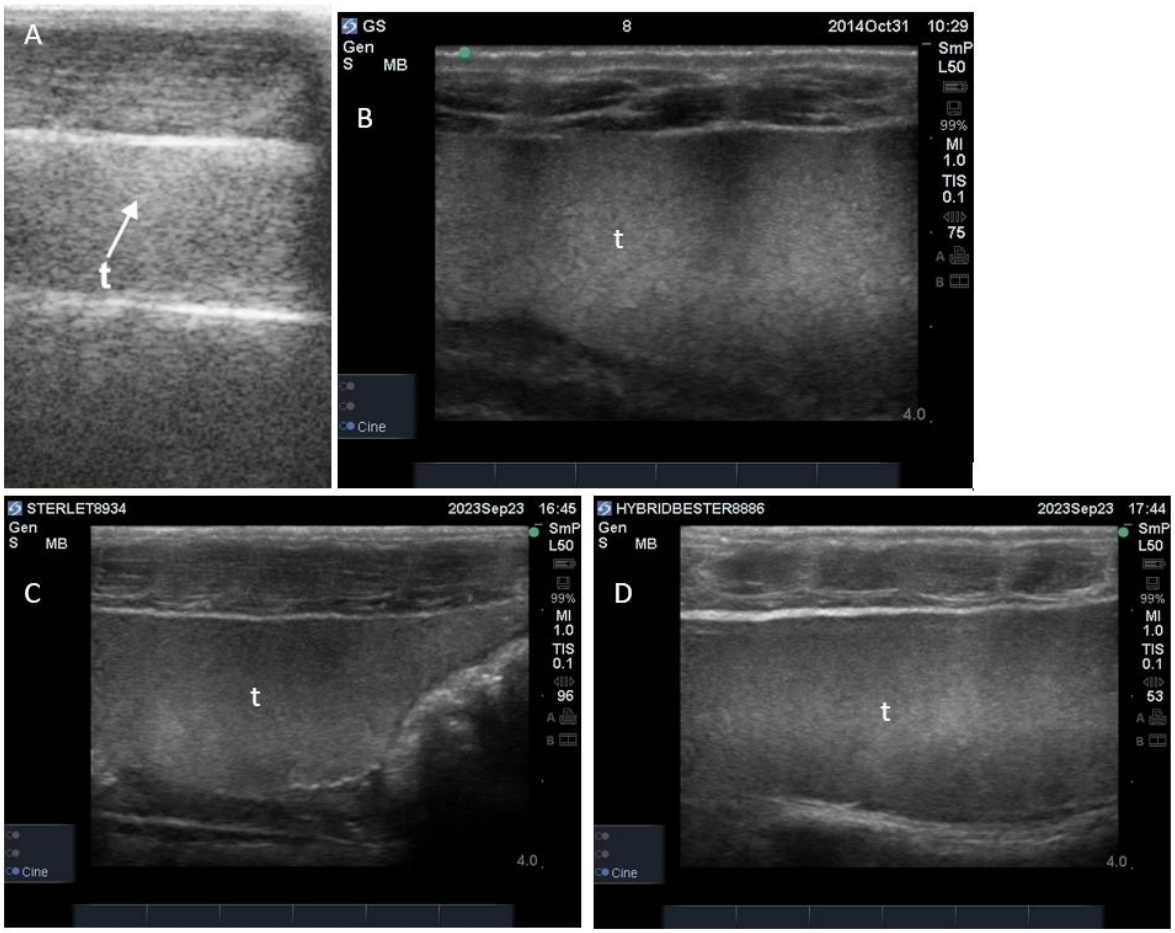
Males



Annex Figure 8.2.7: *A) Longitudinal sonogram of spermatogonial proliferation stage (immature or regenerating) in Huso huso male (t – testis, ft – testicular fat, convex transducer), B) longitudinal sonogram of spermatogonial proliferation stage (immature or regenerating) in Acipenser gueldenstaedtii male (t – testis, ft – testicular fat, linear transducer), and C) transverse sonogram of spermatogonial proliferation stage (immature or regenerating) in Acipenser baerii male (t – testis, ft – testicular fat, linear transducer) (Sonogram credit: M. Chebanov and E. Galich (A, B, and C).*



Annex Figure 8.2.8: *A) Longitudinal sonogram of late spermatogenic Acipenser nudiventris male (t – testis, ft – testicular fat, linear transducer), B) longitudinal sonogram of late spermatogenic Acipenser transmontanus male (t – testis, linear transducer) with apparent lobular structure (Sonogram credit: M. Chebanov and E. Galich (A) and J.P. Van Eenennaam (B)).*



Annex Figure 8.2.9: *A) Longitudinal sonogram of ripe Acipenser stellatus male (t – testis, linear transducer), B) longitudinal sonogram of large-lobed testis in Acipenser medirostris male in the fall that spermiated the following the spring (t – testis, linear transducer), C) longitudinal sonogram of large-lobed testis in Acipenser ruthenus male in the fall (t – testis, ft – testicular fat, linear transducer), and D) longitudinal sonogram of large-lobed testis in Huso huso x Acipenser ruthenus male in the fall (t – testis, linear transducer) (Sonogram credit: M. Chebanov and E. Galich (A), J.P. Van Eenennaam (B), and T. Friedrich (C and D)).*

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Chapter 9: Spawning and Gamete Processing

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9.1 Introduction

There is a general similarity in the neuroendocrine regulation of reproduction between teleosts and chondrosteans (Figure 9.1; Doroshov *et al.* 1997; Moberg *et al.* 1995). Two forms of gonadotropin-releasing hormone, a mammalian GnRH1 (mGnRH) and a chicken GnRH2 have been identified in sturgeons (Lepretre *et al*. 1993; Lescheid *et al.* 1995; Sherwood *et al.* 1991). Synthetic mGnRHa is effectively used to induce ovulation and spermiation in sturgeon and paddlefish which acts on the pituitary gonadotropes to regulate synthesis and release of two pituitary gonadotropins (stGTH I and stGTH II). The stGTH I and stGTH II are molecular and functional homologues of mammalian follicle stimulating hormone (FSH) and leutenizing hormone (LH) in *Acipenser baeri*, *A. gueldenstaedti*, and *A. sinensis* sturgeons (Ceapa *et al.* 2002; Hurvitz *et al.* 2005; Quérat *et al.* 2000). The FSH controls the initiation of a gametogenic cycle and vitellogenesis in females (Moberg *et al.* 1991; Moberg & Doroshov, 1992; Moberg *et al.* 1995), and LH stimulates events resulting in gamete maturation and ovulation and spermiation (Moberg *et al.* 1991; 1995). Little is known about regulation of gonadotropin release in sturgeon compared to teleosts (Zohar *et al.* 2010), although several studies have identified the inhibitory role of dopamine on male spermiation in *A. transmontunus* (Pavlick & Moberg, 1997), and *A. stellatus* (Rzemieniecki *et al*. 2004; Rónyai, 2009; Alavi *et al*. 2012), no studies to our knowledge have identified inhibitory role of dopamine in female sturgeon.

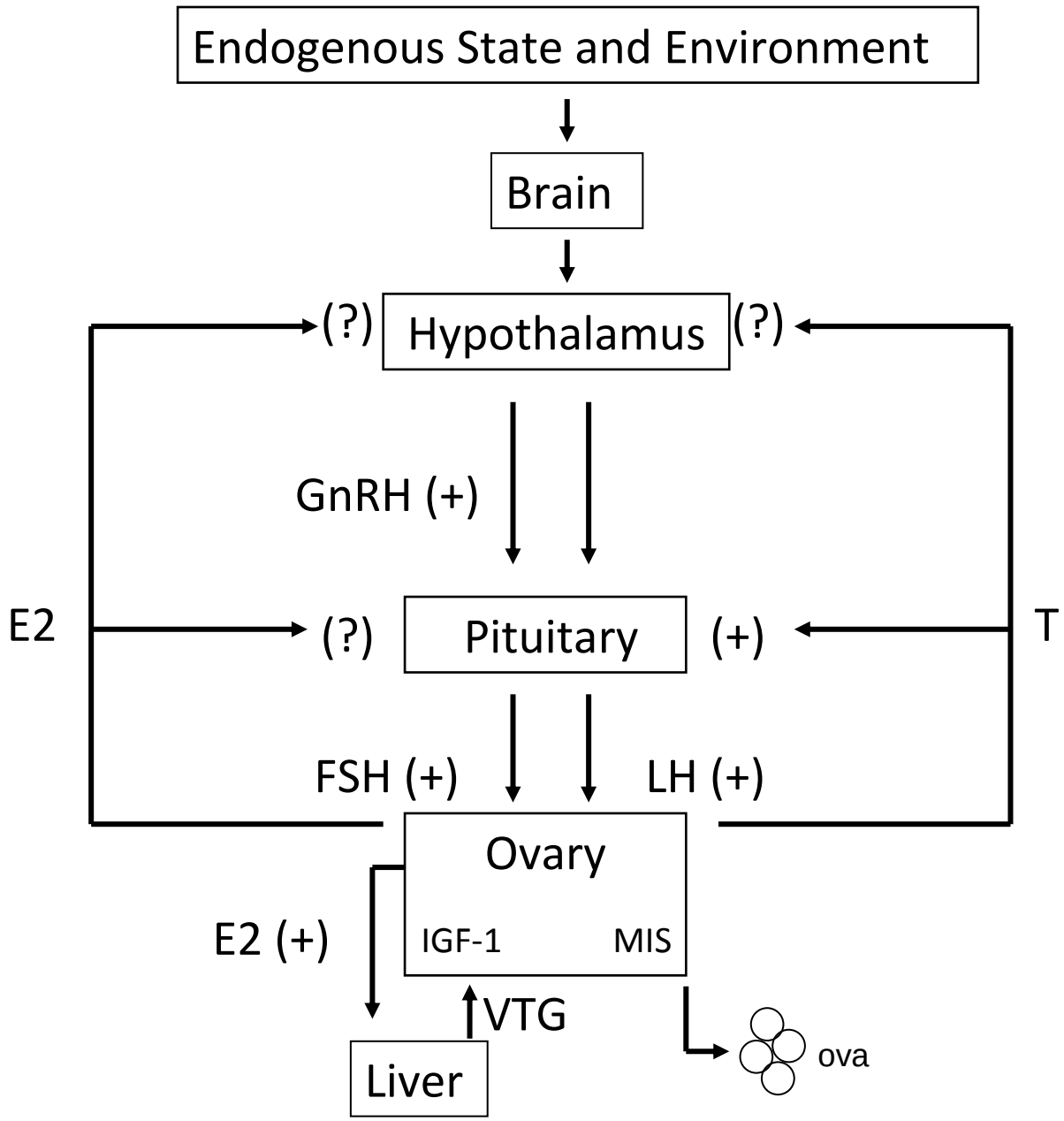

Figure 9.1: *Hypothalamo-pituitary-gonadal axis in sturgeon females. The GnRH (mammalian GnRH1) stimulates the synthesis and release of follicle stimulating hormone (FSH) or leutenizing hormone (LH) and is used for induction of ovulation and spermiation in captive breeding of sturgeon. The FSH controls development and growth of ovarian follicles and vitellogenesis (vitellogenin, VTG) by stimulating secretion of estradiol-17β (E2). The LH stimulates oocyte maturation and ovulation by stimulating synthesis of the maturation inducing steroid (MIS). The positive and negative feedback of sex steroids (testosterone (T) and E2) on the hypothalamus and pituitary are not well known, except of the positive effect of the exogenous T on accumulation of FSH and LH in the pituitary (modified from Webb & Doroshov, 2011).*

9.2 Hormonal Induction of Spawning

Hormone administration has been used to induce ovulation and spermiation in finfishes for almost 100 years, and the first attempts used ground pituitaries (common carp = CCP, sturgeon = SP) from reproductively mature fish, which contained gonadotropins. The use of pituitaries for induction of spawning in sturgeon was most popular through the early 1970's, but after that spawning induction methods began using the newly discovered GnRH, which induces the secretion of the fish's own gonadotropin from the pituitary. Soon after, the development of a highly potent, synthetic analogue of GnRH called GnRHa constituted the next generation of hormonal manipulation therapies (Zohar & Mylonas, 2001). The most common GnRHa used to induce spawning in sturgeon are the des-Gly^{10}- (D-Ala^{6})-GnRH and the des-Gly^{10}-(D-Phe^{6})-GnRH.

Note that the GnRH and LHRH refer to the same hormone, and the different nomenclature exists based on regional preferences. The hormone was initially referred to as LHRH because of its role in regulating the release of LH from the pituitary in the final stages of maturation. As research continued, it was found that it also stimulated the release of FSH, and to reflect the broader scope of its utility, the hormone began to be called GnRH to encompass its role in regulating both LH and FSH release. The analogue, GnRHa, is now becoming more commonly used in scientific literature, although it is still often called LHRHa and is sold in many places as LHRHa (e.g. Syndel).

Surfagon has been used for hormonal stimulation of *A. stellatus* as this species is less sensitive to GnRH as compared to other anadromous species (Chebanov *et al.* 2004). For *A. stellatus* spawning, CCP injections are used, or at stable spawning temperatures very high doses of surfagon (≥40 µg/kg) may be used.

9.2.1 Spawning Tanks

Spawning capable males and females from the pre-spawning holding regime (Chapter 8) are typically moved into designated spawning tanks at the time of first injection. The spawning tanks, already at the optimal spawning temperature with > 70% oxygen saturation, should be covered or have a screen in place and be large enough, based on the species, so the fish can freely swim around the tank.

Although not always done, it is recommended that one injected female and at least one injected male be placed together in a spawning tank. The other injected males to be used during the spawning session can be placed together in a different tank. It is important to understand the ovulatory latency for the species at different spawning temperatures once females have been hormonally induced to ovulate, to ensure tank spawning does not occur with the male in the tank unless desired. One of the first reported possibilities of pheromonal communication between pre-spawning male and female sturgeon was by Kynard & Hogan (2002). The mixing of males and females pre- and post-injection could help initiate a more natural ovulation by an interaction between sexes, as the components of the ovarian fluid,

including free and conjugated forms of sex steroids, can play a significant role in sturgeon pheromonal communication that regulates reproductive behavior (Bayunova, 2016; Bayunova *et al.* 2011; Kasumyan & Mamedov, 2011; Vizziano *et al.* 2006). Social interactions can have major consequences on spawning for both female and male fish (Mylonas *et al.* 2010). Dettlaff *et al.* (1993) reported that during natural spawning, in the presence of a male, ovulation proceeds and portions of eggs are released, but under conditions of keeping males and females separately, females do not display spawning behavior and unreleased ovulated eggs can accumulate in the body cavity resulting in *in vivo* aging of oocytes. Because oocyte aging is considered a factor in reduced egg quality and in the increased incidence of spontaneous autopolyploidy (see Chapter 6), at least in *A. transmontanus*, holding female and male broodstock in the same spawning tank is considered a best hatchery practice (Schreier *et al.* 2021).

9.2.2 Spawning Induction Administration and Dosages

Hormonal injections of CCP, SP, and GnRHa have all been used to stimulate oocyte maturation and ovulation in females and spermiation in males. Pituitary doses and the time interval between injections for several Eurasian species vary with water temperature (Table 9.1), and the priming dose can be increased at higher oocyte polarization indices (Chebanov & Galich, 2011). Males receive a single dose at approximately 1/2 or 1/3 the concentration of the female's total dose, and the injection may be given before the female priming dose, at approximately the same time as the females receive their priming dose, or at approximately the same time as the females receive their resolving dose, depending on the species and water temperature. The resolving dose in females is given approximately 7-18 hours after the priming dose, depending on species and water temperature (Table 9.1).

Hormone injections are given using a 1.0 to 3.0 cc syringe with a 22 to 25 gauge needle (2.5-3.8 cm long). The larger bore needles are sometimes needed for the "thicker" pituitary suspensions, while thinner bore needles can be used for GnRHa, which completely dissolves in saline. Injections are usually administered intramuscularly in females between the lateral and dorsal scutes and between the pectoral fin and midsection of the fish (Figure 9.2). Males may be injected intramuscularly or intraperitoneally. If injecting intraperitoneally, the injection is administered between the pelvic fins and midsection of the fish. Injections can be given while the fish are in a stretcher and are being moved from the broodstock holding tank to the designated spawning tanks or injected underwater while in the spawning tanks.

A female's resolving dose may be injected underwater as the fish is already in the spawning tank. No more than 1.0 cc of solution should be administered at an injection site. If the injection volume is more than 1.0 cc of solution, the total dose can be split into smaller injection volumes and administered at different sites.

Figure 9.2: *Intramuscular injection of hormone to induce spawning in Scaphirhynchus albus (Photo credit: M. Ilgen).*

Table 9.1: *Relationship between total pituitary dose, time interval between priming and resolving injections in females and water temperature for four species of sturgeon (modified from Chebanov & Galich, 2011). The priming dose increases for females that have a higher polarization index (PI): PI 0.04=10%, PI 0.05=13%, PI 0.06=15%, PI 0.07=18%, PI 0.08=20%, PI 0.09=23%, PI 0.10-25%, PI 0.11=25%, PI 0.12=28%, PI 0.13=30%.*

Species/ Water Temperature (°C)	**Sturgeon Pituitary (mg/kg)**	**Common Carp Pituitary (mg/kg)**	**Time Interval Between Injections (hours)**
Acipenser gueldenstaedtii	2.5	4.0	18
10-12	2.0	3.0	15
12-14	1.5	2.5	12
14-18	1.0	1.5	9

>18			
Acipenser ruthenus			
10-12	4.0	6.0	14
12-14	3.5	5.0	12
14-16	3.0	4.5	10
>16	2.5	3.5	8
Acipenser stellatus			
13-16	2.5	4.0	14
16-19	2.0	3.0	12
19-21	1.5	2.5	9
>21	1.0	1.5	7
Huso huso			
9-12	2.5	4.0	16
12-15	2.0	3.0	12
15-16	1.5	2.5	12
>16	1.0	1.5	10

In North America, it is common to use GnRHa at a dose of 10-20 µg/kg of female weight, and males are typically given a single injection of 5-10 µg/kg of fish weight. The female dosage is given in two injections: a priming dose equal to 10% of the total dosage, and a resolving dose equal to 90% of the total dosage, that is usually administered 12 h after the priming dose. Males are typically injected at the time of the females' first injection. Hochleithner & Gessner (1999) reported total dosages of GnRHa of 30 µg/kg for females and 80-100 µg/kg for males, and Chebanov & Galich (2011) reported even lower doses of GnRHa for induction of ovulation in *A. gueldenstaedtii* and *H. huso*. The primer dose was 0.3-0.5 µg/kg body weight, followed by a resolving dose, 8-12 h later, of 0.5-1.0 µg/kg, and *A. stellatus* were induced to ovulate after a single injection of 0.5-1.0 µg/kg. Males of all species were administered a single dose of 1-2 µg/kg of body weight.

The effects of different hormonal treatments on ovulation, fertilization and hatching success have been assessed in several species. Injections of CCP alone or after a priming injection of GnRHa in captive *A. transmontanus* had low fertility and hatch rates. These data resulted in the shift to the current use of GnRHa alone for hormonal induction of ovulation in this species. The general low quality of eggs obtained from using pituitary injections was also reported by Chebanov & Galich (2011). GnRHa is easier to prepare and inject than pituitary extract (product volume can be greatly reduced), and there is stability of hormone activity and less risk of adverse immune reaction in fish as pituitary gland contains a cocktail of hormones). However, ovulatory success to GnRHa is not the same for all species, for example, in *A. ruthenus* it is low, therefore CCP is used for this species.

9.3 Gamete Collection

Spermiation begins approximately 10 h after injection, and milt is generally collected 18-24 h after hormonal injection. It is recommended that males be injected at least 12 h before females to allow time for determination of sperm viability before ovulation occurs. In some facilities, milt is collected and evaluated immediately after the first ovulated eggs are found and in other cases milt is collected the day before expected ovulation. Collecting the day before allows for injection of additional males if only a few males spermiate and allows for detailed evaluation of milt quality using computer assisted semen analysis software (see sperm collection, storage, and assessment below).

Checking for ovulation every 1-2 h begins approximately 4-6 h before the earliest expected ovulation and then hourly. Ovulated eggs can be examined more closely by using a fine mesh aquarium dip net connected to a long pole for ease of scraping along the tank bottom for collection. It is better to begin checking earlier rather than later so a female ovulating early is not missed because the eggs could become overripe if not collected in a timely manner. Ovulatory latency is usually 10–24 h after the resolving injection and is species-specific and water temperature dependent. Good quality eggs will be uniform in appearance and relatively firm and sticky, depending on how long they have been on the tank bottom. Marbled colored eggs (swirls of black, white, yellow) could be a sign of overripe eggs.

After observation of the first released ovulated eggs, there is a period of time to wait before collection of eggs, to ensure complete ovulation. The amount of time to wait until egg collection begins will depend on species, body size (larger females take longer), water temperature (colder water results in longer ovulatory latency), and the number and rate of eggs being released into the tank or upon capture. If the rate of egg deposition increases from the time of first observation through the following 60 min, the female is typically ready for egg collection. If a female has only released a few dozen eggs after waiting an hour or more, the individual should be gently maneuvered into a tube-net, rolled ventral side up, and palpated for a large-soft abdomen indicating abundant ovarian fluid and ovulated eggs. Often, when handling a female, the capture and the back-and-forth body movement will result in release of hundreds to thousands of eggs from the urogenital pore, clearly indicating a fully ovulated female. Occasionally a female will be fully ovulated but does not release any eggs into the tank. In this scenario, after the expected time of ovulation has passed, the individual females should be captured and examined as described above. Lack of visible ovulation has primarily been observed with females held in their own separate tank. It has not been well studied but enough observational data is available to recommend holding at least one injected male with the female for pheromonal stimulation.

After the longest latency time has passed, any female that does not ovulate can be resampled following the same procedures for obtaining ovarian follicles for calculation of oocyte PI (see Chapter 8). Ovarian follicles that are soft and marbled

indicate atresia is occurring and that the female was likely injected too late after the last assessment. Oocytes with the GV still intact indicates that the female did not complete maturation likely because of the failure of temperature water regime, stress, dopamine inhibition, or was injected at too high of an oocyte PI. If oocytes quickly underwent GVBD but ovulation did not occur, meiosis was interrupted (the second meiotic division did not occur), which could be due to stress or incomplete hormonal processes.

Ovulating females and spermiating males should be captured and moved using a type of soft mesh dip net or tube-net to a stretcher or cradle to ensure fish welfare is maintained or, while carefully working directly in the spawning tank, the individual male or female should be guided directly into a stretcher or cradle, lifted out and moved to the gamete collection area. In some instances, gamete collection may occur in the same tank to ensure that handling time is reduced. Sufficient staffing levels are recommended for larger sturgeon species to ensure the physical stresses of the process on the individual are minimized during capture and while restraint is occurring during gamete collection. Collection of milt does not typically require anesthesia, but some facilities may choose to anesthetize large female when collecting eggs via minimally invasive surgical technique (MIST) or celiotomy (see Chapter 8).

9.3.1 Collection, Storage, Cryopreservation, and Quality Assessment of Milt

Collection of milt from large males (> 7 kg) is done using a 30-60 cc syringe with a 5-8 cm length of tubing. The tubing diameter should be slightly smaller than the urogenital opening so not to cause any trauma to surrounding tissue when inserted. Smaller sized males can be hand stripped by flexing the body and directing the milt stream into a dry bowl (Chebanov & Galich, 2011).

Individual males are placed ventral side up in a stretcher or cradle with a hose of freshwater placed above the mouth which helps calm the fish as it ventilates its gills with water. Anesthesia is not typically used, as the entire procedure only takes a couple minutes. During collection, the activation of sperm with water must be avoided. The area of the urogenital opening is gently blotted dry with a rag or paper towels, the tubing inserted just 2-5 cm into the urogenital opening and using the syringe plunger slowly extract milt (Figure 9.3). Common mistakes are inserting the tubing too far and withdrawing the plunger too quickly, as both can suck the walls of the spermatic duct into the tip of the tubing and possibly lead to contamination of the milt with blood. An alternative is to collect milt via the tubing directly into a plastic beaker. For this purpose, flexible tubing of about 25-30 cm is used and the beaker is placed below the fish to benefit from gravity. A gentle ventral massage can be performed at the same time. Milt with obvious contamination of blood, bile or other impurities should not be used.

Figure 9.3: *Milt collection from the urogenital pore in male Acipenser oxyrinchus (Photo credit: J. Gessner).*

After milt is collected, the syringe full of milt is slowly ejected into a large volumetric flask or beaker to keep a thin layer (< 0.5 cm) exposed to air. Place the containers directly into a refrigerator (4 °C) if available, or else into an insulated container maintained at a similar temperature with wet ice or gel packs. It is also recommended to not place containers of milt directly on wet ice. This storage will keep the milt viable for a typical spawning session (approximately 4-5 h), and any longer than this, the milt should be rechecked for quality (see below) before using. Alternately, a syringe is filled just half-way with milt and the other half filled with oxygen. The syringe is inverted to mix the oxygen and milt and then stored lying flat in a refrigerator or in a chilled/insulated container. Make sure that no water comes in contact with the milt prior to use to ensure that the sperm are not activate.

In some cases, it is necessary to store the sperm for several days to 1-2 weeks. To maintain high survival and ability to fertilize eggs, it is best to store the sperm in a sealed container filled with oxygen (Dilauro *et al.* 1994) or a 1:1 mix of oxygen and air (Chebanov & Galich, 2011) that is placed in a refrigerator (4 °C) type environment with daily replenishment of oxygen. Using this method, fertilizing capacity has been obtained for up to 2 weeks (Conte *et al.* 1988; Dilauro *et al.* 1994; Ciereszko *et al.* 1996). The use of various extender solutions has also been utilized to improve the fertilizing ability and storage time of collected sperm (e.g. Mims, 1991; Linhart *et al.* 1995; Billard *et al.* 2004; Park & Chapman, 2005) but are primarily used in conjunction with cryopreservation (see below).

Sturgeon sperm cryopreservation is important for preserving genetic material, especially for sturgeon species that are threatened or endangered, and methods

with different levels of success have been reported and reviewed (e.g. Billard *et al.* 2004; Cabrita *et al.* 2010; Yamaner *et al.* 2015; Chandra & Fopp-Bayat, 2020). However, it is beyond the scope of this publication to go into method details as research continues to improve and refine the technique. Numerous approaches for sturgeon have been reported focusing on different compounds or technical parameters used in cryopreservation, such as dilution with extenders, cryoprotectants (most commonly dimethyl sulfoxide, ethylene glycol, glycerol, methanol, dimethyl acetate), dilution rates, equilibration time, size of packaging, slow or rapid freezing, addition of antioxidants, thawing parameters, and using activation media (Yamaner *et al.* 2015; Igna *et al.* 2022; Kolyada *et al.* 2023). One of the more recent reported successes with cryopreserved sperm was by Fopp-Bayat *et al.* (2023) using *H. huso* males to cross with female *A. ruthenus*.

Sperm quality must be evaluated prior to use. Collected sperm will vary in concentration (<1 to 10 billion per cubic centimeter of milt; the color of non-fat milk to whole cream), and the average duration of motility is 2-5 min (Chebanov & Galich 2011). There are three ways sperm quality has been evaluated: the subjective technique, the objective technique, and the Persov's scale.

The subjective technique involves activating a small, diluted sample (1:50) on a microscope slide with hatchery water at the same temperature as the male was held at and estimating 1) the percent of motile sperm within a given microscope field at 30 s post-activation and 2) estimating time to 90% immotile (not moving in a forward motion). This is typically repeated 3 times to confirm the observations and to take an average of the three data points. In general, sperm with over 80% motility at 30 s post-activation and having over 2 min of motility duration (time to only 10% motile) is used for egg fertilization. However, if males are limited in availability or are needed to fit a specific genetic plan, sperm with 40-60% motility for only 1.0-1.5 min could be used.

The objective technique involves activating a sample, typically using dilution mediums and activation solutions, under a microscope with a high-resolution video camera, connected to a computer where it undergoes analysis by computer assisted semen analysis (CASA) software, like CEROS II Hamilton-Thorne (Gilroy & Litvak, 2019a; 2019b) with details of the procedures to analyze spermatozoa activity provided in Gilroy & Litvak (2019a). During the last 15 years, this technique has become the method of choice for studying fish sperm motility (Gallego & Asturiano, 2019) and has been used on numerous sturgeon species, e.g. *A. gueldenstaedtii* (Igna *et al.* 2022), *A. baerii* (Barulin & Shumski, 2022), *A. ruthenus* (Xin *et al.* 2020; Dzyuba *et al.* 2021), and *A. fulvescens* (Larson *et al.* 2020). It should be noted that the calibration of the device, in particular the number of frames per second, is essential for analysis (Caldeira *et al.* 2019).

Besides estimating the percent of motile sperm, the Persov's (1941) scale (translated in Chebanov & Galich, 2011) is used to score motility type. The point

scale below is evaluated after diluting a sperm sample at a ratio 1:20–1:50 (sperm:hatchery water) while observing the sperm under a microscope. Ideally, sperm that score 4-5 points are used for fertilization, but sperm that score 3 points can be used if needed.

5 Points =	rapid forward motion of all spermatozoa observed.
4 Points =	rapid forward motion of most spermatozoa observed (but zigzagging and oscillating movements of spermatozoa are evident).
3 Points =	rapid forward motion of some spermatozoa but zigzagging and oscillating movement prevails, and some immobile spermatozoa can be seen.
2 Points =	forward movement is almost absent, some oscillating movement is observed, the percentage of immobile permatozoa is high (up to 75%).
1 Point =	all spermatozoa are immobile.

9.3.2 Collection of Ovulated Eggs

There are several non-lethal methods for collection of ovulated eggs (manual stripping, minimally invasive surgical technique (MIST), and celiotomy). The technique selected will depend on the species, body size, and approximate quantity of eggs needed from each female. Stainless steel or plastic bowls of various sizes are used for holding the eggs from each female until fertilization with a general guideline of a 1-3 cm layer of eggs for each bowl, though it may be much less depending on the genetic plan and number of males to be used for each female. A thin layer of ovarian fluid should cover the eggs so they do not dry out, and any excessive fluid should be poured off before fertilization. Eggs should be fertilized promptly so their temperature does not rise or fall beyond 1-2 ^{o}C, from the female spawning temperature. Eggs from multiple females should never be mixed for fertilization (see Chapter 8).

