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FOURTH INTER-GOVERNMENTAL MEETING ON THE ESTABLISHMENT OF THE CENTRAL ASIAN AND CAUCASUS REGIONAL FISHERIES AND AQUACULTURE COMMISSION

Issyk Kul, Kyrgyzstan, 22 – 24 June 2011

DRAFT TECHNICAL GUIDELINES FOR STURGEON HATCHERY PRACTICES AND MANAGEMENT FOR RELEASE

Note: These draft Technical Guidelines are being finalized by FAO and the World Sturgeon Conservation Society (WSCS) at present. The current version presented to this meeting should not be considered a final draft yet. Comments and observations made by the meeting will be taken into consideration in the final version. Some sections of this draft document (those marked with an asterisk *) will be checked again and the whole document will undergo a thorough editing before publication. Missing references will be included and wherever possible the text will be improved to facilitate the readers' understanding of the subject.

At this stage, the Fourth Intergovernmental Meeting may wish to consider the following options (either individually, or in combination), as a way forward on this subject of the "Technical Guidelines for Sturgeon Hatchery Practices and Hatchery Management for Release" in the region:

1. To take note of the draft Technical Guidelines (as presented to the meeting) - but consider that further action by the Commission on this subject will not be required.

2. To discuss, review and adopt the draft Technical guidelines, request the experts to finalize the document, and to pass the final draft version forward to the Inaugural Meeting of the Commission for official endorsement by the Commission.

3. To request the Technical Advisory Committee (TAC) of the Commission - at its first meeting in 2012 - to look into ways in which the Technical Guidelines might best be followed up at the national and regional levels.

These draft Technical Guidelines have been produced under a partnership composed of the following organizations:

Technical guidelines on sturgeon hatchery practices and hatchery management for release

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Preparation of this Document

These Technical guidelines have been prepared by Mikhail Chebanov (South Branch Federal Center of Selection and Genetics for Aquaculture, Russian Federation) and Harald Rosenthal (World Sturgeon Conservation Society, Germany), with the collaboration of Phaedra Doukakis (United State of America), Joern Gessner (Germany), Mohammad Pourkazemi (Iran), Raymon van Anrooy (FAO) and Patrick Williot (France) on the basis of comments, inputs and suggestions received on the various drafts of these Technical guidelines at workshops held in Atyrau, Kazakhstan, 15-18 April 2009 and Wuhan, China, 25 October 2009. Selected sturgeon hatchery and sturgeon biology experts and other stakeholders from academia, national and international research institutions, governmental organizations and international non-governmental organizations (INGOs) were invited to these workshops.

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ABSTRACT

(to be prepared)

Acronyms and Abbreviations

BMPs	Best Management Practices
BOD	Biochemical Oxygen Demand
CaspEco	The Caspian Sea: Restoring Depleted Fisheries and Consolidation of a Permanent Regional Environmental Governance Framework
CCRF	Code of Conduct for Responsible Fisheries
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CWT	Coded Wire Tags
DNA	DeoxyriboNucleic Acid
FAO	Food and Agriculture Organization of the United Nations
GEF	Global Environment Facility
GV	Germinal Vesicles
GVBD	Germinal Vesicle Break Down
GnRHa	Analogues of gonadotropins-releasing hormones
HIV/AIDS	Human Immunodeficiency Virus /Acquired Immune Deficiency Syndrome
HUFA	Highly unsaturated fatty acids
IHE	Institute for Hydro-Ecology (China)
ILO	International Labour Organization
ISS6	6th International Symposium on Sturgeon
INGOs	International Non-Governmental Organizations
IUCN	International Union for Conservation of Nature
IUCN/SSG	IUCN Sturgeon Specialist Group
IUU	Illegal, unregistered and unreported fishing
LLTHFB	long-term low temperature holding facilities for breeders
LLTUHB	Long-term low temperature unit for holding of broodstock
MS-222	Tricaine Methanesulfonate
P	number of pectoral fin rays
PI	(oocyte) Polarization Index
PIT	Passive Integrated Transponder
SES	Speed of Erythrocyte Sedimentation
PCB	Poly-chlorinated biphenyles
PPT	Parts per thousand
SPGP	Sturgeon pituitary glycerol preparation
SIS (medium)	Siberian Sturgeon (medium)
S(Lat)	number of Lateral Scutes
STR	Spawning Temperature Regime
SV	number of Ventral Scutes
TCP	Technical Cooperation Programme (FAO)
TFESSD	World Bank Trust Fund for Environmentally and Socially Sustainable Development
UNDP	United Nations Development Programme
UV	UltraViolet light
V	number of Ventral fin rays
violet ”K”	$C_{24}H_{28}N_3Cl$
WSCS	World Sturgeon Conservation Society

CONTENTS

1. INTRODUCTION

1.1 The need for guidelines

Governments of the Caspian Sea littoral states indicated in various occasions in recent years that rehabilitation and management of sturgeon stocks in the Caspian Sea is a priority issue for them. One of the critical aspects in the rehabilitation of the sturgeon stocks is the restocking of sturgeon species in the Caspian Sea; sturgeon hatcheries play an important role here.

While the sturgeon hatchery sub-sector is slowly being rehabilitated after the collapse of the Soviet Union in 1991, a large part of the experience, knowhow, and specific knowledge on sturgeon hatchery practices and management in the Caspian Sea basin has disappeared. Numerous experts have left the sector in the 1990s and due to reduced governmental funding of (State) hatcheries the production reduced and technologies that were introduced elsewhere did not follow suit in the region.

In recent years it has been recognized that stocking of hatchery-produced progeny may include the risk of altering the genetic structure of the natural stocks while also producing juveniles not fit for survival in nature. Therefore, the entire culture process, and the strategy for conservation culture must be critically re-assessed to adjust the design of rehabilitation programmes and subsequently the design of hatcheries and the mode of their operation to serve the specific release purposes, of which there are several:

- release for ranching purposes (a strictly commercial activity, addressing primarily a terminal fishery, however, without any consideration on species conservation)
- release for stock enhancement purposes (strictly in support of overall fisheries in the region, supporting an aquaculture-based fishery)
- release for compensation of lack of recruitment (stabilizing population size, including a certain component of species conservation)
- release for re-introduction (re-stocking; re-building of stocks either already extinct or at the brink of extinction; this is primarily a conservation objective)

The recognition of the fact that these four specific objectives require very different approaches has led to the conclusion that based on well-established knowledge for conventional hatchery operation for any fish species, there is a need to revisit and re-assess the existing hatchery methodologies in terms of their appropriateness for future sturgeon rehabilitation programmes. The pertinent issues related to this re-assessment will be addressed in this document. The main emphasis of the present guideline has been placed on the last issue (re-introduction; re-stocking)

As a consequence of these new considerations, the recent impetus given by the Caspian Sea countries to the sturgeon hatchery sector in particular, in terms of funding and other support, cannot be absorbed well by the sector, in particular when addressing specifically the above cited scenarios. Many managers and technicians have not been properly trained in the wide range of aspects of sturgeon reproduction, breeding, restocking and re-establishment of self-sustaining populations. Practices applied in some hatcheries conflict with ecological, environmental and socio-economic objectives of the countries concerned.

For example, numerous projects and activities in recent years have aimed to increase the release of sturgeon in the Caspian Sea basin, in order to rehabilitate the stocks in the wild.

Many have failed to achieve their outputs foreseen or only achieved a small part of what was aimed at and this may have numerous reasons. Simple measures, such as simply distributing the fingerlings for release over various locations may not be effective.

In order to assist in understanding the requirements for hatcheries that are intended to serve conservation culture, one has to clearly emphasize the **fundamental differences** in hatchery operations for **commercial aquaculture** and for **culture for release** into natural waters.

Unfortunately, over the past century, sturgeon stock rehabilitation programmes had simply adopted common commercial aquaculture technology and ignored the need for challenging the culture methods whether they are optimal to produce progeny that has been adequately selected, raised, acclimated or trained for fitness for survival in the wild.

The Governments of the Caspian Sea Littoral countries increasingly recognize the limited efficiency of their restocking programmes. They requested the Food and Agriculture Organization of the United Nations (FAO) to support training in sturgeon hatchery operations. In response, FAO teamed – up with the World Bank and UNDP and conducted a hatchery training workshop in Atyrau, Kazakhstan in April 2009. Considering the fact that only a limited number of stakeholders can be trained through a workshop approach, and as the subject is of interest to a much wider (Global) audience, the three partners decided to develop, in close collaboration with the WSCS, IUCN/SSG, CaspECo, IHE and ISS6 the current technical guidelines.

1.2 Aim and Scope of the Guidelines

These Technical Guidelines have been developed specifically for use by sturgeon hatchery managers, fisheries managers, fisheries policy makers and other authorities responsible for fisheries and aquaculture development. They are targeted particularly at those who are involved in the senior management of sturgeon hatcheries, those who commission or manage hatchery feasibility studies and investment studies, and those who are involved in sturgeon restocking projects and programmes.

These Technical Guidelines do not aspire to and cannot function as a sturgeon hatchery manual.

These Technical Guidelines aim to increase global awareness, technically guide and build capacity about the best-practices currently available in sturgeon hatchery management, by providing senior and mid-level sturgeon hatchery staff a practical tool for modern sturgeon hatchery practices and management. These technical guidelines focus on hatchery practices that are aimed at reproduction and growth of fry and fingerlings for restocking objectives, in contrast (in some cases) to aquaculture objectives.

Because of the importance of the differences between the aims for aquaculture production and for re-establishment (re-stocking) we wish to specifically address the major goals of both culture objectives (the fitness for performance in the two different target environments).

The objectives for Aquaculture production focus on (1) fitness of the cultured specimens of any species in the aquaculture system and (2) meeting consumer preferences. Therefore the entire operation aims at

(a) maximum survival (producing high numbers)

(b) fast growth (producing big biomass in the shortest time possible)

(c) produce disease free specimens and/or resistant to disease (avoid any losses)

(d) achieve high food conversion efficiency (reduce production costs; no need to train to hunt for food)

(e) produce good meat composition/quality (provide health food and meet consumer expectations)

(f) manage all year round spawning (allow continuous culture, totally independent from natural life cycle)

(g) produce fish hardy in handling (allowing easy management for sorting and life transportation)

(h) achieve good processing characteristics (allow easy processing for added value products to suite the markets)

In contrast, none of the target objectives mentioned above for commercial aquaculture of sturgeons or any other aquatic species apply for cultivation for release.

Certainly, as briefly addressed in chapter 1.1, cultivation for release may also serve different purposes which may not necessarily have a strong conservation element. This has also to be clearly understood when planning culture systems that produce juvenile fish for release.

For example, **Cultivation for Ranching** does not have a conservation element in its strategy but wishes to serve a terminal fishery where ownership is preferably predetermined (individually, as fishing community or national). As such ranching aims at providing fish for the market and not necessarily supporting natural stocks. In fact, the species to be ranched may not necessarily be a native one. Ones the ranching activity is terminated, the fishery is also terminated because the objective does not target for an establishment of self-sustaining populations (although this may happen).

Cultivation for stock enhancement (either for r- or k-selection species). Again, the major objective is to support the fishery by enlarging the otherwise self-sustained but potentially overfished population to a size beyond what natural recruitment can achieve. The species may not be at all threatened with extinction.

Cultivation to compensate for lack of recruitment. This may apply in areas where a species has lost a major portion of its supporting habitat, either as spawnning ground or nursery area. Here, a strong conservations element has to be incorporated in the operational strategy as the released fish must have the identical fitness (genetical, physiological, behavioural) in line with the natural population so that there is no risk of any outbreeding depression.

Cultivation to re-establish a highly endangered or extinct species in its former natural habitat has the highest conservation element in its strategic programme. Here, the final product and endpoint of the programme is the establishment of a population having the same performance traits as the former natural population so that – in the long-term – the cultivation for release can be terminated ones self-sustaining populations have been established in the historic range.

Obviously, the objectives of cultivation methods for re-establishment (or re-introduction; re-stocking) aim mainly at imitating – as close as possible – the natural environment in which the cultured specimens are to be released, providing the natural temperature, light and water quality conditions (including natural diurnal, seasonal cycles), and other essential natural environmental cues. Thus, specimens raised for release are totally unfit for performing well in commercial aquaculture systems and visa-versa. At best, surplus production of fertilized eggs and early yolk-sac stages derived from release-culture can be provided for commercial aquaculture, however, this does not necessarily work the other way round. We wish to specifically emphasize these differences as many aquaculturists providing stocking material for the put-and-take fishery believe that the same methodology would be adequate for conservation culture – it is not!

Even when considering culture methods for release, It should be recognized that not all technical procedures and suggested strategies can be applied everywhere, but those involved in the subject should strive towards implementing the guidelines to the extent conditions allow.

1.3 Normative and methodological basis for these Guidelines

The normative and methodological basis for these Technical Guidelines is provided by the "Code of Conduct for Responsible Fisheries" (FAO, 1995).

Some articles of the Code are directly associated with the objectives of these Technical Guidelines, for instance:

"States should promote the use of appropriate procedures for the selection of broodstock and the production of eggs, larvae and fry."
(CCRF Article 9.3.4)

Selection of broodstock. Selection of broodstock should be based on, *inter alia,* the performance of the fish in a culture environment, the desired breeding programme, the genetic profile of the broodstock, and economic and environmental considerations. Production of eggs, larvae and fry will depend upon sound hatchery and grow-out management, after selection of appropriate broodstock.

Breeding and genetic improvement. While considerable improvements have been made in cultured stocks through genetic selection and breeding programmes, few fish farmers have the required training and experience to do such work efficiently and without significant losses of genetic fitness. For such reasons it is advisable to establish specialized facilities for the development of improved stocks and the production of seed. Where this is not practicable, farmers should try to keep genetic diversity high (Tave, 1995):

- by breeding an adequate number of specimens of the species of concern so as to fully preserve its genetic diversity,
- by using breeders and eggs from the entire spawning season so as to cover all population traits,
- by avoiding full-sib or parent-offspring mating, and
- by keeping careful records on all production parameters so that in retrospect the entire production lineage can be tracked.

Decreased hatchability, decreased fertility, increased deformities, increased diseases and decreased survival in the hatchery or later in the natural environment (after release) may be signs of inbreeding and loss of genetic diversity. They may be signs of other management and handling problems as well and this is why comprehensive records of all system specific factors are necessary to determine the most probable cause(s) of the problem (FAO,1997).

"States should, where appropriate, promote research and, when feasible, the development of culture techniques for endangered species to protect, rehabilitate and enhance their stocks, taking into account the critical need to conserve genetic diversity of endangered species." ***(CCRF Article 9.3.5)***

Sturgeon hatcheries play a key role in safeguarding these highly endangered species. If properly managed in line with the suggestions laid down in this guideline, they can contribute to the re-establishment and protection of these endangered species and may also serve stock enhancement programmes. States should carefully consider the provision of support to the development of appropriate culture techniques for endangered species. The use of specific hatcheries for the temporary protection and breeding of endangered species is considered to be a valuable facet of *ex situ* conservation. While such *ex situ* conservation is often necessary in the face of immediate environmental threat and the potential loss of valuable species or genetic resources, the preferred method for endangered species protection is *in situ,* i.e. habitat rehabilitation and the amelioration of the threat to the species.

Breeding of endangered species. The purpose of an endangered species breeding programme is to produce an organism that can be released into nature once the threat to its survival has been alleviated (Johnson and Jensen,1991) to the extent that one can hope a self-sustaining population can be re-established at least in the long run. Breeding efforts should try to optimize the natural genetic variability in the species:

- by establishing broodstocks (of adequate size and composition) as founder population, in a properly planned manner, in order to minimize the loss of genetic diversity (hatchery brood stock composition must be based on the diversity exhibited by wild stocks);
- by avoiding direct or induced inbreeding; that is, avoid breeding strictly related individuals and avoid releasing stocks mostly composed by related animals (obtained by one or few reproductions) that are possibly going to mate in the environment;
- by using breeders of the native population if possible, in order to avoid outbreeding depression;
- by avoiding intra- and inter-specific hybridization;
- by identifying the susceptibility towards selection and adaptation processes in captivity in order to avoid "captive selection".

Genetic technologies can be utilized:
- to determine the taxonomic status of an endangered species on which a brood stock will be built
- to perform an initial analyses on the natural populations of native species in order to identify the conservation units to be separately managed;
- to identify genetically compatibility or appropriateness of the selected broodstock
- to reconstruct a population of males and females of an endangered species using gametes from one sex of the endangered species and a modified gamete of the other sex from a closely related, and presumably non-endangered, species
- to ensure a ready supply of sperm through cryopreservation from endangered or closely related species (see above) so as to enhance the probability for successful egg fertilization because of the risk of having not always gametes of both sexes readily available in each season. Particularly in sturgeon species close to extinction the allee-effect may also affect hatchery operation.

Where feasible and known, species that are in imminent danger of becoming endangered should be studied and managed to reduce the threat in their natural habitat. As a safeguard, sperm or live individuals could be conserved *ex situ* while management efforts to improve their chances of survival in nature are underway. The collection of species for this *ex situ* conservation should not threaten the viability of the natural population.

Apart from the above mentioned articles (originating from FAO, 1997), these Technical Guidelines will support States to implement Article 9.3.1" States should conserve genetic diversity and maintain integrity of aquatic communities and ecosystems by appropriate management" of the Code of Conduct for Responsible Fisheries.

The guidelines will also support the implementation of the strategy for sturgeon conservation as presented in the recommendations of the "Ramsar Declaration on Global Sturgeon Conservation" (Rosenthal et al., 2006), the Action plan for the conservation and restoration of the European Sturgeon (Council of Europe, 2008) and national level action plans and strategies in the same line.

Although it is believed that this manual will assist in improving common operational practices in hatchery management, it has also to be emphasized that employing best available technology and bio-production strategies alone are by far not sufficient to enhance the chance for success to re-establish or enhance populations of highly endangered sturgeon stocks, unless a much broader approach by all involved is taken. As has recently been pointed out by Lorenzen et al (2010), a responsible approach to re-establishing self-sustaining populations must include an broader integrated strategy within fisheries management systems across national borders with emphasis on a "......stakeholder participatory and scientifically informed, accountable planning process". Likewise, Bell et al (2006) already emphasized that "......the pre-occupation with bio-technical research at the expense of objective analysis of the need for he intervention, and failure to integrate the technology within an appropriate management scheme..." has prevented the success of such hatchery and release programmes. "The dynamic interactions between the resource, the technical intervention and the people who use it" (Bell et all., 2006) have been equally responsible for past failures to re-establish dwindling populations as the lack of knowledge on the optimum strategy for release to achieve good survival. Thus, all stakeholders have to recognize the need for a simultaneously undertaken integrated approach to interventions through hatchery release programmes, including mainly habitat restoration efforts, mitigation measures to counteract river fragmentation, improved stock assessment methodologies and strict enforcement of tight fisheries regulations (e.g. closed fishing seasons, catch quotas, and substantial penalties for violators of the rules at equal level in all jurisdictions of the Caspian basin.

1.4 General Principles

These "Technical guidelines on sturgeon hatchery practices and hatchery management for release" have been developed on the basis of three key principles:

- to contribute to the conservation of the natural gene pool of sturgeons in the Caspian Sea basin (and potentially elsewhere);
- to enhance the efficiency of hatchery-bred fingerling release programmes into the Caspian Sea basin in terms of improved fitness for survival when released.

- to build functional cultured broodstocks that are maintained to meet long term release objectives for either re-establishment, restocking or stock enhancement objectives

The implementation of these principles requires:

- to implement genetic monitoring programmes, including DNA-technology of wild breeders used for controlled reproduction and establishment of captive broodstocks, and development of optimal crossing protocols (mode of mating) to prevent inbreeding and outbreeding depression of populations;
- to establish live gene banks and sperm cryobanks of sturgeons from different river basins;
- to apply optimized technologies for sturgeon reproduction to support genetic biodiversity, including different intra-population ecological forms (hiemal, vernal, summer-spawning) where such variety in forms exist;
- to promote the implicit use of non-lethal methods for obtaining eggs from wild sturgeon females;
- to reduce stressors and risks during all handling, rearing and health management operations of breeders on the basis of non-traumatic (e.g. ultrasound techniques and other methods (computer-assisted video image analysis; Hufschmied et al., 2011) to diagnose weight gain and growth as well as sex, maturity stages and state of internal organs and systems.
- implementation of innovative biotechnology of larvae and fingerlings rearing to decrease the mortality rate during all stages of the life cycle and release into natural environment;
- preparation of fingerlings for release into natural water bodies through optimization of size, age, place and timing of release.

2. STURGEON BIOLOGY AND SPECIES IN THE CASPIAN SEA BASIN*

The diversity of sturgeon species in the Caspian Sea basin is relatively large compared to other regions in the world. There are five species that can be found in the basin: Russian sturgeon, Ship sturgeon, Stellate sturgeon, Beluga and Sterlet. Some are rather common in some areas of the basin while others are endangered. Each of these species has its own characteristics, feeding and spawning behaviour. In this chapter each of the species is discussed.

Russian sturgeon (*Acipenser gueldensdaedtii* Brandt). This species currently spawns in the Volga and Ural Rivers in the North Caspian Sea and may also spawn in Kura River and Sefid Rud River in the South Caspian Sea. A complicated pattern of spawning migrations includes spring and autumn runs and seasonal forms are reported in the Caspian Sea. Individuals migrating in spring enter freshwater just before spawning; they tend to spawn in lower reaches of rivers. Individuals migrating in autumn overwinter in rivers and spawn the following spring further upstream.