Manual stripping collects fewer eggs than other techniques and should be initiated 2-3 h after the first eggs are released into the spawning tank allowing time for the Mullerian ducts to fill with ovulated eggs. A female should be removed from the spawning tank as quickly and calmly as possible and either be placed into an anesthesia tank or stripped immediately. Depending on the size of the female and how many eggs were released during capture and initial handling, 0.3-1.0 L of eggs can be collected during the first stripping. This quantity of eggs may be entirely sufficient depending on the hatcheries genetic plan (see Chapter 6) or production and stocking targets. Subsequent stripping could be done approximately every hour. The disadvantages of manual stripping are the labor-intensiveness, the repeated handling stress, its long duration, and lower quality of eggs in subsequently later stripping events. To strip eggs from an ovulated female, place her on her back, tipped slightly to either the left or right side. Hands should be placed over the ovaries on the abdomen and gentle pressure anterior to

posterior should be applied. Once the fish is freely expressing eggs, a slow anterior to posterior pressure applied directly to 20-30 cm of the abdomen immediately anterior to the vent will often sustain the flow of eggs. Generally, 2,000 to 80,000 eggs can be quickly removed from a female using this technique.

The most widely used and very effective technique to collect ovulated eggs when in a hatchery setting is the MIST, which requires the careful insertion of a scalpel into the urogenital opening, cutting through the oviduct (Figure 9.4), and then manual stripping the ovulated eggs (Podushka, 1986). This technique has been used successfully with *Polyodon spathula* (Štěch *et al.* 1999; Mims *et al.* 2004); *A. stellatus* (Bani & Banan, 2010,); *A. persicus* (Pourasadi *et al.* 2009; Kabir & Bani, 2011); *H. huso* (Kabir & Bani, 2011; Aramli *et al.* 2014); *A. baerii*, *A. gueldenstaedtii*, *A. ruthenus* (Chebanov & Galich, 2011). The small size of *A. ruthenus* does present a challenge for using the MIST and should be considered if choosing to employ this technique.

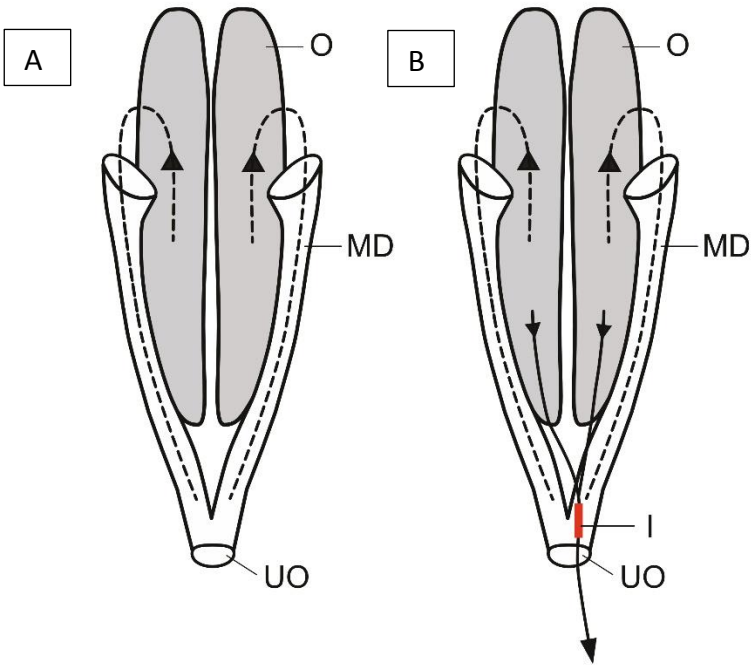

Figure 9.4: *Schematic drawing illustrating the pathway of A) ovulated eggs from the ovaries (O) in the body cavity of sturgeons through the Mullerian Ducts (MD) and out the urogenital opening (UO) during natural spawning and B) ovulated eggs from the O in the body cavity through the MD and an incision (I) made by the minimally invasive surgical technique (MIST) technique in the MD close to the UO (modified from Podushka, 1986 by Heidrun Eichhorn).*

An anesthetized female is first placed ventral side up in a stretcher or cradle. For larger sized species, a finger is then inserted into the urogenital pore to stretch the opening, then quickly removed and a scalpel (e.g. a number 11 straight blade) is carefully inserted blade-side up about 1-5 cm (according to size of the fish) into

the urogenital pore. A 1-3 cm incision is made through the ventral wall of the Mullerian duct (Figure 9.4). When cutting internally, be very careful not to cut laterally towards the GI tract. The exact size and type of surgical scalpel will depend on the species, body size, and, most importantly, the diameter of the urogenital opening.

Ease of manual stripping through the MIST incision will depend on the amount of ovarian fluid and amount of ovarian and adipose tissue that could block the incision. Often a surgical spatula or long surgical blunt-nosed forcep can be inserted to help keep the incision unblocked and keep the eggs flowing freely. Up to 80-90% of the ovulated eggs can be collected in 5-15 min. Females recover fully from this procedure in 3-4 weeks, and it has been used for more than 20 years with many species. Some individual females have undergone MIST and been stripped more than seven times using this technique (Chebanov & Galich, 2011).

The MIST was compared with a modified version using a polypropylene catheter (external diameter 10-14 mm) inserted directly into the urogenital opening and pushed past the Mullerian duct valve and into the Mullerian duct region. Eggs can then be stripped out through the catheter (Szczepkowski & Kolman, 2011). The authors reported similar amounts of time to obtain similar quantities of eggs from both techniques for *A. baerii*, *A. gueldenstaedtii*, *A. baerii* x *A. gueldenstaedtii*, and *H. huso* x *A. ruthenus*. The advantage is no scalpel is introduced, and it has been successfully repeated on individuals up to three times. Disadvantages were similar with MIST in that ovarian tissue can block the catheter, females with little ovarian fluid are more difficult to strip, and species like *A. ruthenus* have a urogenital opening that is too small to use this method.

The collection of ovulated eggs can also be done through an abdominal incision typically called caesarian surgery (e.g. Burtsev, 1969; Doroshov *et al.* 1983; Conte *et al.* 1988; Mohler, 2003; Van Eenennaam *et al.* 2004) or key-hole surgery (Parandavar *et al.* 2006), and this technique is still used at aquaculture facilities in North America. After anesthetizing the female, a 6-8 cm incision is made in the mid-body to lower third region of the abdomen, approximately midway between the abdominal midline and lateral scute line depending on the species and body size. The incision should be made in the region that has about 1.0-1.5 cm muscle thickness to ensure good vascularization for healing and for effective suturing. After the incision is made, the eggs are removed typically with a plastic spoon of various lengths. Caution is used when collecting in the anterior portion of the body cavity where the liver, gall bladder, spleen and other vital organs are located. Egg removal is completed in 10-20 min with approximately 70-80% of the eggs collected.

The incision is closed by internal and external sutures to ensure the peritoneum is closed and to help with apposition and rapid healing. The first is an internal suture used to bring the peritoneum and bottom half of the muscle together, and the second is an external suture for the top part of the muscle and skin. The internal

suture is made using single interrupted stitches with a PDS #0 suture and a swaged-on CT-2 taper needle. The external stitches will use the same material but with a thicker (#1) suture and a larger swaged-on CP-1 or CPX cutting needle for cutting through the tough skin. The best external suture is a special tension suture pattern called "far-near-near-far" pattern (Knecht *et al.* 1987). The advantage to this suture is that it apposes the skin edges and provides a degree of tension with the far and near suturing. After suturing, an injection of antibiotic may be administered (e.g. 200 mg/mL of oxytetracycline at a dose of 5 mg/kg body weight) for 1-3 days. The best to use is the slow-release oily formulation for cattle (e.g. Zoetis, Liquamycin® LA-200®). The females should be allowed to recover in a separate clean tank for approximately 4 weeks, and earthen ponds or raceways are not recommended due to potential pathogens from the soil-water interface.

Initial years of this surgery had some post-surgery mortalities related to improper suturing and prolonged exposure to anesthesia. However, with improved suturing and anesthesia monitoring, the occurrence of post-surgery mortalities, at least in *A. transmontanus*, is very rare (Van Eenennaam *et al.* 2004). A modified version of this technique is to make a smaller (3-5 cm) incision, about 5 cm anterior to the urogenital opening and slightly off center to not hit the lower GI tract, and then hand strip the eggs out of this incision (Williot and Chebanov, 2018).

Both of these techniques are most applicable to broodstock that remain captive and are held under optimal water quality hatchery conditions for recovery, but they are not recommended for wild-caught broodstock from threatened populations, especially if they are to be released back into the wild.

The quality of ovulated eggs can generally be assessed by visual examination of the uniformity of coloration, shape, absence of marbled early atretic or overripe eggs, and presence of abundant, clear ovarian fluid. Time to eggs first becoming sticky after exposure to hatchery water may also be used as an egg quality assessment (Chebanov & Galich, 2011), but the optimal time to stickiness of high-quality eggs will vary between species and with water temperature and would thus need to be established at each hatchery. Gorbacheva (1977), translated by Chebanov & Galich (2011), reported the optimal time to stickiness for *A. gueldenstaedtii* and *A. stellatus* eggs were 8-19 and 5-12 min, respectively, while time to stickiness of overripe eggs was only 4-6 and 2-4 min, respectively.

9.4 Egg Fertilization

In the past, pooled milt from several males was often used to fertilize a bowl of eggs to ensure the highest fertility rate as possible, but the probability of one male with the best quality milt fertilizing most of the eggs was high. Today, hatchery genetic plans call for the total amount of eggs collected from a single female to be equally divided between bowls, based on the number of males to be crossed with that female, creating full-sib matings. Review Chapter 6 for genetic considerations prior to collection of gametes and fertilization.

Fertilization of eggs is usually done within 5-30 min after collection, depending on how long it takes to collect all the eggs and divide them between bowls. The eggs should be maintained at the spawning temperature and covered with a thin layer of ovarian fluid until fertilization. Good quality eggs can still have good fertilization success, even after delaying fertilization for 2-10 h, if the eggs are held under a layer of ovarian fluid in low light and maintained at or below spawning temperatures (Dettlaff *et al.* 1993; Gisbert & Williot, 2002; Linhart *et al*. 2016). Note that holding ovulated eggs for too long before fertilization may lead to spontaneous autopolyploidy (Schreier *et al.* 2021).

Insemination of eggs is usually performed using the semi-dry method (Dettlaff *et al.* 1993; Chebanov & Galich, 2011) but care must be taken to remove any excess ovarian fluid prior to addition of milt because excessive amounts can interfere with insemination. If the ovarian fluid is very abundant and viscous, it is best to quickly (for 10-30 s) rinse the eggs in hatchery water to remove the fluid or use a colander to drain off the ovarian fluid/water, which will improve fertilization rates (Van Eenennaam *et al.* 2008; Feledi *et al.* 2011). Milt (approximately 10mL/kg of ovulated eggs is diluted 1:200 with hatchery water (e.g. 20 mL:4000 mL depending on the volume of the bowl), and then without delay, the diluted sperm is added to the bowl of eggs.

The eggs and milt are gently mixed for 1-3 min or until just a few eggs start to stick to the sides of the bowl. Some female's eggs become sticky more quickly than others, and some species, like *A. sterlet*, become sticky often within 30 s. It is likely that most eggs capable of being inseminated are fertilized within the first 30-60 s (Chebanov & Galich, 2011). The insemination solution is poured off, and a de-adhesion suspension is immediately added to the eggs.

9.5 Egg De-adhesion Techniques

Sturgeon eggs have an outer glycoprotein matrix (jelly coat) that hydrates upon contact with water at spawning and needs to be either covered with fine particles or chemically removed to prevent clumping during incubation. Although there have been various suspensions used to cover the sticky coating, including talcum, milk powder, starch, mineral silt (bentonite), and pottery clay, the most effective and popular have been blue clay and Fuller's Earth (Chebanov & Galich, 2011; Van Eenennaam *et al.* 2004). The use of whole milk powder can lead to an increase in bacterial infestation, and the use of mineral silt can cause rupture of the chorion if crystalline material is present. The approximate proportion of dry material is 100-300 g per 4-5 L of hatchery water, which is mixed by hand and then poured immediately and gently into the bowl of fertilized eggs. The eggs and de-adhesion solution are very slowly and very gently mixed by hand for large quantity of eggs (>1 L) or by using a feather for small quantities of eggs. Being very slow and very gentle is crucial as it has been reported that mixing eggs too vigorously during de-

adhesion can lead to variable rates of spontaneous autopolyploidy (Van Eenennaam *et al.* 2020).

The amount of jelly coat formation varies between females and between species, thus the length of time for egg de-adhesion will vary from 30-60 min. The process is also temperature dependent such that cooler water temperatures require a longer de-adhesion process. The de-adhesion suspension is changed every 10-15 min to maintain temperature and dissolved oxygen at optimal levels.

De-adhesion can be automated by using an upwelling flow (Monaco & Doroshov, 1983; Chebanov & Galich, 2011) or an aerated container (Dettlaff et. al. 1993), but these systems could possibly be too vigorously mixing the eggs, which has been shown to induce spontaneous autopolyploidy in *A. transmontanus* (Van Eenennaam *et al.* 2020). However other species may in fact be more resistant to spontaneous autopolyploidy, and thus the use of upwelling and aerated containers may not be an issue.

Methods for chemical elimination of the jelly coat are also available but are not as commonly used due to the potential of over treatment and resulting embryo mortalities. The most common have been urea, sodium chloride, tannic acid, and sodium hypochlorite (Kowtal *et al*. 1986; Bouchard & Aloisi, 2002; Feledi *et al.* 2011; Pšenička, 2016). Interestingly, the study by Pšenička (2016) illustrated the damaged chorions of treated eggs from the use of both sodium hypochlorite and tannic acid, which shows that prepared de-adhesion mixtures should be checked for optimal pH (6.5-7.5) prior to use to be sure it is not too acidic or basic for the fertilized eggs.

9.6 Tank Spawning

Hormonally induced *A. medirostris* have been successfully tank spawned since 2009, utilizing outdoor temperature controlled 3.7 m diameter/0.7 m deep circular fiberglass tanks containing 2-3 males and a single female (Van Eenennaam *et al.* 2012). The tank was kept turbid with constant addition of Fuller's earth using automatic belt feeders, and eggs were collected from the tank bottom with a fine mesh dip net every 1-3 h. The combination of low numbers of eggs released at each spawning bout, the exposure of fertilized eggs to Fuller's earth, and the fact that the adhesiveness covered only about one third of the egg lead to minimal clumping of eggs (Van Eenennaam *et al.* 2012). Conceptually, a designed spawning tank with a false elevated bottom with proper sized openings, so fertilized eggs could fall through into some type of silting system could be highly successful.

9.7 Embryo Incubation

After egg de-adhesion is completed, the de-adhesion material must be rinsed off by repeatedly adding clean incubation/hatchery water to the bowl, mixing, and then pouring off the water/de-adhesion material until all the material is gone. The clean eggs are then placed into egg incubation containers.

A sub-sample of the clean eggs should be measured volumetrically (average count of 3 samples of 5-15 mL of eggs) so the estimated quantity of eggs per mL can be calculated. The total volume (mL) of eggs placed in each container is then recorded for determining "fecundity" of each ovulated female. Alternatively, a gravimetric method of weighing and counting 3 subsamples of eggs and weighing the total amount of eggs collected can be used but requires draining the water from the eggs prior to weighing.

Various types and sizes of incubators are used to hold sturgeon embryos from post-fertilization through hatching. The most common systems utilize either horizontal (Yuschenko system, Osetr system) or vertical (Weiss jar, McDonald jar, Eagar upwelling jar) flows of water (see Chapter 10, Chebanov & Galich, 2011). Ideally, UV light-sterilized ground water should be used that is semi-recirculated, with a chiller if needed to maintain optimal incubation temperature. Oxygen levels should be at greater than 70% saturation. In general, the incubation systems should be in low light sections of the hatchery (< 100 lux) and never in direct sunlight, which can lead to developmental abnormalities (Chebanov & Galich, 2011). It should be noted that eggs of some species may survive better in specific incubation systems. For example, it was found that the McDonald jars, typically used for *A. transmontanus* embryos, were too vigorously tumbling the thin-chorion *A. medirostris* embryos resulting in high mortalities compared to the gentler upwelling Eagar incubators (Van Eenennaam *et al.* 2008).

9.7.1 Embryo Densities

The volume or number of eggs placed into an incubation system will depend on the size of the section/box/jar and species egg size (Chebanov & Galich, 2011), and in general, a Yushchenko section can have 150,000-260,000 embryos, an Osetr box 100,000-250,000, a 6-L McDonald jar or Eagar upweller 20,000-65,000, and an 8-L Weiss jar 20,000-35,000.

9.7.2 Monitoring Embryo Incubation

Continuous (or at least twice daily) monitoring of the water supply for flow, temperature, and dissolved oxygen at optimal levels is critical during embryo incubation. Water flow rates through the incubation system will depend on the system and stocking density of embryos used. Initial flow rates are always lower during the initial sensitive stages (late cleavage and gastrulation to early neurulation) and are then almost doubled after neurulation (50-70 h post-fertilization depending on temperature). For the Yushchenko systems, initial flow

rates are 1.2-2.4 L/min/100,000 eggs and increased to 3.3-5.5 L/min/100,000 eggs. The Osetr systems are initially at 2-3 L/min/100,000 eggs and increased to 4.5-6.2 L/min/100,000 eggs at later embryonic stages. McDonald and Weiss jars are initially at 3-5 L/min and increased to 7-10 L/min for approximately 1,000-1,200 mL of eggs. Upwelling incubators, due to the diffusion of water flow through 5-6 cm of marbles and then a perforated stainless-steel plate (2 mm openings), utilizes higher flow rates (15 L/min/1,500 mL of eggs) but creates a gentler upwelling of the embryos.

Although proper UV treatment of water should reduce the incidence of fungal outbreaks from the genus *Saprolegnia*, twice daily removal of unfertilized eggs and dead embryos is important. When fungus begins on a dead embryo or unfertilized egg, it can quickly spread to live embryos. The advantage of the upwelling incubation systems is that any dead embryos that do become infected with fungus will be more buoyant than the live embryos and can be siphoned out of the incubators. This reduces the need to chemically treat the eggs for fungal infections, although there are several antifungal therapeutics that have been used on sturgeon eggs (see Chapter 11).

9.7.3 Estimating Egg Fertilization and Hatch Rate

Fertilization success is calculated at the 2nd or 3rd cleavage (4 or 8 blastomere stage), and the time for sampling depends on water temperature. Timing of sampling for fertilization success at different temperatures is available for many species (e.g. *A. gueldenstaedtii*, *A. stellatus*, *H. huso*, and *A. ruthenus* (Dettlaff *et al.* 1993 reprinted in Chebanov & Galich, 2011); *A. fulvescens* (Wang *et al.* 1985), *A. transmontanus* (Beer, 1981; Wang *et al.* 1985); and *S. albus* and *S. platorynchus* (Kappenman *et al.* 2013). Sampling for other species is also available A random sample of 100-200 eggs should be collected from each incubator and examined visually or under a dissecting microscope. All unfertilized, dead, activated, and polyspermic eggs are removed, and the number of normally fertilized embryos in the total number of eggs in the sample counted to give the percent fertilization rate. Similar sampling and processing can be done at the late neurulation (Stage 23) to the stages of heart rudiment formation (Stages 26-28) (Dettlaff *et al.* 1993) which can typically give a good estimate of hatch rate in good quality eggs.

9.7.4 Duration of Embryogenesis

The chronology of embryonic development was presented by Dettlaff *et al.* (1993) and reprinted in Chebanov and Galich (2011) and is available for many other sturgeon species (e.g. Beer, 1981; Wang *et al.* 1985; Kappenman *et al.* 2013). The duration of embryogenesis is species and temperature dependent ranging from 50-100 h for *A. stellatus* and 160-240 h for *H. huso* (Chebanov and Galich, 2011). The temperature should be maintained within the optimal species-specific range and close to the optimal value.

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Chapter 10: Rearing of larvae, fry, fingerlings, and juveniles for release

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10.1 Introduction

The target of rearing fish for release in *ex situ* programs is the release of animals that mimic the abilities of wild progeny with regard to high genetic diversity, traits to survive under natural conditions, ability to reproduce successfully and homing behavior to the targeted waterbody. Therefore, *ex situ* rearing conditions should mimic conditions in the wild as much as possible, taking increased mortalities through natural causes, such as hydrological events or predation, out of the equation. Enriched rearing conditions stimulate brain plasticity and adaptability while environmental cues of the river of release are important for imprinting and adaptation to local pathogens. The application of enriched rearing conditions can be implemented in a variety of life stages, unfortunately, no conclusive studies have yet addressed the cumulative effects of enriched vs conventional rearing through a series of subsequent life stages.

10.2 Application of enriched rearing

Various means of enriched rearing can be applied, while not investigated yet in their cumulative effects, nevertheless enable beneficial traits in the fingerlings for release. Underlying experiments are described in more detail in Chapter 12. While further research and experiments may be required to fully understand some of the processes, to goal is to provide means for enrichment activities mimicking natural conditions best possible and applicable under hatchery conditions and operations. The application and feasibility of single enrichment activities have to be evaluated according to the local situation and can be grouped into different factors.

10.2.1 Water source and quality

While underlying mechanisms are not yet fully understood, the use of river water during the first life stages is considered a prerequisite for imprinting and hence homing behavior. In addition, the provision of river water can add other environmental clues and challenges for juveniles, such as changes in sediment load and turbidity, variations in water temperature and quality, olfactory traces of predators and prey, the possibility of natural prey items being supplied through the water, etc. A thus enriched environment exposes the animals to a multitude of traits which are barely replicable in captive conditions otherwise. On the other hand, the approach can pose challenges and even risks to hatchery operation (e.g.

water quality, algae blooms, hygiene), requiring failsafe mechanism and back-up options to guarantee the safety of the animals.

10.2.2 Tank design and hatchery operation

The light regime should follow the diel rhythm and tank colors and covers such as opaque acrylic sheets can be used to simulate lighting conditions similar to the natural environment and are easy to implement in the hatchery to foster timely melanophore reaction as adaptive response to changing environmental conditions (see Chapter 12).
Flow velocity variations and different flow patterns through structures in the tanks only require minimal additional worktime during hatchery operations and can significantly increase stamina and swimming capacity of individuals (see Chapter 12). Ideally structures can be removed, cleaned and varied in their location

The provision of substrates mimics conditions in the wild, as many prey organisms of sturgeons are typically buried in the sediments (see Chapter 12), and provision of feeds within sediments can be used to train fish accordingly. While easily implementable in natural ponds or raceways with earthen bottom, the use of gravel and sandy substrates in tank environments has it challenges with regard to hygiene, removal of feces and dead animals and has to be evaluated according to the local conditions.

The availability of substrates has also shown to be beneficial for energy content within eleutheroembryos in initial studies. Baker *et al*. (2014), Boucher *et al.* (2014, 2018), and Gessner *et al.* (2009) have shown that adapting the rearing conditions by simulation of near natural habitat features through the provision of gravel or other substrates, supported tigmotactic behavior in these life stages, resulting in differences in larval behavior, swimming performance, altered fatty acid composition, increased stress levels, altered growth and increased energy reserves. As a result of providing coarse substrate for eleutheroembryos, a delay in dispersal behavior (swim up and drift) prior to exogenous feeding was described (Gessner *et al.* 2009). Furthermore, settling fry also preferred structured substrate, minimizing energy expenditure. Timing of onset of feeding is impacted by the rearing conditions applied. By only providing eleutheroembryos with substrate until onset of feeding, swim up and dispersal as well as onset of feeding occurred up to 24 hours later than in substrate free rearing. Furthermore, the energy reserves of the individuals were greater and as such the onset of exogenous feeding had a wider window of opportunity. Interestingly, the individuals that were exposed to a lack of substrate were larger (10% increase in length) but revealed lower energy contents.

10.2.3 Feeding

The prolonged or additional administration of live or frozen feeds and a variation of feed items (see Chapter 10) as well as varying feeding times with high activity can be used to train fish to a multitude of prey items and avoid domestication effects through sole administration of compound diets as observed in salmonids. While similar effects have not yet been documented for sturgeons, benefits of natural feed items for acclimatization to natural prey and also with regard to the physiological development are highly likely. Still, potential risks depending upon feed source (pathogen transmission, environmental pollution) and economic feasibility have to be addressed on a case by case basis.

10.2.4 Egg Incubation

Under controlled conditions, facilitated reproduction, including the application of breeding plan, the selection of breeders, and the collection and admixture of gametes in the fertilization process play a vital role for the quality of offspring, as outlined in Chapter 7.

The differences in the incubation techniques applied with regard to the impact upon the embryos differ widely since the natural embryonic development would take place in an egg that is attached to the substrate, where it stratifies and the embryo polarizes. This incubation process could only be closely resembled in hatching trays while all other methods only differ in the degree of agitation that the embryo experiences. A systematic study on the effects of the different incubation techniques on the quality of offspring is still lacking.

10.3 Hatching and holding of larvae

10.3.1 Dispersal and conditions of larval holding

The onset of hatching is characterized by the occurrence of single floating free embryos in the incubation system. The hatching larvae are transported to the rearing tanks. This can happen by autonomous dispersal of the animals from the used incubation system using flow and light, utilizing the phototactic behavior of the fry. Otherwise the larvae are collected in a hatching tank and are subsequently manually dispersed into the rearing tank. In this case collection of the embryos should happen several times per day during the hatch period in order to separate the healthy larvae from egg membranes, dead eggs, individuals with anomalies and collecting debris from river water timely. Another possibility is to use hatching troughs equipped with barriers to retain the egg membranes. Larvae are moved from those troughs to clean ones with a syphoning system.

With large numbers of larvae, enumeration is hard to perform individually with regard to effort as well as handling trauma to the animals and therefore the count is routinely determined by rough estimation. Commonly the numbers are estimated by comparison with a container holding 500 individuals) or by weighing. A few norms related to density of larvae, water level and water quality in tanks or trays are presented in Table 10.1. If the number of larvae exceeds the available

hatchery space, it is not recommended to increase given density numbers but to either disperse the surplus to other facilities or release at suitable locations (See Chapter 14).

After stocking of larvae into the subsequent rearing tanks, remaining membranes, dead eggs and individuals with anomalies should be removed subsequently over three days using rubber siphon or forceps.

Table 10.1: *Maximum larval stocking density in tanks and trays per m² (modified from Chebanov & Galich, 2011).*

Parameter	**Density**
Stocking density, individuals/m^2	
Very large species	4 000
Large species	5 000
Medium species	6 000
Small species	7 000

Figure 10.1: *Tanks for larval holding and larval grow out (Photo credit: J. Gessner)*

Figure 10.2: *River water rearing system for larvae and small juveniles (Photo credit: J. Gessner).*

10.3.2 Behavior during endogenous feeding

Some behavioral traits of larvae during the first days of life should be noted. After hatching, they disperse in the water column, making periodic movements up to the water surface and then drifting down to the bottom of the tank. During natural spawning, such a behavior of sturgeon larvae lets them, firstly, avoid siltation and secondly, by running along the current, reach the zones with high concentrations of food organisms (Chapter 2). At transition to branchial respiration and at the stage of alimentary system formation a period of aggregation-can be observed in captive conditions in which larvae are resting at the bottom of the tank, aggregating in dense clusters. This behavior may be associated with the search for shelter in the wild, and was not observed in larvae reared in tanks with sediments (Gessner et al. 2009, Boucher et al. 2014). If these aggregations are located in zones with poor water supply, mortality induced by oxygen deficiency may occur as oxygen consumption is several times higher during this period as compared with embryonic development (Zhukinsky 1986).

The mass mortality of larvae at this stage may be related to the hatchery quality of eggs and unfavorable rearing conditions. Upon reaching these stages, larvae with morphological defects such as developmental anomalies of the respiratory organs and/or digestive tract are not capable of further development and die. The overall mortality during the period of endogenous feeding should not be higher than 5–10 percent (depending on species). Therefore, sampling of larvae (30–50 live and dead individuals) should be performed every three days to control the development of the larvae and evaluate their quality (Figure 10.3).

Figure 10.3: *Sample collected to assess quality of larvae during the periods of larval holding in tanks (Photo credit: T. Friedrich).*

It is essential to provide consistent control of seasonal temperature and oxygen conditions. The regular cleaning and timely increase of mesh size of the filter screen at the outlet of the tanks is of importance. In the course of larval growth, the mesh size of the outlet screen should be gradually increased from 1 to 7 mm depending upon the size of the larvae to prevent impingement and escapement.

10.3.3 Transition to exogenous feeding

Transition to exogenous feeding is associated with changes in the respiration, metabolism, growth rate and survival of sturgeon larvae. At the onset of exogenous feeding, the cellular partition that closes the passage from the oral cavity to the gullet in larvae resolves and simultaneously the melanin (fecal) plug is extruded from the anal opening. These pigment plugs are visible as small black extrusions on the tank bottom. At the moment of initiation of exogenous feeding, prelarvae that have been in a quiescent state exhibit bottom grouping while searching for feed. In conventional hatchery protocol (Dettlaff et al. 1993), the appearance of single melanin plugs serves as an indicator to initiate first feeding, which should be performed at melanin plug extrusion in 2–3 percent of larvae. The period of melanin plug extrusion can take about 3–4 d. Untimely feeding may cause damage and loss of larvae; this is most characteristic for larvae of carnivorous sturgeon species (*Huso sp.*). At the same time, past experience has shown that administration of feed in small doses stimulates the transition to

exogenous feeding and significantly increases both survival ability of larvae and growth rates. The opinion that melanin plug extrusion may not be a criterion for timing of feeding initiation has been expressed by Gisbert & Williot (2002) and Williot *et al.* (2005). The time of transition to exogenous feeding depends on the water temperature and energy expenditure (Table 10.2.).