Most spawning sites have been lost due to dam construction. The Caspian basin lost 70 percent of spawning grounds since the 1950s mainly due to the construction of hydroelectric power stations; the Ural is now the only river in the basin with unregulated flow. High levels of pollution (from oil and industrial waste) in the Caspian Sea basin have altered hormonal balance and increased the number of hermaphroditic fish. Pollution levels are now decreasing since the collapse of the Union of Socialist Soviet Republics (CITES, 2000).

The average age of parents of current cohorts, is estimated to be 15 years under natural circumstances, but due to the variety of threats faced by this species the generation length has become some 12 years in the Caspian Sea. Females reproduce every 4-6 and males every 2-3 years, males reproduce for the first time at 8-13 years, females at 10-16. In April-June when the temperature rises above 10°C the reproduction period starts. Larvae drift on the currents in the rivers; juveniles then move towards shallower habitats, before migrating to the sea during their first summer. They remain at sea until maturity. The species feeds on a wide variety of benthic mollusks, crustaceans and small fish.

The species wild native population has undergone a major decline. Despite relatively high stocking of fingerlings in the sea, the catch of this species by fisheries has fallen some 98 percent in the last 15 years, particularly from the early 1990s onwards. According to FAO fisheries statistics (FAO 2009) global catches fell from 4,250 tonnes in 1992 to 67 tonnes in 2007. Khodorevskaya et al. 2009, reported similar declines for catches in the Caspian Sea basin from around 14 500 tonnes in the early 1980s to less than 1 000 per year in the period 2000 to 2008. The estimated spawning stock biomass in the Volga has also drastically declined, from 22 200 tonnes (1966-70) to 1 000 tonnes (1998-2002). Average number of spawners (1 000 individuals) passing fishery zones to the spawning grounds in the lower Volga (per year) has declined by 88 percent when comparing the averages of the period 1962-75 to the period 1992-2002.

Persian sturgeon (*Acipenser persicus* Borodin). This species is most abundant in the southern part of the Caspian Sea. In the past, Persian sturgeon could be found in most rivers around the Caspian Sea. However, currently the species only ascends lower courses of Iranian rivers, Volga and Ural and may enter the Terek and Kura occasionally.

Persian sturgeon spawns in strong-current habitats in the main courses of large and deep rivers on stony or gravel bottom surfaces. Juveniles remain in riverine habitats during their first summer. Males reproduce for the first time at the age of 8-15 years, females at an age of 12-18 years. The age structure of the mature population is diverse. The age ranges for mature females is 6-40 years, 85 percent of the mature females are between 14-18 years of age, and 80 percent of the males are 12-16 years (Moghim, 2003). Average generation length is some 14 years. The mature fish do not spawn every year. Spawning takes place in June-August when temperature rises above 16°C. In the southern Caspian basin the species spawns in the period April-September but reproduction is interrupted in the period June to August when water temperature rises above 25°C. Most individuals migrate upriver in April-May, but some may enter the rivers at other times of the year. In the southern Caspian basin, there is a second run in the period September-October. Juveniles migrate to the sea during their first summer and remain there until maturity. In the marine environment the species feeds on a wide variety of benthic mollusks, crustaceans and small fish.

Iran is the only country in the Caspian region that uses this species for stocking purposes. More than 80 percent of the total sturgeon (re)stocking activity carried out by Iran is with this species. In 1997 some 24.5 million fingerlings and in 2008 about 10 million fingerlings were released to the Caspian Sea (Pourkazemi pers. comm.).

This species has different ecological and biological requirements to *A. gueldenstaedtii* as it prefers warmer waters for spawning and has a shorter migration run. The lack of ability to genetically identify the species in international trade is a potential threat, as Russian and Persian sturgeon (caviar) can be mixed.

The only legal commercial fisheries exploitation of this species takes place in Iran (mainly originating from hatchery reproduced stocks). The catch data from Iran shows that in the period 1960/65 to 2006 the annual catch declined with 75 percent-82 percent. The catch has continued to decline since 2006 but official data are not available. The decline in catch reflects a decline in abundance even though there are fisheries regulations in place and catch efforts are reduced (Pourkazemi 2006). The Russian Federation has banned the commercial catch of this species in the Caspian Sea since 2000. The quota allocated in 2007 for scientific catch of this species was 8 tonnes. It is difficult to distinguish a decline of the wild populations due to the long term stocking of the species. However, it is suspected that the native wild population has declined by over 80 percent in the past three generations (est. 42 years) as all the wild populations have almost disappeared, except from the populations of restocked individuals from Iran. There are only occasional records from catches in the northern Caspian basin (in 2008 100 immature individuals were caught in the northern Caspian basin (Mugue pers. Comm.).

Illegal, unregistered and unreported (IUU) fishing, poaching, pollution (oil, industrial pollution, agricultural pollution and domestic wastes) and habitat loss across the Caspian region are the main threats for the conservation of this species.

Ship sturgeon (*Acipenser nudiventis* Lovetsky). This species is currently only found in the Caspian Sea Basin, where it ascends in the Ural River (where it naturally reproduces). It also occasionally ascends the Sefid Rud River, where seven fish were caught in 2002. This species spawns in strong-current habitats in main courses of large and deep rivers on stone or gravel bottom materials. Males reproduce for the first time at 6-15 years of age, females at an age of 12-22 years, with an average generation length of 15 years. In general there are two migration runs, in spring and autumn. Individuals migrating in autumn remain in the river until the following spring to spawn. Females reproduce every 2-3 years and males every 1-2 years in the period March - May and at water temperatures above 10°C. This species has the highest relative fecundity of all sturgeon species (Chebanov pers. comm.). Most juveniles move to sea in their first summer and remain there until maturity. Some individuals remain in freshwater for a longer period. The species feeds on a wide variety of benthic fishes, mollusks and crustaceans.

There are few catch data available on the commercial exploitation of this species. In Kazakhstan 12 tonnes were caught in 1990, 26 tonnes in 1999; in Iran 1.9 tonnes was caught in 1990 and 21 tonnes in 1999 (Traffic, 2000), and 1 ton in 2005/6 with 0.5 to 1 percent of total sturgeon catch in Iran belongs to this species (in past 20 years) (Pourkazemi pers. Comm.). According to Commission of Aquatic Bioresources of the Caspian Sea (CAB) since 2001/2 export quota for caviar of ship sturgeon is zero - for all Caspian range states.

Illegal fishing (poaching), over harvesting, by-catch and habitat loss and destruction, along with dams construction, water abstraction and drought has led to the loss of spawning habitats/ground, have caused massive population declines.

Stellate sturgeon (*Acipenser stellatus* Pallas). This species ascends Volga, Ural, Terek, Sulak, Kura, Don, Septedrud and Gorganrud Rivers for spawning. Its feeding area extends from the shallow water areas in the northern part of the Basin to the Iranian coast in the south. The migration of Stellate sturgeon begins in spring (March-May) to the shallow water areas in the North-western Caspian where the largest concentrations of fish are observed, as well as in the pre-river mouth areas in the eastern part of the Volga River Delta.

Stellate sturgeon spawns in strong-current habitats. In main courses of large and deep rivers, in areas with a stony or gravel bottom substrate. The species also spawns on flooded river banks. If gravel bottom substrate is not available the species spawns on sand or sandy clay surfaces. Juveniles remain in shallow riverine habitats during their first summer. Stellate sturgeon mature first at 6-7 years (males) and 7-8 years (females). The generation length not less than 10 years. Females reproduce every 3-4 years and males every 2-3 years in the period April-September. Upriver migration takes place in spring and in autumn. Migration starts only at higher water temperatures and therefore a bit later than other sturgeon species. Males remain at spawning sites not longer than six weeks and females only 10-12 days. Individuals that have spawned migrate directly back to sea. Yolk-sac larvae are pelagic for 2-3 days and drift with the current. Juveniles migrate to sea during their first summer and remain there until maturity. At sea Stellate feeds on a wide variety of crustaceans, mollusks and benthic organisms as well as pelagic fish. Currently, the greatest number of stellate sturgeon migrates to the Ural River (Peseridi et al., 1986; Dovgopol et al, 1992). Stellate sturgeon stops eating after the beginning of the spawning migration. After spawning the fish directly move downstream to the sea, where they begin feeding actively.

In the early 1990's it was estimated that nearly 30 percent of the Caspian Sea's stellate sturgeon population originated from (re)stocking. Recent estimates are that more than 50 percent of the Caspian Sea populations are from stocking (Pourkazemi pers. comm.). Khodorevskaya et al. (2009) shows that the average numbers of spawners entering the lower Volga per year has fallen from a peak of 230 000 (between 1986-90) to just 50 000 (between 1998-2002), a decline of 78 percent. The decrease of the Catch Per Unit of Effort (CPUE) has been more apparent in the southern part of the Caspian Sea. The stellate sturgeon population has declined from 69.7 million specimens in 1978 to 15.6 million in 2002 and 7.6 million specimens in 2008.

For the whole Caspian basin, the catch of Stellate sturgeon peaked in 1977 with 13 700 tonnes. Since then an almost continuous decline has taken place to 305 tonnes in 2003 (most recent data) (over 97 percent decline in 32 years) (Pikitch et al. 2005). In 2008 the agreed catch quota for A. stellatus for all Caspian Sea countries was set at 240 tonnes, including commercial and scientific catch, and this quota has not been met (Pourkazemi per com.).

Similar to other sturgeon species in the Caspian Sea, illegal, unreported and unregistered (IUU) catch, poaching, overfishing and habitat destruction such as dam construction, water removal, entering industrial, oil and agricultural pollution are the main threat for survival of this valuable species. Dams have also led to the loss of many spawning grounds. In the Volga River some 40 percent of the spawning sites have disappeared (Khodorevskaya et al. 2009).

Beluga (*Huso huso* Linnaeus). This sturgeon species is one of the largest anadromous fish found in the Caspian Sea. Currently the native wild distribution of this species is only found in the Ural River. In the Caspian at least three beluga populations have been identified by microsatellite technique (Pourkazemi 2008). In the past was it the largest fish species in the Caspian Sea, reaching lengths of more than 5 meter and a weight of 1 000 kg. The age of such large specimens, apparently, exceeded 100 years. Currently there are individuals up to 280 cm, weighing up to 650 kg. Average length of females and males are respectively 240 cm and 220 cm, and their weights are respectively 130 and 65 kg. The species reaches maturity at an age of 10-15 years (males) and 15-18 years (females).

The species migrates further upstream to spawn than any other sturgeon. It prefers to spawn in strong-current habitats in main courses of large and deep rivers on stony or gravel bottom substrates. Juveniles remain in shallow riverine habitats during their first summer season. Males reproduce for the first time at 10-15 years of age and females at 15-18 years of age with an estimated generation length of 20-25 years. Mature fish spawn every 3-4 years in April-June. A complicated pattern of spawning migrations includes one peak in late winter and early spring and one in late summer and autumn. In spring the fish migrate from sea just before spawning. Individuals migrating in the autumn season remain in the river habitat until the following spring. Spawning occurs at water temperatures between 6 and 14 ° C and on spring flooded spawning grounds where current of 0.8-1.2 m / second exist. Most spawners participate in the late winter/spring run in the Volga River (80 percent), whereas the late summer/autumn run is dominant in the Ural River. Yolk-sac larvae are pelagic for 7-8 days and drift with the river current. Juveniles migrate to sea during their first summer and remain there until maturity. Spawning numbers for the Volga river reduced from 26 000 (annual no. spawners) in 1961-65 to 1 800 in 1996/1997 (Khodorevskaya et al. 2000).

Some natural reproduction of the species still occurs in the Volga and Ural Rivers. However, at present the abundance of beluga is extremely low. Currently nearly 100 percent of beluga in the Volga are hatchery reared, but there is some evidence of spawning at the remaining spawning grounds (Khodorevskaya et al. 2009).

Restocking programmes are ongoing for this species. However these do not compensate for the loss of natural reproduction and the populations continue to decline (CITES 2000). Annual number of fingerlings released into the Volga river was 0.4 million in 1951, 13.1 million between 1966-70 (average per year); 19.4 million between 1981-85, 11.3 million between 1996-2000 and 3 million between 2001-2005 (Khodorevskaya et al. 2009). The Report of 28th Session of the Commission on Aquatic Bioresources of the Caspian Sea (CAB) states that the total fingerling release of beluga in 2008 was 2.93 million from Russia, Iran and Kazakhstan.

Despite intensive restocking of this species in the Caspian Sea basin (91 percent of each generation is estimated to come from hatchery stock) the annual catch in the northern Caspian Sea has drastically fallen. Average catches in the Caspian in the following periods were 1945-55 (1380 tonnes), 1956-65 (1283 tonnes), 1966 - 1975 (1623 tonnes), 1976 -1985 (849 tonnes), 1986-1995 (506 tonnes), 1996-2003 (61 tonnes) according to Doukakis et al. (in press – need to update), which shows a decline of 95 percent. The official catch statistics support this trend, as they show that the species was abundant 1938 and then stable to late 1980s, with the major decline starting from 1990 to present showing a decrease of 90 percent in the past 60 years (see Khodorevskaya et al. 2009). The agreed beluga catch quota for all Caspian Sea (2007/8 - 28th session of the Commission) was 99.8 tonnes, this quota was not reached.

Various environmental factors influence the distribution of the sturgeon species in the Caspian Sea. One factor is water temperature, as mature beluga prefer water temperatures not exceeding 30°C. The mature beluga spend the spring and summer mostly in the northern and middle parts of the Caspian Sea and then move southwards to spend the winter in the southern areas; a seasonal migration which coincides with the highest densities of food organisms. Mature individuals of beluga are less sensitive to low temperature than the immature, as they feed in the northern part of the Caspian Sea under the ice, in early spring and autumn for much longer, delaying their arrival at the feeding grounds in the southern areas. With water temperatures decreasing beluga narrows down the range of depths at which it feeds. Immature individuals in spring and autumn prefer the more desalinated sea areas. In summer the highest concentrations of beluga can be found in areas where the salinity ranges between 3 and 7 ppt. The largest concentrations of beluga in the northern Caspian occur during the migration of its main prey organisms (herrings, kilka, gobies, roach, etc).

Global fisheries statistics show that there has been a 93 percent decline in catch of beluga from 520 tonnes in 1992 to 33 tonnes in 2007. The average catch declined by 65 percent when comparing the period 1992-1999 (211 tonnes) with the period 2000-2007 (74 tonnes) (FAO, 2009). The number of beluga annually entering the Volga dropped from 26 000 (1961-65) to 2 800 (1998-2002), a decline of 89 percent in 33 years (Khodorevskaya et al. 2009). Only 2500 beluga migrated upstream in the Ural in 2002 (Pikitch, et al. 2005).

Dam construction, disappearance of spawning grounds (by 88 -100 percent), poaching, overfishing at sea and in estuaries and rivers, by-catch from other fishing activities and pollution are the major threats to the survival of this species in its natural environment. These threats have led to the largest and most mature specimens being removed from the population and reduction of natural reproduction to almost insignificant levels (Krassikov and Fedin, 1996). In the Ural River current fishing rates are four to five times above sustainable levels. Due to the longevity of the species there is evidence of pesticide contamination, leading to many fish health problems, including a reduction of reproductive success (Gessner, J. pers. comm.).

Sterlet (*Acipenser ruthenus* Linnaeus). This freshwater species inhabits the Volga River and Ural River. Its population in these rivers has decreased by nearly 40 percent between 1990 (116 tonnes) and 1996 (80.6 tonnes) (CITES, 2000).

This species usually found in flowing water in relatively deep parts of the rivers. When the water levels rise the fish move to flooded areas to feed. The species spawns in strong-current habitats on gravel substrate bottom, rarely on gravel-sand bottom. Males reproduce for the first time at an age of 3-5 years and females at an age of 5-8 years. Siberian populations mature at a later age: 7-9 years (males) and 9-12 years (females). Average reproductive age is about 10 years. Females reproduce every 1-2 years and males every year in April-June when temperature rises above 10°C. There was a migratory population with large-growing individuals in the Volga River until the end of 19th century, with their feeding grounds in the northern Caspian Sea and moving upriver in autumn. The species feeds on a wide variety of benthic insect larvae and mollusks. Overfishing, poaching, habitat destruction and pollution are the most important threats to the conservation of the species. Pollution, including oil products, phenols, PCB's and mercury has threatened the species stocks in the Volga River system and in Siberian rivers.

3. HATCHERY DESIGN AND LOCATION

3.1 Selection of the hatchery location

Guideline 3.1:
Sturgeon hatcheries destined for producing fish for release should be located in places that are in accordance with national and local level planning and legal frameworks in order to obtain the respective permits as is required for any hatchery application. However, they should be constructed in environmentally suitable locations; local and legal authorities must be made aware that granting a permit for a hatchery will include protection of the site and close-by environments to prevent any other industrial or rural development that could harm the culture facility (e.g. pollution). Selected locations should allow efficient use of the available land and water resources while avoiding (as much as possible) any environmental impacts (e.g. conserving local biodiversity).

Justification:
Inappropriate siting and location of hatcheries has caused production failures, reduced efficiency in restocking, conflicts with other resource users and loss of investment. Planning for a hatchery site should take into consideration the local area plans, and foresee land and water use practices of neighbours which may affect positively or negatively the hatchery functioning. It should be recognized by the respective licencing authorities that other resource users (e.g. agriculture sector, tourism, fisheries, transport and hydropower) will have to face restrictions so as not to impair or threaten the objectives and operation of the hatchery while also protecting the nearby ecosystem services on which the species to be released will depend. Specific care should be taken when establishing sturgeon hatcheries in ecologically sensitive habitats.

To better foster co-existance of various human activities, it may be useful to retain certain buffer zones and habitat corridors between the sturgeon hatchery and other users of habitats and water resources.

Implementation guidance:

While choosing the location for a hatchery, the following practical prerequisites should be considered:

- water source characteristics (sufficient water availability and discharge options for an equal amount, especially in low-water seasons [summer/winter], water quality in line with species requirements, water level and as much as possible excellent quality (bacteria-free), ground water availability would be preferable.;
- distance from the capture sites of parental fish (preferably less than 25-30 km), to avoid long and stressful transportation of wild breeders;
- distance from the infrastructure (e.g. nearest settlements and transport systems, road, railway or airport);
- distance from an energy supply source (possible power lines routing);
- appropriate distance to fry release locations (to reduce stress and mortality as well as allowing early exposure (acclimation) to water conditions of receiving waters); Alternatively, an acclimation site would be required near the sites of release of the fingerlings.
- level of ground waters which should not influence full seepage and drainage of pond beds;

- level of Rivers and/or Caspian Sea must be considered as well as surges to prevent flooding;
- protection against overflow;
- on-site or nearby availability of impermeable pond construction materials (dikes, bottom sediments). There are many construction guidelines available for conventional aquacultural ponds since pond aquaculture has been practiced for millennia and many engineering manuals have been published. The construction of pond culture systems for sturgeons should take advantage of the existing basic knowledge of such guidelines and manuals;
- a good access road for transport of supplies and fish;
- prior use of the site (it is important to know what activities have been carried out at the site before, in particular with regard to contamination of the site itself or of surrounding habitats. In critical cases such knowledge may prevent chosing the site).

3.2 Design of the hatchery

Guideline 3.2:
During the past few decades, hatcheries for teleosts and sturgeons have seen quite some design improvements with regard to optimized system layout, the use of safety devices, and monitoring equipment as well as specific materials. These modern designs and construction techniques should be used as much as possible when establishing new sturgeon hatcheries. Therefore, advantage should be taken of past experiences and modern technologies that take into account not only the requirements of sturgeon reproduction, broodstock keeping, fingerling nursing but also system layouts and materials that permit the efficient (worker friendly) operation and management of the hatchery, while also integrate the hatchery into the local environment, causing a minimum of possible disturbances to the surrounding ecosystems.

Justification:
Many of the sturgeon hatcheries that were designed under Soviet rule are still functioning. However, the design of basins and tanks used, the layout of recirculation systems, heating systems, water quality monitoring and feeding systems is often not optimal, sometimes employing materials that may corrode or release harmful substances (e.g. softeners of plastics). As a consequence the risks involved in production and reproduction are higher and these systems use more manpower, electricity and water than modern hatcheries. Similarly, hatcheries that were build in the past for other species and are being modified for sturgeon reproduction often need adjustments in design of their essential units in order to meet the required performance output needed today.

The strict separation and/or isolation of specific units within the hatchery is a highly recommended design feature, permitting good risk management as to the operational control of processes as well as hygiene and health conditions. The separation of units is an essential design feature to be strictly employed in the construction of new hatcheries while in older facilities some improvements may be achieved by building barriers between onsite-units. Simultaneously, product flow controls may be arranged to force separate handling of units.

There is a need to protect hatcheries by sufficiently high walls or fences around its periphery, preventing the entrance of unauthorized persons and wildlife.

Implementation guidance:

When designing a new sturgeon hatchery it is important to:

- Determine the production capacity target and specificities of production (e.g. final size of fry/fingerlings to be released; broodstock size and holding requirements, e.g. number of tanks for year-class separtion, separate handling of males and females);
- Incorporate modern technical equipment that permits to modify light intensity and daylight regimes, water and air temperature control and maintenance as well as modifying the water flow and current speed in tanks, particularly when recirculation systems are used in which hydrodynamics through biofilters are rate controlled and need to be constant. Sufficient by-pass options in the recycle flow can help solve the problem),
- daily cleaning and maintenance work requirements must be considered in the layout of the system (e.g. easy access to all parts, sufficient space between units and fittings). Here, comprehensive advice on adequate materials that allow to keep proper hygienic conditions in all "corners" of the system (all parts such as pipes and fittings) can be obtained from the EU regulations for certified aquaculture hatcheries.
- selection of adequate construction materials (e.g. durability and toxicological aspects) also for the equipment and facilities;
- Allow for future increase in scale of the hatchery (e.g. ensure sufficient land area and water supply);
- Assess system design needs in response to the prevailing environmental factors (e.g. site location, water supply, weather conditions –see also section 3.1 above). These considerations are essential as they will have an noticeable impact they on the production efficiency of the hatchery;
- Ensure that both technical and economic aspects of the design and future operations are taken in consideration; Although it is always important to keep production costs to a minimum, there are costs that cannot be avoided without risking full success and these costs are clearly justified;
- Ensure to always monitor and control the entire production procedures throughout the process. Without a comprehensive monitoring programme, the performance cannot be reliably assessed. However, decisions on future improvements on operational modes and infrastructure investments require sound and solid monitoring data ;
- Take into account opportunities to conserve biodiversity and encourage re-establishment of natural habitats in the design process of the hatchery;
- Minimize creation of degraded areas such as unused soil piles and borrow pits through adequate landscaping, in particular when considering the previous recommendation on biodiversity conservation;
- Design dykes, canals and infrastructure in ways that do not adversely affect hydrology. Substantial progress has been made in dealing with such environmental issues by international aquaculture research and inter-governmental working groups on Environmental Interactions of Aquaculture (e.g ICES Working Group on Environmental Interactions of Mariculture; EIFAC Working Party on Fish Farm Effluents) and extensive guidance can be obtained from numerous publications by these organisations and by professional aquaculture societies and inter-governmental as well as regional organisations (;
- Separate effluent discharge points from inlet canals to reduce self pollution and maintain biosecurity. Again, the pertinent scientific literature of recent years provides a wealth of information for various site-specific aspects. These publications should be consulted when designing sturgeon hatcheries;

- Consider the working conditions (and living conditions, if relevant) of farm labourers, engineers and daily management. Also, lots of experience is available from commercial aquaculture on these issues and planners and engineers would be well advised to consult the respective literature.

3.3 Hatchery structural elements

Guideline 3.3:
Sturgeon hatcheries should comprise all necessary production, transportation, control, monitoring and management systems, which would allow for a suitable living environment and conditions for the fish as well as suitable working environment for the hatchery workers. Again, for many of the detailed requirements, much can be learned from commercial aquaculture practices while specific adjustments being made in line with the specific requirements for culture systems designed to produce fish for release (see Chapter 1.1 and 1.2).

Justification:
Modern sturgeon hatcheries often cater for many of the required services and inputs themselves. This means that the whole process, from collection of broodstock to the release of fingerlings is controlled and monitored to generate an optimal output in terms of volume and quality produced, while taking into account important issues such as fish welfare and the wellbeing of the hatchery workers.

Implementation guidance:
Modern sturgeon hatchery systems comprise the following elements (units):
- collection, transportation and holding, including long-term low temperature holding facilities for brood fish (LLTHFB) with re-circulated water systems (Also called recirculation aquaculture systems – RAS). It has to be emphasized that the design of such recirculation systems are different than for commercial aquaculture systems. They must permit to manipulate water quality in a predictable manner so as to imitate the natural conditions of water bodies to which the produced progeny is to be released;
- egg extraction, fertilization and incubation(here advantage can be taken of the many available methodologies available in commercial aquaculture while minor adjustments may be required);
- grow out of larvae and fry (tanks, trays);
- live food production (methods may vary because of site-specific opportunities, including intensive, highly controlled (predictive) culture units and/or semi-natural “mesocosm”-systems);
- fry transportation;
- laboratory, warehouse and subsidiary;
- broodstock holding unit with feed preparation farming house

The following structural design aspects should also be considered:
- System layout should allow that Incubation facilities and tanks can be operated either as flow-through or as recirculation units. The latter should be designed as multi-loop systems in which each of the processes (e.g. mechanical and biological filtration), can be operated independently as to their specific hydraulic and mass loading requirements while the flow dynamics in fish tanks can also be operated as demands require without influencing the flow needs in treatment units.

- Equipment for thermoregulation, water degassing and aeration or oxygenation (if required) should be incorporated at sufficient capacity (pre-calculated based on metabolic rates and mass transfer theory models) ,
- Units for disinfection using UV- light or ozonation should always be installed in bypasses and never in the direct flow.
- An LHTFB will enable operation with breeders on a continuous basis, with water thermoregulation, however, one has to recognize that biofilter design for such cold-water systems require extensive dimensions that must be pre-calculated while the rate of recycling will have to be recudes (larger freshwater replacement rates) at temperatures below 10°C as nitrification (in particular) will become slow and inefficient.
- Flow-through ponds can simulate the environmental conditions of natural spawning grounds (substrate, flow velocity, water type and quality), provided the location of such ponds is close to the target river and natural (or historic) spawning sites are in the vicinity .
- The water supply of each hatchery unit should be continuous and independent.
- Ongrowing ponds should be equipped with fish traps and there should be an outflow control system to regulate flow. Furthermore, there have to be specifically designed retainment barriers (multiple screens of various mesh sizes) to avoid accidental escapement of individuals of the respective fish sizes in the system while also preventing accidental release.
- Water quality management and fish health management facilities (laboratory), including methods for egg disinfection are essential (good guidance can be obtained from the EU requirements for certified aquaculture hatcheries).
- Water treatment for the water supply systems in case clean groundwater sources are not available (e.g. sedimentation tanks for pre-treatment, backwash sand-gravel filtration systems, cross-flow filters) for water purification from siltation, retention of debris, invertebrates, plankton species and wild fish, while also preventing the unintentional invasion various developmental stages of fish parasites or their intermediate hosts.
- water supply to ongrowing units should be arranged through sedimentation, net screen systems and other modern mechanical and biological filtration techniques.
- Area, layout and depth of ponds are determined according to hatchery standards. Layout of the pond bed, levelling with the river and supply channel dimensions and slopes should be designed to ensure a rapid 1-2 days filling and draining of the ponds. Appropriate calculations and layout of the water flow systems are decisive factors in determining the adequate water management for any size of ponds, regardless whether operated in flow-through or stagnant mode..

When considering the modernization or partial reconstruction of existing sturgeon hatcheries it is essential to ensure the application of modern technological advancements and the latest knowledge on the needs for management of the genetics and fish welfare as well as for the environmental compatibility needs (including biodiversity conservation in the habitat settings. In case modernisation means total replacement of all hatchery components, the guidance on new and modern hatchery design and construction should be employed to comply with all requirements for a new hatchery destined for producing progeny for release.

The hatchery design and layout should be future-oriented to allow technology improvements such as new methodologies for controlled maturation without exogenous hormonal stimulation, development of ecological compatible methods of reproduction simulation under hatchery controlled conditions, as well as egg incubation in adhesive state (then avoiding de-adhesive treatment and active moving during embryogenesis). Some steps have been undertaken towards using artificial spawning grounds which ensure the optimal hydrological conditions for pseudo-migration of breeders and include the possibility of annual clearance of spawning ground and rearing areas (Appendix 6).

3.4 Economic aspects of hatchery construction and operations

Guideline 3.4:
In the planning for, design and construction of sturgeon hatcheries, which main purpose is the supply of fingerlings for release and rehabilitation of populations in water bodies of their natural range, it is imperative to calculate the costs of construction and future operation realistically to ensure the long-term cost implications of the hatchery operation to society. Such financial plans are commonly needed to obtain bank credits and/or funding from local, national, international or inter-governmental donors as well as from licensing agencies. In case hatcheries are destined for ranching or stock-enhancement (supporting specific clients such as commercial fishery and also providing aquaculture with stocking material) a financial business plan presenting total investment and running costs, as well as amortisation and profits is imperative. Hatcheries entirely destined for rehabilitation of endangered species should – from the beginning – incorporate a plan on how to recover (at least partly) the running costs from beneficiaries ones natural populations have recovered to the extent that a limited and tightly regulated fishery can be re-opened or self-sustaining populations have been fully established.

Justification:
A sturgeon hatchery, like any business, should be economically viable if it is to survive in the long term and if it serves primarily an aquaculture objective, including stocking of water bodies serving a fishery specifically being supplied with recruits. The economic feasibility of the hatchery should therefore be taken into consideration from its planning and design phase. However, hatcheries which are constructed and operate with the sole purpose of supplying fry and fingerlings for restocking purposes, have other goals than making profit and serve society at large. Moreover, some hatcheries, particularly in the Caspian Sea basin, are state owned and have as main objective to support rehabilitation of the sturgeon fisheries in the Caspian Sea basin. In the long-term, contributions to the operational costs should be received from the fishery or the caviar and sturgeon meat industry ones the fishery on recovered stocks will be re-opened.

Sturgeon hatcheries are expensive operations. Considerable investment is required to build a hatchery and finance their operations. The owner (often a State Agency in the Caspian basin countries) must have sufficient working capital to carry out the necessary operations. Before deciding to build a hatchery, one needs to carefully examine all facets of building and operating a hatchery and determine financial schemes that include various potential donors while determining at what level public support and accompanying income from hatchery operations can be generated to achieve gradually an economically viable operation that can be sustained by the owner.

Implementation guidance:

In the planning design, construction and operational phases of a sturgeon hatchery one could learn from the past experience in commercial aquaculture development on how to prepare a feasibility study, develop a business plan and establish operational procedures. Certainly, while the overall principles apply, specific adjustments for sturgeon hatcheries will be needed. It is recommended to:

- Produce (and update when required) a good business plan and carry out an economic- and investment feasibility study.
- Be involved in national planning and programming activities related to sturgeon recovery programmes, as to ensure awareness of the capacity and interests of the hatchery as well as creating a broad understanding of the societal benefits and responsibilities of such an undertaking.
- Avoid to depend (solely) on state subsidies and grants for economic sustainability but gradually seek for accompanying measures that provide some income to defray a certain portion of the running costs (e.g. training programmes, entrance fees for guided visiting tours, a small proportion of fry production for sale to aquaculture operations, etc.) Planning such activities have some small design implications for the construction of a hatchery (e.g. visitor corridor alongside of but isolated from the production units equipped with windows to see all operations of the system as well as an exhibition room .
- Maintain financial records of all inputs and outputs of the hatchery.
- Assign accountability for the hatchery functioning and the use of the budget for inputs required for production to the responsible persons (i.e. the hatchery manager) who has control over the budget.
- Obtain life/accident insurance to cover the main risks involved in sturgeon hatchery activities. There are world-wide only a handful of insurance companies that have their own regulations and technical minimum requirements for hatcheries and this will also apply for sturgeon hatcheries. Therefore, potential insurance agencies should be consulted before planning and designing a sturgeon hatchery so as to guarantee that the requirements for becoming eligible for insurance are also met before starting construction.
- Do not start production if no budget is available for release or restocking purposes
- Make an effort to estimate non-economic value of the hatchery and its produce, including the social, cultural and environmental values that are associated with the hatchery. Particularly the environmental value of restocking of a critically endangered species may be high and offset economic costs for society.

4. COLLECTION AND TRANSPORTATION OF WILD BREEDERS

4.1 Sites and periods of breeders collection

Guideline 4.1: *Sturgeon broodstock should be collected from the natural environment in accordance with international, national and local level laws and regulations, at dedicated sites and over a pre-determined period. As a principle it is strongly advised to catch as many broodfish as necessary to maintain the genetic integrity of the species without ignoring the need to keep the impact on those natural populations at minimum that are highly endangered or at the brink of extinction. In such cases, priority should be given to the use of the available ex-situ broodstocks.*

Justification:
The commercial and scientific catch of sturgeon has been banned in certain parts of the Caspian Basin and in certain seasons. Unfortunately, the national and local laws and regulations on catch of sturgeons and sturgeon broodstock that are in place are often not or only to a limited extent enforced. In general, the broodstock collection is done in a semi-legal environment, justified by scientific research purposes. In practice this means that there is very limited information on broodstock harvests from the wild and most broodstock catches can be considered as Illegal, Unregulated or Unreported (IUU) fishing.

Implementation guidance:
When planning and conducting broodstock collection it is important to recognize that:

- National and local laws and regulations and international agreements should be followed. In specific cases, dispensation should be requested from the respective authorities while following the relevant procedures.
- The initiation of the wild broodstock collection depends on the time of sturgeon migration to the coastal (estuary) area and onset of spawning temperature for each species. This time should be adjusted in accordance with ambient seasonal conditions as are prevalent in different river basins. It is strongly recommended to continuously monitor these conditions in order to regularly update and adjust the estimates on the time window for collecting mature broodfish thereby improving cost-efficient scheduling of the operation.
- Broodstock collection should be performed in rivers during spawning migration, in coastal (estuary) areas using fishing nets and in rivers using trap nets that do not harm or damage the fish.
- In cases where there is a very low number of breeders it is advised to collect even pre-adult animals in other (nearby) areas (e.g. estuaries) only for the purposes of holding the fish for later use for reproduction.

4.2 Transport of broodstock to the hatchery

Guideline 4.2:
The effects of handling of brood fish during and after catching as well as during and after transport need specific attention with subsequent consequences on the welfare of caught broodstock in the hatchery. Handling and transportation stress should be minimized.

Justification:

Sturgeon broodstock become vulnerable to injury and diseases when they are confronted with stressful situations during handling. Its highly beneficial to the hatchery and the fish to do all the needful to reduce stress levels to a minimum. Proper care during catching, handling and transport (by boat, car or hand at the farm) is essential. The Aquatic Animal Health Code 2010 of the World Organization of Animal Health (OIE, 2010) provides key guidance on how to guarantee the welfare during transport of fish. However, adult sturgeons are exceptional large specimens (much larger than most of the teleost fish species handled in common commercial aquaculture). Therefore, adjustments of equipment to handle fish at capture and during transport must be made to meet the dimensional needs and specific handling skills will be needed required for which little expertise is available among most hatchery operators. There is an urgent need for the preparation of a specific manual to assist those involved in handling large, mature and ready to spawn sturgeons.

Implementation guidance:
While recognizing that detailed guidance on this subject is provided in the OIE Code in more general terms, these guidelines would specifically emphasize that:

- The length of time freshly captured broodstock are handled and spends on a transport should be as short as possible. Good planning and organization of the transport by competent (trained) persons is essential to minimize handling stress.
- The density of fish in the tanks or containers used for transport (by boat and truck) should be minimized when a large number of individuals are being transported. In this respect it should be noted that the design of transport equipment should be suitable for the transport, preferably using splash-less tanks at a size that accommodate the full length of the fish while either aeration or oxygenation is provided on board boats or trucks.
- Fish should be transported in the water they were caught in. This water must be temperature controlled, either to maintain the temperature from which the fish originate (water should not warm-up while fish is in transport), or cool the water to reduce metabolic activity.
- Cradles/slings, tanks and containers for broodstock transportation should be rinsed by fresh water before use and subjected to approved disinfection procedures. Stress-less (e.g. “splash-less”) tanks should be used, to avoid mechanical damage to fish and reduce “sea-sickness”. Moreover, to avoid fish damage, the inner surface of containers should be smooth (nor rough surfaces, also easy to disinfect).
- Breeders intended for reproduction with evident signs of abnormalities on the body should be excluded from transport to culture (broodstock) facilities.
- During catch, handling and transport, efforts should be made to maximize safety for the fish and keep the animal out of direct sunlight (e.g. while waiting onshore to be prepared for transport).
- Emergency, contingency and back-up plans should be ready in case any irregularity occurs as to equipment function (e.g. oxygen supply) and handling protocol, in order to guarantee the well-being of the fish throughout the handling and transport process.

In certain cases it can be necessary to transport live sturgeon from one country to another. In such circumstances it is recommended to follow the FAO Technical Guidelines on Health management for responsible movement of live aquatic animals (FAO, 2007) which have been developed to support sections of FAO’s Code of Conduct for Responsible Fisheries (CCRF) as well as the “ICES Code of Practice on the Introduction and Transfers of Marine Organisms” (latest version 2004; see references) which is also endorsed by FAO, incorporating several of its principles into the FAO Technical Paper on “Assessment of freshwater seed resources for sustainable aquaculture (Bondat-Roantaso (Ed.), 2007).

These Technical guidelines give advice on how to reduce the risk of introduction and spread of serious transboundary aquatic animal diseases (TAADs), and although they deal primarily with safe transboundary movement at the international level, they are also applicable to domestic movements between different provinces, (sub)basins, geographical areas or zones of differing disease status. As such the Technical Guidelines on health management for responsible movement of aquatic animals can form a valuable addition to the Technical Guidelines outlined in this document. Additionally, the ICES Code addresses not only health aspects but also ecological considerations on risks on potential ecosystem disruptions from non-indigenous species and/or foreign strains of native species.

5. SELECTION AND MAINTENANCE OF BROODSTOCK

As a consequence of the drastic decline in the numbers of wild breeders the broodstocks built in the hatchery are serving and will continue to serve as prime sources of population replenishment in their previous natural range. Representative conservation of sturgeon species gene pools and intra-specific groups (spatial, seasonal) must be ensured in the established hatchery broodstocks intended for release to either re-establish a sturgeon species in its previous natural range or to replenish natural stocks which are at risk.

Different approaches to establish a broodstock may be applied, depending on specific local conditions. These include the use of domesticated brood fish and immature specimen obtained from natural runs, as well as using eggs, yearlings and fish of older age groups, reared at the hatcheries. Genotypes of broodstock specimens should accurately represent the genetic structure of natural population and complete genetic characterization of all specimens before use will therefore be an essential prerequisite before any of the fish can be used in producing progeny for release programmes (see 5.1).

5.1 Genetic aspects of broodstock establishment and management

Guideline 5.1:
Hatchery populations should be established and managed under principles that capture and maintain the genetic diversity in natural populations. Inbreeding, outbreeding and selection should be avoided in creating and maintaining the broodstock. Individuals from different populations should not be interbred. Genetic management of the broodstock should take place throughout the process from selection of breeders through rearing and releasing. Coordination within and among river systems and countries and establishment of a centralized entity for overseeing and coordinating hatchery practices is recommended. Research into the genetic diversity of wild sturgeons is needed to more accurately manage hatchery practices.

Justification
Genetic diversity is essential to the long-term survival of a species. Individual populations of sturgeons may have unique adaptations and genotypes. This diversity should not be compromised by hatchery practices. Given that there are a large number of hatcheries across the Caspian Sea basin, coordination amongst these entities on genetic practices would be beneficial.

Implementation Guidance:
Some hatcheries currently cultivate captive stocks while others cross individuals captured each year without holding the animals beyond that time. These latter types of hatcheries may

establish captive broodstocks in the future. These implementation guidelines address both circumstances. As there is not yet sufficient information to genetically attribute an individual to its basin or river of origin, research will be needed to implement some guidelines. Caspian Sea nations should thus prioritize research on the genetic structure of sturgeon populations.

When establishing the broodstock, it is essential to be mindful of the geographic origin of the animals used as well as their genetic identity. To preserve the genetic diversity within the species, several important rules should be followed:

- Individual animals (or their gametes) from other basins (e.g., Black or Azov Seas) should not be used in Caspian Sea hatcheries, even in cases where there are only a few breeders of the target species available. This is essential to avoid genetic pollution of the original populations. If the presently established broodstock includes animals from other basins, they should be removed and the progeny from crosses using these animals should not be released under any circumstances.
- All necessary efforts should be made to establish broodstocks for an individual river system that includes only individuals captured in and genetically attributed to the river where the hatchery operates and supplementation will occur. When sufficient broodstock from an individual river is impossible to obtain (i.e. the local population is extinct), animals from the closest river system and/or with close genetic relatedness can be used if absolutely necessary.
- As a best practice, animals from the same run within a river system should be used to create broodstocks that correspond to individual runs. Individuals captured during a certain run should be reared separately from those captured in a different run and only bred with individuals from that run.
- A large random sample of individuals from the wild should be collected at different periods during the spawning run to avoid selection of particular phenotypes and genotypes.
- In cases where it is not possible to capture both a male and female of a species in a given season, and no captive animals (or gametes) from the same river system exist to achieve a cross, it is recommended that the males captured are non-lethally stripped of their sperm for the purposes of cryopresevation. Captured individuals should be retained in captivity for future use when a suitable mate (e.g. female) is captured.

Breeding practices in the hatchery setting should strive to maximize the effective breeding size of the population by breeding as many individuals as possible (ASMFC, 2006). In the ideal case, the effective size of each population group should not be less than 100-250 different age-graded females and males (FAO, 2008). This means having 20-50 individuals effectively breeding (with an equal number of males and females). When unequal numbers of males and females are used, the effective population should be measured using the table in Appendix I. Because it is likely that not all individuals will be responsive to hormone treatment and thus not effectively reproduce, it is recommended that 100 different individuals are bred each year.

In many Caspian Sea sturgeon hatcheries, only a limited number of breeders obtained from natural spawning runs may be available each year. It is therefore recommended that an effective population size of six (with equal numbers of males and females or with adjustments as per Appendix I) be achieved each year and that different individuals be bred each year (St. Pierre, 1999). If less than six unrelated animals effectively and successfully breed in a given year, the progeny obtained from such reproductions should not be released.