Table 10.2: *Duration of sturgeon larval development in several species before transition to exogenous feeding in relation to water temperature.*

Water temperature, °C	**Duration (d) by sturgeon species**							
	A. gueldenstaedtii	***A. stellatus***	***H. huso***	***A. ruthenus***	***A. oxyrinchus***	***A. sturio***	***A. transmontanus***	***A. medirostris***
12	20	–	18	20	-		15-17	
13	18	–	16	16	-		13-14	
15	12	–	12	12	-		11-12	10-15
17	9.5	12	10	10	7	11-12	9-10	
19	8	9	8	-	6-7		7-8	
21	7.5	8	7	-	6			
23	–	6.5	–	-	5-6			

Water supply depends upon achieving the required water quality parameters (>6mg/L O^2, <0.05 mg/L NH4) and tank form as well as the swimming capacity of the larvae (See also Chapter 5). Total exchange of the tank volume should be between 2 - 5 times per hour during the period of transition to exogenous feeding. Sharp variation in water temperature should be avoided. Temperature drops, despite melanin plug extrusion, can cause refusal of larvae to feed due to a delayed process of fat resorption in the alimentary system.

During the period of transition to exogenous feeding, the number of dead specimens tends to increase, mostly due to mortalities of larvae having morphological defects. The most frequently occurring anomalies during the larval period are malformations (of functional, structural and mechanical character) in body shape, in external and internal organs etc. (see the more detailed description given in Chapter 11). Mortality of sturgeon larvae at the transition to exogenous feeding is primarily due to poor quality of gametes and sub-optimal rearing and incubation conditions (Semenov 1965).

10.3.4 Feeding with live prey items

It is common practice to use the following live foods in sturgeon larviculture to stimulate normal growth and formation of the digestive system during the first days of feeding: nauplii of brine shrimp (*Artemia*), small cladocerans (*Daphnia magna, Moina macrocopa*), copepods (Copepoda), branchiopods (*Streptocephalus torvicornis*), rotifers (*Rotatoria*), chironomids (*Chironomus plumosus*), gammarids (Gammaridae), and minced oligochaetes (*white worms – Enchitreus albus*), tubifex (*Tubifex tubifex*) and Californian red worms (*Eisenia foetida*). While it is possible to start directly with compound diets in some sturgeon species (Hung *et al*. 1997; Gisbert & Williot, 2002; Deng *et al*. 2003; Ware *et al*. 2006) others react poorly. It is generally not recommended for fish intended for release programs as it may foster traits not beneficial for survival in the wild and induce artificial selection, favoring animals feeding better on compound diets. It has to be noted, that exclusive use of some live feed types such as *Daphnia sp.* may not work with some sturgeon species e.g. *A. oxyrinchus*.

During the first days of transition to exogenous feeding, it is recommended to lower the water level in the tank when administering live food to 5-10 cm, thus increasing the density of prey organisms, decreasing the energy expenditure of fry while seeking feeds and avoiding loss of live organisms with water draining from the tank. The feeding of larvae starts with nauplii of *Artemia,* minced oligochaetes or a small portion of zooplankton on the basis of 10% of larval weight for stimulation and then according to Table 10. 3.. It is very important to avoid overfeeding during the first days due to uneaten feeds accumulating in the tank and affecting the hygiene regime; thus, small portions of feeds should be used. Considering that it is normal behavior for sturgeon fry to rise to the water surface at night, it is recommended to feed them with zooplankton in the evening, while feeding with oligochaetes, etc. in the morning and afternoon.

The daily live food consumption rate is calculated by taking into consideration the target growth and feed conversion rates (*Artemia nauplii* FQ 3–4; *Daphnia* FQ 6; Oligochaeta FQ 2).

Table 10.3: *The daily live food consumption rate in % of stocked larval weight (modified from Chebanov & Galich, 2011).*

Feed organism	Ratio of live weight (%)
Artemia nauplii	30-60
Daphnia, Moina	60-80
Tubifex sp.	20-40
other oligochaetes	30-50
Chironomous spec.	20-40

Tubifex and other oligochaetes are fed in minced form (the amount depending on the fry weight) diluted with water and administered along the tank wall (perimeter) in two or three portions. The subsequent feed ration is formulated taking into consideration the objective of the fry rearing. Note that the prolonged exclusive use of live food is recommended if further grow out of larvae is intended with subsequent release to the wild.

Figure 10.4: *Automatic feeders for live feed, e.g. Artemia salina (Photo credit: T. Friedrich)*

Frequency of feeding with live food depends on the rate of nutrient digestion, which is species specific. *A. transmontanus*, *A. ruthenus*, *Huso sp.* and probably other species show high degrees of cannibalism over more than 5 h without feeding, hence it is necessary to work in shifts or use automatic feeders suitable for live feeds (see Figure 10.4). Orange or pink bellies after feeding show sufficient food intake by the post- larvae when using *Artemia*. Prior to each feed administration, silt, dead larvae and feed debris should be removed from the tank. This is essential for successful grow out of fry considering the important role of olfaction in sturgeon feeding. In order to correct the daily ration in each tank, the dead larvae are enumerated and subjected to teratological and morphological analysis.

10.3.5 Instructions for Artemia preparation

Artemia cysts can be obtained from multiple sources and locations. It is recommended to use high grade cysts with hatching rates above 90% and conical tanks for easy harvest (see Figure 10.5.) A good hygiene regime is required, such as disinfecting tanks after each harvest, in order to maintain high hatching rates

and avoid nauplii die-offs. It is recommended to start *Artemia* incubation five to seven days before the expected transit to exogenous feeding to be able to start timely feeding. Unused *naupliii* can be frozen as back-up for shortfalls.

Figure 10.5: *Conical Artemia hatching jars (Photo credit: T. Friedrich)*

For incubation:

- Mix freshwater and uniodized/seawater salt in a ratio of 40:1 (add 25-36 g salt/L H_2O)
- Add 1.5-2.5 g of Artemia cysts per L
- Temperature of 26-29 °C (depending on cyst origin. 30 °C should not be exceeded)
- Provide constant aeration and light
- Hatching and harvesting after 24-26 h

After hatch the nauplii can be harvested. It is important to separate live nauplii from unhatched cysts and empty egg-shells, as the intake of eggs/shells by the sturgeon larvae can lead to constipation in the digestive tract and high mortalities. While empty shells float, unhatched cysts will aggregate at the bottom of the tank, with live nauplii in the layer above. By using conical jars, it is possible to drain the different layers, separating the entities. Aeration should be turned off for 15-20 min before harvest. Another time-saving option to obtain very clean nauplii is the use of cysts readily covered with iron particles and using magnets to separative live nauplii from unhatched cysts and shells.

The nauplii can then be rinsed with freshwater in a sieve with a mesh size of 150 µm, then fed immediately or transferred to holding tanks or automatic feeders with cool fresh saltwater to avoid depletion of the nutritional value of the cysts due to high metabolism rates at incubation temperature.

10.3.6 Transition between feed types and further feeding regime

The long-term application of live food (especially of one prey species) is not feasible in terms of economic efficiency and can hamper the further transition of fry to artificial feeds which is normally desired in commercial aquaculture. Therefore, the portion of live food in the ration is gradually reduced from 100 percent to 0–10 percent after 12-15 d with formulated diets replacing the live food.

To train juveniles intended for release to the wild to natural diets, an intermediate step can be the prolonged additional administration of live or commercially available frozen prey organisms depending on the species preferences and juvenile size, such as *Daphnia sp. Mysis sp.* Chironomidae, Chaoboridae, Culicidae, Gammaridae, Tubificinae, adult *Artemia salina* or marine krill until the release. Depending of the release date and duration of rearing, a sole use of these feeds can become economically unfeasible for release programs at a certain point due to the fed conversion rate and may also be logistically challenging due to the high amounts needed. Therefore, a mix of both administration of live/frozen feeds and artificial diets may offer a solution. A weaning to formulated diets at a TL of 50-60 mm and a subsequent ratio of 1:1 frozen food to formulated diets until a TL of 100 mm, succeeded by a weekly reduction to 1:9, considering the different feed conversion rates, has proven to be economically feasible for *A. ruthenus* in a river water rearing facility and did not result in any changes in performance in the captive environment.

Fingerlings destined for broodstock replenishment should be weaned on dry feeds as outgrowing with live/frozen feeds cannot be accomplished in an economical way. Still, occasional feeding with live/frozen feed may contribute to health and gonad development in all species.

Weaning the fish to formulated diets works best with semi moist feed since it resembles the texture of the natural diets. The protein and lipid concentration ranges in larval compound feeds should be 50–60 percent and 9–16 percent, respectively. For accurate ration formulation, one can follow the feed rations recommended by producers of special sturgeon compound feeds.

Generally, compound sturgeon feeds are composed of around 45% high quality raw protein, 10-20% raw fat and 15-20% carbohydrates. However, the rearing targets in commercial farms (fast growth, caviar yield) and e*x situ* hatcheries (fitness in the wild, long term broodstock development) differ and hence the selection of suitable compound feeds has to take the local situation, species and rearing target into account.

Table 10.4: *Feeding outline from fingerling size. Feeding amounts, frequency and pellet sizes may vary between species, temperature and local situation (modified from Chebanov & Galich, 2011; Steinbach, 2021).*[1,2]

Fish size, g	Min. feed rate, %BW/d	Max. Feed rate, %BW/d	Feeding frequency, times/d	Pellet size, mm
0.5-2 g	5	10		0.5-0.8
2-5g	4	5		1.2
5-10g	3.5	4.5		1.2-1.5
10–50	3	4.5	10	1.5
50 –100	2	3	8	2.0
100–200	1.5	2	6	3.0
200–800	0.5	1.2	4	4.5
800–1–500	0.3	0.8	4	4.5
1 500–3 000	0.2	0.8	3	6.0
3 000–5 000	0.1	0.5	2	6.0
5 000 –10 000	0.1	0.4	2	8.0
> 10 000	0.1	0.4	1	8.0-12.0

[1] This feeding advice is a guideline based on an optimal water quality and oxygen levels.

[2] BW = body weight.

For some species such as *A. sturio* however, diet composition remains a bottleneck for successful reproductive broodstock development. Commercial compound feeds need further development for optimal health gonad development and target species specific requirements.

The daily feeding rates for compound feeds are age dependent and are thus calculated every week, considering the temperature, average weight and fish number. The determination of mean weight can be performed on a subsample to adjust feeding rates more precisely. The original stocking number and subsequent mortalities should be documented to calculate feed ratios. At transition to larger particle size, the larger pellets should be gradually mixed with those of previous sizes depending on juvenile size.

After each feeding delivery, checking of the feed consumption should be performed. If a large quantity of uneaten feed is recorded, the feeding schedule and the state of the fish should be checked. The daily rate should then be adjusted after determination of possible causes of the weak feeding activity. Recommended frequencies of feeding and particle sizes are presented in Table 10.4.

Small automatic feeders (Figure 10.5.) equipped with a control for regular supply of feed into the tanks have been routinely used at large hatcheries. After each feed administration, the rate of feed consumption should be monitored. If a considerable amount of uneaten feed is found, feeding regime as well as the state of the fish and the daily ration are checked for possible causes of weak feeding activity.

Regardless of feed types, it has to be made sure that particles do not float on the water surface as several sturgeon species then tend to feed inverted from the surface, swallowing air in the process, which can lead to air bubbles being entrapped in the body. Fish with air bubbles often develop malformed spines, leading to the loss of ability to feed and hence mortality.

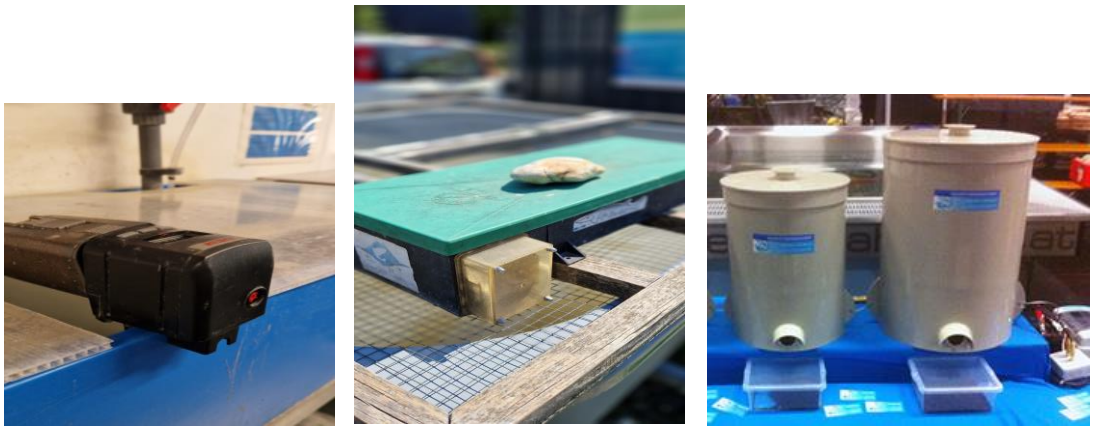

***Figure 10.5**: Different types of automatic feeders for dry feed administration (Photo credit: T. Friedrich)*

10.3.7 Control of feed consumption and fry sorting

In the process of grow out, the stocking densities and size structure of the sturgeon in each tank should be monitored. Once substantial variations in sizes within one tank can be observed due to increased feed competition, sorting should be performed. The timely sorting allows:

- increased growth rate;
- decreased size variation of fry;
- improved FCR, hence better feed availability for fry; and
- decreased trauma associated with feed competition.

Sorting of fish very much depends upon the design of the *ex situ* program (e.g. keeping families/cohorts separate or not) and the available tank infrastructure (especially if small mobile systems at the river banks are used). While there are

automatic graders for sorting fish, it is generally more practical to separate the different size classes visually by hand, especially in smaller streamside facilities. It has to be pointed out, that fish with slower growth and hence poorer performance in the hatchery may not automatically perform poor upon release in the wild. Therefore, if fish are being kept for broodstock replenishment, it is advised to keep animals from all grades.

10.3.8 Tank rearing and stocking densities

Sturgeons are benthophagic fishes with an active feeding response. This is manifested through their feed search behavior during which the fish search the available habitat by moving in concentric circles during which the bottom is investigated by contact with the barbels. The stocking density of sturgeon in tanks, net cages and small ponds are given in Chapter 5, Table 3. The numbers given are maxima derived from commercial production, for juveniles intended for release and systems running with river water, it is recommended to operate at 50-70% of the given values.

At the given stocking densities, it is essential to provide a water exchange rate of tanks of 2-5 times per hour at transition to exogenous feeding, up to 10 times per hour at rearing up to 2–5 g. Oxygen content in this case should be above 6.0 mg/L; concentrations under 3.0 mg/liter cause death in early life stages. The threshold concentration of ammonium should be as low as 0.05 mg/L. Excessive free ammonia resulting from pH elevation during the process of fry rearing causes serious cases of autotoxicosis, which can manifest as gill necrosis and damage to the skin and fins and can lead to mass mortalities.

The rearing of fingerlings generally follows the given feeding regimes and stocking densities but may be adapted to species specific traits and requirements as well as to local conditions. As most species jump frequently, tanks have to be covered with lids or nets. In order to simulate diffuse light conditions at the river bed, opaque acrylic sheets can be used as lids. Handling of the animals should be reduced to the necessary minimum and follow along the outline given in Chapter 4.

10.3.9 Rearing for broodstock replenishment

If the e*x situ* program depends upon or intends to build a captive broodstock, it is necessary to maintain the genetic diversity of the progeny. Hence a share of each reproduction will be kept to act as broodstock replenishment. In accordance with different conservation units and population segments (see Chapters 6 and 8) it is necessary to differentiate the various families. In order to take a random sample of each family, it is recommended to only separate the broodstock candidates prior to the release of the cohort. Ideally a 50:50 sex ratio is maintained. While sexing in most species was not possible at young ages in the past, the use of genetic sex markers (Kuhl *et al.*, 2021) now enables more targeted and efficient broodstock replacement but requires differentiation of individuals through individual tagging. Double tagging, such as color tagging with VIE for families/cohorts and PIT tags

for the individuals can ease hatchery operation (see also chapter 13 on tagging). Numbers of animals to be maintained to replace one fish, considering mortalities are given in in Chapter 5. For species on the very brink of extinction or with very long maturation cycles, the numbers to be kept can be significantly higher. On the other hand, later surplus animals can be included in release programs or shared with other entities. It is recommended to divide broodstock replenishment to several locations as an additional fail-safe measure.

10.4 Rearing of broodstock

Replenishment of broodstock by offspring of controlled reproductions follows the general principles of sturgeon farming (Chebanov & Galich, 2011; Hochleithner & Gessner, 2012; Williot *et al*. 2018) and can be carried out in tanks, raceways or ponds, with stocking densities like those of broodstock in Chapter 8 and under conditions that prioritize welfare of the animals. Safety and risk reduction protocols are critical to safeguard the different genetic entities. As the main goal is the provision of high-quality gametes, special emphasis must be given to the feeding regime of future broodstock candidates.

There is information available on sturgeon nutrition, especially for juveniles (e.g. Amrkolaie, *et al*. 2012; Hung 2017; Falahatkar, 2018; Lee, *et al*. 2021, 2022; Iegorov, et al. 2022), including probiotic, prebiotic and synbiotic supplements (e.g. Hoseinifar, *et al*. 2016; Mocanu, et al. 2022; Puri, et al. 2022). In general, during the immature and long duration pre-vitellogenic phases of female gonadal development a high protein (42-52%) and high fat (15-18%) diet is commonly used to ensure initial fast growth to reach the size/age when vitellogenesis is about to begin. However, it is then important to change the feeding regime to lower fat content diets (8-10%) to minimize excessive fat accumulation in the gonad (Fedorovykh, et al. 2015; Gille, *et al*. 2017). Chebanov & Galich (2019) reported that sturgeon maintained on high fat diets may have delayed onset of vitellogenesis or disturbances in gametogenesis, such as gonads with proliferating adipose tissue but very little oocyte development (see Chapter 11, Annex 11.2). However, this dietary regime may not be optimal for all sturgeon species, as continued lipid levels of 16-18% were reported to promote *Acipenser sinensis* ovarian development through vitellogenesis and spawning (Du, *et al*. 2018; Leng *et al*. 2019). Although the primary focus has been on female gametogenesis the future male broodstock should also be fed a lower fat diet as they begin to mature. Delayed gametogenesis can also occur when sturgeon are fed diets that are unbalanced in polyunsaturated fatty acids, such as, a ratio of omega-3/omega-6 fatty acids of less than two. There are numerous studies on the positive effect of different dietary fatty acids on growth, for different species of sturgeon (e.g. Li, *et al*. 2017; Falahatkar, *et al*. 2018; Wang, *et al*. 2019; Jafari, *et al*. 2024) and their impacts on sperm, egg and offspring quality (e.g. Luo, *et al*. 2015; 2017).

The use of ultrasonography (see Chapter 8) can be used to identify the females and males that have gonads with a high proportion of adipose tissue (Annex 8.2)

so they can be moved to low fat diet tanks. This assessment and sorting of females could significantly reduce delays in final maturation and reduce the proportion of mature females with high fat ovaries. In addition, overfeeding must be avoided which can contribute to excessive fat accumulation in gonadal tissues and a day of fasting per week is regarded as beneficial (Steinbach, 2021). It can also be favorable or even required to supplement formulated diets with natural items. When available, excess fish (e.g. Cyprinidae, Gobiidae, Percidae) from pond farming or fisheries for predatory sturgeons such as *Huso sp.* as well as invertebrates from commercial harvest can be used to rear animals for broodstock replenishment, however security of supply as well as possible adverse effects of the administration of live or frozen natural diets such as quality issues, transfer of parasites or pathogens as well as environmental pollution have to be taken into consideration.

10.5 Monitoring, hygiene and health control

The provision of adequate water quality and hygiene regime is the prerequisite for healthy animals. Water quality parameters follow the standards of cold-water aquaculture (Chebanov & Galich, 2011; Hochleithner & Gessner, 2012) with the most important parameters given in Chapter 5.

Strict daily documentation of several water parameters such as temperature as well as mortalities, fish transfers, feeding regime, cleaning activities help to identify problems and improve hatchery operation.

Weekly tests of alarm- and back-up systems ensure that the systems are up-and running during emergency situations. A cleaning schedule for filter systems, intake structures and piping can solve problems before they occur. Frequency has to be adapted to local conditions (frequency of floods, high organic load, high sediment load, etc.).

Regular observation of the fish is crucial in order to identify irregularities which can lead to problems and hence its necessity cannot be overstated. Unfortunately, daily hatchery routines and maintenance often leave little time, therefore it is recommended to make time in the schedule to focus on watching the animals. Fish with unusual behavior or altered external appearance can easily be detected and can be inspected more closely. For larvae, as well as smaller animals <3 cm TL it is recommended to check a subsample of 20-100 animals on a white laboratory tray or dish for behavior and appearance every three days. Regular supervision through local veterinarians either through visits of the facility and periodic provision of a few animals to a veterinarian lab for routine controls on pathogens and parasites should be budgeted for a best-practice modus operandi.

Hygiene standards are obligatory and must be executed carefully. This concerns daily cleaning of tanks, disinfection of equipment and hands and prevention of cross-contamination between rearing units. Dead fish, debris, uneaten feeds and faeces should be removed at least twice daily. During the summer months it is

recommended to clean the biofilm in the tanks on a daily basis, while in winter once to twice per week may be sufficient depending on fish sizes, stocking densities and water temperatures. An option is the use of a sponge soaked in 0.01% peracetic acid solution and wiping the surfaces of the tanks slowly from the inlet to the outlet. During the early rearing phases cleaning has to be conducted very carefully in order not to crush any larvae. During the yolk-sac-stage photoactivity can be used to lure the larvae to certain spots for easier facilitation of the cleaning process.

The use of chemicals and disinfectants is subject to local laws and regulations. In the case of a stream-side hatchery, in most cases it is necessary to obtain a permit for water use, further specifying usable treatments. See also Chapter 11 on fish health.

Gear such as handnets, sponges, tubes etc. should be disinfected for at least one hour with a suitable disinfectant and then rinsed with water after each use or before being transferred to another hatchery unit. Ideally, often used gear is assigned to individual tanks and not transferred in between.

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Chapter 11: Health Assessments and Treatments

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11.1 Introduction

The objective of this chapter is to provide a general overview of potential diseases and health issues that may arise in an *ex situ* facility while holding and rearing sturgeon. Pathogens are found among both wild and captive populations of sturgeon. The severity of the diseases that can manifest from pathogens can range from mild effects with perhaps minor impacts on growth to diseases that can cause significant mortality. The majority of sturgeon diseases (environmental, viral, bacterial, fungal, protozoal) are similar to those affecting other cultured finfishes, and their diagnostics, prevention and treatment are reviewed in detail in many published works (e.g. Dar *et al.* 2022; Hadfield & Clayton, 2021; Smith, 2019; Austin, 2012; Noga, 2010).

An important component in health assessment of your fish is the required daily observations of normal fish behavior in each tank at your facility. Depending on the type of rearing systems utilized, this may be accomplished by an initial facility walk through while checking on each tank for water flows, aeration, clogging or broken internal standpipes, etc. or it can be done in combination with another daily activity, like flushing the tank or adding feed to feeders. Once the facility staff are experienced in seeing the normal behavior of their sturgeon species, the clinical signs of disease (see descriptions in Tables below) are usually quite apparent. Always check for decreased feeding activity (waste feed on the tank bottom), which could be an initial sign of the onset of a problem.

Biologists with a general knowledge of fish diseases and access to reference texts and a basic laboratory with a compound microscope can identify most parasites and some bacterial infections, but in most cases, especially for viruses, diagnosis requires analysis of moribund individuals taken to a properly equipped fish disease laboratory or the submission of tissues samples (e.g. collected from large broodstock).

11.2 Prevention and Management

The priority in any fish culture facility is to prevent disease outbreaks in the first place by implementing biosecurity measures, sanitation and disinfection protocols, and reducing any environmental and handling stressors, including:

- Use of quarantine tanks for any new fish brought to the facility, with subsequent monitoring and/or disease diagnostic testing, depending on the facility and program guidelines/requirements.

- Implement rigorous disinfection protocols, including foot baths before entering and exiting each building.
- Avoid cross-contamination of equipment (e.g. nets, tank scrubbing poles, gloves, waders); use disinfection baths before reusing in a different tank.
- Reducing pathogens in the water source. Whether ground water or surface water, treating with fine filtration and then UV for disinfection will significantly reduce the pathogen load. A recirculating aquaculture system (RAS) should have a disinfection system installed after the mechanical and biological filtration. Maintain UV sterilizers by regular bulb changes, as the manufacturers recommend, and schedule regular cleaning of the quartz sleeves.
- Regular flushing or siphoning of tanks for removal of organic matter (feces and waste feed). For smaller larval and juvenile tanks, the standpipes, tank sides and bottom should be scrubbed as needed (when algae, biofilms are obvious), although larger (> 3 m diameter) tanks can usually be designed for self-cleaning.
- Prompt removal and disposal of dead or moribund fish.
- Provide optimal water quality parameters (see Chapter 5).
- The RAS systems have unique protocols to maintain optimal water quality (e.g. regular removal of captured solids, backwashing filters, proper aeration of biomedia).
- Maintain low stocking densities.
- Live feeds are an important source of nutrients and are needed for training fish for release, but they may also be a source of pathogens.
- Store commercial feeds in cool and dry conditions and use before expiration dates to avoid potential loss of nutrients and buildup of mold or other toxins.
- Minimize and optimize handling and transport of fish.
- Apply health assessments using established "normal" hematological and biochemical reference value intervals (Annex 11.1) and by using ultrasonography diagnostics (Annex 11.2).

11.3 Environmentally Caused Diseases

The most common non-infectious/environmental diseases are related to water quality and include dissolved oxygen, gas supersaturation, temperature and pH stress, and toxicity from ammonia, nitrites, nitrates, chlorines, heavy metals, hydrogen sulfide, and pesticides (Hadfield, 2021a). Prevention of these environmental issues is addressed through facility design, and diagnosis can occur through water quality testing and monitoring (Table 11.1).

Table 11.1: *Clinical signs, diagnosis, and prevention of common environmental diseases in sturgeon culture (modified from Hadfield, 2021a). Sudden and extreme changes in the environmental conditions can result in high rates of mortalities in both flow-through and recirculating aquaculture systems (RAS).*

Environmental Disease	Clinical Signs	Diagnosis	Prevention
Low Dissolved Oxygen	Lethargy, flared opercula, gasping at the surface.	Routine monitoring with a dissolved oxygen meter.	Maintain adequate aeration, and/or direct oxygenation. Consider packed column aerators, aeration cones or Venturi oxygen injectors.
Gas Supersaturation	Gas emboli visible in the skin or fins, lethargy.	Use of a total gas pressure meter.	Use of a degassing apparatus which is required for groundwater sources.
Temperature Stress (primarily elevated temperatures)	Increased or decreased activity, decreased feeding.	Daily water temperature monitoring.	Add heat exchangers or chillers, change to a cooler water source.
pH Stress (primarily in RAS)	Increased activity or tremors, rapid breathing, increased mucus production.	Routine monitoring of pH and alkalinity.	Partial water changes, add calcium hydroxide or sodium hydroxide slowly and monitor closely.
Ammonia Toxicity (primarily in RAS)	Lethargy, inappetence, rapid breathing, increased mucus production.	Routine monitoring of total ammonia nitrogen and then calculation of the unionized form based on pH and temperature.	Daily removal/flushing of organic matter, increase nitrifying bacteria biofilters (in RAS), reduce stocking densities. Short-term: adding zeolite (freshwater systems).
Nitrite Toxicity (primarily in RAS)	Rapid breathing, tan or brown gills.	Routine monitoring with commercial test kits.	Denitrification filters should be planned for the maximum bioload.
Nitrate Toxicity (primarily in RAS)	Lethargy, inappetence, rapid breathing, gills pale or tan-colored.	Routine monitoring with commercial test kits.	Denitrification filters should be planned for the maximum bioload.
Heavy Metal Toxicity	Variable: lethargy, loss of equilibrium, inappetence, rapid breathing, acute or chronic mortalities.	Water sources should be tested in a commercial lab prior to use.	Water high in heavy metals should be run through activated carbon, zeolite, or other ion-exchange filters.
Hydrogen Sulfide Toxicity	Lethargy, inappetence, rapid	Distinct sulfide (rotten egg) smell.	Groundwater should be degassed and aerated

	breathing, acute mortalities.	Levels can be measured with test kits.	prior to use. Pond water sources should be monitored for bottom sediment stirring and mixing of anaerobic conditions.
Pesticide Toxicity (usually runoff into ponds)	Inappetence, erratic swimming, seizures, rapid breathing, acute mortalities.	Monitor runoff into ponds from nearby agriculture fields.	Prevent runoff from entering ponds (e.g. higher levees, bypass channels).

11.4 Parasites

Sturgeons are hosts to many ectoparasites, many of which are common to other species of fish (Hadfield, 2021b). Protozoan parasites (single cell eukaryotes) often infest the skin and gills of sturgeon and are detected in fresh scrapings of the skin or mounts from sampled portions of gill lamellae. More common parasitic diseases in sturgeon are caused by *Trichodina* spp. *Chilodonella*, *Costia*, *Epistylis* (Radosavljević *et al.* 2019) and an extensive review on the numerous potential parasites of sturgeon was conducted by Bauer *et al.* (2002). Most recently, Barzegar *et al.* (2023) reviewed 26 known parasitic protozoan species from freshwater fish across Iran, including the bloodborne *Haemogregarina acipenseris* and *Trypanoplasma acipenseris* in both *Acipenser persicus* and *A. stellatus*. These authors also reported the less commonly found (for sturgeon) *Ichthyophthirius multifiliis* in these two *Acipenser* species and *Huso huso*.

Metazoan parasites (multicellular eukaryotes), including species of Monogenea, Trematodes, Cestodes and Nematodes, have been found only occasionally in sturgeon (Bauer *et al.* 2002), but most recently, a *Sacphirhynchus albus* mortality event at an *ex situ* hatchery was due to the Monogenean (flatworm) *Gyrodactylus conei* (Leis *et al.* 2023). Some of the more common clinical signs and treatments for parasites are given in Table 11.2. Any immersion treatments that have not been successfully used before should be tested with a small number of fish in a separate tank to ensure the dose and duration do not result in any direct mortalities, as some species and life stages could require lower dose treatments. Appropriate personal protection equipment must be used when handling any hazardous chemicals, such as formalin. Prophylactic UV treatment of the facility water at 26-318 mJ/cm^2 @ 254 nm will inactivate many parasites (Yanong, 2009).