Inbreeding should also be avoided by ensuring that closely related individuals (e.g., siblings and half-siblings) are not crossed. Rotational breeding schemes can be used to minimize mating between related individuals (Kincaid, 1977). This will require that breeding lines are tracked through generations (e.g., through proper and reliable (long-lasting) tagging methods and full record keeping) and this can be best accompanied through molecular genetic analysis of the captive stock. In the absence of genetic information, inbreeding can be avoided by crossing different age-grades and crossing females and males captured at different locations during different periods of the spawning run. Different adults should be spawned every year. When possible the selection of mating pairs should be optimized to preserve rare alleles. However, this can only be an interim procedure. Full molecular genetic analysis of broodstocks should be vigorously promoted as soon as possible.

When there is a limited supply of broodstock, several steps can also be taken to avoid inbreeding:

- A breeding plan can be created that maximizes the number of crosses undertaken (factorial mating). This involves dividing gametes from several individuals and making all possible crosses between males and females (see Kapuscinski and Miller, 2007).
- When unequal numbers of each sex are available, the gametes from the individuals from the less numerous sex should be crossed with all of the gametes from the sex in excess. It is essential that each cross be conducted separately so that the individual lines can be tracked, sperm competition minimized and the relative contribution of each cross to the resulting population that will be released can be understood.

It is preferred that the crosses conducted each year are of wild and not domesticated broodstock. Wild individuals should be captured, tagged and genotyped each year, stripped of gametes and released back into the wild (except in cases of highly endangered species and individuals with rare alleles should be retained in the captive broodstock). In cases where an adequate broodstock cannot be obtained from the wild, crosses with captive (hopefully not fully domesticated) females and wild males can be used, and vice versa in case of lack of males; it is desirable to use also a rotational breeding scheme in these scenarios, incorporating at least 5-10 percent of wild breeders into the broodstock (Bartley et al., 1995).

Detailed records should be kept of crosses performed and the resulting surviving progeny and their genetic identity (i.e., attribution to family line) be documented while also the approximate number of individuals released from the different crosses should be recorded. The risk that some selection will occur after fertilization through to the fingerling stage and may therefore alter the relative percentages of progeny from different crosses in the population ultimately released. Therefore, complete record keeping is essential for a retrospect analysis on success and failure of release programmes.

Several hatcheries will likely exist in a given river system. These hatcheries should operate collaboratively, sharing broodstock when appropriate. As the establishment of broodstocks requires technical expertise and a sufficiently equipped hatchery it may be preferable to concentrate efforts into one facility in each river basin. This facility would be able to deliver fertilized eggs to “secondary” on-rearing facilities which are located closer to release sites. In order to safeguard the broodstock and protect against unforeseen risks, however, a “duplicate” broodstock should be housed at one other hatchery within the region.

Given that the genetic resources of the Caspian Sea sturgeons are shared, establishing a basin-wide legal framework on genetic practices and protocols in hatchery management is strongly recommended and seen as an essential part of any monitoring and assessment programme. A regional body should harmonize practices among nations, track breeding lines and share genetic resources when appropriate. The coordinating body could set guidelines for standardized tagging and molecular genetic techniques to be used by all hatchery in the Caspian Sea basin. Such information should be linked to the database mentioned in the preamble of this section. The body could further support additional genetic research on population structure of Caspian Sea sturgeons, which is a necessary first step in accomplishing sound supplementation guidelines. A range wide genetic database is also needed to be able to attribute captured individuals to a river of origin.

In cases where broodstocks have been already established under conditions that do not comply with those recommended above, these stocks should be genetically tested to find matches with newly established broodstocks. Until these captive stocks are characterized, they should not be used for supplementation.

5.2 Other biological criteria to establish captive broodstocks

Guideline 5.2:
Establishment of a sturgeon broodstock base at a hatchery should be conducted on the basis of a proper breeding plan, including evaluation of optimal species and age structure of the broodstock. All age groups should be represented in the broodstock. Each fish group should be marked by tags of corresponding series number and related information recorded and registered in the pedigree documentation (diary). At selection of fish for becoming part of the broodstock, the individuals with typical exterior characteristics and, without anomalies having high gamete quality should have preference.

Justification:
Spontaneous building of broodstock without long-term science-based planning would result in a loss of natural population genetic diversity in the sea and would not risk the success of continuous hatchery stock enhancement.

Implementation guidance:
It is important that an optimal age structure and size of the broodstock should be reached at the hatchery, in order to fulfil the restocking objectives.

The breeding plan should be planned on the basis of:
- species of sturgeons used at broodstock establishment,
- number of intra-population groups,
- age of sexual maturity and inter-spawning intervals, and
- productive capacities of the hatchery (at first stage of broodstock establishment).

Criteria for fish selection for broodstock development include the following:

- Quality of gametes in mature fish: uniformly pigmented eggs, regular shape, weight and size correspond to the mean species specific values, timely sticking to substrate (5-15 min) when subjected to water, transparent ovarian fluid; sperm with spermatozoa concentration not less than 3 billion/ml and their duration motility for 200 s or above.

- It is recommended to start selection of fish intended for stock rehabilitation from fry of 5 g weight onwards.

- Prior to selection of juvenile fish for being used as broodstock an evaluation of the fitness indices of the juveniles should be conducted with the use of express techniques.

- Within each genetically homogeneous group, weak specimens with different abnormalities, and irregular body coloration should be culled. Then the further selection of broodstock fish from all size- and age graded groups should be performed in such a way that an optimal age and size structure of the broodstock base will be established.

5.3 Broodstock holding and handling

Guidelines 5.3:
Sturgeon broodstocks should be held in special units (ponds, large tanks, cages) with each having an autonomous water supply. Each species, intra-specific and age groups, as well as mature females and males should be kept separately. The holding of broodstocks should be performed under conditions that are as similar as possible to the conditions in the natural environment of the fish and handling should be minimized in time and frequency.

The handling of the broodstocks (grading, fitness assessment, monitoring of development of biomass and the reproductive systems etc.) should be conducted with minimal possible exposure to stressors.

Justification:

Due to lack of space or proper facilities and as a consequence of limited knowledge of how to hold sturgeon broodstock, many hatcheries hold the sturgeon broodstock presently under sub-optimal conditions. As a general rule, the unit for broodstock holding should provide not only for the bare survival of the fish, but also ensure fish welfare. In this respect it should be noted that it is important to hold the broodstock at low stocking density levels or in individual hatchery units. Holding of the broodfish elsewhere, outside the hatchery location, may lead to increased fish health risks and impede the control of their fitness indices and selection for optimal mating.

Implementation guidance:
When holding sturgeon broodstock and handling the broodfish, it is important to keep in mind the following aspects:

- Earthen or concrete ponds of small to medium sizes (minimum 0.01 ha), as well as cages installed in natural water bodies should be preferably used for broodstock holding.

- Pond stocking density of the broodstock in the holding units concerned should be considerably lower than those applied for commercial rearing of corresponding species and age groups of sturgeons.

- Brood fish that have taken part in the spawning season (campaign) should be held at an aclimation unit for at least 2-3 months, providing a low stress environment (low stocking density, natural photoperiod, minimum ambient factors influence, optimal hydrochemical parameters and water supply). In the acclimation unit the fish can be monitored better and are subject to acclimation procedures, including sanitary control measures, to prevent possible health implications but also adjustments to behavioural cues such as interactions with con-specifics and feeding.

- Natural or artificial wintering (vernalisation of 2-4 months at a temperature 4-6^{o}C) is necessary before final maturation of breeders (Kazansky, 1981; Chebanov et al. 1999, 2002) for successful spawning.

- The use of non-invasive methods (e.g. ultrasound diagnostics) when handling the broodstock is highly preferred above invasive methods, as the latter may cause excessive stress, skin abrasions with subsequent infections, reduction in general health conditions and additional physiological stress.

5.4 Adaptation of wild fish

Guidelines 5.4:

Adaptation of breeders and immature sturgeon captured in natural water bodies to the hatchery conditions (including maturation in fresh and brackish water and feeding on other life and formulated feeds) is an essential component of sturgeon hatcheries aimed at restocking.

Justification:

Recognizing the dramatic decline in wild captured breeders of sturgeon in the Caspian Sea Basin, their adaptation to artificial conditions of holding is important. Adaptation allows for building productive broodstocks with a genetic structure which is similar to that of the wild population. In fact adaptation is required to keep the broodstock alive, ensure the fish welfare and contribute to successful restocking and the natural genetic diversity conservation of sturgeons.

Implementation guidance:
In order to ensure success of any sturgeon broodstock adaptation activities, the following aspects are important:

- Adaptation of wild fish should be preferably be performed at low water temperatures (10-15^{o}C) at a high levels of oxygen in the water and under conditions of a natural photoperiod.

- The technological scheme of wild fish acclimation to captive conditions in the hatchery comprises the following elements:
  - alive collection of gametes from breeders using standard protocols in terms of handling and hygiene;
  - gradual transition of caught fish from life food to composed diets;
  - holding at the hatchery until (repeated) maturation has been achieved;
  - attempts being made to repeated utilization of mature breeders.

- At the beginning of the process of acclimation wild fish should be trained to feed on natural food (fish, mollusks, worms, crustaceans) with gradual transition to pasture-like mixtures, containing animal components and commercial (mixed) feeds.

- The following methods of efficiency of transition to formulated feeds enhancement should be used:

- training of conditional reflexes to take formulated feeds by using a domesticated fish as a decoy;
- appetite increasing by medicaments - triiodothyronine, biostimulators (Chebanov et al., 2004), food attractants and taste stimulants (Kasumyan 1999, Kasumyan and Døving,2003);
- forced feeding of fish by pasture-type feeds using catheters.

- It is recommended to use non-lethal express ultrasound methods for monitoring of the alimentary system (Chebanov, Galich, 2009).

- Wild fish that do not accept formulated feeds within 90 days after the adaptation has started should be released to natural water bodies or culled.

5.5 Monitoring of the sexual structure of the broodstock

Guidelines 5.5:
The state of gonad development as part of the sexual structure (composition) of the sturgeon broodstock at any of the hatcheries should be accurately determined and monitored throughout the lifetime of the captive stock.

Justification:
Monitoring of the sex structure is necessary for the continuous controlled reproduction and elevation of the effective captive population size. Without accurate and up-to-date information on the stages of sexual maturity of all the sturgeon individuals on the site it will be difficult to establish an effective reproduction programme or management plan.

It is important to build towards a final sex ratio and number of breeders following a reproduction programme or plan, taking into consideration the hatchery objectives in terms of number and size of fingerlings to release.

The sex structure of the stock should be corrected after the fish reach maturity stage using express ultrasound technique of sexing and staging. The maturity stage is reached at different bodyweights and different age, depending on the sturgeon species (Table 1).

Table 1. Age at first maturity and corresponding weight by sturgeon species (based on practical experience onsite farms).

Species	Rearing at seasonal temperature	
	Body weight (kg)	Age (year)
Russian sturgeon	1.5-3.0	2-3
Stellate sturgeon	2,0-2,5	3-4
Beluga	8.0-10.0	4-5
Ship sturgeon	2.0–2.5	2+-3
Sterlet	0.3-0.6	2-2+

The following factors should be considered when deciding on the sexual structure of the broodstock;
- sexual differences in gametosomatic indices and age of puberty for each species;
- diverse age of puberty for females and males;
- inter-spawning intervals for females and males;
- maximum possible time of spawning for individual females.

Implementation guidance:
In order to be able to reach reproduction and restocking targets, sufficient fingerlings and non-mature fish of different age groups will have to be held in the hatchery for the purpose of building and maintaining a continuously effective and reproductive cohort. Thus, the sexual structure of the broodstock will have to be monitored regularly.

For rearing of one breeder to maturity, on average, the following number of juvenile fish will have to be raised to achieve the objective (Table 2):

Table 2. Number of juvenile fish of each age group to be held at the hatchery for each breeder to be produced for the reproduction programme.

Age	**Number, ind.**
Fingerlings, mean body weight 1,5 – 3 g yearlings	160 - 200
1 year +	16 - 24
2 year +	8 – 12

The effective population size for gradual replacement of a long-term broodstock (rejuvenation) depends on the number of mating individuals. In order to decrease the number of fish (for release) of the same origin, it is necessary to provide equal replenishment of males and females to each subsequent generation.

Within the overall hatchery plan a “broodstock reserve” should be kept which is preferably composed of 30 percent mature females and 10 percent males of the required number of breeders.

5.6 Monitoring and control of the broodstock

Guideline 5.6:
The hatchery should monitor and control the biological, morphological and reproductive characteristics of the broodstock on a frequent basis to ensure the quality and physiological condition of the broodstock.

Justification:
Frequent physiological monitoring of the broodstock (2-3 months interval; but more frequently during final maturation) enables the hatchery to avoid cases of inbreeding depression (late maturation, decrease in reproductive indices and disease resistance) and will help to ensure breeder quality and good fish health conditions.

Implementation guidance:
Monitoring and control of the hatchery broodstock is an essential requirement for each sturgeon hatchery. It is recommended for the hatcheries in the Caspian Sea Basin that:

- Broodstock monitoring is performed during spring and autumn assessment. It is important that the spring assessment is completed before spawning temperature will be reached.
- In the course of the assessment, morphological and biological indices, as well as the physiological and ichthyo-pathological status of the fish should be evaluated and recorded and any fish with evident developmental malformations should be subject to culling.

- All the broodstock data should be recorded in special diaries and individual genetic passports (standardized format for comparative analysis in all Caspian sturgeon hatcheries), where all initial data on reproductive characteristics of females, as well as all the changes observed during the course of monitoring should be recorded. On the basis of these data the culling of the individuals should be performed that do not match the requirements of the broodstock destined for reproduction to produce progeny for release in species conservation programmes.
- All the genetic data and information going along with the identification (Barmintseva et al., 2003) and the standardized individual breeder passports (Safronov, Krylova, 2004) should be recorded in the hatchery database and preferably also in a regional hatchery database (Register of sturgeon breeders) available for all hatcheries of the basin to serve long-term overall assessments on performance characteristics leading to success or failure so as to allow regular consultations and upgrading of the procedures. Therefore, these data and related record requirements must be standardized in order to allow common usage in the process of breeding programmes development and in terms of effective assembling of breeders mating pairs.
- Each fish should preferably be marked by individual tags of two different types (external and PIT-tag) to avoid possible loss.

5.7 Cryopreservation of sturgeon sperm

Guideline 5.7:
Sperm Cryopreservation should be considered as a generally applied and useful tool for conservation of sturgeon species, especially for rare and endangered species. However, one has to realise that this strategy takes care only of the paternal gene pool. Unfortunately, it is yet not possible to cryopreserve oocytes.

There are various techniques available on how to control the cryopreservation process, the storage condition monitoring and the thawing process, including the application of cryoprotectants and diluents. The pertinent literature should be consulted to develop an own specific protocol of which some basics are outlined below.

Justification:
Sperm Cryopreservation is being applied in some countries and the method has been proven to be a useful tool for conservation of at least the paternal fraction of the gene pool of rare and endangered sturgeon species. Individual hatcheries could make significant contributions by incorporating further research programmes on optimization of procedures, but through national and regional collaboration much more impact on improved methodologies can be achieved. Overviews on procedures, protocols and present success in fish gamete cryopreservation can be found in two recent volumes of the first and second International Workshop on Fish Sperm Biology, summarizing the global expertise on the subject (Alavi, et al. 2008; Rosenthal, et al., 2010).

Implementation guidance:
Cryopreservation of Sturgeon sperm can be a useful tool in the conservation of the species. In order to implement cryopreservation measures in an effective manner it is recommended that the following principles and standards are followed:

- It is important to ensure the genetic purity of the brood fish used; the use of hybrid brood fish must be strictly avoided.
- In case brood stocks are collected from coastal, estuaries or even in the shallow waters of the Caspian Sea it is necessary to know the genetic origin of such species

and therefore, tissue samples for accompanying genetic identification should be taken. In case the expected genetic identity cannot be confirmed, the cryopreserved sample has to be destroyed.

- There is a definite need to work in a clean environment when extracting semen for cryopreservation from individual fish. All equipment used in preparing sperm samples for cryopreservation must be disinfected to minimize the risk of bacterial contamination of the sperm sample. One has to recognize that contaminated samples will ALSO preserve the potential pathogens accidentally cryopreserved along with the sperm cells.
- The mixing of sperm of various brood fish should be strictly avoided. To be able to trace genetically every single individual in all long-term breeding programmes is a prerequisite strongly requested in the recommendations of the Ramsar Declaration on Global Sturgeon Conservation,.
- Keep the sperm of each species separate and store it independently. Therefore, systematic labelling which allows long-term and logical following breeding lines of decades can be possible (see specific instruction below).
- Additionally, record and register all morphometric and genetic characteristic of broodstock.
- Hold the collected breeders in the best possible condition (O_2, temperature, pH, light), and limit exposure to any kind of stress.
- Use only the very high quality of spermatozoa regardless whether for direct fertilization, short-term storage or cryopreservation (based on pre-determined criteria such as density, spermatocrit, motility or progressive movement and its duration, and velocity; see some details below). For this purpose a specific manual is required that standardizes handling time (in seconds and minutes) because handling of a large number of samples require well-prepared logistics to minimize the variability of results by uncontrolled experimental handling times.
- For short-term storage (hours) find a good temperature (around 4°C). Maintain the samples in contact with air but avoid dehydration. The design of right extenders is a crucial undertaking and optimum composition is not yet known for all sturgeon species. The choice of a good container type (tubes, Eppendorf vails; plates), depending on sperm volume). Also, to collect “clean” spermatozoa, catheterization is recommended. Further, using cathethers, sperm from the same testis should be collected repeatedly in several minutes interval.
- Sperm quality evaluation (both for fresh sperm and post-thawed sperm should include firstly sperm volume and density of spermatozoa. Ones the right cryopreservation media have been designed (ionic composition, % cryoprotectant(s) added, pH, and medium osmolality) the optimum sperm-cryoextender dilution ratio has to be determined and recorded. Valuable guidance can be found in the review by Cabrita et al. (2010).
- When freezing, a freezing protocol must be designed and must be recorded. The same holds for the thawing protocol.
- Register the code for each tube and its numbering for storage and establish a computer databank including the following information: (a code number that allows easy identification of the species, its origin (catch station, date of capture, date of reproduction), kind of hormonal treatment, and the sperm performance/quality characteristics tested prior and/or parallel to cryopreservation (e.g. percentage of sperm motility, fertilization rate).
- Subjective evaluation after spermatozoa activation under the microscope is only useful as an initial orientation before a thorough quality test starts. An overview on the state of the art is given by Fauvel et al. (2010). To make a precise evaluation of Sperm

performance, CASA (Computer-assisted Sperm Analysis) software should be used as a more reliable methodology, although the method is relatively expensive (e.g. software + microscope + video camera). However, we are dealing with rare, valuable and highly endangered species. The use of CASA needs also a standardization effort (times, dilutions, frames captured per second, etc) but this can be employed to standadize all other aspects of sperm handling and use (example see Hatef, et al. 2010). Basic parameters to measure using CASA are percentage spermatozoa and spermatozoa velocity (VCL and VAP) but also flagellum beat frequency is increasingly used.

- Also, ASMA software (Assisted Sperm Morphometry Analysis) (Marco-Jeminez et al 2008) is increasingly used to determine spermatozoa morphology (rate of anomalies) as a useful tool in order to check the effect of changes of extender osmolality and other operational factors.
- Also, three replicate samples should be thawed 24 hours after cryopreservation and used in an immediate fertilization trial to assess the initial fertilizability of cryopreserved spermatozoa for later comparison of the long-term storage effects on performace. The eggs fertilized with cryopreserved sperm should be observed in certain time intervals, e.g. 24, 48, 72, or 96 hours, to count fertilization rate, abnormalities in ontogenetic development and mortality of early stages)
- Store the cryopreserved sperm in the appropriate storage tank and change the liquid nitrogen in following predefined appropriate standards. Modern equipment allows to automatically monitor the level of liquid nitrogen in storage tanks and either provide an alarm at low levels or automatically refill from a general liquid nitrogen storage tank.
- Avoid the frequent opening of the lid of liquid nitrogen containers. Any need for opening of storage containers should be registered in a protocol, including the date and time of opening as well as the duration.
- Ensure that those hatchery staff working on the cryopreservation of sturgeon sperm are well-trained in handling of equipment and monitoring of cryopreserved samples.

At the Caspian Sea basin level it would be important that the following is undertaken in the field of Sturgeon sperm cryopreservation:

- Estimate the total demand for sperm of each sturgeon species and by hatchery.
- Allocate a proportion of sperm sample for a sperm gene bank and long-term storage.
- Update in regular intervals the methods and technology on sperm cryopreservation and gene banking ones such bank has been established.
- Develop a Caspian Sea basin sturgeon sperm cryopreservation strategic plan, supported by a regional level plan which is strongly supported by national institutions of Caspian Sea abutters.

Based on the nature, capacity, demand and objectives of each country in relation to sturgeon sperm cryopreservation, it is necessary to develop a comprehensive strategic regional plan. Such a plan should be linked and coordinated with national plans. The objective of the plan should clearly be determined by the end users. It is important, for instance, to determine whether the cryopreserved sperm will be used for restocking of wild stocks, for aquaculture or for an overall sperm gene banking exercise serving general conservation issues.

Since sturgeon resources of the Caspian Sea are considered to comprise shared stocks of five littoral states, it is important to develop a joint regional programme for cryopreservation of sperm, storage and for the exchange of sperm between hatcheries. Under a regional programme the hatcheries can exchange their experiences and achievements and benefit from knowledge of international centres and scientific institutions.

Certainly, it has to be acknowledged that sperm cryopreservation is only a part but important component of preserving the gene pool while the maternal component will have to be taken from live broodstocks. Research should be encouraged in cryopreservation techniques also of the maternal gene pool.

6. TAGGING OF STURGEON

6.1 Tagging of wild sturgeon and sturgeon at the hatchery *

Guideline 6.1:
Hatcheries should tag broodstock sturgeon caught in the wild as well as sturgeon raised at the hatcheries to enable identification, increase information and monitoring, and reduce handling related stress.

Justification:

In sturgeon restocking practices, having information on the origin of wild breeders and location in the river where natural spawning occurs is highly important.

Record keeping by tagging application as well as finding correlation between various information such as reproductive biology, genetics, fisheries return can play an important role in sustainable management of sturgeon stocks.

The only reference number for monitoring and tracking of fish is provided by tagging. The tag is generally fixed to the fish to last for many years; therefore it is necessary to select the best appropriate tag to cause minimum damage and injury to the fish as well as ensure the highest possible retention on the fish.

Implementation Guidance:

In terms of tagging of sturgeon the following criteria and activities are recommended:

1) Use of appropriate tags (preferably internal tags, PIT tags and external tags)
2) Register all necessary information linked to the tags in a database including: Species, sex, catching station, date of catch, total weight and total length, fin tissue (for genetic analysis), and time of breeder's migration.
3) All information related to reproduction should be recorded such as: Eggs weight (kg), Number of eggs in each gram, Fertilization and hatching rates and male fish tag number.
4) Beside external and internal tags, the genetic tag or DNA fingerprint for each cross should be obtained (e.g. using microsatellite analysis).

Because of the dramatic decline of wild breeders of sturgeon in the Caspian Sea, conservation of broodstock is highly recommended. In reproductive stages and seasons all eggs should be removed without killing the fish using micro caesarean techniques. Breeders may be used for several years and therefore the internal tag (PIT tag) may provide a suitable option for fish at the hatchery. In order to prevent inbreeding and also to increase genetic variability and avoid of any crosses between close relatives it is highly recommended to design a genetic ID for each specimen using appropriate tags. Tagging of wild brood stock will assist in the identification of the genetic origin as well as increase possibilities for behavioural studies of broodstock and offspring performance.

6.2 Sturgeon tagging procedures and criteria

Guideline 6.2:
Tagging of sturgeon should be practiced following clearly defined procedures and criteria which minimize handling stress and avoid negative effects on fish welfare while also ensuring high retention of tags over a long period of time.

Justification:
It is important to determine the proper tagging procedures and criteria for monitoring fish inside the hatchery but also ensure adequate tagging techniques that can endure for time periods on fish ater release. The tagging methods as well as the type of tag information that is to be recovered from the tags should be kept up-to-date and follows standards that are widely accepted and applied in the Caspian Sea basin.

Implementation guidance:
Hatchery operations that require obligatory tagging of the fish involved include the following:

- collection of broodstock, aiming at separation of broodfish in terms of the period of spawning run;
- collection of fish intended for broodstock replacement, aiming at group identification;
- collection of fish in the course of assessment, aiming at individual identification of specimens;
- release of fingerlings into natural water bodies, aiming at monitoring growth and survival rate control and evaluation of production efficiency**.**

Materials and methods used for tagging and marking operations should match the following criteria, regardless the objective of the operation:

- Provide the lowest possible level of fish injury risks during the tagging procedure;
- Ensure a minimal effect on the hydrodynamical properties (swimming capabilities etc) and fish survival rate after tagging and during its life cycle;
- Ensure a high retention of tags (depending on tagging objective during a certain period or through all of the life cycle);
- Allow for a fast tagging procedure;
- Allow easy tag detection in and on the fish concerned;
- Provide the possibility of applying non-lethal techniques to read the information from any tag
- Provide the possibility to perform ex-situ tagging.

Recognizing that there are many tags available, it is important to select a type of tag that suits the purpose and objectives of the tagging activity, as well as fish characteristics.

The following tag types can be distinguished:

1. internal tags
    - PIT tags
    - magnet tags (Coded Wire Tags (CWT))
2. external tags
3. dye marks and tattoos

4. resection of fin parts and scutes.

It is recommended that small fish (juveniles) should be marked (mostly by bath). Use of different markers should be tested before any generalisation and a control group should be kept under farm/hatchery conditions. Tagging should be applied for larger fish only.

7. WATER QUALITY AND SUPPLY *

7.1 Access to and availability of water *

Guideline 7.1:
Hatcheries should provide good quality water (in terms of O_2 content, pH level, and temperature) and sufficient supply to the sturgeon in the hatchery throughout the production and re-production process and the water quality must be monitored continuously to ensure fish welfare and proper conditions for the fish.

Justification:
Water quality determines to a great extent the success or failure of any fish culture and hatchery operation. Therefore, it is a very important issue to be considered. Sturgeons have been cultured in wells and surface waters of varying water quality. General water quality parameters for sturgeon hatcheries are shown in Appendix 2.

Implementation guidance:
In view of the fact that failures in water quality and volume supplied can result in near immediate mortality of sturgeon at the hatchery, the following aspects should be taken into consideration:

- The inlet water supplied to any hatchery must be free from all harmful substances. Pre-filtration of the water may be required before any disinfection is carried out to eliminate larger particles of organic matter. If particles larger than 30 µm can be removed the size of the disinfecting equipment can be greatly reduced and the abilities of the units can be optimized. Following the removal of these particles that can absorb the ozone or ultraviolet light and waste energy, a cost-effective treatment could be accomplished. For pre-filtration purposes, either sand filters or micro-screen filters can be used.

- Ozone gas could be used for disinfection of the water before supply to the fish tanks. Ozone gas is a highly effective disinfectant and its effectiveness is determined by the dosage, the length of contact time, and the presence of other materials that may consume the ozone. Since at higher concentrations it may cause fish health problems, residual ozone must not be present after the treatment process.

- Ultraviolet disinfection of the water is clean and safe, leaves no residuals to cause problems and poses no possibility of overdosing. The only human health hazard is the possibility of direct exposure to the lit lamps, which should be avoided. UV light has a wide spectrum of wavelengths but the germicidal wavelength is 254 nm, which all low-pressure UV lamps produce. The size of the UV equipment should be based on the water flow rate, dosage and the transmissivity of the water.

- In case of poor quality associated with the water supply source, the feasibility of quality improvement measures should be considered. Some improvements could include: temperature change, acidity neutralization through liming, solids and iron deposition.

- Protection against incidental pollution of inlet water should be provided if possible and monitoring of inlet water should be conducted continuously.

- Sturgeon fry can be susceptible to gas bubble disease when water is supersaturated with dissolved gases (more than 105 percent). Therefore, hatcheries should use adequate degassing technology to drive off excess dissolved gases.

- Applied norms of water quality and supply should meet the international (EU Water Framework Directive etc.) and national (for example, Water Code of Russian Federation) water laws and regulations.

- The application of recirculation aquaculture systems (RAS), particularly for the LLTHFB should be promoted in order to allow for better monitoring of water quality, ensure stable water quality, reduce risks of contamination/pollution from elsewhere and minimize the demand for water. In addition, recirculation systems will reduce water costs for the hatchery and costs of wastewater/sewage water release in certain situations.

8. FEEDING AND FEED QUALITY

Guideline 8.1:
Sturgeon diets used should meet the energetic and nutritional requirements of the respective life cycle stage while considering the digestive abilities of the fish.

Growth, fitness and health of the fish produced should match those of con-specifics growing under natural conditions or be superior to it. Characteristics to describe the required criteria have to be developed on a species specific basis, allowing differentiated interpretation of the rearing performance. Furthermore, adaptations are required based on the respective life cycle phase, since feed size, feed consistency and nutrient requirements reveal drastic differences between these.

Justification:

Feeding together with water quality and facility design is among the most important elements determining the success of the rearing process.

Optimal feed quality improves the condition and therefore the suitability of the fish for release. Cost only plays a secondary role in this process, while also the ease of the supply must not be a driving force for feed selection. It should be recognized that rearing of larvae intended for broodstock supplementation requires a different rearing practice than growing fish for release.

8.1 Feeding fish for release

8.1.1. Feeding during pond rearing

Pond rearing is a practical and suitable measure to raise juveniles under semi-natural conditions, allowing exposure to quasi-natural fluctuations of several environmental factors while having less tight control of culture conditions than in indoor facilities.

Justification:
Feeding during pond rearing generally depends upon a natural food chain within the pond. There are many guidelines and manuals for conventional extensive fish culture available that describe in detail the handling and management of ponds over an annual production cycle. Such ponds can be operated either as stagnant ponds (with intermittent water exchange) or as flow-through systems with a modest water exchange rate (to avoid washout of nutrients and loss of food-chain organisms via the outlet). The operational schemes may have to be adjusted, depending on local conditions (e.g. soil quality, inflow water quality, seasonal temperature, and other factors).

Implementation guidance:
Timely preparation of the ponds, prior to production is necessary. Drying, wintering, ploughing, and fertilizing the ponds as well as inducing plankton blooms should be carried out based upon the methods well described and established for both, ponds in sub-tropical and temperate climates. Supplemental feed supply mainly depends upon the preparation of the ponds and the time provided for plankton development. Stocking densities for fry are in principle well defined but will be site- or pond-specific and should be handled depending on the productivity of the pond, the previous utilization and the season. This requires continuous monitoring of the abundance of food organisms in such ponds. This is not an easy task and requires standardized sampling at a number of representative sampling points (depending on the size of the pond, including at least the four corners and two stations in the middle, using a vertically operated plankton net (hauled at comparable speeds from the bottom to the surface). To avoid time-consuming counting of plankton organisms, settling volume (4% formalin fixed) could be used as an initial measure while brief checks on the presence of key species should be recorded.
Supplemental feeding in pond rearing should only be used under severe shortcomings in natural production. In this case the administration of natural diets is based upon the live food production methods summarized in FAO (2001) and also described in several manuals and protocols for Artemia and rotifer production for teleost, crustacean and oyster hatcheries. However, it is economically not feasible to continuously have a large-scale cultivation of food organisms in operation. Food shortages in ponds occur often unexpected rapidly. In order to be able to respond in time to such events, a small fully equipped unit to keep stock cultures for rapid inoculation of a set of available tanks (incubators for Artemia cysts; stock cultures for at least three key microalgal species commonly used in commercial hatcheries; accompanying laboratory equipment) should be incorporated into the design of a sturgeon hatchery. It usually takes 2-3 days to have such inoculations reaching the exponential growth phase ready for harvest. Logistics for such operations should follow existing protocols, including linear programming.

8.1.2. Onset of feeding under controlled conditions
Onset of feeding in most cases of large scale sturgeon production is carried out under controlled conditions in confined rearing units with water supply and defined water exchange rates to assure the balance between metabolite dilution and good retention of life food organisms (or micro-pellets). At low exchange rates, often oxygen is supplied either in the instream water or in the culture tanks directly. Under these conditions, feed has to be administered to maintain energy requirements sufficient for activity metabolism and growth of the larvae. Feed particle sizes must be appropriate to match the gape size of the larvae and must reveal a behaviour that matches food search patterns of early juveniles.

For initial food supply upon onset of exogenous food uptake in larval sturgeons, cultured plankton organisms are provided. Mainly nauplii of *Artemia salina* are used for this purpose due to the ease of storage of the encysted eggs, easy hatching technology and well-established standardized procedures for preparation of this live food source and good success in most sturgeon species. Quality of commercially available cysts vary greatly with year and origin and advice from professional services (such as the International Artemia Reference Center, Ghent) should be sought for. Alternatively, wild caught zooplankton (Cladocera, Copepoda) can be used, however, the risk of introducing parasites and disease agents for which planktonic crustaceans may act as intermediate hosts showed be recognized through pre-cautionary checks of plankton samples prior to application. Also for very small larvae the utilization of *Brachionus* grown in captivity has been proven to be a good starter diet in many marine fish cultures. Feed administration of live feed is carried out in providing a density of 2-4 *Artemia* per ml of culture water. High density Artemia nauplii stock cultures should be supplied almost continuously to replace the uptake and flushout of nauplii through the drain in order to maintain a fairly constant density of food organisms in the culture tank, since the larvae are highly susceptible to starvation in case food density is getting too low. Also dead food organism and left over feed items have to be removed from the tanks regularly (or continuously through appropriate drain design) to maintain acceptable sanitary rearing conditions and avoid bacterial and fungal growth.

Apart from easy production of *Artemia* and *Brachionus*, both species allow easy supplementation with essential fatty acids and vitamins . There are well-documented standard protocols for these nutrient and HUFA-PUFA enrichment procedures which have been well backed-up by numerous scientific publications. These standard protocols developed for aquaculture should also be employed, including considerations on maintaining stock cultures for Brachionus strains (inoculation with local strains is preferred) and Artemia cysts origin (see also under 8.1.1) as well as suitable culture technology (incubation jars, culture tanks, aeration systems, harvesting and cleaning methods).

An excellent “Manual on the production and use of live food for aquaculture” has been published by FAO (Lavens and Sorgeloos, 1996) which offers detailed protocols for various food organisms, although in recent years numerous improvements (particularly in instrumentation and automation) have been reported in the pertinent science literature.

Timing of the first phase of the feed supply largely depends upon the feed organisms used in the second phase. Mainly the size and the feeding behaviour of the larval or juvenile sturgeons determine the time of transition. Once the larvae are in transition from planktonic to benthic feeding they are ready for a change in diet. Particle size of the diet should match the mouth gape of the fish either through selection of small-sized organisms or by cutting them up into suitable fractions. Transition from one type of feed organisms to another is best performed with gradual increase of the proportion of new feed items in the diet over an extended time period (depending on species; up to 14 days).

8.1.3. Ongrowing to fingerling size

During the rearing period in tanks the supply of food organisms should cover a wide range of species. Nauplii and adults of *Artemia salina,* small Cladocera (*Daphnia*, *Moina*), Copepods, small *Streptocephalus*, Gammarids (e.g. *Pontogammarus maeoticus*), minced Oligochetae (*Enchitrea*), *Tubifex* and earth worms can be used as foods. In case of Beluga it is recommended to use eggs and larvae of cyprinids or anchovy.

The methods of live food rearing have been initially elaborated and widely used in the aquaculture industry and many recommended procedures are practiced (Bogatova et al.,1975). Besides the many reports in the scientific literature, a detailed description of the live food production is presented in the respective FAO Technical Guidelines for Responsible Fisheries – Aquaculture Development: 1. Good aquaculture feed manufacturing practice (FAO, 2001).

The daily rations should be determined based upon life cycle stages, the size and the fitness of the individuals, considering water quality, oxygen availability and temperature. Feeding rates are best determined through ad libitum feeding. Feeding intervals should not exceed 2 hours. The size of feed particles depends on the fish species and the actual wet weight.

Some constraints of live food utilization are encountered such as difficulties in supply, potential vector for pathogens, complicated and costly storage. The improved fitness and reduced time required to acclimate fish upon stocking are generally well worth the extra effort.

8.2 Feeding of larvae selected for broodstocks *

Justification:
Rearing of larvae intended for building a broodstock or supplement (enlarge) an existing broodstock requires a different rearing practice than growing fish for release. Here, live food should be administrated over a limited time span only, to allow timely weaning to formulated diets. The long-term live food application is not feasible in terms of economic efficiency and will hamper further transition of larvae to formulated feeds.

Implementation guidance:
The time span for live feed administration can vary considerable from a few days to a few weeks depending upon species, feed source and rearing techniques applied. Ideally, the weaning process can take place during the first days after the transition from yolk-sac stage to active feeding. For the weaning process, the proportion of live food in the daily food ration should be gradually reduced from 100 percent (first day of feeding) to 5-7 percent (12-15 days of feeding). In several species, the fish that do not readily adapt to the formulated diet are undergoing a second cycle of weaning. Similar procedures are applied only over a shorter period of time for the transition between different feed types and/or pellet sizes.

Caution has to be taken, that the alteration of feed source is not associated with a selection in the weaned fish. More intensive genetic monitoring should be applied to identify the genetic background of mortalities and growth suppression upon weaning trials.

For formulated diets, the protein and lipid concentration in larval feeds should be 48-60 percent and 8-16 percent accordingly. This composition is altered with increasing fish size and age. In larger fish, protein contents of 42 percent are sufficient, while optimal lipid concentrations are below 15 percent. Digestible carbohydrates are kept close to zero, since carbohydrates lead to increased fat deposition in the liver.

The feeding rations largely depend on the size of the fish. In early life stages up to 6 percent of dry feed based upon body weight are considered effective. These rations are reduced with size to reach 3 percent at 10-20g live weight. Good feed quality meeting the requirements of the fish should not produce pathological or clinical reactions (liver fat content) and if fed in reasonable rations, should lead to feed coefficients of 0.5 – 0.75 for fish up to 200g.

8.3 Feeding at acclimation of wild fish *

Justification:
The integration of adult or juvenile wild fish into the broodstock has to be accompanied with a transition of these fish from live to formulated diets in most cases.

Implementation guidance:
Upon transfer into controlled rearing conditions, the fish are maintained at optimal temperatures for feeding. Initially, the fish are offered a variety of natural prey items to overcome the barrier to feed under the new conditions. Subsequently, after feeding has been established, pasture-like feed mixtures of live fish and formulated diets are used for a transition period. In some species it has been beneficial to adapt the fish to dry diets by filling small fish or squids with the semi-moist diet to increase acceptance. Also, in order to increase the efficiency of wild fish domestication and ease the transition to formulated diets, it is advisable to use different attractants (anise, vanillin, chironomid extract or *Tubifex* water).

The final transition stage is to reduce the water contents in the semimoist diet. This can be carried out in repeated steps until all fish readily accept the pelleted diet. Feed composition for grow-out is reported to vary between seasons with regard to optimal fatty acid requirements. Also improvements in feed composition concerning palatability, adaptation to species specific requirements are long waited for.

8.4 Feeding of breeders *

Justification:
Diets and feeding regimes for breeders differ markedly throughout the maturation process. Initially, the phase of somatic growth and building up of reserves required high proportions of digestible energy. During the final stages of maturation (i.e. hibernation) feeding may cease completely. Feeding of brood fish should be carried out using special feed formulations that resemble the composition of the eggs to ensure normal gonad formation. Species specific requirements have to be considered to allow optimal performance.

Implementation guidance:
Breeders have a high energy demand during the time of vitellogenesis. Nevertheless, excess energy supply usually results in accumulation of excess body and gonad fat adversely impacting maturation. Fatty acid requirements have been documented to differ from those of salmonids. Especially long chain PUFA (polyunsaturated fatty acids) are considered essential in the diet, and so are amino acids. The energy balance of the broodstock diets should be adjusted to nutritional requirements of the fish at this stage of gonad maturation (Shcherbina, Gamygin 2006). In preparation of maturation, feeding rates of 0.3-0.5 percent of body weight are administered.

A few (2-3) months prior to the spawning period, in coherence with a natural or controlled decrease in water temperature, the feeding of fish should be stopped. This should correspond to the end of the 3rd-4th stage of maturity (period of the vitellogenesis completion).

8.5 Evaluation of feeding efficiency

Justification:
The feed conversion efficiency of the brood fish should be monitored in regular intervals (on a 3-6 months basis by weighing and measuring the fish. Feed conversion efficiency is defined as the amount of dry weight of consumed feed versus biomass gain in wet weight per day).

Thus, the daily amount of feed administered must be registered,. Poor feed conversion rates indicate either physiological problems or insufficient quality of the feed administered while inadequate composition of feed may increase the nutrient load of the tank water (particularly when feed disintegrates fast and is not consumed immediately) and subsequently the nutrient and suspended solid load in the effluent will also increase. To validate the feed conversion efficiency the control of the true feed consumption should also be verified by documenting the percentage of uneaten feed. This is technically not easy in large tanks but modern tank design allows for sediment traps inside the outlets so that most of the uneaten feed can be captured during regular daily service work.

Implementation guidance:
For monitoring feed conversion efficiency several methods are presently available, the choice depending on tank or pond system design and feeding strategy. In case of automatic feeding, the feeding rate and the number of proportions of the daily rate can be timed using computer control and automatic delivery systems which also monitor the amount delivered and recalculate the daily ration based on programmed daily weight gain estimates (based on established length-weight relationships). Recent developments (Hufschmied et al. 2011) show promising results using three-dimensional underwater image analysis to determine sturgeon size and weight inside the tank so that fish do not have to be handled at all. Depending on size (number of fish) of the broodstock, many operators may prefer hand feeding in small units. In case of cage holding, video systems could monitor the sinking rate of pellets and stop feeding when a certain rate of pellets are passing the bottom while not being taken up by the fish (demand feeding). Besides, the ultrasound diagnostics can also used monitor fish growth while simultaneously determining the state of gametogenesis (maturity stages, the ratio of generative and adipose tissue in the gonads, identification of possible hermaphrodites) and the state of the liver (Chebanov & Galich, 2009).

If a large proportion of uneaten feed is observed, the feeding technology, water quality and the health status of the fish should be checked. Stress caused by handling, light, temperature or water quality changes, vibrations, noise should be reduced to a minimum. In such cases, the daily ration should be reduced to avoid bacterial contamination of the rearing unit and to eliminate additional risks in case of weak feeding activity.