Table 11.2: *Potential clinical signs and treatment guidelines for protozoan and metazoan diseases in sturgeon culture. Treatments are through a prolonged immersion (or bath) that are repeated as needed. Ensure that the treatments are approved within the region and prescribed by a veterinarian where appropriate.*

Clinical Signs	Treatments	References
Inappetence, flashing, erratic swimming, fin and gill damage, hyperactivity, increased mucus production, increased ventilation rate, hemorrhagic areas (erythema) or skin ulcers, secondary infections may occur.	Formalin (37%, Formacide-B, Parasite-S®), 100-200 mg/L for 1 h. Chloramine-T, 2.5-20 mg/L for 1 h. Iodine-free salt (NaCl) 0.5-1.5% for 1 h. Hydrogen peroxide 3-100 mg/L for 30 min – 1 h.	Vergneau-Grosset & Lair, 2021 Leis *et al.* 2023 Vergneau-Grosset & Lair, 2021 Hochleithner & Gessner, 1999 Vergneau-Grosset & Lair, 2021

11.5 Bacterial Diseases

Most bacterial diseases reported for sturgeon are opportunistic infections of gram-negative bacilli (*Acinetobacter* spp. *Aeromonas* spp. *Flavobacterium* spp. *Pseudomonas* spp. *Citrobacter* spp. *Vibrio* spp. *Yersinia* spp. *Pasteurella* spp. *Serratia* spp.), gram-positive bacilli (*Mycobacterium* spp. *Bacillus* spp.), and gram-positive cocci (*Streptococcus* spp. *Lactococcus* spp.) (e.g. Kayiş *et al.* 2017; Santi *et al.* 2019; Hadfield, 2021c; Chinchilla *et al*. 2023).

Antibiotic treatments in sturgeon culture are primarily delivered orally using medicated fish feed and require an antibiogram before treatment. Immersion treatments have been successfully used on small juveniles with bacterial gill disease (*Flavobacterium* spp.; Table 11.3), but this requires large volumes of water, and the potential human health and environmental issues when handling and disposing must be considered. Sturgeon will often have reduced feeding activity as a bacterial disease proceeds, so early diagnosis and treatment are important to ensure the infected fish consume the medicated feed. Long-term or repeated use of antibiotics must be considered carefully, as there is the potential for anti-biotic resistance to develop. Prophylactic UV treatment of the facility water at 4-22 mJ/cm^2 @ 254 nm will inactivate many bacteria (Yanong, 2009).

Table 11.3: *Potential clinical signs and treatment guidelines for bacterial diseases in sturgeon culture. Ensure that the treatments are approved within the region and prescribed by a veterinarian where appropriate.*

Clinical Signs	Treatments	References
Lethargy, abnormal behavior (floating, immobile), inappetence, hemorrhagic areas (erythema) or skin ulcers on various parts of the body (Figure 11.1), gill damage, pale or dark body color, pale gills, fluid accumulates in the body cavity, increased mucus production, poor body condition (emaciation), secondary infections (e.g. viral, fungal parasitic) are common.	Immersion: oxytetracycline 10-100 mg/L, 1 h, for 4 d. Immersion: hydrogen peroxide 60-110 mg/L, 1 h, for 5 d. Immersion: chloramine-T 20 mg/L, 1 h, for 4 d. Diet: florfenicol 10-15 mg/kg of fish for 10 d. Diet: oxytetracycline 60-80 mg/kg of fish for 10 d. Diet: oxytetracycline 10-15g/100kg feed for 10 d. Injection: oxytetracycline, 40 mg (active ingredient)/kg, same dose booster injection 2 d later. Injection: oxytetracycline, 100 mg (active ingredient)/kg, same dose booster injection 2 weeks later. Injection: florfenicol, 67 mg/kg, same dose booster injection 2 weeks later. Injection: Amoxicillin, 10 mg/kg.	Goodwin *et al.* 2005 Rach *et al.* 2000 Gaikowski *et al.* 2008 Vergneau-Grosset & Lair, 2021 Durborow & Francis-Floyd, 1996 Hochleithner & Gessner, 1999 Mohler, 2004 Webb *et al,.* 2016 Webb *et al.* 2016 Webb *et al.* 2016

11.6 Fungal Diseases

Bauer *et al.* (2002) reported the most common fungal pathogens of sturgeon to be *Saprolegnia* spp. *Achlya*, spp. *Dictyuchus* spp. *Aphanomyces* spp. and *Zeptolognia* spp, with the most frequent fungal diseases in sturgeon caused by Saprolegniaceae during during embryo incubation.

One of the most recently identified emergent fungal diseases in sturgeon (especially *A. transmontanus*) is *Veronaea botryose*, which is the causative agent of systemic mycosis known as "fluid belly" (Yazdi & Soto, 2021). The authors recommend proper disinfection of equipment and facilities due to the lack of effective prophylactic and therapeutic methods to combat this fungus. They suggest a dose of at least 75 mg/L iodine for 5 min, 390 mg/L sodium hypochlorite (household bleach) for 5 min, or 3.75% hydrogen peroxide for 30 min as being sufficient. Another recently reported infection was the identified yeast species, *Candida manassasensis,* which resulted in mass mortality of *A. baerii* (Kim *et al.* 2022).

Fungal disease during embryo incubation can be controlled when using upwelling incubators, as the infected embryo is more buoyant and can be siphoned out of the incubator before it spreads to other embryos, which can eliminate the use of chemical treatments. However, this method of removing infected embryo is not as effective for Yushchenko and other horizontal-style incubators (Ghomi *et al.* 2007). Prophylactic UV treatment of the facility water at 10-40 mJ/cm^2 will inactivate many *Saprolegnia* spp. (Hadfield, 2021d).

Table 11.4: *Potential clinical signs and treatment guidelines for fungal diseases in sturgeon culture. Ensure that the treatments are approved within the region and prescribed by a veterinarian where appropriate.*

Clinical Signs	Treatments	References
For eggs/embryos, they appear covered in whitish-grey cotton wool. For fish, skin lesions are grey or white, raised, and with a cotton wool appearance. Inappetence, erratic swimming or loss of equilibrium, and gill damage can also occur.	Embryos: hydrogen peroxide (30%) at 1 ml/L, 10 min/d, as needed. Embryos: hydrogen peroxide (35%) at 0.5 ml/L, 15 min/d, as needed. Embryos: formalin (37%, Formacide-B, Parasite-S®), 0.8-1.5 ml/L, 30-45 min/d, as needed. Embryos: Pyceze (bronopol, 2-bromo 2-nitropropane 1,3 diol), 50 mg/L, 30 min/d, as needed. Fish: hydrogen peroxide (35%, Perox-aid®), 75	Ghomi *et al.* 2007 Bouchard III & Aloisi, 2002 Rach *et al.* 1997; Abtahi *et al.* 2006 Sudova *et al.* 2007 Hadfield, 2021d

	mg/L, 1 h, every 48 h for 3 treatments. Fish: chloramine-T, 20mg/L, 15 min/d, for 18 d. Fish: Pyceze (bronopol), 20 mg/L, 30 min/d, up to 14 d. Fish: Iodine-free salt (NaCl), 5-15g/L, 1 h/d, as needed.	 Ghazvini *et al.* 2012 Sudova *et al.* 2007 McEnroe & Cech, 1985

11.7 Viral Diseases

Reports on viral infections in sturgeon have increased due to improved testing and biomolecular methods to identify the causative agent, although a virus can still be challenging to diagnose as there is often a lack of specific clinical signs. The risk of introducing a virus into a facility can be reduced using appropriate isolation and quarantine protocols, and transmission to and from wild and cultured fish is possible with all viruses and is often known as spill-over or spill-back (Hadfield, 2021e). The two main groups of sturgeon viruses, sturgeon nucleocytoplasmic large DNA viruses and herpes viruses, will be described here while the less common *Adenoviridae*, *Rhabdoviridae*, *Nodaviridae*, *Birnaviridae*, and *Reoviridae* have been reviewed by Mugetti *et al.* (2020a).

There are no vaccines available for any of the viruses listed below and the best treatment, as described above, is to prevent an outbreak in the first place to the extent that it is possible. Often a viral outbreak is not noticed until secondary infections occur (bacterial, fungal, parasitic), and those can be treated as described above. If the behavior of a potential virus is observed in a tank of fish (lethargy, abnormal behavior, lack of feeding), try to improve water quality (e.g. increase oxygen levels, water turnover) and check that all other water quality parameters are optimal. Feeding can be reduced or stopped depending on feeding activity observed and then slowly resumed after the virus has run its course. Treatments of salt are often used to reduce stress, increase mucus production, and help to heal damaged skin tissue. Dosages (5-15 g/L for 1-3 h) will vary depending on species and life stage, as small juveniles are less tolerant than adult fish. Prophylactic UV treatment of the facility water at 100-150 mJ/cm^2 @ 254 nm will help to inactivate many viruses (Yanong, 2009).

11.7.1 Sturgeon Nucleocytoplasmic Large DNA Viruses (sNCLDV)

These are the most numerous and heterogeneous group of viral agents causing disease in sturgeon (Mugetti et al, 2020a), including:

- White Sturgeon Iridovirus (WSIV) was first reported on several northern California *A. transmontanus* farms (Hedrick *et al.* 1990), with high stocking densities and stressors (e.g. pump failure, frequent handling and transport) identified as factors for the disease onset (Georgiadis *et al.* 2001). The clinical signs are primarily lethargy and lack of feeding (La Patra *et al.* 1994), and other species of *Acipenseridae* can also be susceptible.
- Missouri River Iridovirus (MRSIV) is associated with *Scaphirhynchus albus* and *S. platorynchus*. Symptoms included lethargy, emaciation, skin and fin inflammation (Mugetti *et al.* 2020a).
- Shortnose Sturgeon Virus (SNSV) was initially identified by LaPatra *et al.* (2014) as an irido-like virus and was later verified and named SNSV (Clouthier *et al.* 2015).
- British Columbia White Sturgeon Virus (BCWSV) was identified by Raverty *et al.* (2003) and symptoms included inappetence, lethargy and lack of reactivity.
- Namao Virus (NV) was identified in hatchery reared *A. fulvescens* and the symptoms included inappetence, anorexia, erratic behavior, skin redness, and excess gill mucus (Clouthier *et al.* 2013).
- Acipenser Iridovirus-European (AvIV-E) outbreaks have occurred is several European countries involving *H. huso, A. baerii, A. gueldenstaedtii, A. naccarii, A. stellatus, and A. ruthenus* (Ciulli *et al.* 2016; Bigarré *et al.* 2017; Mugetti *et al.* 2020b). Symptoms can differ between outbreaks, species and body size, and can include anorexia, lethargy, erratic or swimming impairment, skin ulcers, excessive gill mucus, and swollen abdomens causing them to float upside down (Mugetti *et al.* 2020a, 2020b).
- Frog Virus 3 has been rarely reported for *Acipenseridae* but was identified in a mortality outbreak of *S. albus* juveniles (Waltzek *et al.* 2014). Clinical signs included high rates of mortality (95%) with haemorrhagic lesions on the skin.

11.7.2 Herpesviruses

These belong to the *Alloherpesviridae* family and are the next most common viral diseases in farmed and wild sturgeon (Mugetti *et al.* 2020a). Symptoms vary from few or no obvious skin lesions to small whitish blisters or patches to obvious skin ulcers (Figure 11.1 and 11.2), and include the following:

- Acipenserid Herpesvirus 1 (AciHV-1) was first reported in mortalities of captive *A. transmontanus* juveniles but with no external clinical signs (Hedrick *et al.* 1991).

- Lake Sturgeon Herpesvirus 1 (LSHV-1) identified from four rivers in Wisconsin was determined to be like AciHV-1, yet distinctly different, and Walker *et al.* (2022) proposed the name lake sturgeon herpesvirus.

- Lake Sturgeon Herpesvirus 2 (LSHV-2) was isolated from infected wild *A. fulvescens* (with grossly apparent skin lesions) from the Lake Huron and Lake Erie watersheds and was similar to LSHV-1 (Johnston *et al.* 2022).

- Acipenserid Herpesvirus 2 (AciHV-2) was first reported from farmed *A. transmontanus* ovarian fluid, with the fish showing no clinical signs (Watson *et al.* 1995), but more commonly, ulcerative skin and fin lesions are present that sometimes end up with a co-infection of bacteria or fungus (Figure 11.3; Soto *et al.* 2017). This virus has been verified throughout North America and in other species including *A. brevirostrum* and *A. fulvescens* (Kurobe *et al.* 2008; Lapatra *et al.* 2014). It has also been identified in European species (e.g. *A. baerii*, *A. ruthenus* x *H. huso* hybrid) (Doszpoly *et al.* 2017; Shchelkunov *et al.* 2009).

- Acipenser Herpesvirus 3 (AciHV-3) was recently detected in hatchery-reared *A. fulvescens* displaying skin lesions that resembled blisters on the ventral surface of the pectoral fins and abdomen, but no unusual mortality was observed (Clouthier *et al.* 2023).

- Cyprinid Herpesvirus 3, also known as Koi Herpesvirus, has been reported in *A. oxyrinchus*, *A. gueldenstaedtii*, and the hybrid *A. ruthenus* x *H. huso* (Kempter *et al.* 2009). The fish had clinical signs of weak swimming and haemorrhagic lesions at the base of the fins, with pale to grey gills covered with a grainy deposit.

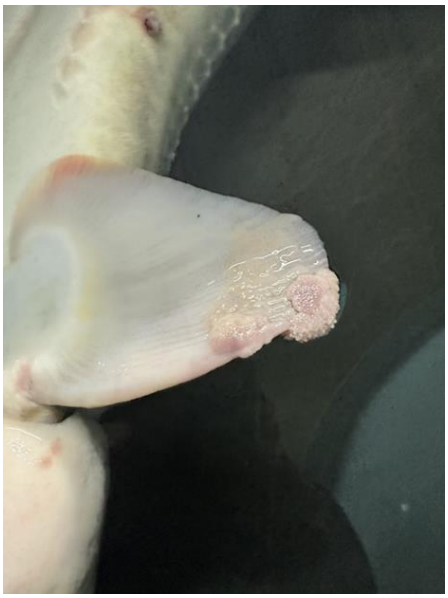

Figure 11.1: *Fin and skin lesions on a juvenile Sacphirhynchus albus diagnosed with pallid sturgeon herpesvirus (Photo credit: M. Webb).*

Figure 11.2: *Skin lesions associated with Acipenserid herpesvirus 2 infection on a juvenile Acipenser transmontanus (Photo credit: R. Hedrick).*

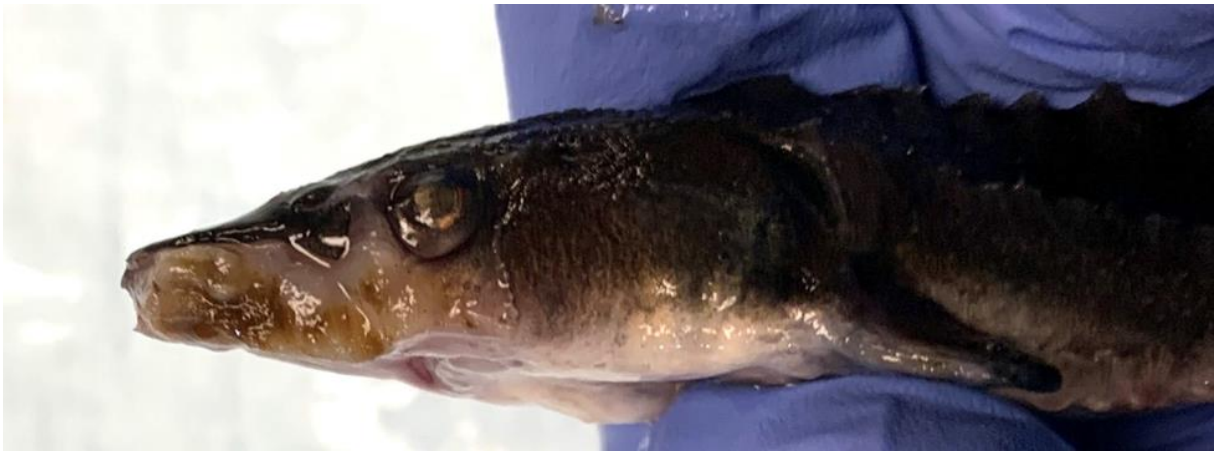

Figure 11.3: *Ulcerative skin lesion on the rostrum of a juvenile Acipenser transmontanus, diagnosed with a co-infection of Acipenserid herpesvirus 2 and Flavobacterium columnare (Photo credit: E. Soto).*

11.8 References

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Annex 11.1. Health Assessment using Hematological and Plasma Biochemistry Indices

The use of hematologic and biochemistry analyses of blood and plasma could help to identify changes in the health of sturgeon, and these diagnostic tools could also be useful in identifying the early stages of a disease outbreak or for assessing a response to a suboptimal environmental condition.

The problem is the very limited number of "normal" reference value intervals available for sturgeons, and only a few studies provide a comprehensive set of analyses (Table 11.1.1 and 11.1.2). But more importantly to consider is the numerous extrinsic (e.g. water quality, season of the year, feed contents, capture and handling stress) and intrinsic (e.g. species, population, age, life stage, sex, captive or wild) factors that can shift the "normal" reference intervals established. In addition, past studies have used different types of analytical procedures, different statistical analysis (e.g. parametric versus robust methods to determine reference intervals), and different presentation metrics (e.g. number of monocytes per microliter of blood or as a percent of total white blood cell count) making comparisons between and within a species challenging (see Table 11.1.1 and 11.1.2 for the variability). The other limiting factors of using blood and plasma indices is the required handling/stress to collect the blood samples, and the cost involved in having a commercial laboratory conduct the analyses. If a program is going to pursue using this diagnostic tool, it is recommended that each facility establish their own "normal" health assessment indices for their species under their culture or wild conditions for different life stages and sex to be able to compare to potential or identified "unhealthy" individuals.

There are a variety of reference intervals (some with only a few parameters measured and some more extensive) available for wild and captive sturgeon: captive (Sepúlveda *et al.* 2012) and wild *A. fulvescens* (Sepúlveda *et al.* 2012; DiVincenti *et al.* 2013), captive *S. albus* (Djokic, 2020), captive (Knowles *et al.* 2006) and wild (*A. brevirostrum* (Matsche & Gibbons, 2012; Matsche *et al.* 2013), captive *A. gueldenstaedtii* (Cassle *et al.* 2021), wild *S. platorynchus* (Sepúlveda *et al.* 2012), captive *A. oxyrinchus oxyrinchus* (Matsche *et al.* 2014), wild *A. stellatus* (Shahsavani *et al.* 2010), captive *A. persicus* (Bahmani *et al.* 2001; Milad *et al.* 2016), captive *A. schrenckii* (Shi *et al.* 2006), captive *A. sinenis* (Shi *et al.* 2006), captive *H. huso* (Bahmani *et al.* 2001; Asadi *et al.* 2006), and captive *A. naccarii* female x *A. baerii* male hybrids (Di Marco *et al.* 2011).

Table 11.1.1: *Hematology reference intervals for some species of sturgeon. Packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC; a measurement of the average amount of hemoglobin in a single red blood cell (RBC)), white blood cell (WBC), ratio of total neutrophils to total lymphocytes (N:L). Data are from: [1]Matsche et al. 2014 (combined data from three different facilities), [2]Knowles et al. 2006, [3]Matsche & Gibbons, 2012 (combined sexes and data from all years), and [4]Cassle et al. 2021.*

Analyte	**Captive *Acipenser oxyrinchus*[1]**	**Captive *Acipenser brevirostrum*[2]**	**Wild *Acipenser brevirostrum*[3]**	**Captive *Acipenser gueldenstaedtii*[4]**
PCV (%)	21-26	26-46	23-56	16-34
Hemoglobin (g/dL)	5.0-6.2	5.7-8.7		3.9-9.7
RBC count (x 10^6 cells/µL)	0.90-1.24	0.65-1.09		
MCV (μm^3)	196-256	307-520		
MCH (pg)	45-68	66-107		
MCHC (g/dL)	22-27	15-30		
Total WBC count (cells/µL)	21,000-33,700	28,376-90,789	2,028-8,749	5,440-13,770
Total lymphocytes (cells/µL)	12,500-22,700			1,650-7,500
Small lymphocytes (cells/µL)	11,400-20,600	9,063-56,656	178-1,614	
Large lymphocytes (cells/µL)	800-1,700	2,122-10,435	0-1,342	
Neutrophils (cells/µL)	1,600-7,900	3,758-33,592	459-5,208	850-4,470
Monocytes (cells/µL)	300-1,000	0-7,137	0-410	63-560
Eosinophils (cells/µL)	0-4,600	0-1,544	0-522	20-1,640
Thrombocytes (cells/µL)	28,300-40,700	32,205-122,179		
N:L ratio	0.12-0.58	0.68-1.03	0.21-3.63	

Table 11.1.2: *Plasma chemistry reference intervals for some sturgeon species. Aspartate transferase (AST) in units per liter (U/L), creatine kinase (CK), lactate dehydrogenase (LDH), plasma alkaline phosphatase (ALP), creatinine phosphokinase (CPK). Data are from: [1]Matsche et al. 2014 (combined data from three different facilities), [2]Knowles et al. 2006, [3]Djokic, 2020, and [4]hybrid sturgeon Acipenser naccarii female x Acipenser baerii male; Di Marco et al. 2011 (data are the 2.5-97.5 percentiles).*

Analyte	**Captive** ***Acipenser oxyrinchus*[1]	**Captive** ***Acipenser brevirostrum*[2]	**Captive** ***Scaphirhynchus albus*[3]	**Captive** **Hybrid Sturgeon[4]**
Total Protein (g/L)	27-39	27-53	24-35	18-72
Albumin (g/L)	10-15	8-17	8-14	8-25
Glucose (mmol/L)	0-1.6	2.1-4.1	2.6-4.6	2.6-13.3
Urea (mmol/L)	2.4-7.0			0.7-1.1
Calcium (mmol/L)	1.9-2.0	1.7-3.0	1.3-3.1	1.9-7.2
Phosphorous (mmol/L)	2.6-3.6	1.6-2.6	2.6-4.8	1.7-5.8
Sodium (mmol/L)	135-151	124-141	108-128	135-142
Chloride (mmol/L)	116-118	106-121	90-114	87-121
Potassium (mmol/L)	2.6-3.4	2.9-3.7	2.0-3.7	1.7-2.5
Globulins (g/L)	17-24	18-37		
Osmolality (m0sm/kg)	258-299	232-289		
Creatine (mg/dL)		0.0-1.4		0.01-0.57
Total bilirubin (mg/dL)		0.0-0.1		0.15-1.86
Cholesterol (mg/dL)		42-133	64-199	40-238
AST (U/L)	110-277	90-311		17-406
CK (U/L)	10-840			389-5,559
LDH (U/L)	1,811-4,223		90-434	724-4,040
ALP (U/L)		47-497	52-203	39-415

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Annex 11.2. Health Assessment using Ultrasound Diagnostics

11.2.1 Introduction

In *ex situ* holding and breeding of sturgeon species that are in danger of extinction, monitoring the individual state of the internal organs of broodfish and the subadults of long-lived and late-maturing sturgeon species is important. Some of these non-contagious disorders are associated with hereditary causes, feeding with artificial feed, water quality and exposure to toxicants. The difficulty in preventing or identifying these diseases is often the lack of any external manifestations for a long period of time.

11.2.2 Diagnostics

Chebanov & Galich (2011, 2018a, 2018b, 2019) provide the detailed descriptions of the procedures for assessment, identification, and visualization of the main signs and degree of pathological anomalies and diseases of various sturgeon species based on the interpretation of ultrasound sonograms. It should be noted that only a trained and experienced ultrasound technician in sturgeon anatomy can accurately identify some of the disorders described below, especially the diseases of the internal organs.

This Annex will review the most common disorders that are currently used in Russia as a basis for culling specimens from *ex situ* broodstocks in accordance with a new regulatory document (Ministry of Agriculture of the Russian Federation, 2020).

11.2.3 Common Disorders

11.2.3.1 Fatty ovarian tissue and decreasing reproductive capacity

Fish held at a constant high temperature and fed a constant high fat diet often have degeneration of germinal tissue and its subsequent replacement with fatty/adipose tissue. A sonogram reveals imitation egg-bearing ovigerous folds that are predominately adipose tissue (Figure 11.2.1).

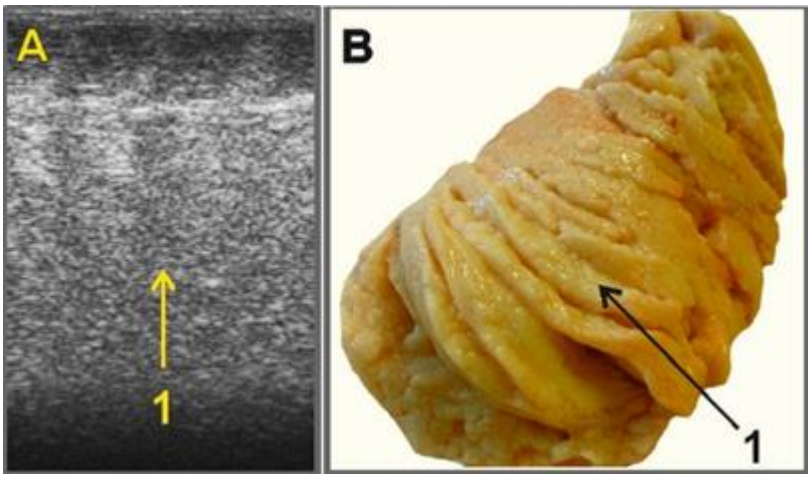

Figure 11.2.1: *Frontal sonogram of ovary of Acipenser baerii with fatty degeneration (A) and a necropsy fragment of the ovary (B), arrow 1 indicates fatty ovarian tissue (Photo credit: M. Cebanov & E. Galich).*

The incidence of fatty degeneration of Russian sturgeon gonads in the Volga-Caspian region in the mid-1990s was quite high, ranging from 8.0-12.3% of the fish sampled (Romanov *et al.* 2001). Another sonogram of an ovary with excessive adipose tissue shows that the ovary has lost its typical structure (Figure 11.2.2).

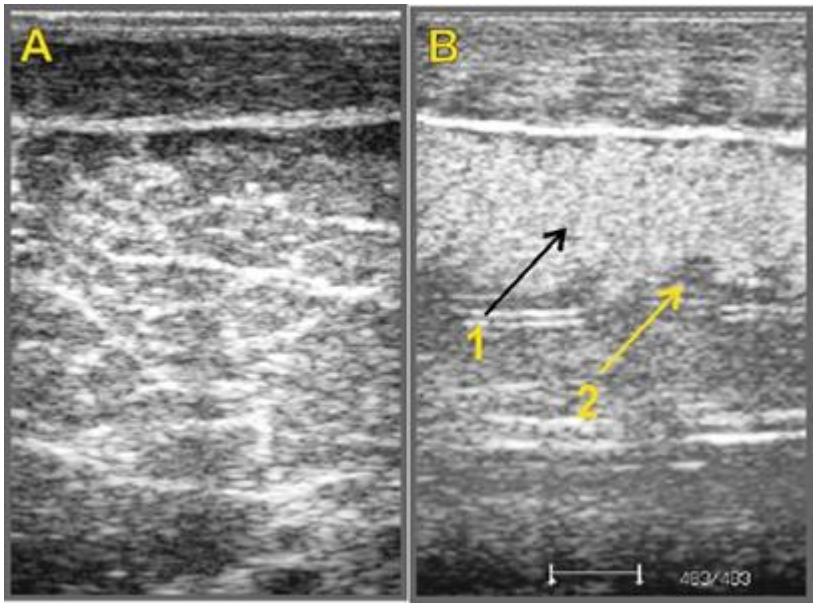

Figure 11.2.2: *Sonogram of a female Acipenser ruthenus ovary in a state of degradation (A) and a normal previtellogenic ovary (B): arrow 1 indicates ovarian tissue (whitish), and arrow 2 indicates ovarian fat (dark areas) (Photo credit: M. Cebanov & E. Galich)*

11.2.3.2 Asynchronous gonadal development

A serious disorder of gonadogenesis is the asynchronous development of the ovarian tissue (Figure 11.2.3). Kornienko (1998) reported its occurrence in the Azov basin and discussed how it could lead to a decrease in fertility and most likely the skipping of the next potential spawning. If this condition is prevalent on a sturgeon hatchery, it indicates unfavorable conditions for fish and the urgent need for optimization of rearing conditions (e.g. feed and feeding regime, fish density and grading, seasonal changes in temperature or wintering) and then subsequent ultrasound monitoring of these fish, because depending on the degree of asynchronous development, this is not always a reason for culling females.

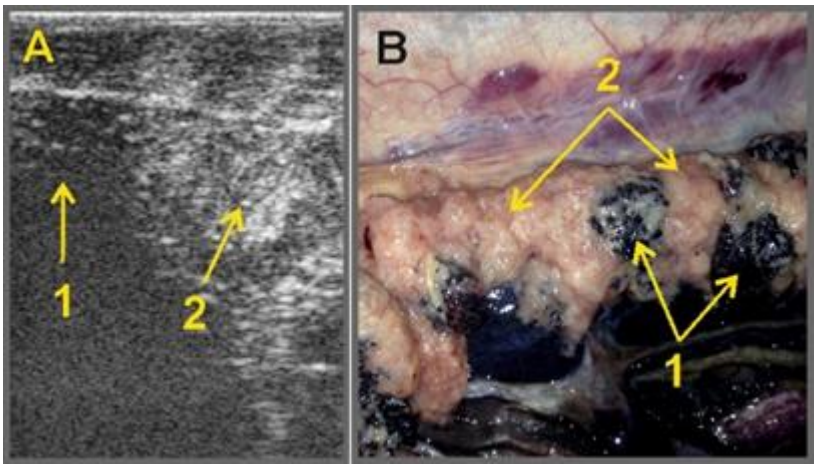

Figure 11.2.3: *Asynchronous development of an Acipenser baerii ovary. The sonogram (A) and the necropsy view of the ovary (B) reveal post-vitellogenic regions of the ovary (1) and early vitellogenic regions (2) (Photo credit: M. Cebanov & E. Galich).*

11.2.3.3 Atresia of spawning capable ovarian follicles

In recent years in the Azov hatcheries, the sensitivity of mature *A. gueldenstaedtii* females to warming temperatures has resulted in the increased incidence of follicular atresia. Clearly, one of the factors influencing this is climate-related warming and an increase in average monthly water temperatures during the winter and spring months of 1.5-2.0 °C. Timely, accurate identification of atresia in maturing fish is important for sturgeon hatcheries, and the use of ultrasonography has been used to identify this condition.