8.6 Quality and safety of feed *

Justification:
Standards of quality are generally determined in accordance with FAO technical guidelines (FAO, 2001) and/or national codes of best practice and guidelines (Shcherbina, Gamygin, 2006).

Composed feeds may contain components that are not, or only to a limited extent, consumed by fish in their natural habitat. Carbohydrates are such feed ingredients, which are adversely affecting the sturgeon physiology. Also, the feed – especially if stored under suboptimal conditions - may comprise substances that can negatively affect the fish organism state. These include products of lipid peroxidation and metabolites of microorganisms (fungi and bacteria). A significant microbial contamination of feed changes its chemical composition, substantially reducing nutritional value, and leading to the accumulation of toxic products. Furthermore, bacterial and fungal contamination might adversely affect the micro flora in the digestive tract of the fish, leading to inflammations or gas development which can lead to the loss of equilibrium and subsequently death of the fish.

Therefore, special attention should be paid to the safety of feed, particularly to the microbiological characteristic, terms and conditions of its storage. Also in locally produced feeds, the use of antioxydants such as vitamin C should be considered.

To improve safety in aquatic animals and aquatic animal products the Aquatic Animal Health Code 2010 was developed by OIE to provide for relevant aquatic animal health measures. The Code also provides recommendations that address aquatic animal health hazards in aquatic animal feed. A key objective is to prevent the spread, via aquatic animal feed, of diseases from an infected country, zone or compartment to a free country, a free zone or a free compartment.

Implementation guidance:
FAO published various recommendations relevant to terrestrial and aquatic animal feed, such as the Technical Guidelines for Responsible Fisheries – Aquaculture Development: 1. Good aquaculture feed manufacturing practice. FAO 2001; Draft Good Practices for the Animal Feed Industry – Implementing the Codex Alimentarius' Code of Practice on Good Animal Feeding, IFIF/FAO [In preparation]) and there is a Codex Alimentarius Commission (CAC) standard (Code of Practice on Good Animal Feeding [CAC/RCP 54-2004]). The application of these publications is recommended to improve standards of aquatic animal feed safety.

Although the Key considerations relevant to aquatic animal feed apply mainly to commercial aquaculture, they are also relevant for hatcheries producing fish for release and this holds in particular to the potential risk of disease radiation. include the following:

1. Concentration of aquaculture establishments heightens the risk of disease transmission, whether the pathogen enters the culture system via feed or other means.
2. Historically, animal proteins used in feed were mainly sourced from the marine environment, due to the nutritional needs of aquatic animals and for reasons of economy. This practice increases the risk of disease transmission, especially when aquatic animals are fed live or whole aquatic animals of the same or related species.
3. The usage of feed in moist form (moisture content equal to or greater than 70 percent), semi-moist form (moisture content between 15 and 70 percent), and dry form (a moisture content equal to or less than 15 percent) implies different levels of risk due to the processing applied to the feed.
4. The increasing use of live and moist feed increases the risk of pathogen transfer and therefore, the live food production line must be operated under highly controlled hygiene for which there exist several manuals, particularly in salmonid farming systems from which a lot of insights can be gained for sturgeon hatcheries.
5. Hazards may be transmitted from feed to aquatic animals via direct or indirect means. Direct transmission occurs when the cultured species consumes feed containing a pathogenic agent while indirect transmission refers to pathogens in feed entering the aquatic environment or infecting non target species, and thereby establishing a mechanism for indirect infection of the species of commercial interest. Pathogens that are less host-specific (e.g. white spot syndrome virus, *Vibrio* species) present a greater risk of indirect transmission as they can establish reservoirs of infection in multiple species.
6. The expression of disease may be facilitated by culturing species under intensive and novel conditions. Also, it is necessary to conduct research and develop new feed (and feed ingredients) that are appropriate to the species and its culture system.

9. SELECTION OF BREEDERS FOR CONTROLLED REPRODUCTION

Proper selection of breeders for controlled reproduction is of key importance for sturgeon hatchery practices. Three aspects are of special concern, which include the following: control of seasonal propagation, spawning induction, and timing of maturity and female examination.

9.1 Control of seasonal propagation

Guidelines for 9.1.
Sturgeon hatcheries should control the seasonal propagation to increase the effective use of broodstock under controlled reproductive conditions.

Justification
At present cultured broodstock provide the only chance for rehabilitation of the wild populations, through restocking. The long process of gametogenesis and non-yearly oogenesis requires long-term management to plan reproductions from sturgeon broodstock under controlled hatchery conditions.

The traditional hatchery bio-technologies imply the use of breeders within a short period of time. Such operational strategies do not allow for breeding all the intra-specific groups. Certainly, this might be appropriate for common commercial culture to serve the market for human consumption, however, this is less suitable for sturgeon cultivation reproduced for restocking of natural populations. The long-term holding in brood fish facilities (LTHBFF) allows to solve these problems and to use more efficiently the production capacity of the hatcheries relative to the objectives in order to conserve the genetic and ecological structure of natural sturgeon populations.

Implementation guidance
The control of seasonal propagation would generally include the following aspects:

- Long-term holding in LTHBFF of sturgeons at a pre-spawning temperature regime depending on the species or race.
- Transition of the mature fish to spawning temperature regimes (STR), based on the natural system of temperature variations and duration corresponding to holding of sturgeons of different species and races (Chebanov et al., 2004).
- Shift the sexual cycle of wild “hiemal“ sturgeon breeders to an earlier season. Obtain winter and early spring progeny from spring run migrants with the use of thermo regulated systems and a recirculated water supply.
- Selection of brood fish to be held in LTHBFF to initiate reproduction should be mainly based on the oocyte polarization index (PI).
- Fish of both sexes should be brought together to obtain better results.

9.1.1 Determining stages of gonad maturity

Different techniques can be used to identify sex and determine the maturity (both in wild and domestic fish) during autumn and spring assessment. Gonad status and ovarian follicle size are the main criteria for determining maturity in the various techniques.
These techniques can be divided into two groups.

- surgical (requiring surgical intervention) – biopsy, laparoscopy (Matsche et al., 2011), endoscopy etc. (Conte et al., 1988; Williot and Brun, 1998).
- non-invasive (do not require surgical intervention) – ultrasound diagnostic technique (Chebanov et al., 2009).

Selected fish have to be divided into three groups depending on their spawning potential:

- early ripe gametes (reproduction to be promoted through the use of thermoregulation);
- spawning at seasonal temperatures (use of natural temperature cycle);
- delaying egg maturation at completion of the reproductive cycle (delaying by long-term holding of breeders at somewhat lower temperatures)

9.1.2 Criteria of ripe females selection and pre-spawn holding of breeders

Prior to the spawning initiation, the feasibility of breeders to produce ripe gametes should be evaluated using basic prediction criteria (Kazansky et al.,1978; Williot 2002) as follows:

- appearance (normal drawing, absence of marble appearance illustrating over maturation);
- absence of lipids in the sample (presence might illustrate ovaries under maturation);
- homogenous oocyte size;
- PI values should be lower than 0.1, better lower than 0.05;
- In Vitro Maturation Competence (IVMC), measured by counting the boiled ovarian follicles of which the envelopes of the nucleus broke down (GVBD, Germinal Vesicle Break Down) at a rate of over 90 percent.

The efficiency of artificial reproduction primarily depends on the temperature regime applied prior to hormonal stimulation of the fish concerned (Chebanov, Savelyeva, 1996, 1999, Doroshov et al, 1997, Williot et al., 1991, 2000; Goncharov et al., 2009).

9.2 Spawning induction of brood fish

Guideline 9.2:
The most efficient methods to obtain good quality gametes should be applied when inducing spawning of sturgeon, in view of the large differences in sturgeon maturity (e.g. some fish mature late and some fish are non-yearly spawners).

***Justification*:**
Brood fish, especially the females, are generally unable to naturally produce their gametes under hatchery conditions,. The most cost- and time-effective way to obtain ovulated eggs and sperm is to stimulate the fish with hormones.

***Implementation* guidance:**
While the following two methods for inducing the spawning have been successfully applied, it is strongly recommended to use mammalian mGnRH:

- dried common carp or sturgeon pituitary;
- synthetic analogues of gonadotropin-releasing hormone (GnRH)

The reasons for which mGNRH or GnRHa should be preferably applied include the following:

1) The reliability of a constant content of active substance is higher;
2) The absence of any other non-specific organic molecules is certain;
3) The action of the applied substance through the own hypohysis of the treated fish is more certain;

4) Easy availability is certainly a management factor;
5) Easy longer-term storage without loss of activity is possible.
6) Over-dosage is not detrimental (Goncharov, 1998).

Injection strategies vary. As in teleosts, one or two-injections can be used, depending on the maturity stage and on previous management (culture) conditions. Large fish are easier to examine when there are only few fish in the tank. As pointed out under system design, it is therefore advisable to have several tanks available so that fish to be used for reproduction in a particular year can be kept separate at low stocking density.

9.3 Timing of maturity and females examination (latency in ovulation)

Guideline 9.3.
The ovulation and spermiation processes should be initiated post hormonal injection within an optimum time window.

Justification:
In the course of breeding, one of the ultimate tasks is to properly collect ovulated eggs and sperm. In absence of any means to check and fully control the start of ovulation and spermiation, it is difficult to decide when to collect the best quality gametes. It is clear that the collection of the gametes should be performed in the optimum range of time post hormonal injection.

Implementation guidance:
Recognizing the difficulties for hatcheries to determine the optimum time to collect the best gametes, the following suggestions should be taken in consideration:

- The latency in response depends on the water temperature and should be determined according to a species-specific diagram, (Detlaff et al., 1993) (see Appendix 3).
- The presence of ovulated eggs at the bottom of the tanks might be a helpful indicator. The examination schedule has to be organized accordingly.
- For large females the use of ultrasound methods allows to evaluate the process of ovulation without stress causing handling (Chebanov, Chmyr, 2005).

10. SPAWNING AND GAMETE PROCESSING

10.1 Obtaining Ovulated eggs *

Guideline 10.1:
The extraction of ovulated eggs should only be performed using methods that keep the fish alive, support the recovery of the fish and allow to maintain brood fish for future reproduction cycles.

Justification:
In the past, when there was a high abundance of wild breeders, the conventional practice included the collection of ovulated eggs after slaughtering of the females. Under the current conditions extraction of ovulated eggs should be performed using modern minimally invasive techniques of mature gametes collection, which keep the fish alive, consider the fish welfare and it’s re-use in the (re-)production cycle.
The use of minimally invasive techniques for mature gametes collection ensures minimal stressors influence and survival of the broodstock, including the possibility to release the fish

into natural water bodies. Moreover, the re-use of the broodstock is generally a cost effective investment for the hatchery in times when not many wild breeders are available.

Implementation guidance:

In the process of extraction of ovulated eggs it is important to consider the following aspects:

- It is essential to assess the quality of ovulated eggs prior to insemination.
- The method of oviduct incision with further egg stripping has proven to be the prime method of ovulated egg extraction (Podushka, 1986). This approach is less stressful and traumatic for fish. The stripping should be continued till the eggs are freely flowing from the body cavity. If stripping is conducted professionally then there is no need for a second stripping.
- Anaesthesia application at egg stripping take between 2 and 20 minutes; use of anesthetics is particularly recommended for large females (> 30 kg), as those otherwise can hardly be held. In case of even larger females (> 150 kg) it is wise to use laparotomy technique (Burtsev, 1969; Conte et al., 1988) with posterior suturing and application of related adaptation procedures.
- Whatever the size of the fish, a continuous water renewal in the mouth of the fish during the process of ovulated egg extraction should be applied to make the brood fish able to breathe normally, which is a prerequisite for the better recovery.
- The quality of extracted eggs and their fertilization ability should be assessed visually, examining the uniformity of the coloration (surface drawing), regularity of the egg shape, absence of resorbed and activated eggs, plugs, abnormalities, transparency of the ovarian fluid, etc.
- The elasticity of eggs and their ability to get sticky post water exposition (stellate sturgeon 6-12 minutes, Russian sturgeon 8-19 minutes after fertilization) may be also used as maturity assessment criteria.
- The longer time interval from insemination to adhesion is generally an indication of the delay in ovulation, while shorter period one shows the over-maturation of females (Gorbacheva, 1977).

10.2 Collection of sperm and their quality evaluation

Guideline 10.2:
Collection of sturgeon sperm and assessment of the quality should be conducted in a hygienic way, causing minimal stress to the mature male fish involved.

Justification:
It has proven essential to keep stress levels minimal when collecting sperm from mature sturgeon males. Experiences from the past have shown that clean and hygienic handling of the fish while obtaining the sperm will result in better quality sperm and less stress.

The application of the Janet syringe when collecting sperm does not require sperm transfusion into the other containers, and thus avoids its contamination by water or debris and allows to estimate the necessary quantity without additional measuring equipment.

Neglecting of preliminary evaluation of sperm quality (ratio active and motionless spermatozoa, duration of their forward motion etc) can lead to a drop in fertilization rate.

Implementation guidance:

The following steps should be taken in consideration by the hatchery when performing sperm collection and sperm quality assessment activities:

- Capture of mature males should be conducted with minimal stress for the animal.
- The genital pore of males should be carefully wiped off before any sperm collection takes place.
- Collection of milt (sperm) is performed into clean dry containers. At this, ejaculate with evident grumes, bile and other foreign inclusions should be removed.
- Collection of milt (sperm) should be performed with the help of a cannula combined with a Janet syringe (Parauka, 1993) or (alternatively) directly into the graduated beakers by bending the males.
- After the collection of the necessary quantity of sperm, its quality should be assessed properly.
- The short-term preservation of sperm should be performed at a temperature which is not higher than that applied for holding the males. In case of mid-term (few days) preservation, the hypothermic method should be used.

Criteria of sperm quality determination include the following (see also Appendix 4):

1. Motility of spermatozoa by 5-point grade scale. In this scale the sperm assessed as less than 3 points is considered unsuitable for reproduction (Persov, 1975). However, modern methods of precise and reliable quantitative assessment of sperm motility have recently been described by various authors (e.g. Fauvel et al., 2010; Hatef, et al. 2010; Cabrita et al.,2010) and some details have also been described in chapter 5.7 (cryopreservation).
2. Density of spermatozoa per ejaculate volume unit. This characteristic is evaluated visually. The sperm of appropriate quality should have not less than 1 billion spermatozoa per 1 ml.
3. Test of absence of motility without adding water should be used for control the quality of the sample.

In order to provide accurate evaluation of sperm quality, modern methods of flow cytometry allow to measure velocity, trajectory of spermatozoa movement, their concentration, quantity of live and dead cells and other characteristics, using computer software and video monitoring may be used (Billard et al., 1999, Pavlov, 2006 and references cited above). In conventional sturgeon hatchery practices these methods have not yet been widely used, but for conservation of rare and endangered species and male selection for broodstock and sperm cryopreservation their application is obligatory.

10.3 Eggs insemination

Guideline 10.3:
Eggs insemination should be performed using the semi-dry (‘’Russian”) technique in order to ensure minimal losses in fertility of the eggs during the process.

Justification:

The sharp decrease in numbers of wild females used has caused hatcheries to increase the number of males used in the insemination process, to elevate the genetic heterogeneity of populations produced through controlled reproduction.

In order to obtain genetically different graded sturgeon progeny, it is highly recommended to divide eggs, collected from one female into 3-5 portions, and inseminate each portion with sperm from a different male. After insemination, the egg portions collected from the same female may be combined for incubation.

Implementation guidance:
When carrying out sturgeon egg insemination the following guidance should be taken in consideration:

- Insemination should be performed using the semi-dry (‘’Russian”) technique (Detlaff et al., 1993). The semi-dry technique of insemination has been claimed to reduce the probability of polyspermy associated with possible insemination through numerous micropyles common in sturgeon eggs.
- Insemination of sperm should be performed through a sperm solution in water with an approximate concentration of 1:200.This ratio can be slightly different if intensively mixed during 2 minutes, taking into account that only 10-20 percent of spermatozoa will be active after 2 minutes. (Detlaff et al.,1993; Billard, 2000).
- The limiting factor in insemination is loss of fertility of eggs after adding of water. An extra harmful factor for insemination is the presence of coelomic fluid. In order to avoid this harmful factor, a process of two-step insemination could be recommended, by replacing the added sperm solution after 1 minute with clear and clean water.

10.4 Eggs de-adhesion

Guideline 10.4:

In support of good survival during incubation and optimal ontogenetic development of the sturgeon embryos high quality de-adhesion techniques should be applied, although this precedure deviates from the natural embryonic development of sturgeons eggs on river beds.

Justification:

A highly effective de-adhesion method to remove the sticky coating layer from the egg surface is essential and will considerably affect the successive development of embryos and their survival rate. Poorly de-adhesed eggs stick together in lumps, leading to embryo mortality and increased *Saprolegnia* infestations on egg surfaces with subsequent impact on gas exchange, metabolic rate retarded growth, malformations and subsequent length of incubation period (e.g. pre-coccious hatching) and rate of mortality . In order to avoid egg mortality during the de-adhesion process one should provide sufficient oxygen and add fresh water to the mixture applied, although the oxygen consumption rates of early cell stages are relatively low. Eggs de-adhesion should be conducted in special devices when applying various agents. The temperature of the de-adhesion-agent-water mixture should be kept at the same temperature used for the fertilization of the eggs while also being the same when transferring to incubators.

Fertilized eggs deprived from their adhesive coating should be carefully rinsed in fresh water and transferred to incubators. The shape or design of the incubators should be such that dirt can be collected easily and removed without harming the hatched pre-larvae. At de-adhesion any trauma to the egg membranes must be avoided.

Implementation guidance:
When applying de-adhesive techniques it is important to consider the following:

- Mineral mud, blue clay, talcum, milk and tannin may be used as de-adhesive substances.
- In case of the river mud application it is essential to minimize infection through disinfection of the substance prior to use. The duration of the de-adhesion procedure is equal for all sturgeon species and depends on the applied substances (see also Appendix 5 –table to be inserted).
- To reduce egg/embryo mortality it is necessary to add fresh aerated water (for 20 minutes) into the system in the process of the de-adhesion and monitor the air supply. After the completion of the operation, the eggs should be rinsed with water to cleanse the de-adhesive substance residues.
- Water used for rinsing should comply with standard hydrochemical parameters, have high oxygen content and be at the spawning, fertilization and incubation temperature.

10.5. Incubation

Guidelines 10.5:
The holding of sturgeon eggs from fertilization to hatching in incubation systems should be carried out in line with common and established knowledge on the metabolic requirements of sturgeon embryos following similar principles as has evolved over a century in teleost hatchery operations in order to ensure high survival rates and good quality sturgeon larvae production.

Justification:

In general incubators of various types are used to hold sturgeon eggs from fertilization to hatching. The incubator systems can have various designs (conical, horizontal) and be made of cotton, glass, fiberglass or metal (aluminium). Because of corrosion risks and subsequent heavy metal ion release after long-term employment (yeas), heavy metals (e.g. iron) are less preferable construction materials and should be avoided. Whatever material or shape is used, all incubators aim to ensure steady water exchange around the eggs to facilitate optimum gas exchange (e.g. oxygen into the eggs, carbon dioxide release from the eggs). This can be achieved in different ways, either by (a) holding the eggs in a monolayer over which water flows in a well-controlled current (e.g. Heath-tray), (b) eggs are continuously moved and held in suspension by the water flow so they do not stick together (e.g. Zuger-jars), and (c) moving eggs in intervals (mechanically, e.g. Yoshenko-incubators) while water exchange is kept at constant (excess) rates throughout the incubation period. (see also FAO, 2008a). The rate of the incubation system water supply depends on the type of the system and egg developmental stage.

The light level of incubation units is generally provided at low level in accordance with the environmental requirements of the reproduced species, in order to provide maximum survival rate of the embryos and diminish the number of abnormal eggs and larvae. High light intensity is detrimental to most demersal fish embryos and retard hatching when embryos have reached a stage ready to hatch.

Loading of incubation units with eggs with a low fertilization rate leads easily to the development of fungal infections which commonly spread rapidly within the egg batch. As a consequence such infected batches may act as a source of spores which may also get into other incubation containers through the water flow (in cases where incubators are supplied in sequence from the same water source), increasing the portion of infected eggs and mortality rates. Also for this reason it is preferred that incubation units have a separate water supply to minimize transfer risks. However, if incubators are in the same room, transfer of spores occurs also easily via the humid atmosphere and may accumulate at wet points in the incubation room, action as reservoirs. For all incubation units, the fertilized eggs to dead eggs ratio must be calculated to assess the quality of the incubated eggs.

In general, the egg incubation at the upper limit of spawning temperatures negatively affects embryo development, leading to an increase in the number of anomalies and larval hatch rate with lower values of yolk sac resources. At temperatures close to the lower limit of the range, the incubation period tends to increase and the number of preventive treatments needs to increase accordingly.

Implementation guidance:
Although incubators are used for nearly all cultured fish species mistakes are often made in using them, with consequent high losses during the reproduction process.