As can be seen in Figure 11.2.4 in the upper part of the ovary, the echogenicity increases compared to typical sonograms of mature females (Chebanov & Galich, 2011), the rows of ovarian follicles are disrupted (white stripes are the reflection off the chorions), which indicates deformation of some of the eggs, and the light (gray) areas indicate already resorbed ovarian follicles. These females are allowed to complete another cycle of maturation under improved rearing temperature regimes, and culling is advisable only if atresia occurs repeatedly.

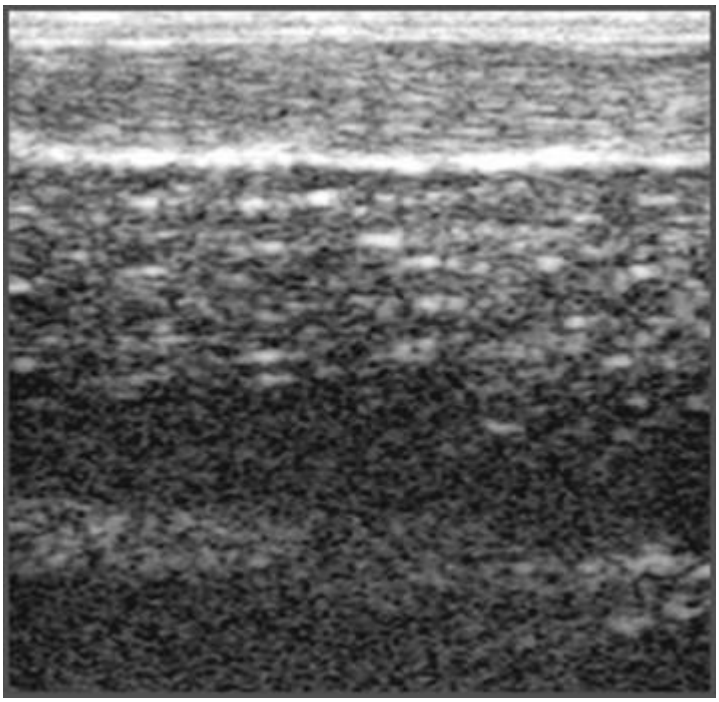

Figure 11.2.4: *Sonogram of an Acipenser stellatus ovary undergoing atresia (Photo credit: M. Cebanov & E. Galich).*

11.2.3.4 Replacement of ovarian tissue with connective tissue

The replacement of ovarian and testicular tissue with connective tissue (Figure 11.2.5) in various sturgeon species in natural water reservoirs was described for *A. gueldenstaedtii* in the Caspian Sea (Romanov *et al.* 1990, 2001; Romanov & Sheveleva, 1992); *A. baerii* (Akimova & Ruban, 1992), *A. gueldenstaedtii* and *A. stellatus* in the Sea of Azov (Kornienko, 1998); and for *A. schrenckii* and *H. dauricus* sturgeon (Koshelev *et al.* 2009). It has been established that this can lead to a decrease in their reproductive ability and potential formation of tumors.

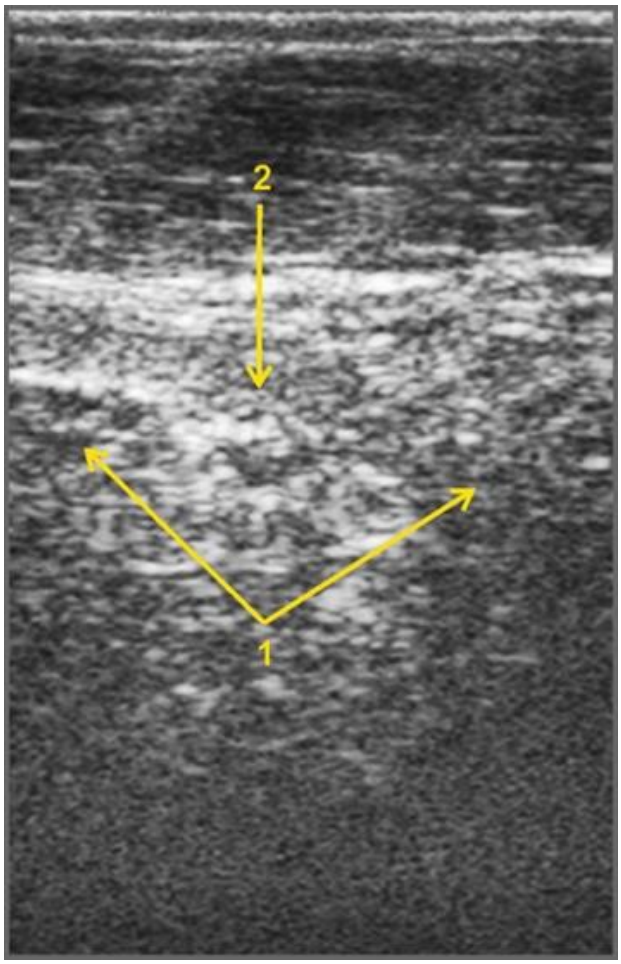

Figure 11.2.5: *A frontal scanned sonogram of an Acipenser gueldenstaedtii ovary with connective tissue (2; whiter streaks) within the ovarian tissue (1; darker regions) (Photo credit: M. Cebanov & E. Galich).*

11.2.3.2 Cysts in the gonads

Cysts are typically filled with liquid and have been observed in the ovaries of 1–3% of the Sea of Azov *A. gueldenstaedtii* (Kornienko, 1998). Those formed in the process of fatty degeneration of the egg-bearing ovigerous folds were organotypic tumors (Moiseyeva *et al.* 1997). The frequency of their occurrence in the Northern Caspian Sea in 1988 in relatively young individuals (5–6 years old) and first-spawning females (16–20 years old) was 8%. In *A. stellatus*, the proportion of females with a similar pathology was 11% (Romanov *et al.* 2001).

Cysts found in sturgeon can be single or multiple (polycystic). As shown in the sonogram of *A. ruthenus* (Figure 11.2.6A), the cyst is visualized as a round anechoic (dark) area encapsulated in an echogenic (white) envelope. However, the decision to cull females from the broodstock depends on the size of the cysts and their number. In cases of numerous megacysts (Figure 11.2.6B), the individual would be culled.

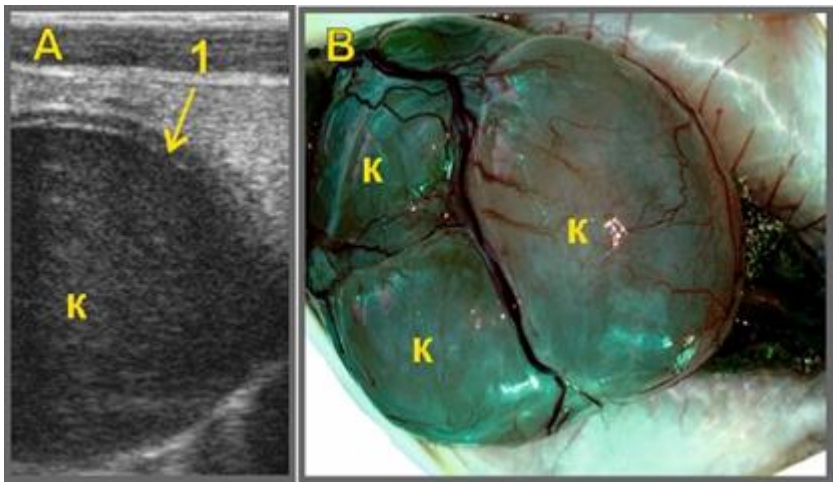

Figure 11.2.6: *Ovarian cysts in Acipenser ruthenus. The sonogram (A) shows the large, dark megacyst (K) surrounded by the white cyst envelope (1), and the necropsy photo (B) reveals a three-chambered, highly vascularized megacyst (K) (Photo credit: M. Cebanov & E. Galich).*

11.2.3.3 Abnormalities of gonadogenesis in sturgeon males

Often in sturgeon hatcheries, the monitoring of male maturation is carried out less carefully than that of the females, which may lead to having fewer males reaching spawnable condition. When holding males, it is important to maintain optimal temperature conditions in accordance with the stage of maturity, proper stocking densities and feeding regimes. It is important to limit the use of feeds with high quantities of soybean products as they can potentially be sources of phytoestrogens that can cause endocrine disorders in males (Bennetau-Pelissero & Le Menn, 2017). Degeneration of the testis is visualized on sonograms as a hypoechoic area with a disturbed structure (Figure 11.2.7), while the medial edge of the gonad is hardly evident due to greater absorption of ultrasound by adipose tissue (Chebanov & Galich, 2009, 2011).

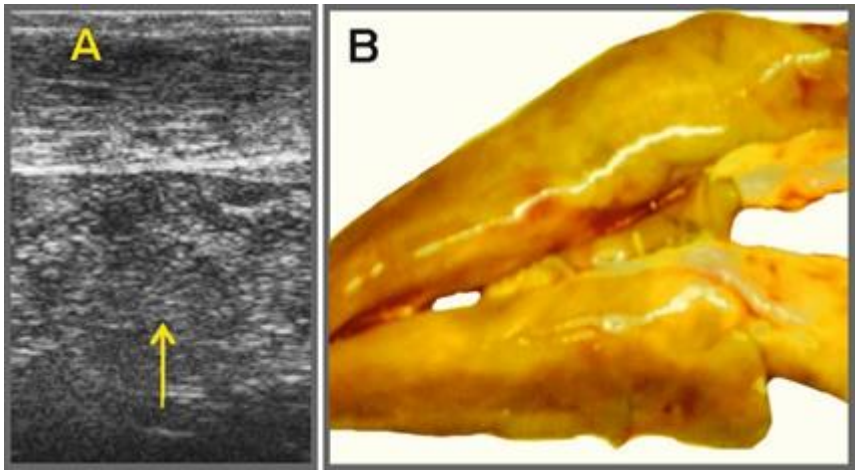

Figure 11.2.7: *Sonogram revealing fatty degeneration (arrow) of testes in Acipenser baerii (A) and a necropsied view of the testes (B) (Photo credit: M. Cebanov & E. Galich).*

11.2.3.4 Intersex

The appearance of intersex sturgeon is usually associated with disruption of hormonal regulation and an increase in endocrine disorders caused by the accumulation of toxic substances. Intersex fish are common at low frequencies in both wild and cultured populations of sturgeons, and the prevalence of intersex sturgeon has been described in the San Francisco Bay (<0.01%, *A. transmontanus*; Chapman *et al.* 1996), the Hudson River (1%, *A. oxyrinchus oxyrinchus*; Van Eenennaam & Doroshov, 1998), and the Mississippi River (3%, *S. platorynchus*; Carlson *et al.* 1985 and 2%, S. *platorynchus*; Colombo *et al.* 2007). The occurrence of intersex sturgeon in natural water reservoirs and sturgeon farms in Russia has ranged from 3 to 10% (Chebanov & Galich, 2017;), and in the Azov Sea basin, the occurrence of intersex sturgeon 25 years ago reached 4% (Kornienko, 1998). Intersex usually reveals itself as areas of ovary and testis (ovotestis) on the same gonad. Less common are fish in which one gonad is ovary and the other is testis.

There are synchronous and asynchronous forms of intersex. The asynchronous (non-functional) form manifests itself, for example, when small areas of testicular tissue are formed in a mature ovary (Romanov *et al.* 2001) or when small areas of ovaries with previtellogenic oocytes are found in the mature testes (Figure 11.2.8). For this reason, in some cases, ultrasound scanning of just small sections of the gonad can lead to erroneous determination of the sex of sturgeon. Scanning the entire length of both gonads is important.

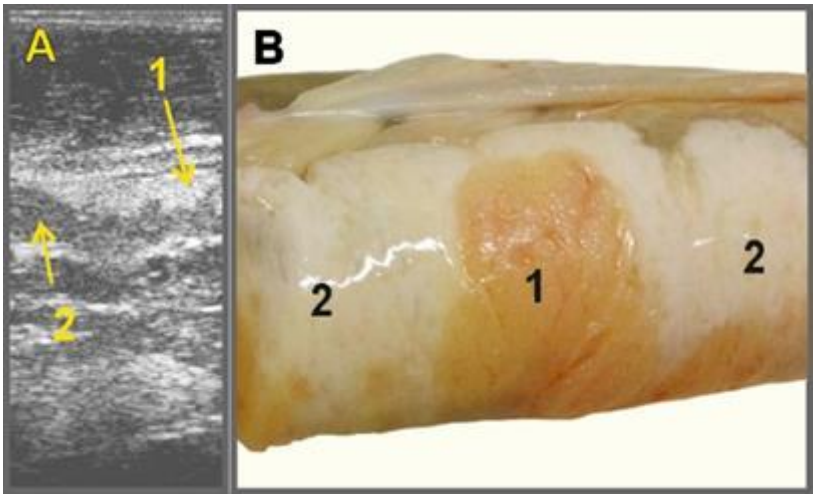

Figure 11.2.8: *An asynchronous intersex Acipenser baerii. The sonogram (A) has distinct regions of ovary (1) and testis (2), and the necropsy photo (B) clearly reveals the ovarian (1) and testicular (2) tissues (Photo credit: M. Cebanov & E. Galich).*

In a synchronous ovotestis (functional form), sections of spawning capable ovary and testes are observed (Figure 11.2.9). The "bilateral" intersex of *A. baerii*, when one gonad is an ovary and the other is a testis, is shown in Figure 11.2.10.

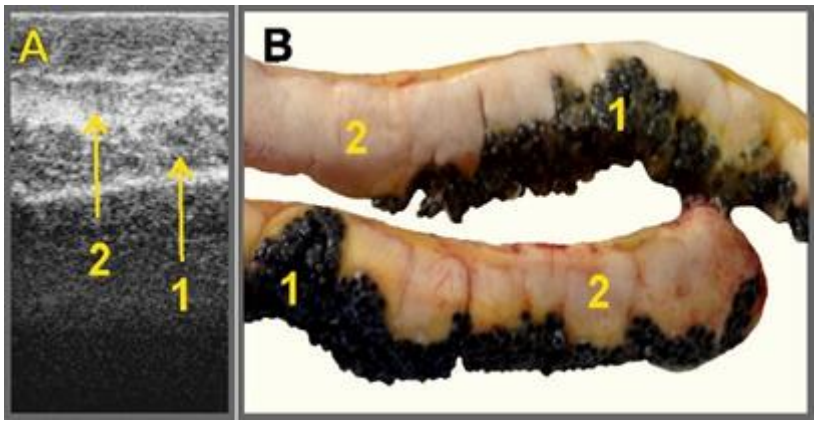

Figure 11.2.9: *The sonogram of a synchronous functional intersex Acipenser ruthenus (A) and the ovotestes necropsy photo. Oocytes (1) and testis (2) (Photo credit: M. Cebanov & E. Galich).*

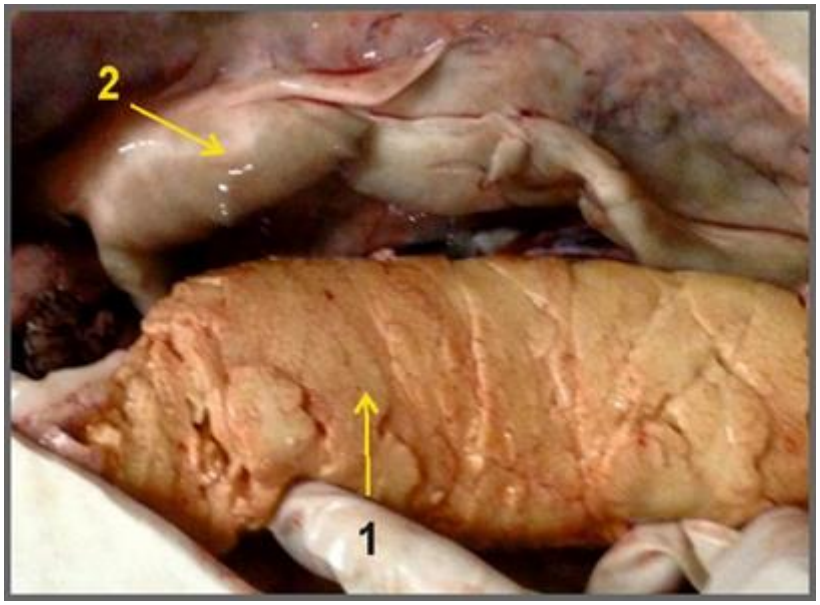

Figure 11.2.10: *Necropsy view of a bilateral intersex Acipenser baerii. Ovary (1) and testis (2) (Photo credit: M. Cebanov & E. Galich).*

11.2.3.7 Intersex with numerous neoplasms

After the resorption of oocytes in intersex fish, connective tissue growths are formed in the testes, which can transform into various neoplasms (Figure 11.2.11), cysts, tumors, and teratomas (Romanov *et al.* 2001). The interpretation of sonograms for such developmental disorders is like that for cystosis (Chebanov & Galich, 2018a, 2018b, 2019).

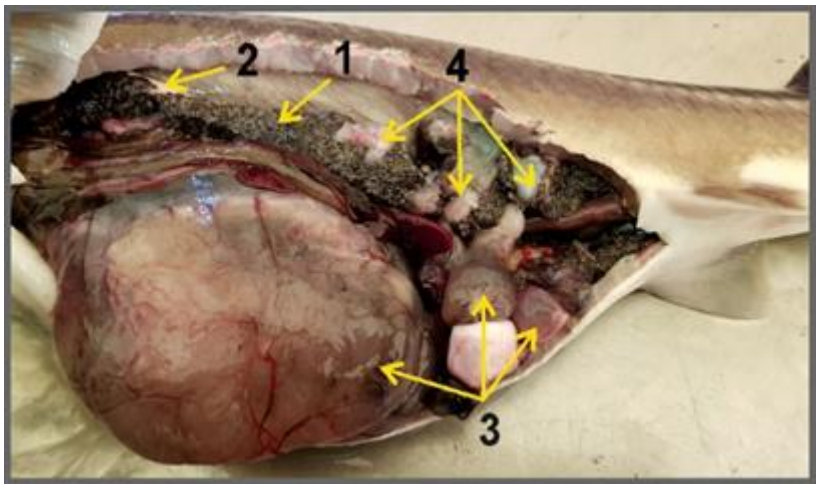

Figure 11.2.11: *View of a intersex Acipenser ruthenus with numerous neoplasms. Ovary (1), testis (2), cysts (3) and other neoplasms (4) (Photo credit: M. Cebanov & E. Galich).*

11.2.3.8 Diseases of the internal organs

The liver plays an important role in the synthesis of vitellogenin, which is required for the normal development of oocytes. Therefore, early ultrasound monitoring of the liver in females, starting from 2–3 years of age, is of great importance for timely treatment or culling of sick individuals. The metabolism of lipids in the liver is affected by the composition of feed, feed rate and the rearing water temperature. Rearing of fish in constant, elevated RAS can lead to fat deposition in the liver, which is caused by the thermal effect on the process of phospholipid conversion (Figure 11.2.12).

Rearing of sturgeon of different sizes or species together (not recommended), especially at high stocking density, can instigate fatty liver in larger fish, which can subsequently lead to cirrhosis, profound shifts in calcium metabolism, and significant fluid build-up in the body cavity (ascites). Therefore, with cirrhosis (Figure 11.2.13), echo-negative ascites is evident around the liver (due to impaired metabolic processes, impaired lymphatic drainage, etc.). Comprehensive diagnostics of the state of the liver of female sturgeons allows timely identification and elimination of errors in fish feeding or culling of fish with severe pathology.

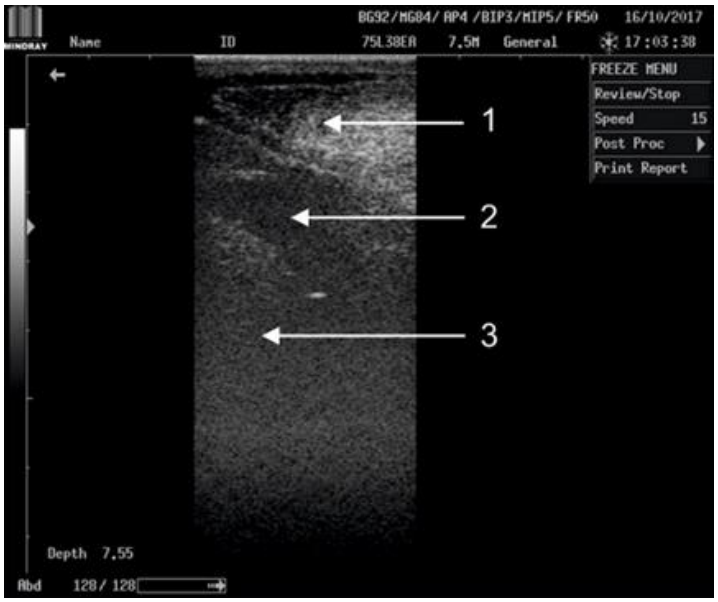

Figure 11.2.12: *Sonogram of the right lobe of a fatty liver (3) of a Acipenser baerii. Muscle layer (1), gall bladder (2) and liver parenchyma (3) (Photo credit: M. Cebanov & E. Galich).*

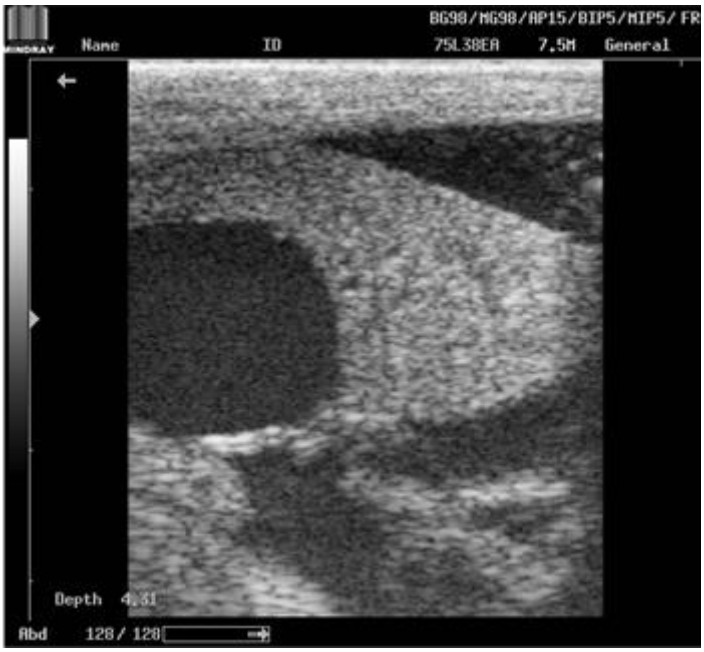

Figure 11.2.13: *Sonogram of liver cirrhosis in Acipenser gueldenstaedtii. Gall bladder (1), liver (2) and ascites (3) (Photo credit: M. Cebanov & E. Galich).*

Abnormalities in the kidneys are potential bioindicators of the level of environmental pollution or possibly some internal parasitism or viruses. A population of 1-year old *H. huso* x *A. ruthenus* (150-300g body weight) on a fish farm had a 1% incidence of unilateral and bilateral abdominal distention, and upon necropsy examination, large nephroblastoma was observed that originated from the posterior kidney (Rahmati-Holasoo *et al.* 2018), similar to Figure 11.2.14A which illustrates a large heterogeneous neoplasm in the posterior kidney of an *A.*

ruthenus. On the sonogram of the kidney area of this specimen (Figure 11.2.14C), a hypogenic (dark) area with bright hyperechoic septa is evident. Ascitic fluid is formed during transudative processes (accumulation of colorless fluid in body cavities and tissues) due to disruption of metabolic processes (Figure 11.2.15) (Photo credit: M. Cebanov & E. Galich).

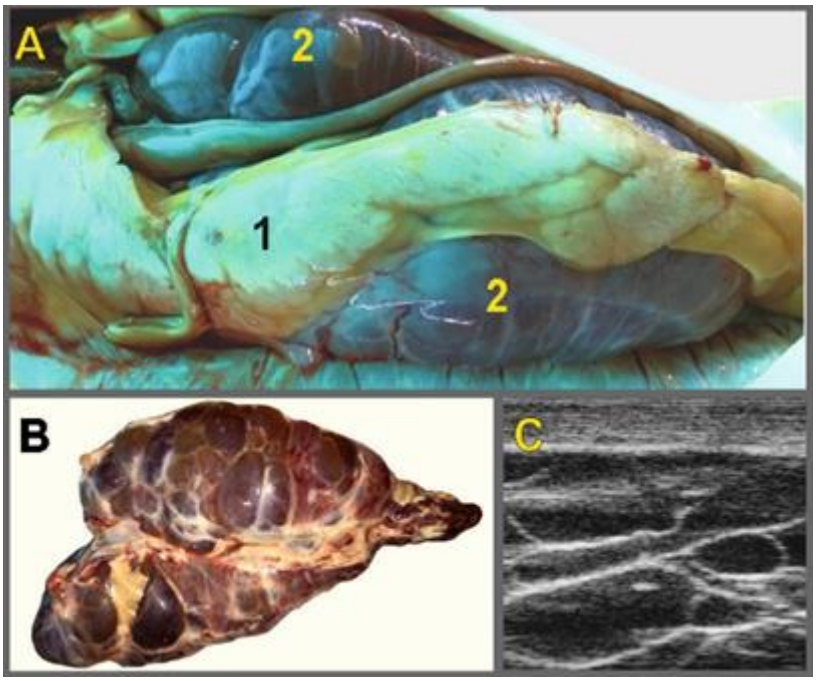

Figure 11.2.14:

A large neoplasm in a male Acipenser ruthenus. A necropsy view of the abdomen (A) reveals the testis (1) and the grossly enlarged neoplasm in the kidney (2). Photo B shows the dissected kidney and the sonogram of the neoplasm (C) (Photo credit: M. Cebanov & E. Galich).

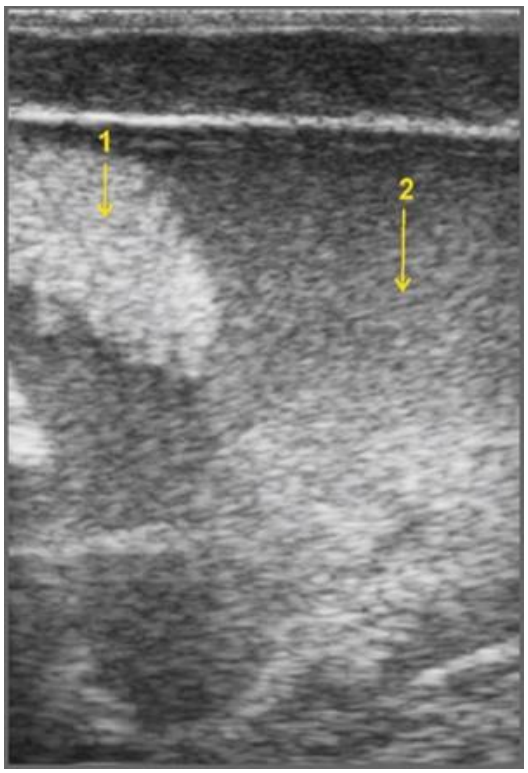

Figure 11.2.15:

Sonogram of a female Acipenser gueldenstaedtii, with ovarian tissue (1) and ascitic fluid in the body cavity (2) (Photo credit: M. Cebanov & E. Galich).

Ultrasound scans of the heart can potentially identify heart pathologies like cardiac obesity (Figure 11.2.16), neoplasms, and pericardial effusion (Figure 11.2.17; Chebanov & Galich, 2018b). In addition, it makes it possible to identify abnormalities in the heart functioning (e.g. bradycardia when reared at high temperatures). Guarda *et al.* (1997) showed that the most likely causes of spontaneous steatosis of *A. transmontanus* during the intensive warm water rearing in Northern Italy were the unavailability of vitamin E and/or the insufficient antioxidant protection in the feed, resulting in the hepatotoxic effect of oxidized fish fat.

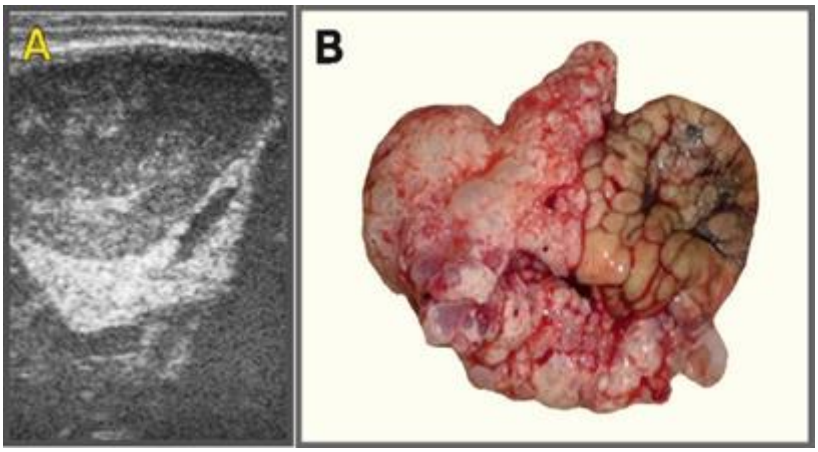

Figure 11.2.16: *Sonogram (A) of a heart from an Acipenser baerii with a fatty ventricle, and the necropsy photo (B) of the removed heart (Photo credit: M. Cebanov & E. Galich).*

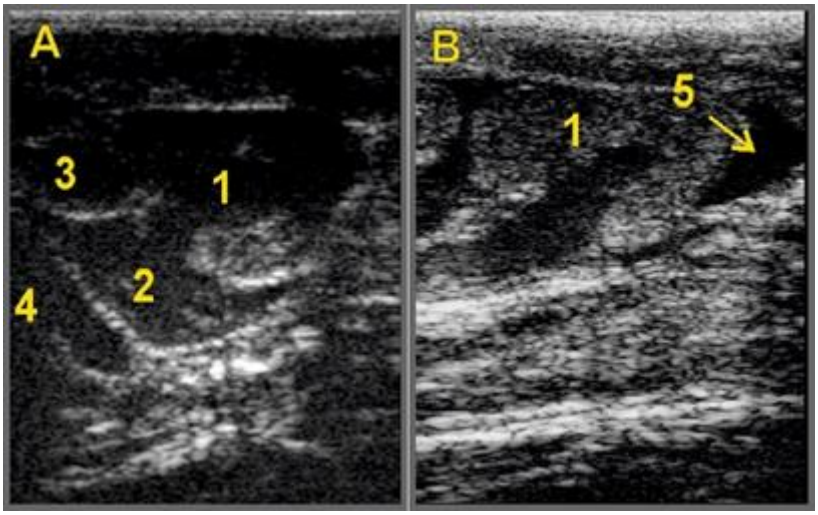

Figure 11.2.17: *Sonograms of a normal heart of Acipenser ruthenus (A) and Huso huso heart with effusion in the pericardium (B). Ventricle (1), atrium (2), conus arteriosus (3), venous sinus (4), effusion (5) (Photo credit: M. Cebanov & E. Galich).*

The early diagnosis of neoplasms (tumors) with ultrasonography can allow a hatchery to possibly isolate these individuals into a separate "hospital" tank and conduct scheduled examinations to follow the tumor(s) progress, although there are no established treatments for neoplasms in sturgeon. In fact, there have been very few cases of any type of surgical removal of tumors or chemotherapy used

on fish (Ferraro *et al.* 2024). An example of a sonogram from an *A. baerii* ovary with neoplasms is presented in Figure 11.2.18.