It is important to consider the following aspects when using incubators for holding sturgeon eggs from fertilization to hatching:

- Incubation units are in most cases not operated all year round but seasonally. Before restarting the use of a unit it is strongly recommended to check all system components including walls, windows (being protected by mosquito nets to prevent insects entering the incubation hall), floors and corners of the room. In fact the entire unit should be disinfected before start-up.
- the water inlet and outlet of the system should also be checked, their completeness and functionality (valves etc) and the state of the incubation sections (boxes). The water supply system should be rinsed with water, and the supply pipes and other water-transporting systems should be disinfected and thereafter rinsed with disinfected and/or filtered fresh water once again. The water supply should be adjusted depending on the incubation system type. Surface water should be avoided as much as possible. Preferrably ground water should be used as this is bacteria free and helps to avoid from the start the use of antimicrobials.
- For small quantities of eggs (sterlet for example), MacDonald jars could be very efficient incubation systems. Also, for quality assessment incubation of eggs to monitor the quality more precisely for females from which mass incubation has been started, such incubators can provide well-controlled standard conditions. Thermoregulation of water upwards should be coupled with degassing to avoid gas supersaturation (remove excessive nitrogen) has to be combined with UV sterilization of water in the incubation unit.
- The light level of the incubation system should correspond with the fish species' environmental requirements (stellate sturgeon – 20-100 lx, Russian and ship sturgeons – 10-20 lx. (Kasimov, 1987). Higher illumination can cause increased level of malformations and mortality of embryos.

- The incubated eggs quantity assessment should be performed at eggs loading into the incubator by volume or weight measurement and on the basis of anticipated oxygen demand at specific ontogenetic stages in relation to the water supply volume (calculating the carrying capacity of each individual incubator). It is possible to assess the weight of eggs (post ovulation) at collection and estimate the total number of eggs by weighing samples of known numbers of eggs. Alternatively, as commonly practiced in marine fish hatcheries where individual fish release several million of eggs, a quick estimate without damaging the eggs is measuring the volume of eggs after fertilization.
- During the process of incubation “around the clock” observation is necessary to ensure regular water supply is guaranteed. This procedure should be necessary only in conventional hatcheries where modern monitoring and electronic control as well as alarm equipment has not yet been incorporated.
- The removal of dead eggs should be arranged frequently, the time interval of inspections to be decided daily anew, depending on the daily mortality records (increase in frequency needed immediately when increased mortality occurs). .
- If the UV bactericidal disinfection and thermoregulation is not sufficient or result in non-optimum quality of eggs, preventive treatment by appropriate preparations should be applied (in the case of open water systems) (the method of constant treatment using low concentrations of violet ”K” ($C_{24}H_{28}N_3Cl$) during almost all incubation period has proven to be most effective) (Mamedov, 2000). In case UV disinfection is repeatedly insufficient, a system design error has to be assumed and a re-assessment of the UV unit and its capacity must be undertaken as soon as possible while the capacity will have to be adjusted accordingly.
- In case ground water is not available in sufficient quantities and surface water supplies have to be used (as the only supply or partially), there should be sufficient pre-treatment of this water through appropriate methodologies such as mechanical filtration (e.g. settling basins, travelling screens to remove large debris; backwash filters).
- The duration of the incubation period depends on the thermal regime and the specific sturgeon species; it should range from 1000 to 1500 degree-hours. It is necessary to maintain species specific temperature regimes for incubation within the mean values range.
- The quality of water in the incubation system should meet the general hatchery requirements (FAO, 2007) as also flagged out in the regulations for EU hatcheries certification to produce fry for stocking aquaculture facilities outside the watershed system of the hatchery location.
- The shape or design of the incubator should be such that waste particles (dead eggs, empty egg cases, debris and settling particles) can be collected easily and removed without harming the eggs during incubation and also the hatched larvae.

10.6 Hatching

Guideline 10.6:

The hatchery should ensure proper conditions in the incubators (see previous chapter), which allow newly hatched pre-larvae to swim freely to the accumulator or enable hatchery staff to collect the hatched pre-larvae from the incubator with minimal handling efforts.

Justification:

Hatching usually expands over a relatively long time, the duration of which is temperature dependent but also strongly influenced by the exchange of the interstitial water between eggs and the gas transfer efficiency across the egg membrane. Therefore, the occurrence of the first freely swimming pre-larvae in the incubation system is considered to be the onset of hatching. Hatching is not an ontogenetic stage but a physiological state (Rosenthal and Alderdice, 1976). Thus, hatching enzymes will be released when oxygen demand within the egg outstrips the supply. Also, during and immediately after hatching, oxygen demand increases due to increased activity. In order to prevent pre-larval mortality associated with lack of oxygen, it is necessary to collect the pre-larvae timely from the incubation containers and larval collectors. Illumination has proven to an important factor which determines the speed of the hatching of pre-larvae as well as their directed movement; certainly in accordance with species specific peculiarities of phototaxis.

Implementation guidance:
The following aspects should be considered at this stage in the re-production process:

- The length of incubation period of sturgeons depends on the water temperature, which preferably should be maintained close to the average values of the species specific optimal range. Usually, hatching intensity in a cohort of embryos in incubators follow a Gausz-distribution with a few larvae appearing early, the bulk hatching almost simultaneously and several appearing late. Egg incubation at the upper limit temperature can negatively affect embryonic development, leading to increase in number of abnormalities and yield of larvae with lower yolk deposit. At temperature close to the lower limit, the prolonged incubation is observed and hence more prophylactic work is needed. The duration of hatch of embryos from eggs takes 24-48 hours on average, however, one should try to optimize incubation conditions so that hatching time is shorest and hatching size fairly uniform.
- The counting of collected larvae from large-scale incubators can be performed using the weight method (batch weighing) and then estimating the total number. While such a crude method is sufficient to assess management needs for ongrowing work in the hatchery, more precise methods are needed to assess the quality of progeny. Therefore, for quality assessment of eggs and larvae derived from controlled broodstocks, incubation three replicate small batches (min 150 eggs per replicate) from each females with precise counting of fertilized and hatched eggs need to be performed. After hatching, the pre-larvae obtained from mass incubations should be transferred into proper tanks for further weaning. The hatch rate should be calculated separately for each female (in case of separated incubation) or form each sturgeon family (in group or cohort incubation of eggs mixed from several females).
- The collection of hatched larvae from the incubation systems is generally performed using a harvesting net, siphons or special traps.

11. REARING OF LARVAE AND JUVENILES IN TANKS *

11.1 Holding of pre-larvae during the period of endogenous feeding *

Guideline 11.1
In the pre-larvae stage the hatchery should provide a suitable environment for the pre-larvae, which preferably should include the use of clean water from the river to which they will be released.

Justification:

Taking into account that homing (a return of mature breeders to spawn in their native river) is the objective of the hatchery stock enhancement programmes, the holding of pre-larvae and larvae in ground (artesian) water or in any other alien water is highly undesirable despite possible economical and other considerations (e.g. favourable ground water quality and temperature). Despite the fact that still there is not any strong evidence about homing in sturgeons, it is desirable to apply the precautionary approach on this subject, which is promoted by the FAO Code of Conduct for Responsible Fisheries (FAO, 1995). As a consequence of the possible olfactory imprinting formation in pre-larvae prior and at the transition to active feeding (Boyko, 2008), the holding of the pre-larvae should therefore be obligatory in water from the river to which they will be released after the rearing.

Implementation guidance:
The following aspects should be considered at this stage in the re-production process:

- Appropriate design of the (pre-)larvae keeping unit is important and dead larvae should be removed at the hatchery. Constant water temperature, degasation and low rate of water exchange and low water depth (20 cm) should be also ensured.
- Holding of pre-larvae and grow out of larvae are generally performed in concrete or plastic round tanks or trays with a surface area of 1-4 m^2 and water depth of 17-20 cm. The stocking density before the start of active feeding can be rather high and amount to 20 thousand individuals per m^2.
- Prior to larvae stocking, the system of water inlet and outlet should be checked and rinsed; the bottom and wall surfaces should be disinfected and rinsed with fresh water.
- If the traditional technology is applied, the minimum water flow rate (1-3 l/min) should be set during the first 24 hours of pre-larvae holding (one time per three hours). Then this rate should be increased to 8-14 l/min (with not less than twice per hour a complete water exchange).
- During the period of endogenous feeding, when pigmentation of pre-larvae is not developed, it is necessary to maintain low illumination during the egg incubation, thus ensuring differentiated approach to rearing of various sturgeon species (Kasimov, 1987; Chebanov et al., 2004).
- During the first 24 hours of pre-larvae holding, it is necessary to remove membranes of the eggs and un-hatched and dead embryos. Further removal of dead larvae should be performed 1-2 times per day. Dead, as well as larvae infected by *Saprolegnia* should be collected using a siphon and removed. In order to detect cases of invasive diseases, parasitologic examination of the larvae should be performed once each 3-4 days or more frequently.
- The time of the larvae transition to active feeding should be monitored visually by controlling their behaviour (Detlaff et al.,1993). It should be noted that pre-larvae, being initially in the quiescent state (swarming), will be dispersed along the bottom of the tank in search of food after starting active feeding.
- Optimal thermoregulation results not only in a considerable increase in survival and decrease in the occurrence of morphological and functional anomalies, but also enhances the performance of artificial reproduction, especially at successive stages of pond rearing of fingerlings.
- The importance of the pre-larval yolk sac size and shape evaluation in the course of hatchery and ecological monitoring of hatchery-bred fingerlings can hardly be overestimated. Pre-larvae with a small size of the yolk sac (the endogenous resources) do not grow and develop during one of the most important stages, the

transition to exogenous feeding. On the other hand, a too large volume of the yolk sac generally negatively affects the formation of the feeding functions, leading to delay in excretory functions of the epithelium. The index of yolk sac deformation in pre-larvae is the height-length ratio. It is normally ranged from 0.55-0.69., while for malformed (pear-like or oval) yolk sacs this ratio is as low as 0.29-0.44 (Belyaeva, 1984).

- The duration of the endogenous feeding period depends on the water temperature and on average ranges from 7 to 10 days. At the onset of the exogenous feeding it is necessary to increase water exchange in the tanks and ensure a high concentration of dissolved oxygen in water.
- The preferred stocking density of larvae at the start of exogenous feeding depends on the species. It has been experienced that at lower stocking density (700 – 1 000 thousand individuals/m^2) in different species, the growth rate and survival of larvae increases, especially when using only formulated feeds.
- Weight measurement is recommended to take place every three days, after which the daily ration of feed to be supplied should be adapted accordingly.

11.2 Rearing of larvae for broodstock replacement

Guideline 11.2

The hatchery should, in case it is required to raise larvae and fry for broodstock replacement and establishment at the hatchery, initiate the conditioning of the larvae and fry as soon as possible.

Justification:

While it is recognized that it is not preferred to raise larvae under aquaculture conditions to broodstock in support of future sturgeon rehabilitation and restocking programmes, this sometimes cannot be avoided. Early training and transfer of larvae feeding behavior to formulated feeds, maintaining of optimal stocking densities and timely grading (sorting) of fry are important factors that determine success of the development of the broodstock.

Implementation guidance:

The following aspects should be considered when rearing larvae for broodstock replacement:

- After a short-term (1-2 days feeding with *Artemia* nauplii), it is recommended to use starter dry feed, with low stocking densities, following the existing methods of commercial sturgeon aquaculture (Ponomarev et al., 2002; Chebanov et al., 2004; Nekrasova, 2006).
- It is necessary to conduct regular size grading of larvae and fry.
- Transition of fry to granular feeds should be performed gradually, from 10 percent of its share in the ration during the first days of feeding to 100 percent after 10-12 days. The size of feed particles is species and size specific. Gisbert & Williot (2002) reported that it is possible to not use nauplii, but start with compound diets directly.
- Observation of larvae and small juveniles' behaviour is useful to detect unusual situations and stress factors in the tank.

11.3 Rearing of juveniles for release into natural water bodies *

Guideline 11.3

Hatcheries aiming at restocking of natural water bodies should prepare the juvenile sturgeons trough training and adaptation to natural conditions, to increase survival after release.

Justification:
Experience from sturgeon restocking and rehabilitation programmes over the last decades has shown that training and acclimation of juvenile sturgeon before release can increase survival rates of the released fish significantly.
The rearing of juveniles for release into natural conditions requires the use of ponds for better adaptation to feeding through natural feeds and to learn how to survive in an environment with predators. In addition, short-term raising of the fish (at fry and fingerling stage) in tanks could be necessary; such raising should take place in compliance with modern (combined: tanks – ponds) hatchery technology.

When raising fry or fingerlings in the tanks, for future release in ponds and rivers and the sea, it is necessary to provide rearing conditions as close as possible to the natural aquatic environment. Experience has shown that short-term raising of fry and fingerlings in tanks considerably increases the survival rate of fry and fingerlings at their successive rearing in ponds.

Application of different types of live feeds, especially at the onset of active feeding helps to increase the level of thyroid hormones in the tissues due to including of the hormonal "pool" of the live feed (Boyko et al., 1993, 2002; Boyko, 2008). This is tightly bound with the decrease in the frequency of morphological anomalies and formation of olfactory imprinting towards chemical stimuli and will determine the homing fidelity (return to the native river) of mature sturgeon brood fish later.

Implementation guidance:

In order to raise fry and juveniles with proper fitness indices that increase survival rates when releasing the fry, fingerlings and juveniles in ponds and in natural water bodies, it is necessary to take the following aspects in consideration:

- Provide for a natural photoperiod (Ruchin, 2007) at the same or preferably a higher level of illumination, corresponding to species specific peculiarities of sturgeons (Kasimov, 1987). In general, one can observe an increase in swimming speed of fingerlings at high illumination. Moreover, to decrease the negative influence of the stressors, while conducting hatchery operations (sorting, feeding) or fry monitoring, it is wise to use red light with frequency 680 mmk. The sturgeons do not perceive the light of this frequency (Sbikin, 1974);
- Maintain an astatic thermal regime with a daily amplitude of 4 -5^{o}C or thermogradient field; this stimulates more intensive energy exchange and higher survival rate of the fry (Konstantinov et al, 2007);
- Creation of a water flow in the tanks, which enables the fry to train their swimming capabilities and improve the adaptive performance of their central nervous systems (Nikonorov, Vitvitskaya, 1993; Kozlov et al., 1989);
- The use of live feeds (nauplii *Artemia*, rotifers) enriched with ω-3 HUFA, will improve survival and growth of fry and enhance stress resistance.

11.4. Fry and fingerlings monitoring and quality control

Guidelines 11.4:
Monitoring of the fry and fingerlings to ensure a high quality at the time of release of the juveniles in the natural aquatic environment should be carried out frequently by use of standardized methods. The use of fitness indices and environmental stressors assessments is recommended.

Justification:
The ecological optimization of reproduction and rearing of fry and fingerlings intended for release into the Caspian Sea and other natural aquatic environments requires continuous monitoring of quality of hatchery produced progeny. Monitoring should be conducted not only prior to release into natural environment, but also throughout all the technological cycle of rearing. In the course of monitoring, it is necessary to control the compliance of all indices with the standard values.

A decrease in the so-called “fitness indices”, expressed in lower resistance to diseases, lower resistance to extreme environmental influences (Lukyanenko et al., 1984) and malformations of fish reproductive systems, can lead to reduction in number of breeders that will be suitable for reproduction. The aim of hatcheries that reproduce and raise sturgeon for restocking is to generate fitness for survival in the wild. This requires unifications of protocols and of sturgeon hatchery management on the basis of sound standardized handling (with minimization of stressors affect), rearing, training and evaluation of fitness indices of fry and fingerlings, as well as proper release of juveniles into natural water bodies (Agh et al.,2007).

Implementation guidance:

The following tests to assess the quality and fitness for survival of the sturgeon progeny are recommended:

- Selection of larvae after the hatching on basis of species specific behavioural response to changes in the water depth (only viable larvae are capable to make “swim up” and “drift down” motion);
- Estimation of shape and size of the pre-larvae yolk sac;
- Measurement of the swimming capacity of larvae during the period of their transition to active feeding allows to evaluate their physical endurance, general formation of the body, gills and swimming capacity and water current resistance (Khodorevskaya et al., 2009);
- Assessment of the physiological state of larvae and fry based on the melanophores (pigment cells) background reactions reflects the state of the neuro hormonal system. This determines the ability of larvae, fry and fingerlings to exhibit protective coloration and thus to survive in natural aquatic environments (Krasnodembskaya, 1994);
- Teratological analysis of larvae and fingerlings of different species, allows for the evaluation of frequency of morphological anomalies in progeny, obtained at hatcheries from wild and domestic breeders (Galich, 2000, Chebanov et al.; 2004; Atlas, 2004; Goryunova et al., 2000);
- Evaluation of adaptive abilities of the fingerlings on the basis of the central nervous system development using an “open space” test, makes it possible to assess the level of locomotory activity, the response to external stimuli and the fitness to survive in natural conditions (Nikonorov, Vitvitskaya, 1993).
- Express analysis of the physiological development stage of larvae, fry and fingerlings allows for the assessment of tolerance to extreme values of the basic abiotic stressors, such as high water temperature, salinity and oxygen deficiency (Lukyanenko et. al., 1987).

Evaluation of the sturgeon fingerling development stability on the basis of fluctuating asymmetry indices is generally another key element of the monitoring plans and programmes used by sturgeon hatcheries. The evaluation of fluctuating asymmetry in sturgeons is considered an effective and non-lethal method of intra-specific variability and possible decrease in heterozygosity of built population, as well as environmental stressors occurrence (Valentine et. al., 1973). The estimation of the fluctuating asymmetry in sturgeons may be performed using bilateral meristic characters such as: number of lateral scutes (S[Lat]) on the right / left, number of ventral scutes (SV) on the right / left, number of pectoral fin rays (P) on the right/left, number of ventral fin rays (V) on the right and left and some other indices.

12. Rearing of juveniles in ponds

12.1 Ponds preparation

Guideline 12.1:
Prior to the hatchery season the pond (to be used for raising juveniles) should be properly prepared through clearance and planning of the pond bed, disposal of vegetation and application of mineral and organic fertilizers.

Justification:
Good ponds should be inexpensive to construct, easy to maintain and efficient in allowing good water and fish management (FAO, 1995a). Costs, however, will depend on local conditions, particularly on the permeability of soil (seepage) requiring extra preparatory efforts to introduce a clay layer or other modern impermeable materials. Pond preparation is a determining factor in terms of providing good water and system management (including fish handling). Detailed guidance on how to design and construct ponds, prepare the soil and water in the pond for fish culture and on general pond preparation and management can be found in the FAO Training Series (FAO, 1995a; FAO, 1985; FAO, 1994).

Implementation guidance:
It is recommended to follow the guidance on pond preparation provided in the FAO Training Series. The growth of sturgeon fry to juveniles in earthen ponds is a sensitive and important stage in any restocking program. In the ponds the fry and fingerlings, beside learning to find and feed on live food, are learning how to prey from natural food resources; their feeding behaviour is formed. Before fry introduction into the pond, it is necessary to make sure that the fry are in a healthy condition and are disease free.

12.2 Development of the feed potential and phyllopods extermination

Guideline 12.2:
When preparing the pond and managing the pond environment for the juvenile sturgeon it is important to exterminate phyllopods (Phyllopoda) where possible and ensure the development of natural food supply in the pond.

Justification:
The main source of feed for sturgeon fry and fingerlings in the earthen ponds consists of live food. It is highly important to have an appropriate pond preparation schedule in order to make best possible use of the productive season for growth of the phytoplankton and zooplankton in the earthen pond. Early release or late introduction of larvae and fry into the pond (e.g. when the temperature is less than 15 ºC or above 28-30 ºC) results in high mortality because of limited live food availability. It is therefore necessary that the hatchery manager has a detailed plan for pond preparation and management in accordance with regional climatic conditions and considering past experiences.

Implementation guidance:
In addition to the guidance provided in chapter 8 (Feeding and Feed quality) the following aspects should be considered:

- When developing the natural live food feed base in the pond it is required to identify the most temperature resistant phytoplankton and zooplankton available in the region, produce zooplankton and supply it during shortage periods to the pond, and use additional formulated commercial feeds to compensate for any feed shortages.
- It is recommended to apply, along with conventional feeding activities, additional measures to enhance feed organism biomass (zooplankton and benthos) and establish a proper species composition including:
  - gradual (step by step) filling of ponds with application of organic fertilizers and *Daphnia* culture;
  - introduction of necto-benthos feed organisms (mysids, Gammarids, captured in the coastal areas of the sea and at future locations of fingerlings release) and exterminate the phyllopods (Phyllopoda).
- There exist few methods of phyllopods extermination:
  - o pond liming (at standard rates recommended for conventional extensive pond culture systems) or – in an emergency - chlorination (may be applied exclusively if any other method application is impossible, however, environmental regulations must be strictly followed, e.g. release of chlorinated pond water to the environment only after dechlorination; respective facilities must be available in such cases);
  - o biological ameliorators application;
  - o repeated pond flushing (with rapid water drainage and refilling of ponds);
  - o earlier filling of the ponds (the possibility of this depends on the climatic conditions).

12.3 Stocking of fry and juvenile production in the ponds *

Guideline 12.3
Hatcheries should ensure a suitable aquatic environment for stocking and production of sturgeon fry and juveniles in ponds, including preventive measures against predators.

Justification
It is highly important that fries are strong and fit with high growth performance prior to release in the pond. The stocking of the ponds should be performed following the relevant methodical guidance. The fry being nursed in the tanks are generally transferred in flasks or other aerated containers with water.