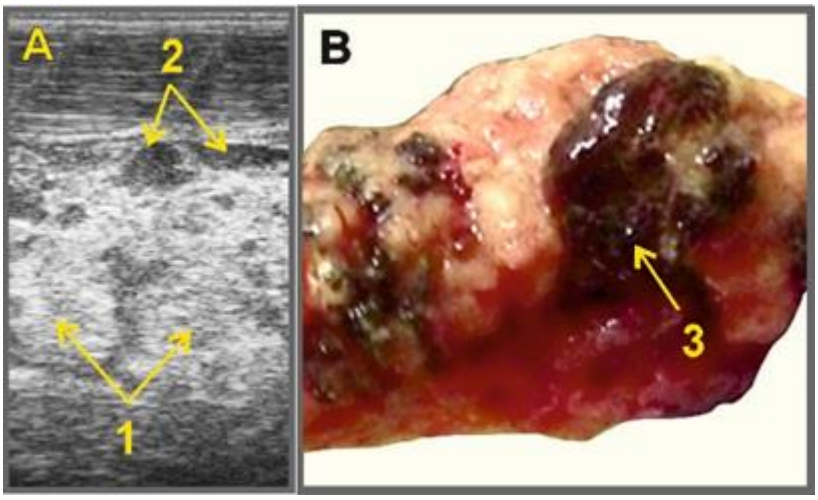

Figure 11.2.18: *Neoplasm (3) in the ovary (B) of an Acipenser baerii. The sonogram (A) identifies some hyperechoic inclusions (1) and hypoechoic inclusions (2) (Photo credit: M. Cebanov & E. Galich).*

In addition to the cases reviewed above, ultrasound scanning also allows to quickly identify disorders of the digestive system, such as an intestinal obstruction (ileus) and other diseases (not manifested externally). Some of those have been described earlier (Chebanov & Galich, 2009, 2011, 2018b). The use of ultrasonography to conduct health assessments of valuable broodstock, especially those that are endangered, could non-invasively identify some internal diseases (anomalies) and potentially allow time to implement measures for their possible treatment (e.g. change culture conditions, diet, water quality) or lead to the decision to cull fish with serious untreatable pathologies.

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Chapter 12: Assessment of fitness for release

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12.1 Introduction and rationale for fitness assessments

The term fitness, developed within evolutionary biology, corresponds to the adaptive capacity of an individual. While there is no universal definition of fitness (Stearns, 1986), growth and survival are currently the most considered criteria for a sturgeon.

The performance of a fish results from a combination of their genetic properties and the effects of the rearing environment. Limiting domestication is a key objective of this guideline since it can negatively affect the performance and survival of individuals after release, and therefore impact the status of natural populations (Araki *et al*. 2008; Sulak *et al*. 2014). Domestication may decrease resistance to diseases (Salonius & Iwama, 1993) decrease adaptation to environmental fluctuations (Johnsson *et al*. 2014), induce reproductive system abnormalities (Rzepkowska, *et al*. 2020), as well as cause behavioral deficiencies (Shumway, 1999, Kellison *et al.* 2003; Huntingford, 2004). In their natural habitats, fishes experience environmental challenges and adapt their physiology and behavior to cope with them effectively. Much of this flexibility is supported and influenced by cognition and neural plasticity (Ebbesson & Braithwaite, 2012; Salvanes *et al*. 2013).

To improve the responsiveness of fish that have been reared under hatchery conditions, several approaches to improve their behavioral plasticity and their resulting adaptability have been developed. Enrichment of the rearing environment (e.g. variability of rearing conditions) has been the prime target over the years (e.g. Crossman *et al*. 2011; Johnsson *et al*. 2014; Carrera-García *et al*. 2016; Anderson *et al*. 2022; Fazekas *et al*. 2023; see Chapter 10).

It is proposed that fitness related metrics can serve as indicators of performance for juveniles produced within *ex situ* programs. To evaluate the potential of the juveniles produced, tests must be established that address relevant metrics and provide meaningful results that are indicative of their ability to survive upon release.

The assessment of the fish for months and years after release is an ambitious target that requires a detailed experimental design and substantial effort to discriminate different treatments in the hatchery and then the subsequent performances in the wild, through recapture and monitoring programs. The performance of the fish produced should be determined using criteria that provide

an indication of the adaptability in the wild, to improve hatchery rearing practices and thus the quality of the fish to be released.

These assessments may include:

- Assessment of different rearing environments (e.g. enriched vs traditional),
- Assessment of performance at different life stages, for different genetic groups, and year classes,
- Incorporation of post-release monitoring results into the *ex situ* program to continually improve rearing methodologies.

12.2 Performance based metrics and indicators

A list of fitness related metrics including the most common survival and growth parameters, as well as morphological, behavioral, and eco-physiological characteristics are presented below. The metrics proposed are commonly used as a proxy for fitness for pre-release in different species, but their relevance may not have been fully verified (Chebanov & Galich, 2011), and their appropriateness may vary according to the life stage and metrics considered.

In order to be applied in an *ex situ* program, the tests need to be relatively easy to conduct, repeatable, and address the objectives. They can be part of the routine facility practices (e.g. survival and growth monitoring) and be assessed directly by hatchery staff, but some tests can be more difficult to implement like, swimming performance and measuring metabolic rate, which requires experienced staff support, specialized equipment, and possibly government permits. To be able to compare the performance between year classes, it is important to have standardized tests, which should also be adjusted based on post-release monitoring results or new research results.

12.2.1 Survival

Survival represents the direct response of fish to the hatchery environment and practices, and the quality of the progeny. While important for the output of the hatchery, it may not predict success in the wild since the behavioral properties of the individual to survive in any of the given conditions will vary considerably (see Chapter 3).

In the hatchery, daily accounting of dead and moribund fish and an estimate of survival rate is a routine practice. For enriched rearing in extensive structures, such as ponds, the daily mortality cannot be measured precisely but assessments through periodic subsampling, like seining fish from part of the pond, for measuring growth and estimating survival, could be carried out periodically during the rearing process. These metrics can be compared with post-release monitoring results when the growth and survival success of the released cohorts in the wild have been evaluated.

12.2.2 Growth and body condition

The growth of the fish under hatchery rearing conditions can be calculated as an absolute growth rate between different time points, or as a comparison of growth relationships (e.g. weight-length; Fulton condition factor) among different groups or hatchery environments. The regular assessment of weight and length during rearing is common and should be completed on an individual facility level. This allows the relative condition of different rearing groups or environments to be compared directly.

Growth and condition factor of the fish following release indicates the adaptation to the wild environment, as well as the availability of the food base. A larger size or higher condition factor of an individual at release can result in higher survival (e.g. Allen *et al*. 2006; Justice *et al*. 2009; Baker & Scibner, 2017; McDougall *et al*. 2020). The verification of this context requires substantial post-release monitoring over a long period of time to ensure differences between different year classes and environmental conditions can be evaluated. This data can be used to compare performance post-release in the wild, to the same cohorts maintained in the hatchery for release at larger-sizes or to be used as future captive broodstock, depending on the specific *ex situ* program objectives.

12.2.3 General morphology: malformations, yolk sac and body shape

Morphological metrics have been developed during Soviet times as indicators for the adaptability and ultimate survival of the released fish produced in hatcheries, as summarized by Chebanov & Galich (2011). Since their assessment focused mainly on the Ponto Caspian species, comparable information is lacking for other species. The significance and the applicability therefore should be verified to ensure relevance for your species. The assessment of the morphological metrics can be simplified by sampling the fish just prior to release, reducing the amount of repeatedly handling. Indeed, this is a common practice for existing hatcheries to weigh, measure, and tag juveniles prior to release (see Chapters 13 & 14).

Collection of morphological metric data prior to release (e.g. body shape and size, scute shape, color) can also be simplified by photographic documentation during embryonic and juvenile development, for all families and rearing conditions. This provides the advantage of being able to go back to re-analyze the pictures once new parameters of relevance are identified. This task can be carried out by the hatchery staff or with other support teams (e.g. scientific/university, non-government or government agencies). Open-source image analyses for measurements are also available (e.g. ImageJ).

12.2.3.1 Malformations

Malformations are an indicator for genetic or rearing-related issues adversely affecting embryo development. Several symptoms have been described in detail (Akimova *et al*. 2004) and the underlying causes for malformations were reported in some cases (e.g. Igumnova, 1985; Ruban *et al*. 2006; Kim *et al*. 2018). The developmental processes involved have a genetic and/or an environmental aspect

that contribute to the phenotype observed. As such, the utilization of malformations is considered more of a quality assessment for selection of broodstock, as well as the assessment of rearing practices in the hatchery. While the symptoms have been described for several species of sturgeon, their impact upon fitness and survival in the wild remains unclear.

For larvae and juveniles, the most prevalent body abnormalities include malformation of the head shape; curvature of the body and tail; underdevelopment of pectoral fins; structural abnormalities in the fin fold; absence of one or both eyes; shortened opercula; underdeveloped barbels; schistasis of the nasal bridge; and underdeveloped olfactory pits (Akimova *et al.* 2004). However, the degree of impact of these deformations upon fitness varies (e.g. schistasis or absence of one or both eyes being more detrimental, than shortened opercula or pectoral fins).

Pectoral fin curl has been reported within the *Scaphirhynchus albus ex situ* recovery program, and has been found to affect swimming performance and station-holding efficacy (Adams *et al*. 2003; Wilga & Lauder, 1999) and can also affect downstream poststocking dispersal (Oldenburg *et al*. 2011). Other abnormalities such as oedema, skeletal axis deformations and heart malformations have recently been reported to be indicators of oxythermal stress (Delage *et al.* 2020).

12.2.3.2 Yolk sac shape

For yolk sac larvae, the height to length ratio of the yolk sac (normal range of 0.55–0.69) has been described as an important indicator of larval abnormalities (Chebanov & Galich, 2011, Figure 12.1).

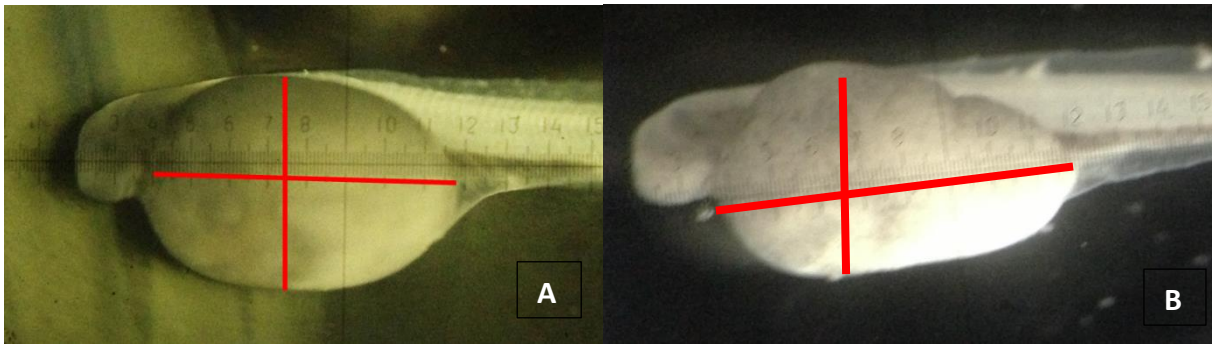

Figure 12.1: *Height-length assessment of an Acipenser stellatus yolk sac in a normal embryo (A) and an embryo with a malformed yolk sac shape (B) (Photo credit: M. Chebanov).*

For malformed (e.g. pear-like) or if the yolk sac is too small, this ratio decreases to 0.29-0.44 (Belyaeva, 1983). Despite the individual and species-specific variability in yolk sac shape, a small yolk-sac indicates that endogenous reserves could be insufficient to provide normal growth and development until transition to external feeding, while too large of a yolk-sac (>0.70) during the differentiation of the alimentary system can delay the secretory function of the epithelium (Gerbilsky, 1957; Bogdanova, 1972).

12.2.3.3 Body shape

Apart from malformations, another indicator of adverse impacts during ontogeny, is the overall body shape of the fish, which can influence performance such as swimming capacity and stamina. It has been noted in several studies that predator presence increases gross morphology in Percid and Gasterosterid fishes resulting in a longer, deeper caudal region and a shallower, shorter anterior region, which increases burst swim speeds (Walker, 1997; Walker & Bell, 2000; Spoljaric & Reimchen, 2007). In *A. ruthenus* it was described that pre-anal fin length and pectoral fin length were larger for wild specimens (Lenhardt *et al.* 2012) but it has yet to be determined how this may affect fitness and survival. In young of the year *A. oxyrinchus*, the body height, as well as the protrusions of the scutes, were stimulated by exposure to predator scent (Gessner *et al.* 2023), a response that is common in cyprinids and other families of fish (e.g. Langerhans, 2010; Domenici *et al.* 2008; Brönmark & Miner, 1992; Andersson *et al.* 2006; Johansson & Andersson, 2009; Poléo *et al.* 1995).

12.2.4 Behavior

The development of behavior is closely linked to the environmental conditions during early ontogeny. Variable environmental stimuli enhances neural development and behavioral plasticity (Johnsson *et al.* 2014; Salvanes & Braithwaite, 2006), rendering them important for conservation rearing. However, metrics linked to behavior can be complex to assess. They require video tracking, specialized equipment (e.g. DanioVision, swimming chambers) and computer-based programs (e.g. EthoVision XT, BehavioQuant) to quantify the behavior assessment (Papadakis et al. 2012). More recently, artificial intelligence could start to provide tools for these complicated analyses.

Further metrics such as personality types: bold versus shy, (e.g. Balaban-Feld *et al*. 2022; Rajput *et al*. 2022; Sales *et a*l. 2023) or predator recognition (e.g. Steindler et al. 2020; Lau et al. 2021), are not proposed here since they are quite difficult to be implemented.

12.2.4.1 Evaluation of adaptive abilities in the "open space" test

The "open space" test is designed to assess fitness of juveniles based on their behavioral response to stimuli (e.g. light and sound of various frequencies), which is the response of the central nervous system to adjust locomotory activity and ultimately increase survival in the wild (Tikhomirov & Khabumugisha, 1997).

Up to ten juveniles can be placed in a round aquarium ($\geq$ 1 m diameter), with its bottom divided into eight triangle-shaped sectors and sub-divided with one inner circle (Figure 12.2) and the number of bottom lines crossed by a fish during different stages of the test (Table 12.1) recorded. Video with EthoVision XT software makes counting the lines crossed by each fish much easier, although direct observation could possibly be used with less fish per test and having one dedicated individual observing and counting for each fish.

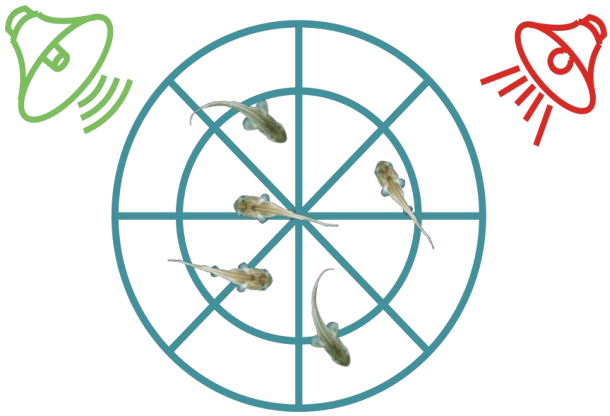

Figure 12.2: *Schematic drawing of the "open space" test set up, with segments being marked on the tank bottom to assess locomotor activity of the fish after exposure to light or noise stress (Photo credit: M. Cebanov & E. Galich).*

As the fish adapts to the new tank during the first 3 min, the approximate locomotory activity (AA, number of lines crossed (#)/min) is determined by recording the number of lines crossed by each fish, and the average taken for all fish tested. When the locomotory activity becomes more or less constant, the mean number of lines crossed during the next 2 min is considered as the background activity (BA, #/min).

Table 12.1: *Chronology of the Open Space test using noise and light as stressors.*

Time, min	**Stages of the test**
1 - 3	Adaptation of the fish to new tank conditions
3 - 5	Post-adaptation period
Exposure to low-frequency noise	
5 - 7	Observation of noise response
Exposure to high frequency noise	
7 - 9	Observation of noise response
Exposure to constant light (5000 lux)	
9 - 11	Observation of illumination response
Exposure to flashing light (5000 lux)	
11 - 13	Observation of illumination response

Following the successive exposure to noise and light, the respective reactivity (RA, #/min) is calculated based on the average number of crossings, for all fish, during the next 30 s after the stressor. The relative activation (IA%) and reactivity (IR%) indices are determined based on the absolute indices (AA, BA, RA) and are calculated as follows:

IA% = AA/BA × 100% = activation index.
IR% = RA/BA × 100% = reactivity index.

where:
AA (#/min) = approximate locomotory activity.
BA (#/min) = background locomotory activity.
RA (#/min) = reactivity following exposure to a stimulus.

Comparative analysis of the locomotory activity using the "open space" test has been established for *A. stellatus* juveniles from a domesticated (farmed) source and wild source (collected from the Sea of Azov, Chebanov *et al.* 2008), and are presented in Table 12.2. The farmed fish had consistently lower reactivity indices as they were likely accustomed to various light and noise sources in their hatchery environment, compared to the wild caught fish.

Table 12.2: *Reference values for the "open space" test results from offspring of domesticated and wild A. stellatus (after Chebanov et al. 2008). AA = approximate locomotory activity (# of lines crossed(#)/minute(min)), BA = background locomotory activity (#/min), IA(%) = activation index (RA/BA × 100%), IR1(%) = reactivity index during the first 30 s after exposure to low-frequency noise, IR2(%) = reactivity index during the first 30 s after exposure to high-frequency noise, IR3(%) = reactivity index during the first 30 s after the exposure to constant light, IR4(%) = reactivity index during the first 30 s after exposure to light flashes.*

Group	AA(#)	BA(#)	IA(%)	IR1(%)	IR2(%)	IR3(%)	IR4(%)
wild	34.5	13.2	261.3	110.2	132.9	99.5	89.7
domesticated (farmed)	39.7	19.7	201.5	88.9	71.3	57.5	42.9

12.2.4.2 Escape response

The test for an escape response assesses the responsiveness of the central nervous system to rapid environmental changes. It allows monitoring of the locomotory activity upon the exposure to strong sensory stimuli (e.g. visual, tactile, hydrodynamic). The locomotor response is considered a proxy for the ability to avoid predators (Tikhomirov & Khabumugisha, 1997; Chebanov & Galich, 2011). Several antipredator behaviors have been described in fish, including freezing or escapement (e.g. Godin, 1997; Smith, 1997; Petersson & Järvi, 2006), and training experiments can lead to improved antipredator behavioral responses (Mesquita & Young, 2007).

Escape movement upon stimulus can be measured as was described previously for the "open space" test. Applying video tracking software provides increased numbers of parameters to be analyzed, such as total distance covered, swimming velocity, and turn angle, before and after the stimuli (Millot *et al*. 2009; Benhaïm *et al*. 2013; Carrera-García *et al.* 2016).

12.2.4.3 Exploratory behavior

The rearing process and the associated environmental cues shape the cognition and the behavior of fish, and an individual's response to new stimuli largely depends upon past experiences and the reactions they triggered. The response of

an individual upon the encounter of a new environment potentially has a significant impact upon the probability of predator encounter. Flight upon predator presence is the ultimate response, while different behavior types are impacting the probability of the encounter in the first place. Cautious fish reveal a lower encounter probability than bold fish. It has been shown that fish from enriched rearing, being exposed to temperature, pH, O_2 fluctuations as well as heterogenous environment (obstacles) are more cautious in a new environment (Gessner et al. 2023). The assessment of behavior in a new environment can be tested in a y-shaped maze (Figure 12.3), recording the preference for sheltered or open areas and the exploration of new, confined regions of the tank. While the results have been closely correlated with the expression of genes in the telencephalon region of the brain (Cámara-Ruiz *et al.* 2019), this assessment would require the fish to be euthanized and as such does not lend itself for mass assessments but only for experimental verification of treatment effects. For this reason, and the fact a highly specialized laboratory would be needed, establishing any gene expression is not included as a recommended criterion.

Increased boldness results in more movement in open spaces and as such increases the chances of finding food but is also associated with increased predation risk. While general differences were noted between groups reared under different conditions (Gessner *et al.* 2023), these results are revealing high variability due to different personality types of individuals (Roy & Arlinghaus 2022; Beukeboom et al. 2023; Fudali & Pietrzak 2024), but due to the lack of reference values for wild sturgeon species these fitness assessments are also not currently recommended.

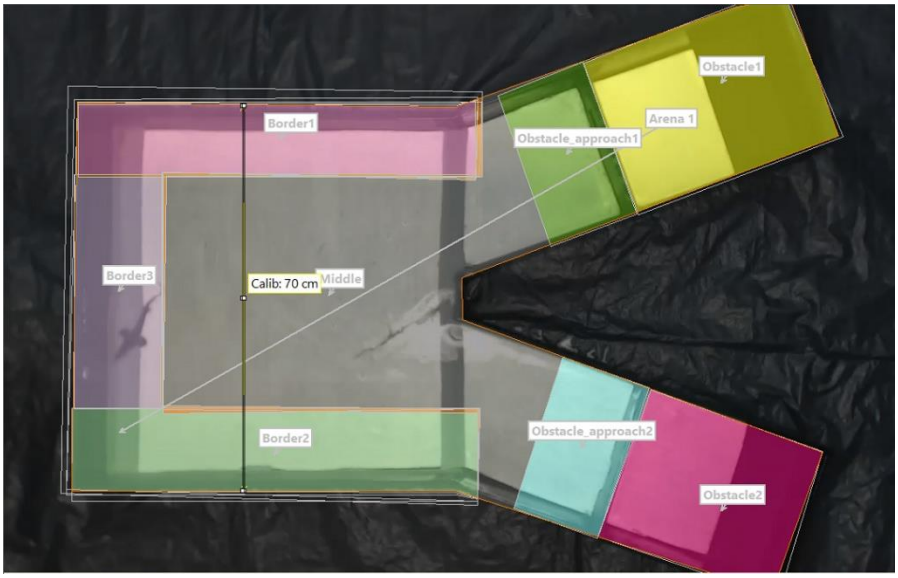

Figure 12.3: *Maze for behavioral testing of juvenile sturgeon. The borders of the open and protected spaces, as well as the areas separated by obstacles (vents) are color-coded (Photo credit: M. Radominska).*

12.2.4.4 Feeding behavior (novel prey test, buried prey test)

Prompt food uptake following release is an important factor to ensure acclimatization to the new environment and to provide sufficient nutrition, to meet the increased energy demands of food searching and aversion of predators. Prey organisms for juvenile sturgeon are typically associated with, or buried within, the sediment.

To evaluate feeding behavior, tests in the hatchery can be implemented as novel prey tests (Carrera-García *et al.* 2016) or buried prey tests (Acolas & Gesset 2014; Cámara-Ruiz *et al.* 2019; Gessner *et al.* 2023). Video analysis can be applied to determine the onset of food search activity and the total number of attempts at feed uptake over time. The effectiveness of the search and feeding is verified by quantifying the number of feed items consumed. The sooner the feed search behavior starts, and the more targeted the feeding attempts are (overlap of prey sites and feeding attempts), the better the fish are prepared for release and subsequent survival. This approach requires the development of a set of metrics for each species, and the conditions under which the experiment takes place will define the thresholds for effective and ineffective feeding.

The search and acquisition of food in the sediment can be enhanced in the hatcheries by providing live prey, and by training juveniles to search for prey at the bottom (e.g. training schools, see Cámara-Ruiz *et al.* 2019). Since sturgeon are bentho-demersal bottom feeders, distribution of the feed on the tank bottom results in increased effectiveness of food uptake (Cármara-Ruiz *et al.* 2019), which has also been verified in flounder species (Takahashi *et al.* 2013). This method, furthermore, has the potential to also limit avian predation in the wild since fish when released will not search for food at the surface.

12.2.5 Ecophysiology

12.2.5.1 Swimming capacity and metabolic rate

The development of ecophysiological metrics such as stamina during swimming also depends upon the rearing conditions encountered during ontogeny. The swimming capacity (i.e. the duration an individual swims at a certain speed) does not only relate to the training it encountered regarding current velocity, but also to the variability of other rearing conditions such as temperature fluctuations (e.g. Deslauriers & Kieffer, 2012; Yuan et al. 2017; Brandt *et al*. 2021).
Swimming performance can be divided into different categories such as constant or sustained and burst swimming. For survival in a new environment, the burst swimming capacity is most important since it determines the ability of the individual to avoid or escape a predator. Swimming performance could be assessed by direct observation, but video tracking is more commonly used to quantify behavior while using a swimming chamber, that is adapted to the size of the fish being tested.

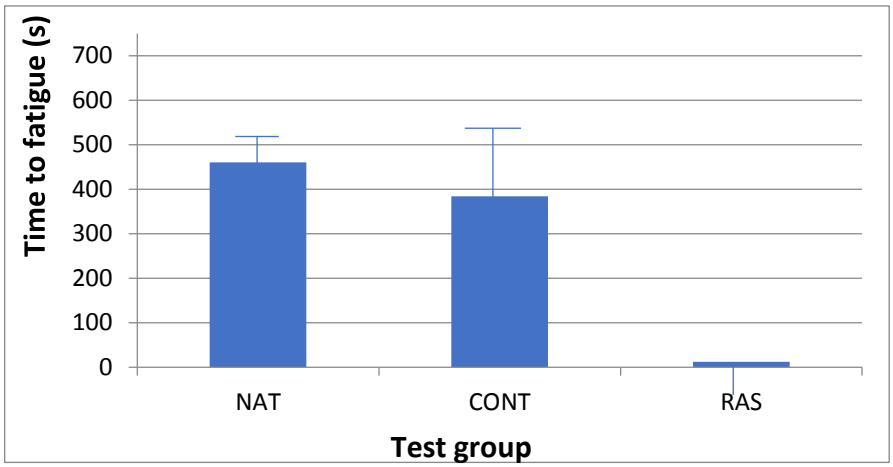

Figure 12.4: *Duration of swimming (time to fatigue) in juvenile A. oxyrinchus under near natural (NAT), control (CONT: well water flow through) and recirculating aquaculture systems (RAS), at a current velocity of 3 body lengths/second (after Gessner et al. 2023)*

These tests provide information about the critical swimming speed (U-crit values), tail beat amplitude and frequency (e.g. Allen *et al*. 2006; Baker *et al*. 2014; Yuan *et al*. 2016; Kieffer & May, 2020). The results can be compared between different batches of offspring, and under different rearing conditions. While swimming chambers do not have to be airtight, if oxygen consumption (pre- and post-exercise) and metabolic rates are to be determined then an airtight respirometry chamber is required (e.g. Yuan *et al*. 2017; Yoon et al. 2021; Deslauriers et al. 2023; Zillig et al. 2024). Since swimming and metabolic performance are not only size and age related, the thresholds for fitness also need to be determined for each species and life stage separately. Due to the specialized equipment and laboratory conditions needed, the applicability of these tests under hatchery conditions is limited.

12.2.5.2 Adaptation of pigmentation

The reaction of melanophores (pigment cells) is indicative of the responsiveness of the neuroendocrine system (Yarzhombek & Zhukova, 2019) to light and changing background color (see Figure 12.5). Since the melanophore reaction is an adaptive response to changing environmental conditions, it allows the fish to blend in with its environment (Svanback & Eklov, 2011), thus reducing predator contact and enhancing survival in the wild (Krasnodembskaya, 1978, 1994) .

Figure 12.5: *Setup for the pigmentation and melanophore response challenge test (Photo credit: M. Cebanov & E. Galich).*

In sturgeon, primary (diurnal) and secondary (background) melanophore reactions were studied at different larval stages. The primary reactions are daily changes of melanophores during changes in light intensity between day and nighttime. The secondary reactions of melanophores are to the background (white or black) of the containers (tanks) in which they are tested and begin to appear at 10 dph and are clearly manifested at 25 dph.

To assess the aggregation and dispersion of melanophores a scoring system with five levels from 1 (minimal) to 5 (maximum extent of melanophore extension) has been developed in the Ponto Caspian species to describe the melanophore response (Figure 12.6), and the response of melanophores varies between body regions. It has been shown that its response is highest when tested at 6.5 dph at temperatures of 21-22° C on the rostrum and lateral body surface, or between 11 and 25 dph in the pectoral fins (Krasnodembskaya, 1978, 1994; Galich, 2000; Galich & Chebanov, 2004). The larvae were tested in plastic containers (~10 L) with either light-colored bottom and sides, or dark-colored bottom and sides (Figure 12.5). The scoring for the melanophore reaction needs to be verified on a species-specific level, regarding both timing of the responsiveness and the extent of the response.

The period of complete adaptation to a light background in a healthy sturgeon larva takes 45-60 min, while adaptation to a black background takes 75-120 min. The dermal melanophores of the head are first to change, then the melanophores of the lateral surface of the body change (Krasnodembskaya, 1978).

To avoid low dissolved oxygen in the water, which can lead to persistent aggregation of the pigment regardless of the background coloration, the water needs to be aerated and the oxygen levels maintained at > 70% saturation. The optimal time for these experiments is about 12 to 2 pm, to prevent the overlap of "primary" (diurnal) reactions, which could confound the results.

The assessment aims at having 70% of the fish after the transfer to white tanks reveal a melanophore index of 1 or 2, and after exposure in the black tank 80% of the fish should reveal a melanophore index of 4 or 5.

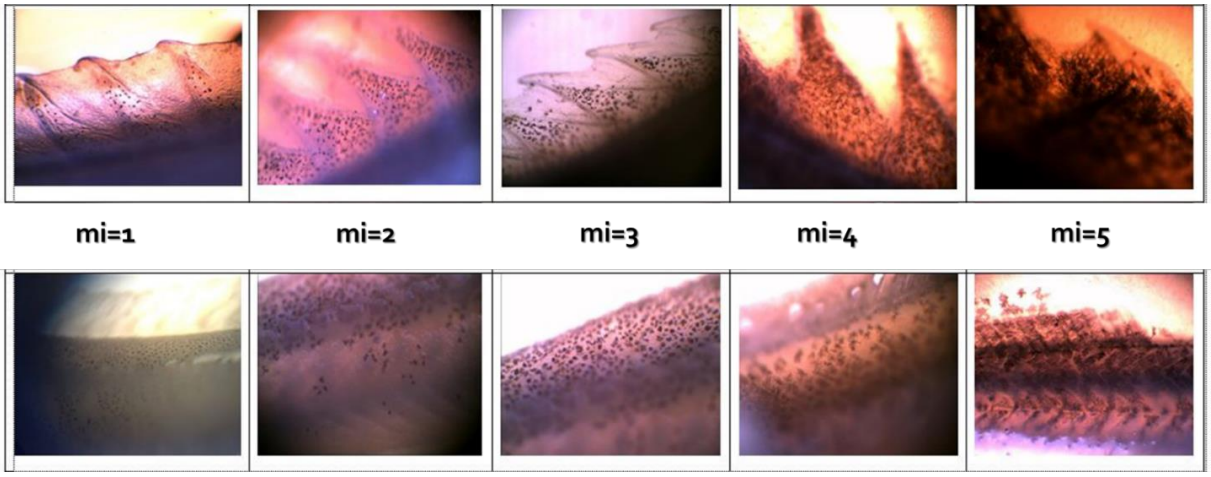

Figure 12.6: *Index to characterize melanophore development to light/dark tank coloration for juvenile (26 dph) sturgeon with mi=1 being minimal pigment aggregation (light body color) and mi=5 being maximum dispersion (dark color) on the dorsal scute surface (upper row) and the lateral body surface (lower row) (Photo credit: E. Galich).*

12.3 Synthesis of the metrics proposed to assess fitness before release

In Table 12.3, the metrics proposed to assess fitness are summarized. Some of the metrics are already part of the rearing practices in most hatcheries (e.g. monitoring survival, growth, and malformations) which makes them relatively easy to implement, provided that their measurement is standardized and that each group (i.e. genotype, cohort, rearing practice) is assessed independently.

The assessment and interpretation of behavior is still under development and reliable metrics are still unavailable for most species. We have presented some other possible metrics to implement and interpret, but they do require some specialized equipment (Table 12.3, yolk sac shape, melanophore reaction, feeding and swimming behavior, and escape response).

We suggest carrying out assessments a few days or weeks before release, except for the parameters that are already part of the rearing protocols (e.g. survival, size/growth and malformations) to have a reference base for the fish condition at release. For fish released older than larvae, we acknowledge that it would be relevant to have test results at different stages of the ontogeny, since some skills can be acquired in a stepwise manner, which could become part of an ongoing research and development program.

Assessment of fitness related metrics compliments the breeding plans that aim at maintaining as much diversity as possible (see Chapter 6). The results can be used

as an assessment of the phenotypic plasticity of the different crosses obtained in the hatcheries under different rearing practices.

Thresholds to absolutely determine the impact of the metrics tested upon survival in the wild are lacking, and so the fate of fish with low performance in the tests described remains unclear. Therefore, their exclusion from being released into the wild cannot be fully justified at this time, especially for species at risk of extinction, but more importantly, training schools can indeed have a beneficial effect on the post release behavior (Camara-Ruiz *et al.* 2019) and should be implemented when possible. Establishing "release" or "not-to-be-released" thresholds for some of the metrics described will be a long-term process and needs to be established for individual species and different life stages.

Impacts upon behavior can also be manifested during mate choice and fertilization processes (Kynard *et al.* 2011), incubation of fertilized eggs and rearing of free embryos (McAdam*,* 2011, Boucher *et al*. 2014, Gessner *et al.* 2009) and during rearing of juvenile phases (Carrera-García *et al.* 2016, 2017). These impacts have the potential to adversely affect the adaptability and as such the fitness of an individual or group of fish. And so, a methodical stepwise approach is necessary to determine the degree of alterations that are impacting the potential adaptability throughout ontogeny.

In cases when supplementary stocking is carried out while the natural population is still present, the quality of the fish released can be compared to their wild conspecifics. To optimize the assessment of fitness related metrics, there is a need to compare the metrics measured under enriched and standard rearing practice with those observed in wild conspecifics. However, the availability of such controls is severely limited for many endangered species, and so we need to rely on some theoretical assumptions of what metrics might be beneficial regarding fitness. Utilization of data from other sturgeon species will be inevitable to initially establish and adopt basic principles that have shown improvements in these other species, until species specific reference values are established.

Overall, to determine the performance of the fish released, close coordination with the monitoring of the population in the field is critical, to effectively evaluate the impacts at different life cycle phases, and ultimately develop improved rearing technologies. The most relevant criteria to measure before release should be decided collectively within the specific *ex situ* program, to focus efforts on metrics considered most promising or relevant.

Table 12.3: *Synthesis of the parameters that can be used to assess fitness related metrics. Ease of assessment: +++ = no specialized equipment required, and should be part of routine hatchery protocols; ++ = some equipment required (e.g. dissecting scope, fish tanks/containers, maze, video camera); + = specialized equipment and software needed (e.g. swimming or respirometry chambers, EthoVision).*

Fitness related metrics	Parameters measured	Purpose/ Relevance	Ease of assessment	Comments
Performance under captive conditions				
Survival	Enumeration of daily mortalities to calculate daily survival for different life stages during the rearing process.	Evaluation of the group performance under rearing condition. Low survival in the hatchery condition may not imply a low survival in the wild and vice versa.	+++	No reference values.
Size / Condition / Growth	Individual weight or weight per batch, individual length, Fulton condition factor.	To compare values between batches (genetic or rearing conditions) or to wild conspecifics of the same age. Conditioned fish may experience lower initial mortality after releasing due to elevated reserves, allowing for a longer period until active feeding.	+++	No reference values.
Morphological/morphometric characteristics				
Malformations	Malformation of head shape, curvature of body and tail; underdeveloped pectoral fins; absence of one or both eyes, shortened opercula, underdeveloped barbels, schistasis of the nasal bridge, underdeveloped olfactory pits, pectoral fin curl.	Evaluation of genetic suitability of crosses and rearing conditions.	+++	Reference values for Ponto Caspian species (Akimova *et al.* 2004). Implication for post release effects is variable or lacking.
Yolk sac shape	Height to length ratio of yolk sac: 0.55 - 0.69 0.29 - 0.44 > 0.70	 Normal development. Nutrient deficient or malformed. Excess nutrients.	++	Development indicator, but post release effects unknown.
Fitness related metrics	**Parameters measured**	**Purpose/ Relevance**	**Ease of assessment**	**Comments**

Behavior				
Open Space Test	Motility in response to visual and acoustic stimuli.	Testing responsiveness to environmental cues as a proxy for fitness.	++	Reference data for *Acipenser stellatus*.
Escape response	Reaction to an external stimulus.	Assess fish response to a stimulus as a proxy for antipredator behavior.	++	No reference values.
Exploratory behavior	Utilization of a new environment.	Exploring new environments makes fish vulnerable to predation, but certain behaviors can decrease predator encounters.	++	No reference values.
Feeding	Ability to find and eat buried preys and/or novel preys.	Decreases the time of adaptation to feeding in the wild, which will increase survival.	++	Behavior can be enhanced by training schools with buried feed over 10 days (Carmona-Ruiz *et al.* 2019).
Eco-Physiology				
Swimming capacity and metabolic rate	Swimming performance, position holding, maximum swimming speed, metabolic rate associated with swimming.	Ensure fish can maintain position in the current or escape from predators in the wild.	+	Swimming speed can be increased by temperature fluctuations and structural diversity.
Pigmentation	Light exposure test; melanophore reaction.	Ensure fish released can adapt to the background color. Adaptation of the melanophores helps camouflage from predators, especially during nocturnal migration.	++	No reference values.

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Chapter 13: Tagging and Marking of fish prior to release

M.L. Acolas, T. Friedrich, J. Gessner

13.1 Introduction

Identification of certain groups of fish or recognition of individual fish requires the fish to be marked or tagged. This is critical for any *ex situ* fish that are released to distinguish these fish that were stocked from fish born in the wild, in order to allow monitoring of the stocked individuals. Tagging can be implemented at the hatchery before release or in the field during the monitoring of the population. Tag monitoring studies always requires the recapture of the fish, to be able to make use of the information collected from the tagged individual. The recapture can be directed (research) or accidental (bycatch). The latter case requires the implementation of an information campaign to ensure fishers are aware of the tagging program and of what data they should collect, and where to report the data.

Tagging methods have to be adapted to the species and to the fish size. Depending on the purpose (e.g. individual identification, mass marking, temporary marking), one needs to choose the best method.

Tagging or marking fish involves treatment and handling, which may disturb and possibly stress the fish, so careful handling is most important when undertaking such procedures (see Chapter 4). From a general point of view, the scientific research objective, difficulty of the tagging method (what level of training is required for the technician(s)), retention rate, country regulations concerning animal welfare, and cost have to be considered when implementing the tagging program.

There is an abundant body of literature, which deals with methods, limits and advantages of tagging fish (e.g. Begout *et al.* 2016; Bridger & Booth, 2013; Hastein *et al.* 2001; Macaulay *et al.* 2021). Ideally the behavior, growth and survival of tagged and untagged fish should be similar.

External tags and marks can be used for visual identification, whereas internal passive or transmitting tags or marks usually require specialized equipment for detection and identification. This chapter summarizes the methods that have successfully been implemented in sturgeons or other fish species. Because of all the different types of marking and tagging methods, the literature should be reviewed, to determine if there has been any reported impact on behavior or fish health, for the type of marking/tagging chosen. This is especially important for external colored marks or tags, as the predation risk could be increased.

13.2 External marks

An external mark may be defined as a visible mark on the exterior of the fish that is used to identify individual fish or to distinguish between groups of fish. External marks may be natural such as phenotypic traits (Stringwell *et al.* 2014). Morphological traits like scute pattern, bent fins or other individual characteristics can be helpful for identifying individuals on a small scale but require standardized photography of animals. In general, they are not always easy to assess, pictures and further morphometrics analysis would be required and may be subject to interpretation.

For external dye marking, Alcian blue dye can be applied on the ventral surface of the fish (Sanchez-Lamadrid, 2001) or on the pectoral fins. Such marks are often simple, inexpensive and quick to apply, but provide limited information due to limited variability of marks available for different families of fish and the short retention time of the marks.

External marks such as fin clipping have been used in several species. Since fins are regenerated it can only be used for a temporary marking of families or year classes. Moreover, injuries of the fins can conceal the mark and fin clipping can reduce the maneuverability of the fish, thus we do not recommend this method.

13.3 External tags

External tags are visible structures that are attached to the fish by piercing tissues (Winter, 1996). The advantage of such tags, which may carry an individual code, batch code or visible instructions, is the ease in detection without specialized equipment and the versatility of individual segregation. External labels have to be easily visible, allowing observation during handling and facilitate qualified reporting by fishers (bycatch).

External tags include ribbons, threads, wires, plates, discs, dangling tags, straps or T-bar anchor tags (McFarlane *et al.* 1990; Morgan & Walsh 1993). By permanently penetrating the skin, which means the tag may provide a potential access route for infection. Furthermore, depending upon size and mounting technique, they have the potential to adversely affect swimming motion and swimming speed. Depending upon color and movement in motion, they could also attract predators. Fouling can be observed in external tags, hampering readability, especially in freshwater. As such, the tag should be adapted to the conditions and the size of the fish to be tagged.

In the hatchery, the use of external tags should be limited due to the risk of abrasion in the tanks and infections, but in the natural environment it can be useful to easily detect recaptures. Thus, fish can be externally tagged at the hatchery a few weeks or days before release.

Before inserting the external tag, skin disinfection is recommended as well as the disinfection of the tag. Diluted (1:10) H_2O_2 or iodine can be recommended but

those substances should not come in contact with fish gills. Floy tags can be applied with an "attachment gun" (Figure 13.1.). In sturgeon, usually the tag is applied at the base of the dorsal fin and is inserted between the radiae. The tag is twisted slightly upon attachment to ensure that the T-bar has been set in place correctly. The application in the fin base instead of the musculature provides a highly increased retention rate.

Wire-On-Tags (WOT) can be inserted using a hollow needle through which the wire in inserted. When the needle is removed and the two ends of the wires are twisted to secure the tag (Figure 13.2.).

Figure 13.1: *Floy tag injection on a juvenile A. oxyrinchus (Photo credit: S. Henneberg).*

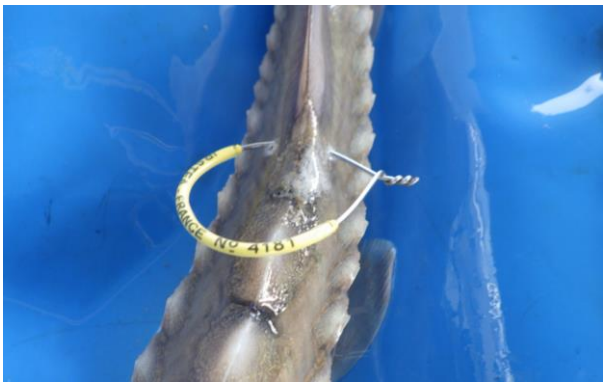

Figure 13.2: *Hall print WOT tag fixed onto a juvenile A. sturio (Photo credit: Inrae, ML Acolas).*

13.4 Internal marks and tags

The need to identify fish, individually or by group, with minimal influence on behavior, health or survival has led to the development of internal tags. In the case of internal tags, the identification of a tagged individual requires specialized equipment (readers or tissue sample analysis). This excludes the fishery from actively participating in the reporting of tagged individuals. On the other hand, internal tags are commonly used in the hatchery to identify fish during the

subsequent grading and handling steps. In these cases, mainly individually coded internal tags are applied.

13.4.1 Chemical Marking

13.4.1.1 Permanent stains

Stains are utilized to mark groups of fish with a chemical that is incorporated into the hard structures of the fish. The fish are immersed in a solution of the stain over a period of time to ensure the stain is incorporated. For this purpose, the fish are exposed to a bath of the chemical of choice (e.g. alizarin red S + NAOH buffer to avoid pH decrease, Oxytetracycline hydrochloride (OTC)) (Fig 13.3).

Figure 13.3: *Balneation tank with oxytetracycline hydrochloride solution (Photo credit: Inrae P. Jatteau).*

Using OTC, a study in shortnose sturgeon confirmed the marks on both sagittal otoliths and first-ray pectoral fin spines one month after a 12 hour balneation in 750-mg/L OTC bath (Crumpton *et al.* 2012). But Loch *et al.* (2011) tagged *A. baerii* at one month old at 300 ppm or 600 ppm OTC and conclude that auto-fluorescence of OTC and ontogenic formation in pectoral fin rays can cause mark detection deficiencies in this hard structure. Alizarin red S solution may provide better results but it is not recommended to rely on pectoral fin rays (Lochet *et al.* 2011). The incorporation of any chemical is usually made by balneation for a certain amount of time, controlling the environmental parameters (pH, oxygen, temperature). Verification of the effect on the batch (absorption of the stain and presence of a clearly discernible mark) is recommended since water chemistry and status of the fish might affect the quality and thus the persistence of the stain.

Detection of the stain is performed under a fluorescence microscope. Since the stains are fluorescent, similar to the age reading, slices of bony material (fin rays, scutes, and otoliths) can be used to verify the presence of florescence in the calcified structures to verify and determine the exact timing of marking. In small fish of less than 15 cm TL the mark is readily visible without removing hard structures when exposing the fish to UV light (380-400 nm) in a darkened chamber.

An advantage of internal marks, such as chemical marking of bony structures, is that large numbers of fish can be marked at an early age. Mass marking has been

used to identify hatchery reared individuals from naturally spawned fish. The staining of juveniles also has some disadvantages. The most obvious drawback is the fact that only batch marking can be performed, and that specialized equipment is required for the analysis. Moreover, the stain can be aggressive and thus affect the nerve endings and receptors for more than a week (Kasumyan, pers. comm.). There are also variable results on retention on non-lethal sampling structures, like a thin section of the pectoral fin ray, and restructuring of the fin ray material at an early age limits the persistence of the stain. Using lethal sampling such as otoliths is not recommended for endangered species in addition to the fact that sturgeon otoliths vary from those of many teleosts in structure and do not absorb the stain in the same way, thus limiting the results obtained by marking. It must also be noted that the disposal of large volumes of antibiotics (OTC) or stain (alizarin red S) can be expensive.

13.4.1.2 Microchemistry

Differences in isotope composition of O, N, S and C are commonly used to determine food web interactions or habitat use (e.g. Boecklen *et al.* 2011; Hobson, 1999). Gradients in certain elements such as Sr, Ba, Ca, Mg, S, and B exist in regions of different salinities, temperatures, and bedrock, and can be used to assess habitat changes (Chang *et al.*2013. Pracheil *et al.* 2014).

Marking by isotopes may occur naturally or by deliberate manipulation of the concentration of specific elements (e.g. Munro *et al.* 2009; Loeppky *et al.* 2020) to create recognizable elemental signatures in the hard structures of fish (e.g. otoliths and fin rays).

In a study on *Acipenser fulvescens* (ages 1-7), elemental signatures in the fin rays of known hatchery-released individuals were quantified to assess whether the ambient water chemistry in a groundwater-fed hatchery would create an elemental signature (Loeppky *et al.* 2020). The concentrations of trace elements, particularly Mn, within the first growth band of hatchery-reared fish were significantly different from those of wild conspecifics, allowing to accurately classify hatchery- versus wild-spawned individuals with 99% success. Induction of a combination of isotopic signatures could also enable hatcheries to track the success of families or stocking groups within a single year-class or across multiple year-classes. Twenty-four hour immersions in Sr-86 and Ba-137 were tested with successful readings on fin rays, two months after marking (Loeppky *et al.* 2020).

The analysis of hard structures to quantify trace element is made via laser ablation (LA) inductively coupled plasma-mass spectrometry (ICP-MS). Spectrometry allows recording the changes in isotope composition over time. The LA allows evaporating material of the hard structure in small increments, allowing the association of the ablation to an age reading in the structure. As such the bony structures serve as a recording of past life history. Special preparations to mount structures for laser ablation must be carried out in preparation of the analysis and need to be done in a specially equiped laboratory. Preparation and analysis must be conducted by a specialist. Otoliths are most often used in microchemical

analyses of fishes since, unlike scales or skeletal bones, there is no potential for resorption or remodeling of this structure. However, otoliths are not the most recommended structure to analyze for sturgeon. Researchers have shown the stability of elemental signatures in fin rays over time, suggesting they are stable structures also appropriate for use in microchemical analyses (e.g. Sellheim *et al.* 2017) as well as scutes (e.g. Altenritter *et al.* 2015).

While the method is relatively easy to apply and has a high retention rate, it only provides batch recognition and requires highly specialized equipment with experienced staff, or else is very expensive if contracted to an outside laboratory.

13.5 Genetic tagging

Genetic tagging is referring to developing genetic markers at the hatchery before releasing the fish. Then when a fish is caught in the wild, using fin clipping and genetic analysis, it can be attributed to the hatchery or even to the parents/cohorts (e.g. Dehaan *et al.* 2008; Roques *et al.* 2018). It requires minimal impact on the fish but needs the development of genetic markers and the use of highly specialized laboratory equipment. Microsatellite or SNP's have been successfully used for sturgeon species (e.g. Dehaan *et al.* 2008; Roques *et al.* 2019).

13.6 Visible internal tags and marks

Tags that are applied subcutaneously remain visible by eye. One example is the visible implant tag (VIT), or the visible implant alphanumeric (VI alpha) tag. Such tags were developed to combine the advantages of external tags with those of internal tags.

VIT are made of plastic strips and VI alpha are made of medical-grade silicone rubber, often with the addition of fluorescent material. These tags are providing printed information and are to be placed in transparent tissue, like behind the eye (Begout *et al.* 2016).

An alternative which can be used for batch tagging only, but allows the tagging of very small individuals, is 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 hours at room temperature it cures into a pliable solid, providing an externally visible internal mark. The fluorescent elastomer is available in several colors, with some being difficult to distinguish. Recognition of individuals is possible through the use of different body locations and colors (Frederick ,1997; Olsen & Vollestad, 2001). To increase the visibility a UV-lamp can be used.

Depending on the number of batches, the tagging plan should be prepared in advance. By combining colors and injection site, several batches can be distinguished. It is recommended to use a two-mark combination per batch in case of an utimely resorption of a mark.

The elastomer should be prepared just before the tagging since the time until it begins to harden is limited. To inject it, the needle is inserted subcutaneously for a few millimeters; the elastomer is injected while starting to retract the needle. Injection is discontinued before removing the needle to avoid the elastomer to expel.

The site of injection most frequently used in previous studies is the rostrum but injection under the eye, in the opercula or within the scutes for small fish are promising (Figure 13.4, Figure 13.5). In *A. oxyrinchus*, Kapusta *et al.* (2015) found a survival rate above 90% and a retention rate of 100% in the rostrum, and retention 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). Kozlowsky *et al.* (2017) tagged fish as small as 5 cm with a 90% retention rate in the rostrum after 70 d; the survival rate was similar with controls but it was low (40%).

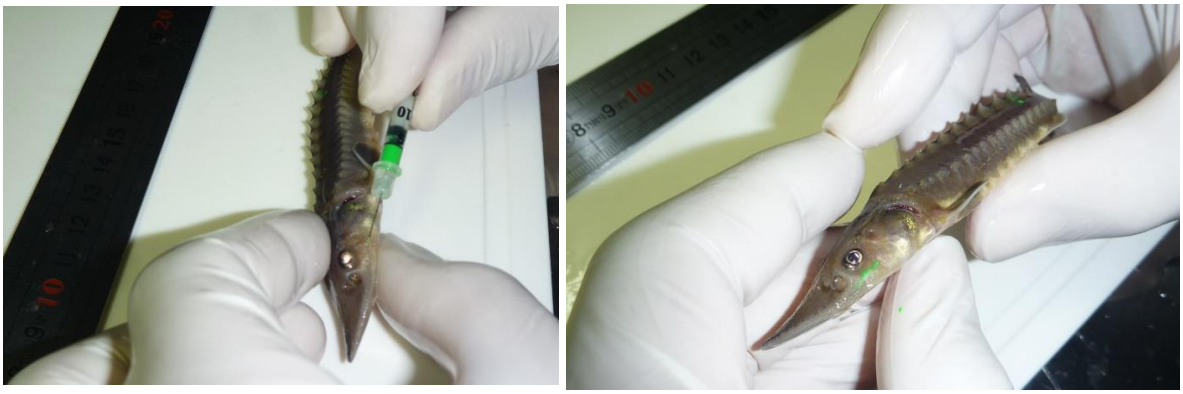

Figure 13.4: *Injection of the visible implant elastomer below the eye in a juvenile A. sturio (Photo credit: Inrae, M.L. Acolas).*

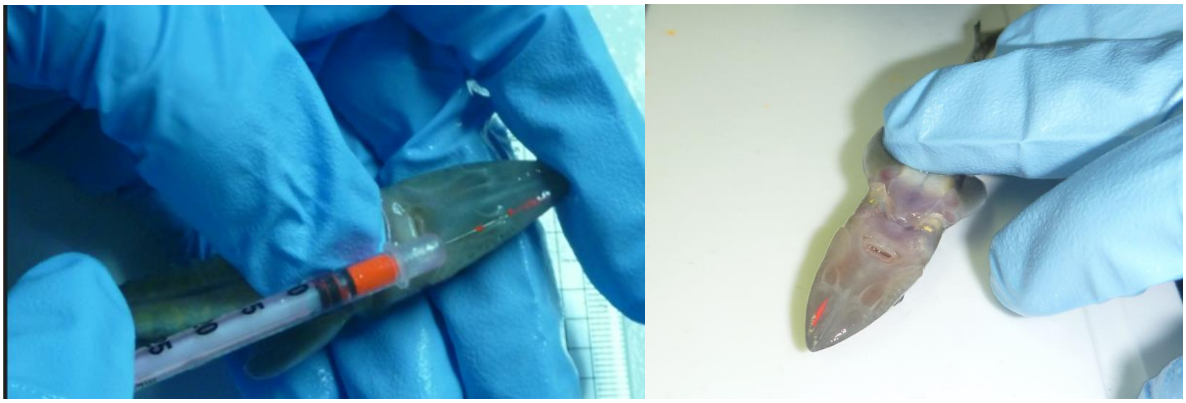

Figure 13.5: *A visible implant elastomer tag applied to the rostrum of a juvenile A. sturio (Photo credit: Inrae, L. Jacob).*

13.6.1 Internal tags

Internal tags are defined as solid objects inserted or injected into tissues or implanted into the body cavity, muscle or cartilage, and can be used to identify individuals or groups of fish. This group of tags are comprised of plastic or glass tubes, metal plates and small pieces of magnetized stainless steel that may have a binary code of Arabic numbers engraved or laser etched on their surface. The coding is either read electronically or mechanically and thus can be identified either by a reader that induces the signal to be emitted (PIT Tags) or by removal of the tag and the inspection under a disectoscope (CWT).

13.6.1.1 Coded Wire Tags CWT

Coded wire tags CWT are small size (0.5–2.0 mm × 0.25 mm) magnetized stainless steel with a unique code. They are normally injected into the snout of a fish, often in combination with an external mark to aid recovery (Schurman & Thompson, 1990). Such tags are extensively used for identifying large numbers of fish and due to their small size can be used on fish of a wide range of sizes.
In sturgeon they can be inserted into the rostrum or under the scutes (USFWS, 2019) or into the first pectoral fin ray (Paraschiv *et al.* 2006). To be detected, a handheld metal detector is required. The major drawback is the need to remove the tag from the fish to retrieve the codes and identify the individual. For pallid sturgeon the CWT can be put on 5 cm individuals (USFWS, 2019). CWTs are also used for several species of sturgeon in the Caspian Sea from as small as 3g (Fadaee, 2007).

13.6.1.2 Passive Integrated Transponder (PIT) tags

RFID (Radio Frequency Identification) tags often called passive integrated transponder tags (PIT-tags), can be read by an external reader transmitting an impulse to activate the tag to emit its code which in turn is collected by the antenna within the reader. Since the tags do not depend upon an internal energy source, they have an unlimited life. Once implanted, they provide a non-invasive means of individual identification (Baras *et al.* 2000; Downing *et al.* 2001; Barbour *et al.* 2012; Gibbons & Andrews, 2004) by hand-held readers and in-stream antennas located on the bottom of rivers or fish passes. Each PIT-tag has an individual alphanumeric code and tag retention is variable, depending on the implant location and species, but is usually high (e.g. Barbour *et al.* 2012; Burnett *et al.* 2013; Thiem *et al.* 2013). The method features several advantages such as individual identification, high retention rate, minimal effect of the tagging procedure, small fish >20 cm can be safely tagged for long term retention, with the major drawback of being not externally visible and the need for appropriate readers to detect them.

For sturgeons the optimal location for PIT tagging is below the 2nd or 3rd anterior dorsal scute of the fish (Briggs *et al.* 2019). To insert the PIT-tag, the use of sterile/disinfected needles is required. For the insertion into the body, different techniques are available. The standard method utilizes an applicator that pushes the tag out of a hollow needle into the tissue. The second option is an applicator (Trovan) that retracts the needle while leaving the tag in place. In both cases it is

recommended to insert the needle with the point on the inside towards the chorda and the opening towards the skin to prevent the needle from breaking the glass body of the tag when the fish muscle contracts. The first option has the disadvantage of the disruption of the tissue when the tag is pushed forward, while the second option has the disadvantage of the applicator being somewhat clumsy and more difficult to handle (Figure 13.6.). After tagging, verification of the tag function and code with a hand reader is recommended.

PIT-tags are available in various sizes, the bigger, the higher the detection range. Individuals above 25 cm can be tagged below the dorsal scutes with a 12 mm long PIT-tag (2 mm in diameter, weight 0.10 g). Moser *et al.* (2020) described that below a total length of 20 cm, the tag retention rate is only 50% for *A. oxyrinchus* when the PIT-tag was placed posterior to the dorsal fin, where tissue growth is least (Moser *et al.* 2020) which was also observed for *A. ruthenus* (Friedrich, pers. comm.). When applying the tag in the dorsal musculature in fish of 18 cm TL the tag retention was >95% in *A. sturio* (Gessner, pers. comm.). In *Scaphirhynchus platorynchus* an 8.4 mm PIT-tag was successfully used for fish exceeding 8 cm in fork length (Schuman *et al.* 2017), when inserted in the abdominal cavity (retention rate above 80%). Very small microtags are also available and could be useful in the hatchery to identified very small fish. A study on *A. baerii* highlighted a retention rate of 77% of microtags in the abdominal cavity for fish of 14 cm, but as the study had a different focus (Carrera-Garcia *et al.* 2017), this result 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 in fixed antennas within streams and hence 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 d 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. fluvescens* but the smallest fish tagged were 60 cm. The region below the pectoral fin is also used for PIT tagging of broodstock (Chebanov & Galich, 2010).

Figure 13.6: *Insertion of a 12 mm PIT-tag into a juvenile A. sturio (Photo credit: Inrae, R. Le Barh) and A. transmontanus (Photo credit: T. Friedrich).*

13.6.2 Transmitting tags

Transmitting tags are usually used in the wild for monitoring purpose. But the fish can be equipped at the hatchery before release or they can be used for behavior assessment in captivity. Transmitting tags can be mounted externally or internally. A large and growing array of electronic transmitter tags is available (see Begout *et al.* 2016 for more general details). Radio tags, can only be used in water of very low salinity. They are useful because radio waves are less affected by physical obstacles, turbidity, turbulence and thermal stratification than acoustic, non-electromagnetic waves. Radio signals also radiate through the water surface and can be detected at great distances, because there is little loss of signal strength in air. Receivers can be placed in boats, aircraft or at land-based listening stations. Radio tags operate at high frequencies (30–300 MHz), so there is little signal drift. Acoustic tags are mostly used in seawater or in deep waters because sound is transmitted over long distances in salt water, whereas radio waves are attenuated very rapidly. Frequencies of 20–500 kHz are used. Pulsed acoustic tags emit signals that can be detected using a simple receiving system comprising a hand-held directional hydrophone, a portable receiver and headphones. Both for radio or acoustic tracking, to get an accurate position of the fish, it requires triangulation using an array of fixed hydrophones or multiple mobile hydrophones (Fredrich *et al.* 2008).

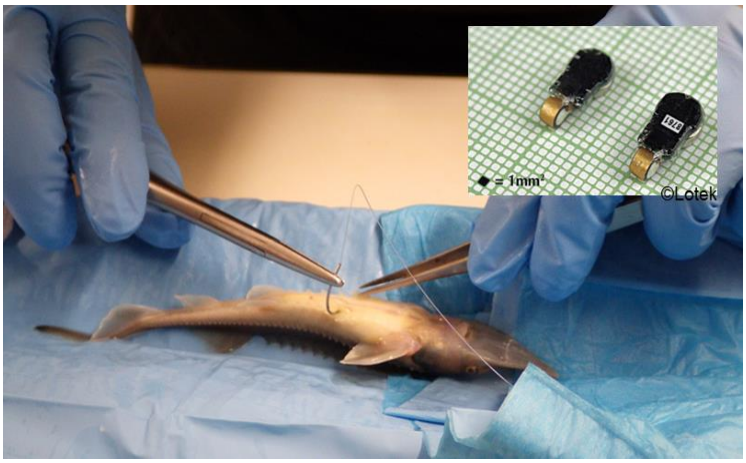

Figure 13.7: *Implantation of an acoustic transmitter tag into the body cavity of an A. sturio juvenile (INRAE M.L. Acolas).*

Apart from signals that identify the position of the fish, some tags carry sensors that collect additional data, such as depth, swimming direction and speed, heart or respiration rate or information about muscle contraction. Such electronic tags are larger than PIT tags and require an internal battery to power the transmitter and microchip. The lifetime of the tag, depends on transmitter size, power supply, range and rate of signal transmission. Microchip technology allows for specific instructions to be placed onto a tag, allowing it to be switched on or off under a given set of conditions. For example, tags may transmit only under certain conditions of water chemistry or light intensity and as such, selective use can increase the longevity of the tags.

13.6.3 Data Storage tags

Tags that are capable of storing measurement data on environmental variables such as temperature, salinity or depth come as archival tags or Data Storage Tags (DST) with or without transmitting capabilities (multi-sensor loggers, tri-axial accelerometers, miniaturized geopositioning systems). The majority of the tags are mounted externally on the fish. They are retrieved either when the fish is caught or they are mounted with a release system that detaches the tag from the fish at a predefined time and floats to the surface to be collected at shore to retrieve the data (pop up tags) or it emits the stored data via a satellite link when reaching the surface (pop-up satellite tags); e.g. Bograd *et al.* 2010; Block *et al.* 2011; Schaefer *et al.* 2011; Hazen *et al.* 2012. The shelf life in these data loggers depends upon battery size, data acquisition intervals and temperature. The logging intervals in most tags are freely adjustable, depending upon the research question to be addressed. Fish can be equipped at the hatchery before being released for monitoring in the wild; such tags can be placed at the base of the dorsal fin or just under the dorsal scutes (Figure 13.8). Under hatchery conditions, these sensor tags either archival or transmitting offer new frontiers for the assessment of fish behavior in controlled environments such as *ex situ* facilities, especially the systems equipped with sensors for heartbeat rate, accelerometers or other vital functions.

Figure 13.8: *External mount of DST Tags on Atlantic sturgeon dorsal fin base and T-Bar anchor tag (left) (Photo credit: U. Schütz) and through the scutes (right) on A. sturio (Photo credit: INRAE M.L. Acolas).*

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Chapter 14: Transport and Release

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14.1 Transportation

The transportation of sturgeons, either within the hatchery, between facilities, or to be released into the wild, should operate under standardized protocols to guarantee safety and animal welfare (see also Chapter 4). The two main transportation methods can be generally classified as open or closed. The closed systems (e.g. plastic bags) are used for eggs, embryos, larvae and small juveniles, while larger juveniles or adults are transported in open systems (e.g. insulated tank(s) loaded onto a truck, and larger tanks onto a transport trailer, Figure 14.1).

The main considerations for successful fish transportation include:

- Size/age of the fish.
- Minimize stress during capture and handling.
- Stocking density during transport.
- Method of transport.
- Duration of transport.
- Maintaining optimal water quality (water temperature, dissolved oxygen, pH, carbon dioxide, ammonia, also see Chapter 5).
- Feeding fish must go through pre-transport fasting for 24-72 h (depending upon size/age).

Depending on the *ex situ* program there may be health status requirements before transport and stocking. The *Scaphirhynchus albus* recovery program requires health certification prior to stocking (USFWS, 2019) which involves subsampling of various tissues from several fish, from each family, that are then sent to a fish disease laboratory for the detection of viruses or bacteria (see also Chapter 11). The general principles of health management for responsible movement of live aquatic animals were previously described in FAO (2007), and basic techniques for fish transportation have been reviewed (Berka, 1986; Wynne & Wurts, 2011; Pandit, 2021). If transportation occurs between different countries, then the appropriate CITES permits need to be obtained.

14.1.1 Transportation of embryos

Plastic bags are routinely used for transportation of eggs, embryos, and larvae, but they can also be used for small numbers of larger fingerlings as well. Plastic bags should be thick (~ 0.08 – 0.15 mm) and have rounded corners, as individuals tend to accumulate in sharp cornered bags which can result in mortalities due to crushing and/or lack of adequate oxygen.
The general procedures for moving embryos in plastic bags include:

- Carefully add the per-determined weight of embryos (Table 14.1) into the bag containing clean, high quality hatchery water at the appropriate temperature.
- The minimum ratio of water to oxygen should be 1:2.
- The ambient air in the bag is released by deflating the bag to the water level and then adding pure oxygen, via a flexible hose connected to an oxygen tank and regulator. Be careful not to fill with oxygen too quickly, or directly into the water, which can create severe turbulence to the embryos.
- Twist the bag closed and secure with a clamp, cable ties, rubber bands or a rubber clamping castration ring (used for livestock).
- Place the bag into a second bag and if needed, add a frozen wet ice or gel pack on top of the first bag and then seal the second bag.
- Place the sealed bags inside a box made of insulating material (e.g. padded polyester, foam plastic, Styrofoam) and tape down the lid. Then place this box into a cardboard box and tape shut the box.
- During the hotter days of the year, it is important that the boxes be protected from direct sunlight and wet ice or gel packs be used, to help maintain optimal water temperature.

During packing, transportation and unloading, it is crucial to avoid prolonged holding of the embryo bags in a motionless state, especially if higher densities of embryos are placed into each bag. With no motion, the bottom layer of embryos has no exposure to oxygenated water which could result in significant mortalities. The duration of possible holding of embryos in a motionless state depends upon the density and water temperature (e.g. from 10–15 min, at 5–6 kg per bag, and 1–1.5 h, at 2–2.5 kg per bag, at 15–17 °C). To mitigate this potential effect, a gentle 45–60 s shaking of the bags every 45-60 min for lower density bags is recommended, and more frequently for high stocking densities. Transportation of boxes of bagged embryos, in most cases, is not an entirely motionless trip, as the trucks, trailers or even airplanes used for transport have enough erratic motion to keep the bottom layers of embryos moving up and around.

The optimal loading density of fertilized embryos in bags depends on their development stage, the temperature regime and the transportation duration (Table 14.1.). Transportation temperatures should always be within the species-specific range and, in general, it is recommended that temperatures do not exceed 20 °C.

Table 14.1: *Recommendations for stocking densities (kg) of sturgeon embryos for transportation in plastic bags (40 L) at different temperatures, and different transportation times (Orlov et al. 1974).*

Temperature °C	**Duration of Transportation (h)**							
	5	**10**	**15**	**20**	**25**	**30**	**35**	**40**
At stages from early gastrulation to lateral plates fusion								
10	6.0	6.0	6.0	5.3	4.5	3.9	3.4	3.1
15	6.0	6.0	4.8	3.8	3.2	2.7	2.4	2.1
20	6.0	5.6	4.1	3.3	2.7	2.3	2.0	1.8
At stages of rotating embryo								
10	6.0	5.1	3.7	3.0	2.5	2.1	1.8	1.6
15	6.0	4.3	3.1	2.4	2.0	1.7	1.5	1.3
20	6.0	3.7	2.7	2.1	1.7	1.4	1.2	1.0

14.1.2 Transportation of larvae

Transportation of larvae uses the same procedures as described above, for embryos. The larvae packed in oxygenated bags can be transported safely for up to 24-30 h. Occasional motion of the bags, and thus the movement of the water surface in the bags, will improve the diffusion of oxygen into the water. During long trips (>20 h), the bags should also be rotated (i.e. vertical to horizontal) to increase oxygen diffusion. If the transportation takes longer than 30 h, the oxygen in the bags should be renewed. Guidelines for stocking densities at 15 °C are shown in Table 14.2. At higher temperatures, lower the stocking density by 25% per 5 °C, and at lower temperatures, density can be increased by 25%. As with embryos, transportation temperatures should always be within the species-specific optimal range.

Table 14.2: *Guidelines for stocking densities (kg) of sturgeon larvae and small juveniles, at different body sizes and transportation times, in plastic bags (40 L), at 15°C water temperature (Orlov et al. 1974; Hochleithner & Gessner, 2012).*

Body weight (g)	**Duration of transportation (h)**									
	5	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**
0.01–0.03	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.12
0.2	0.2	0.2	0.2	0.2	0.18	0.15	0.13	0.12	0.1	0.09
0.5	0.3	0.3	0.3	0.27	0.21	0.18	0.15	0.13	0.11	0.1
1.0	0.5	0.5	0.5	0.4	0.32	0.27	0.23	0.21	0.18	0.16
2.0	0.7	0.7	0.53	0.4	0.32	0.27	0.23	0.21	0.18	0.16
5.0	1.0	1.0	0.7	0.5	0.4	0.33	0.29	0.25	0.23	0.2
10.0	1.5	1.0	0.7	0.5	0.4	0.33	0.29	0.25	0.23	0.2
20	1.8	1.0	0.76	0.57	0.46	0.38	0.33	0.28	0.25	0.23
100	1.8	1.2	0.9	0.85	0.8	0.75	0.7	0.65	0.6	0.5
>500	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

14.1.3 Transportation of juveniles

14.1.3.1 Closed system

Juveniles can also be transported in plastic bags, but primarily when transporting small numbers of smaller sizes. The same procedures for embryos and larvae apply, however the bags, especially when rectangular shaped, should be placed horizontally to maximize oxygen exchange along the larger surface area of water. Due to the sharp scutes in some species, using two bags in double-layered fashion is especially important. Feeding of fish should be stopped for 24-72 h before transport.

14.1.3.2 Open system

Open systems, which are typically used for in-house transportation include various sized buckets, cans, canvas vats, and plastic or fiberglass totes which can be moved by hand, truck, forklift, or other mechanical means. For juvenile transport to other facilities or release sites, live-haul fish tanks are usually made of fiberglass or marine grade aluminum and fitted onto some type of truck transport (see Figure 14.1).

Live-haul tanks are usually insulated to help maintain water temperature. The tanks must be free of any inside sharp edges, and this is especially true for any large or long tanks which usually contain baffles to minimize water surging that can injure fish and potentially create unsafe driving conditions. Some type of oxygenation system is used with diffusers made of either, very fine pore, flat or round ceramic, rubber membrane (ethylene propylene diene monomer) or Bio Weave, fixed to the bottom to avoid the risk of fish (especially smaller sized individuals) getting stuck below the diffusor. Tank size should be appropriate to allow the fish to swim freely and have a large enough dump gate and a full-size lid (able to be held in an open position) to ease loading and unloading. The tank is filled with appropriate water at the designated transport temperature, just before loading the fish, especially during hot days so the water has minimal time for ambient heating. The oxygenation system should be initiated and checked for proper functioning a few minutes prior to fish loading. Stocking density guidelines for transporting different size sturgeon in open tanks are given in Table 14.5.

The long-term transportation of fingerlings can be performed using river/ocean vessels specially equipped with tanks and systems to control temperature and oxygen (Chebanov & Galich, 2011). For example, *A. baerii* fingerlings (3.9 g) were transported 1,800 km, at 15-16 °C for 24 d, from the Abalak Sturgeon Hatchery Farm (Irtysh River, Russia) to the mouth of the river Ob' (Korentovich & Litvinenko, 2018).

Figure 14.1*: Transportation tanks and trailer, with compressed oxygen cylinders, that are used for sturgeons of various sizes (Photo credit: T. Friedrich).*

14.1.4 Water Quality

Water quality must be maintained while fish are stressed and crowded during transportation, and the most critical parameters are water temperature, dissolved oxygen, pH, carbon dioxide, and ammonia. The main factors that affect stress and these water quality criteria are the stocking density and duration of transport

(Harmon, 2009; Falahatkar *et al*. 2012; Sampaio & Freire, 2016; Kurtoğlu *et al*. 2021).
Water temperature is partially maintained by using insulated closed or open systems during transport, but often wet ice or gel packs are needed, especially during the hot summer seasons. A general guideline is that 0.45 kg of ice can lower 7.56 L of water 5.5 °C (Timmons et al. 2002) but the recommendation is to test your closed system, using a temperature data logger instead of live fish, during a mock transport trial over the expected length of transport time. Based on the data collected you can adjust the amount of ice to maintain your desired optimal temperature. For open systems, it is recommended that transport be done during the cooler parts of the day for short trips. For longer distances the temperature should be checked every 4-6 h, and blocks of frozen water from the same source that filled the transport tanks can be added, if needed.

For closed systems, the minimum ratio of water to oxygen should be 1:2 but preferably 1:3, especially for longer transportation times (> 15 h), to ensure adequate oxygen diffusion at the surface of the water. For open systems the oxygenation system, using either liquid oxygen or compressed oxygen tanks, should provide adequate dissolved oxygen rapidly and efficiently, at levels of at least 6 mg/L. Higher levels, near 100% saturation may help fish cope with the physiological stress and ammonia build-up during long transport times. When monitoring water temperature, it is also important to measure oxygen concentrations, so a reliable oxygen/temperature meter is critical.

Carbon dioxide (CO_2), a byproduct of fish respiration, is excreted from the gills and reacts with water to form a weak acid that will decrease the pH of the water. Any type of water disturbance, such as agitators or heavy aeration can help remove CO_2 from the water and any type of opening in the top of the lid in a closed tank can provide the pathway for atmospheric air exchange. Without some type of open vent, a tightly closed lid on a tank could eventually prevent CO_2 from off gassing from the water, if the partial pressure of CO_2 in the airspace above the water becomes too high (Harmon, 2009).

Ammonia levels also can increase because of fish metabolism, and bacterial action on fish waste excreted into the water. To eliminate waste excretion, make sure to stop feeding long enough before transport, to ensure gastro-intestinal tracts are empty. There are some products that are used remove total ammonia nitrogen in transport tanks, including zeolites (microporous crystalline hydrated aluminosilicates) (Ghasemi *et al*. 2018), and they have also been successfully used in closed systems (Mustahal *et al*. 2021).

A general fish transportation guideline is to reduce the water temperature during transport, to a few degrees below your species-specific optima, to reduce metabolic rate and oxygen consumption, increase solubility of oxygen in water, and to decrease ammonia and carbon dioxide production (Luz & Favero, 2024).

There are several other chemical additives that can also be used during fish transport to reduce stress and help maintain water quality. The most common is

salt (NaCl), and for juveniles and adults, it is advisable to add unionized salt (3–5 ‰) during transportation. During the longer transportation times, if the pH drops below optimal levels, sodium bicarbonate (Na_2CO_3) is a fast-acting buffer that could be added at a rate of 1 g/4 L, using a reliable pH meter to verify the increase to an optimum level.

Anesthetic agents have been used to reduce stress during fish transport and handling (Luz & Favero, 2024) but if not used properly, anesthetics can lead to negative consequences, such as acidosis, osmotic stress, insufficient exchange of gases and ions from the water, and respiratory arrest (Zhal *et al*. 2012). Bai et al. (2024) summarized that anesthetized hybrid sturgeon should not be transported longer than 72 h because of the negative physiological and biochemical responses. Use of an anesthetic during transport of juvenile sturgeon is not recommended, primarily because even with a relatively rapid recovery upon release, individuals would be highly susceptible to predation for an unknown length of time until fully recovered.

Some cases of handling, especially when multiple fish are lifted by nets and exposed to the air, can cause excessive mucus production and if this is an obvious problem after loading fish into an open system, the tank should be "rinsed" by replacing water for up to 20–30 min to remove the excess mucus from the transport container (Chebanov & Galich, 2011).

Table 14.5: *Stocking density guidelines for transporting different size sturgeon in open tanks, at different water temperatures. For transports over longer periods of time (>15 h), it is recommended to use even lower densities. (modified from Chebanov & Galich, 2011; Hochleithner & Gessner, 2012; Steinbach, 2021).*

		Stocking density (kg/m³)				
Fish size (g)		1	10	100-200	1,000	10,000
Water temperature	10 °C	50	70	100	200	500
	20 °C	20	30	50	100	200

14.2 Release strategy

The timing and location of release can influence post-release survival, behavior and growth (Anderson et al. 2022). Information regarding, food availability, water quality, and predation risks, at the release locations where the natural cohorts of the population has thrived can help optimize the timing of release, that also incorporates size and age of the fish (Chebanov, 1996; Chebanov & Savelyeva, 1999; Chebanov *et al.* 2002; Chebanov *et al*. 2011).

14.2.1 Larval release

Acclimation to the physicochemical conditions of the water at the release site is required, and temperature, pH, and dissolved oxygen are the most important parameters to measure. Acclimation of fish transported in bags can begin by floating unopened bags in a shaded area of the receiving water for about 30 min. The bags can then be opened and 4-8 L of receiving water added to the bag, and then check both temperature and pH in the bag and continue gradually adding water until the temperature and pH are equalized with the receiving water. The duration of the acclimation depends on the degree of difference between the transport and receiving water. A conservative temperature acclimatization can be at a rate of 1° C per hour, but experience with different species over several stocking events will ultimately determine the rate of acceptable acclimation. Most fish seem to tolerate a rapid drop in temperature better than the equivalent rise in temperature (Noga, 2000).

Cages for acclimation have been tested but require further evaluation to determine if they can improve performance post-release. They have the advantages of allowing the fish to acclimate to the sites for a few hours or days, and allow observation of the fish before their release, in terms of post-transport mortality and normal swimming behavior.

14.2.2 Juvenile release

For open tank systems, an appropriate amount of receiving water can be added to the tank by buckets or a water pump, as the temperature is monitored. Locations for juvenile release should be selected based on the biological requirements of the species and the life phase being released. This information is ideally collected through monitoring of existing wild fish where possible. The primary factors for identification of release sites are the availability of sufficient food organisms, water quality and the state of the habitat, such as, the preferred bottom conditions, shoreline vegetation (potential refuge areas), and presence of predators. Since juvenile sturgeon tend to be more active at night, which may enhance their foraging success (Chiasson *et al.* 1997) and enhance survival, it is recommended to release during dusk or early night hours (Crossman et al. 2011).

Depending on the transport tank design, and the available access for a truck to the release site, the juveniles can be released either through the tanks dump gate (e.g. if it releases directly into the river site that has enough depth, so juveniles do not hit the river bottom) or juveniles are gently netted into transport buckets and then hand-carried, and released into the river in small batches.

Salinity could play an important role in the selection of proper sites for the release as sturgeon juvenile migrations are highly variable and species specific. Some released juveniles may encounter areas of increasing salinity or one of the *ex situ* program goals could be to release them into identified brackish water sites. If this is a goal, then the fish need to be at the proper size and acclimated to the target salinity in the hatchery and/or during transport, before release into brackish water

sites. If juveniles are released into fresh water, but will potentially migrate into brackish water, then the appropriate size at release needs to be considered.

The developed osmoregulatory system in juvenile sturgeon is characterized by the ability to convert from a hypotonic to a hypertonic type of osmoregulation (Krayushkina, 2006), and in the process of adaptation to salt water, activation of the excretory function of the gill's chloride cells occurs, which removes excess univalent ions. The ontogeny of salinity tolerance and the hypo-osmoregulatory changes that occurs in different sturgeon species is primarily size dependent and has been reported for several species (e.g. LeBreton & Beamish, 1998; Cataldi *et al*. 1999; Ziegeweid *et al*. 2008; Amiri *et al*. 2009; Allen *et al*. 2011). Appropriate body size criteria should be established before releasing juveniles into habitats of different salinities.

14.2.3 Release numbers and sizes

Specific release numbers are a critical component of the long-term adaptive management of an *ex situ* program, and should be based on the programs genetic plan (see Chapter 6).

The size and number to release must also take several ecological and logistic aspects into consideration:

- The hatchery capacity and resources for rearing.
- Domestication and epigenetic effects during rearing in the hatchery.
- Existing bottlenecks in the habitat to be released (e.g. food availability, predation risks, fishery bycatch).
- Genetic risks of overstocking and impacts on the remaining wild populations.

When releasing large numbers of early life stages, larvae can be enumerated by volume, weight or individual count. Various automatic fish counters developed for commercial farming are also available and may be suitable depending on life stage and species. They typically use light barriers/infrared light, but their cost makes them only feasible when producing large quantities of fish. To support the post-release monitoring program (see Chapter 15) it is important that all released fish are tagged or marked (Figure 14.2. see also Chapter 13).

The final decision process on release sizes and quantities depends on the local situation and the specific *ex situ* program goals and objectives. In many programs, a mixed approach of releasing various sizes from larvae, age-0 juveniles, and older juveniles (age 1+/2+) has been used (Anderson *et al.* 2022).

Figure 14.2: *Tagged A. oxyrinchus juvenile prior to release (Photo credit: J. Gessner).*

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Chapter 15: Future outlook and important considerations in the success of *ex situ* programs for recovery of sturgeons

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Ex situ measures have experienced considerable change over the past decade with an increasing awareness about the adverse impacts of interventions that solely depend upon the release of juveniles. It is widely accepted now that the prioritizing of habitat protection and restoring habitats where necessary are essential elements of species conservation. The reversal or mitigation of the impacts that led to the species decline must be an overarching principle when facilitating recovery of endangered species. *Ex situ* measures should only be considered in cases when population abundance is insufficient to facilitate recovery and should be implemented in a manner that is specifically addressing the shortcomings in natural recruitment. Nevertheless, *ex situ* measures, including the establishment of captive broodstock, might be necessary where the success of *in situ* measures remains questionable or is uncertain to prevent genetic heterogeneity and as such adaptation potential of the population from being lost. The availability of improved genetic techniques and methodology supports the selection and conservation of rare genotypes and helps establish reproduction plans that avoid the most common genetic drawbacks (e.g. in- and outbreeding).

Improving the awareness of administrators, conservationists, and management agencies around the different options that *ex situ* measures offer, and the prerequisites required to apply them effectively, is one key element to increase conservation success. The second key element is the adaptation of *ex situ* measures to local conditions that consider species-specific biology and their adaptation to the environment where recovery occurs. Scientific experimentation and analysis of the key features and requirements is essential for success during reproduction, rearing, and release. Standardized documentation of conditions and methods applied, adaptations performed, and their outcome is essential to continuing to refine and adapt rearing conditions to be as representative of the natural environment as possible. The same holds true for the documentation of the conditions and results that are associated with processes occurring in wild populations where possible.

Future success of *ex situ* programs depends on an adaptive framework that incorporates monitoring results directly into ongoing decision making informed from working groups comprised of all entities essential to the species recovery (e.g. multi-national; inter-governmental, Indigenous Nations, industry, etc.). While the notion of adaptive management is not novel, the life history characteristics for sturgeons require a management time frame for *ex situ*

programs that exceeds many fisheries managers or scientists' careers. Accordingly, success is predicated on development of objectives for *ex situ* programs that are both clearly established to achieve specific outcomes yet remain flexible to change and allow for the integration of monitoring results on a frequent basis. An ecosystem-based approach to adaptive management is likely most practical for sturgeons but requires considerable collaboration with other regional conservation actions. While the magnitude of results may vary across populations or species, biological responses may be similar due to many species having similar life history and biological requirements. Accordingly, inter-species comparisons and collaborations will only benefit sturgeon recovery. At a minimum, incorporation of information from adjacent programs for other regional species should be considered whenever possible. There are cautionary tales from *ex situ* programs for other species (e.g. Salmonids; McMillan *et al.* 2023) while the rate of learning for sturgeons is remarkably slower in comparison due to their longevity and generation time. Therefore, we recommend *ex situ* programs take a precautionary approach that is founded by best available evidence. This is especially important when it comes to release numbers and the potential for overstocking due to insufficient monitoring data or societal pressures to release rather than cull to specific targets.

Effective management of *ex situ* programs relies on structured post-release effectiveness monitoring that can reduce uncertainty and provide direct feedback to meeting stated objectives. There is a suite of important measures that require evaluation and are well defined in other literature (see Table 2 in Anderson *et al.* 2022) but generally consider factors associated with trends in survival and growth, life stage specific diets, post-release genetic measures, and habitat use. Post-release monitoring results are as important as producing and releasing fish, and *ex situ* programs need to ensure a rigorous monitoring program is incorporated, especially if the effect of different rearing strategies upon post release survival is to be assessed. This includes careful consideration of how fish are marked, where they are released, and how post-release sampling efforts will be balanced over the life of the program. If post-release monitoring is limited due to insufficient resources, *ex situ* programs could consider pausing to allow time for data collection to help direct the next phases of the program. In certain cases where wild populations still exist, this requires more attention to ensure negative effects are not occurring (e.g. growth). It is recommended that effectiveness monitoring is developed in concert with the appropriate entities (e.g. government, academia, industry, Indigenous Nations) to ensure results can inform both modifications to *ex situ* programs but also responses that will inform other conservation actions integral to population recovery (e.g. habitat restoration, flow regulations, etc.). Lastly, we must leverage results across different sturgeon species to facilitate our learning and reduce negative outcomes from *ex situ* programs.

Climate change is expected to significantly impact future recovery of sturgeon and will present challenges for *ex situ* programs and requires urgent attention. The net effect from climate change, existing modification of natural flow regimes from regulation, and increasing water management actions for human interests will

complicate population recovery in some rivers and present multiple bottlenecks for *ex situ* programs. Climate change may complicate *ex situ* facilities water security, influence the thermal profiles at facilities reliant on natural river water, impact wild spawning timing and success (i.e. broodstock or progeny collection), interfere with population adaptation under natural reproduction conditions through a mismatch in larval occurrence and feed availability, negatively impact the success of releases, or question the suitability of *ex situ* for recovery of the species in some cases. Given climate stressors are an immediate global concern, this requires actions by new and existing programs and needs incorporation into overall recovery program design (Ganapathi *et al.* 2020). Similar to effectiveness monitoring, mitigation of climate stressors will be best informed through cross-population and -species dissemination of results and information exchange.

Despite considerable attention on e*x situ* practices for sturgeons in recent decades, a number of uncertainties still require additional research. Imprinting and straying of released fish has been, and remains, and area of concern for most programs. To the extent possible, continued efforts to determine how to reflect natural conditions during rearing remains an overarching component of e*x situ* success. This includes additional research to understand how enhancements to increase complexity during rearing can influence outcomes (e.g. survival, maturation schedules) following release in the wild. Integral to these enhancements includes work to improve diets during rearing. In particular, a better understanding of species-specific optimal feeds during the larval and young of year stages, how to produce natural feed as part of hatcheries infrastructure, and research in the wild to identify prey items as a basis for the development of optimized formulated diets to inform these actions within the e*x situ* program is needed.

To ensure future genetic integrity, measures of genetic diversity at the time of release remains a key research focus, and continued work to describe post-release genetic diversity measures as hatchery-origin fish mature should be an important component of research. In addition, the mechanisms underlying mate choice are poorly understood. The determination of criteria for and the effects of mate choice on fitness of the progeny must be intensified to ensure that reproduction plans are enabled to mimic natural processes for the benefit of the fitness and survival of offspring.

While considerable work has been done to investigate size at release, results have generally favored older, larger sizes as they are more reliably detected in post-release monitoring and allows for predator avoidance. Continued efforts to optimize release size to ensure domestication effects are reduced to the extent possible remains a priority.

The ultimate goal for *ex situ* programs is to not be required anymore. In a simplified approach, it could be postulated that the continued proof of the self-sustaining status of a population over at least half a generation time is the ultimate prerequisite for the termination of the *ex situ* measures. Termination of *ex situ* measures including the infrastructure and the associated work often meets substantial opposition due to the associated socio-economic effects. This

reluctance is further enhanced by the fact that the determination of program end points is complex and depends on restoration and alleviation of other bottlenecks to the population or species viability. *Ex situ* is only one component of sturgeon recovery and cannot be successful without concurrent *in situ* program development and implementation to address other impediments to successful recovery. Even in this case, the fate of the *ex situ* stock remains open since it is a unique resource that would be a backup device in case of adverse conditions that repeatedly affect the population in the wild. If an ecosystem-based approach to recovery is taken, it can reduce socio-economic pressures of closing hatcheries as multi-species objectives can be considered and existing facilities can support other species recovery objectives. Further work to explore the human dimensions of recovering endangered species will be important to ensure a balance between recovery goals and eventual human use for recreation or harvest.

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