Implementation guidance:
When stocking sturgeon fry and fingerlings in earthen ponds it useful to consider the following aspects:

- A proper pond size for fry and juveniles rearing is a 2 hectare pond; at this size the pond can still be properly managed in terms of marinating and improving its hydrobiological and hydro-chemical conditions.
- Fry density in the pond is highly dependent to the species reared and live food availability in the pond. Generally 50-100 thousand fry/larvae of 100 mg. are released

in each hectare of pond. If the pond is kept in a good condition a 70-75 percent survival rate can be expected during the growing season.

- In order to conserve the intra-species population structure and create the necessary environmental conditions (thermal regime, photoperiod, feed availability) for all sturgeon groups, it is wise to perform rearing of fingerlings in ponds in a few cycles during the vegetation season, including:
    - Early stocking of the ponds (with a 20-25 days shift from the traditional dates) is possible using thermoregulation at fertilized eggs obtaining and early rearing of the fry (Kokoza, 2008). This enables the most efficient utilization of production potential of the ponds and considerably expands the season that the fry can grow until release as juvenile;
    - stocking during the traditional period (late April – May);
    - stocking during the second production cycle (July – August) by larger fry (1-2 g) at lower stocking density (10 thousand individual per ha and grow them till a weight of 7-10 g).
- Stocking of the ponds outside the traditional season allows more effective use of production capacity of the hatcheries and it is not necessary to release fingerlings during the summer when temperatures are high to ensure favourable conditions for growth and development in the ponds. This results in high survival.
- Following the release of the fry into the pond, regular biological and chemical monitoring should be conducted, including monitoring of zooplankton biomass by regular sampling (once a week); and fry and fingerling sampling using a small trawl is crucial for assessment of growth and health of the fish.
- During the fingerlings rearing it is necessary to maintain an optimal water level in the ponds. To limit development of filamentous algae, the constant control of hydro-chemical and hydro-biological regimes and ichthyo-patological state of fingerlings should be performed during the whole period of rearing.
- The duration of fry/fingerling rearing in ponds depends on feed availability, but the average number of days is 30 to 35 (in some cases up to 45 days). During this period the fingerlings reach a weight and fitness which is suitable for release in their natural aquatic environment.
- In order to limit mortality and damage to fish from predators (mainly birds and mammals, but also poachers) it is highly recommended to apply preventive and protective measures, such as:
    - Nets and bands (shading) to prevent birds from reaching the water surface;
    - Apply ultrasound scaring systems to scare away larger fish and aquatic mammals (through an acoustic signal);
    - A gas-noise gun is a highly effective for protective measure to scare away predator birds (such as cormorants); one gun can scare away all the birds in an area of 20-30 ha;
    - Use of watch dogs, and appropriate devises (e.g. traps and electric fences);
- To prevent damage to certain types of (endangered) predators (e.g. animal species listed in the red book), the preventive measures applied should be approved by Regional and or national Conservation Authorities.

13. RELEASE OF FINGERLINGS

The release of fingerlings (juveniles) into natural water bodies is the final stage of the technological cycle at the hatcheries that produce fish for stocking/restocking of natural water bodies.

The extended spawning season, spawning ecotypes and run of juveniles to the river mouth are important life history traits of sturgeons. Under the original natural conditions in the Caspian Sea basin, offsprings of different spawning ecotypes migrated downstream towards and into the estuary and the sea at different ages and seasons, which reduced competition and optimized the use of food resources. The construction of dams and weirs in the sturgeon rivers has, to a large extent, rendered impossible, or at least negatively influenced, the maintenance of such a high biodiversity.

In order to conserve the biodiversity of established populations and sustainable use of food organisms in the natural water bodies, it is necessary to conduct prolonged release of different size- and age-graded juveniles.

For the release of fingerlings it is important to prepare them properly for survival in the nature, have suitable tagging procedures in place, select the best sites for release and arrange for suitable transportation to the sites of release.

13.1 Preparing for fingerling release

Size at release is an important issue that needs careful consideration when assessing the potential success of any release programme, whether for ranching, stock enhancement or re-establishment of a species. Lorenzen (2000) considered in his analysis of seven release experiments (including 53 stocking events) the use of survival based on linear length growth with the objective to assess the applicability of alternative release sizes. Although the analysis is based on freshwater teleosts, the principle usefulness of this approach should also be tested with sturgeons since our present practices are mostly based on the rule of thumb rather than sound knowledge on the optimum time-size-release window.

Guideline 13.1

Hatcheries should arrange for proper acclimation and training of sturgeon juveniles to reach fitness when released to the harsh natural conditions. This would greatly support their competitive capacity and subsequent survival in the natural aquatic environment after release. It is important to acclimate the juveniles to the most important abiotic factors they are likely to encounter in their future environment (e.g. temperature profiles, light climates, relevant current velocities and salinity gradients typical for coastal areas.).

Justification

The low level of sensory stimulation of juveniles in the culture environment (primarily in indoor facilities such as tanks or covered outdoor ponds) often leads to sensory depreviation of reared specimens as compared with open pond reared individuals and moreover with specimens developed in the wild., affects the protective behaviour of the juveniles and their capability to form required reflexes (Kasimov, 1980). Therefore, it is highly advisable to provide at the hatchery a set of measures to improve basic fitness characteristics of the fingerlings in order to enhance the stamina and survival of the juveniles in the wild (see above).

Additionally, it might be useful to further develop, through training, the needed reflexes that make the juveniles better adapted to the natural conditions. This “training” can include varying current velocity to gain higher sustained swimming speed and maximum escape burst. Additionally, the introduction of small predators into limited sections of the tanks (or ponds) so as expose fish to their potential enemies. These predators should be sufficiently small so that they may only attempt to attack juveniles but are unable to swallow them, thereby learning to distinguish between friend and foe. Such “training” to environmental cues may be a different levels corresponding the intended size/age of release (e.g. larvae, fry, fingerlings and juveniles.

Implementation guidance:
When preparing for release of fingerlings or juveniles it is important to consider the following issues:

- Provide operational means so that the juveniles will be trained and acclimated to natural aquatic environment conditions of the respective receiving waters, including:

  - exposure to small potential predators prior to release in limited areas of the tanks (ponds) to train "enemy recognition and the corresponding escape response in juveniles;

  - training and sorting of juveniles in hydrochemical trays with regulated current velocity;

  - some feed deprivation of juveniles for 1-2 days prior to release in order to increase the urge to hunt for food immediately after release to the new and unknown habitat.

- It is a good practice to use an "Ichthyotest" or challenge system or a set of tests for "fitness", including exposure (and accompanying evaluation) to slightly fluctuating salt, oxi- and chemical conditions (e.g. testing chemoresistance) in order to assess adaptive capabilities of juveniles (Nikonorov and Vitvitskaya, 1993; Tikhomirov and Khabumugisha, 1997).

- The fingerlings to be released should be collected from the culture units (e.g. ponds) and transferred to specially designed large shaded tanks or cages installed at aerated ponds or circulated channels near or at the proposed final release sites.

- Taking into consideration that the swimming velocity of fingerlings that have been starved during 1 day is higher than that of fed fingerlings, their higher swimming speed facilitates the search for appropriate feeding areas which generally results in higher survival after the release into natural water bodies. If the fish starve for more than 1 day, their salinity resistance tends to decrease. This latter fact should be considered when attempting to stock fish in estuaries and(or) in coastal areas of the sea (Kokoza, 2004; Khodorevskaya et al., 2009).
- When planning for the release, the availability of the required relevant human resources, equipment, maps and records has to be checked as well as the functional state of the needed special equipment. This is necessary to assure that the release and monitoring of the procedure can be commenced using a standardized protocol
- Preferably the release of juveniles should be performed under the control of a "Commission" established by a responsible supervisory body at the regional level. The recording and documenting of fingerlings released should be conducted under the supervision of this "Commission" which cross-links with similar bodies within the region to assure good guidance, exchange of experiences and guaranteeing the comparability of procedures.
- The release of fish produced in hatcheries should be performed only when a valid ichthyopatological certificate has been issued (FAO, 2007) and CITES approval has been obtained.
- Juveniles should be tagged before release in support of restocking impact monitoring and sturgeon research and management purposes. The methods of tagging need to

be pre-determined as well as the minimum number to be tagged. Besides mass-marking methods a certain number (depending on size at release) should be tagged using an advanced (albeit expensive) technique allowing individual tracking

13.2 Selection of best sites for release

Guideline 13.2:
The location of optimal release sites should correspond to biological peculiarities of the sturgeon species under consideration. Sites for release should, therefore, consider habitats and location where normally the natural cohorts of the population thrive. It is therefore essential to chose the time-size-location-release-window in line with the movement and behaviour of the natural population.

Justification:
Studies on the seasonal dynamics of food organisms in the estuary of the Volga River, and other Caspian drainage systems combined with observations on survival and growth of Beluga, Russian and stellate sturgeon juveniles, lend support to new strategies for sturgeon stocking for the Caspian Sea basin. These strategies may include different management options determined by life history of the species, seasonality of reproduction, age and size of juveniles, and climate variations (wet or dry years). The extended timing and variability in sites for release of juveniles will contribute to the preservation of species diversity and provide a more rational concept for release in line with a better use of the food base in the rivers, estuaries, and coastal areas of the Caspian Sea (Chebanov and Billard, 2001).

Implementation guidance:
In the selection process of suitable sites for release it is essential to consider the following aspects:

- compliance with the biological and environmental needs of the juveniles;
- distance from the hatchery;
- transportation infrastructure availability to minimize transport stress (e.g. rough roads) ;
- options to perform releases of juveniles during different times during the day (e.g. interim stress-free holding capacities on-site)**.**

A hydrobiological and hydrochemical study should be conducted at sites of prospective fingerlings release to assess the following criteria:

- sufficiently large habitat area with high benthic feed productivity and its availability capable of supporting the released batch of specimens (state of the bottom – solid sand slightly silted soils, water depth of 2.5 – 5 m, low degree of coastal area vegetation, zoobenthos biomass and size of basic feed organisms) to satisfy the initial nutritional needs of the released fingerlings;
- in order to avoid crowding of fingerlings within limited areas, release into rivers and river mouth areas (coastal areas) should be distributed over considerable space and time based on pre-calculated carrying capacity estimates;
- the number of fingerling predators and pests should be as low as possible;
- match of key hydrochemical factors (temperature, pH, level of oxygen and toxic substances) to specific requirements, absence of thermical and salt stratification diminishing rate of dispersal, localizing area of grow-out and negatively affecting the state of fingerlings (Levin, 1989).

Preferably, identification of potential sites and final selection of a site of release should be performed on the basis of the experimental trial results and related normative guidelines such as the Codes of Best Practices, developed by scientific institutions and approved by the relevant territorial basin fishery inspection authority.

To reduce the inter-specific feed competition and reduce the probability of fish released from the live fish transportation boats being consumed by predators directly after release, juveniles should be distributed in small batches along the gradient of current velocities lower than the cruising speed of released juveniles; mass and long-term release at one location should be avoided. It is necessary to use “scatter” and “trickle” stocking instead of currently used “spot stocking”.

Furthermore, in river stretches with intense shipping, juveniles should be exposed to experimental wave action prior to release into shallow river beaches in order to train escape responses, thereby minimizing the risk to be washed ashore. As has been recently documented with release programmes using allis shad (Alosa alosa), stranding rates are also influenced by wind-generated wave actions and by waves caused by ships. Stranding rates were highest the first seven hours after release (Stoll and Beeck, 2011) but with some pre-experience, the escape response improved and mortality decreased. Such short-term pre-training could be used to improve release strategies.

To increase the survival of released juveniles, it may be necessary to temporarily enclose a certain area at the release site and eliminate any predators prior to release. This will enable the released juveniles acclimatize to the new environment, without being hunted for directly. This approach is very important as it can be observed that the fish, once released, remain immobile for a few hours leaving them susceptible to predators. Alternatively, the culture in semi-controlled hatchery (natural pond) conditions may be expanded to produce larger juveniles (100-500 g) for release that are less vulnerable to predation. In any case, it is recommended to prefer at all stages a step by step acclimation approach while gradually transferring the juveniles from the culture facilities to the receiving waters.

13.3 Transport of fingerlings to release sites *

Guidelines 13.3:
Specialized means of transportation (e.g. appropriately equipped trucks or boats) should be used in order to ensure safe transport, with minimal stress, to the release sites for the juveniles.

Justification:
The schedule of pond draining and fingerling catch and transport should be developed considering the necessity to perform the release in the dark. A release in the dark will considerably decrease the predation pressure and support fingerling adaptation to the new environmental conditions associated with a higher level of protective response and higher velocity swimming of the fingerlings (Budayev and Sbikin, 1989; Levin, 1989).

Implementation guidance:
When transporting the juvenile sturgeons to the sites of release it is essential to consider the following aspects:

- During the loading as well as during the whole transport, the fingerlings should be constantly held in water.
- Loading of fish during the daily peak of temperature should be avoided.
- Juvenile densities in the holding facilities during the transport should depend on the type of transport means, species, size of fingerlings and the conditions provided during

transport (duration, temperature, use of oxygenation equipment etc.) and have to be carefully calculated by personal of the hatchery.

- A sufficient number of transport means has to be made available to cope with the number of fingerlings to be released at any one site.

14. SANITARY AND HYGIENE MEASURES

14.1 MONITORING OF FISH SANITARY CONDITIONS AND HEALTH AT THE HATCHERY

Guideline 14.1:

Monitoring of sanitary conditions and fish health should be performed by the hatchery on a regular basis including daily visual examination of state, behaviour and feeding of fingerlings and broodstock, as well as periodically to carry out a complete external ichthyopatological examination.

Justification:

Fish health monitoring is of critical importance to identify at an early date any potential problems that may arise during the culture period. Generally, diseases are occurring naturally but may become an over-riding problem under culture conditions. They often result from suboptimal rearing conditions, adversely impacting the physiology of the fish and reducing its capability to combat pathogens effectively. Also, rearing under controlled conditions often favours the development of bacterial strains well-adapted to the rearing system, which renders it necessary to monitor the hygienic conditions additional to the regular water quality monitoring of key parameters. This would allow to either develop system-specific prophylactic strategies or recognize early warning signs of unexpected outbreaks to counteract in a timely fashion.

Further, fish develop resistance against prevalent pathogens but might be maladapted to other strains or species of pathogens. If recycling systems are employed, the absence of low-level naturally occurring facultative pathogens may prevent juveniles to gain sufficient immuno-competence so as to cope with the common exposures in natural waters after release. Therefore, the fish health status is considered vital as a prerequisite for release. Furthermore, disease outbreaks with pathogens typical for culture-system can potentially results in (a) lack of fitness of the immune system against disease unavoidable in the receiving waters and (b) converting the cultured juvenile into a carrier, spreading pathogens to natural populations.

Implementation guidance:

To prevent outbreaks of fish diseases in hatchery, sanitary guidance standards should be strictly implemented, including those outlined in the Aquatic Animal Health Code 2009 (World Organisation for Animal Health (OIE) 2009). Specific references should also be made to several other guidelines and protocols such as have been developed by FAO (2007). These standards cover a variety of issues relevant for the maintenance of aquatic animal health through disease prevention and control. Suggested measures are zoning, disinfection, contingency planning, and fallowing which are presented here in modification of the original outline.

The strict adherence to the measures of safe production and adequate sanitary standards should be ensured at each hatchery. Building and reconstruction of facilities should be approved by state authorities of veterinary and sanitary control.

Basic concepts of animal health maintenance and sanitary conditions are comprising the following:

- grow out tanks, ponds and other hatchery units should be cleaned regularly, periodical disinfection is recommended;
- rearing of fingerlings of different age and species should be conducted separately in different facilities and units;
- each production facility should be equipped by its own hatchery equipment, which should be disinfected prior and after the works, and kept at dry and clean places;
- rearing and feeding conditions should be controlled regularly, all observations and operations should be recorded in the special diaries.
- all the entrances in the incubation unit and nursery facility should be equipped by a disinfection barrier effectively maintained on a daily basis,
- transfer of sturgeon within the hatchery should be planned considering the epizootic state of hatchery systems where they have been held,
- transition of fish from hatchery to hatchery should be planned considering the epizootic state of the hatcheries. Transfers during or immediate after disease incidences should be strictly prohibited. Considering the EU regulations for temporary closure

Moreover, the following issues should be considered:

- In case of transfers between facilities, the health status and appropriate quarantine measures are recommended to prevent accidental spread of pathogens to non-adapted stocks. Each hatchery must have a full quarantine station for such purposes.
- Outbreaks of diseases are accompanied with a variety of critical indications sometimes at early infestastion stages. Therefore, regular control of the status of the fish reared allows to respond to such challenges at an early stage when simple metaprophylactic methods still can prevent disease outbreaks
- To improve adaptation to natural conditions and ubiquitous pathogens, utilization of river water is discussed as a means to expose the fish to relevant pathogens early to allow sufficient adaptation of the immune system.
- Relevant protocols should be established. The assessment of the safety in rearing facilities can only be undertaken where treatments are well defined. It may not be necessary to provide full details of the entire treatment or process undertaken. However, any critical steps in combating disease agents of concern should be given in detail. It is therefore necessary that treatments (i) use standardised protocols; (ii) are conducted according to Good Manufacturing Practices; and (iii) that any step in the treatment and subsequent handling of the fish do not jeopardise their safety.
- Contamination risks may be reduced by limiting the number of visitors.

14.2 Daily visual control

Guideline 14.2

In order to prevent development and spreading of diseases within the culture units, a daily visual examination of all fish-holding units should be performed.

Justification:

Daily visual examination by experienced and well-trained personnel is the cheapest and most effective way to identify at an early stage any problems that may emerge in terms of fish welfare, health and sanitary conditions.

Implementation guidance:

During this visual examination, the following criteria should be verified and recorded in a daily diary book:

- fish swimming performance and feeding activity (in comparison to previous days; based on past operator experience. Such observations are subjective and should be made – as far as possible – by the same hatchery operator). ;
- body colour of fish (also in comparison to observation during previous days);
- Abnormal (unusual) production of slime on the fish skin;
- abnormalities in pigmentation around the mouth (potential indicator of yersiniosis),
- abrasive or irregular shape of pectoral fins (potentially indicating cannibalism and/or fungus infections);
- presence of sores, white spots, injuries, blisters, inflammation of the anal area;
- presence of ecto-parasites and/or their cysts on the skin of fish with body;
- gill malformation (hyperplasia, necrosis, erosion), extravasation (hemorrhage), necrotic nidus.

In this context, it is necessary to remove dead or very weak animals from the tanks on a daily basis, since they are one of the main sources for spreading diseases.

14.3 Complete ichthyopatological examination

Guideline 14.3

The overall ichthyo-pathological and physiological state of the fish should be examined once every two months in order to maintain a full record on the history and development of the health status of fish during the entire cultivation period, including seasonal variability.

Justification:

Particularly for sturgeon broodstock it is important to keep track of the health and sanitary conditions of the fish and the culture environment. A complete ichthyopathological examination will provide the hatchery manager with a detailed overview of the sanitary and health situation at the hatchery. It will provide information for a full retrospective assessment of the etiology and development of disease outbreaks or will allow to employ at an early date prophylactic measures to prevent severe outbreaks and losses. Based on these assessments, knowledge-based adjustments to culture operations and facilities can be made in a timely fashion while also the broodstock composition may be changed (e,g, including separation and quarantining of early-on discovered diseased individuals to save the rest of the broodstock) .

Implementation guidance:

The ichthyo-pathological examination should preferably be performed by personnel well trained in fish disease diagnostics. Whenever a disease outbreak occurs (or even when early signs of a disease are discovered through the daily routine observations), a fish veterinarian should be consulted to obtain professional advise. The routine measures may include:

- microbiological study on quality and quantity of pathogenic microflora on scrape samples from skin, fins, oral cavity, and blood (further details to be included);
- diagnosis of the prevalence of parasites as above;
- evaluation of basic physiological blood parameters (red and white blood cell counts, haematocrit etc.);
- evaluation of normal development of internal organs in juveniles, combined with verification of internal parasite and bacterial status.

The results of visual and complete ichthyopatological examination of fingerlings is recorded in special diaries with indication of species, exact dates and time of the study and results of examination. The monitoring should follow the outline provided by the Aquatic Animal Health Code (OIE, 2009).

14.4 Quarantine measures

Guideline 14.4

Quarantine measures must be performed in accordance with the existing international codes of best practice and in accordance with international regulations (OIE 2009, Arthur et al, 2008; FAO, 2008; ICES Code of Practice, 2004).

Justification

Given the difficulty of establishing and maintaining disease free hatchery operations, there may be benefits in establishing and maintaining subpopulations with a distinct aquatic animal health status. Subpopulations may be separated by natural or artificial geographical barriers or by the application of appropriate management practices.

Zoning and compartmentalisation are procedures to define subpopulations of distinct aquatic animal health status for the purpose of disease control. Compartmentalisation applies when management practices related to biosecurity are the defining separating factors, while zoning is defined on a geographical basis. In practice, spatial considerations and good management are important in the application of both concepts since zoning alone might not suffice to separate subpopulations especially in migratory fish.

Implementation guidance:

In addition to contributing to the safety of operation, zoning and compartmentalisation may assist disease control or eradication. Zoning may encourage more efficient use of locally available resources while avoiding the transfer of individuals or even populations while compartmentalisation allows the functional separation of a through biosecurity measures. Following an outbreak of disease, compartmentalisation may allow, to facilitate disease control and/or the resumption of production. However, the potential of employing the hatchery management guideline for certification of disease free hatcheries in several European countries, permitting the transfer of raised juveniles to aquaculture facilities outside the hatchery watershed, may be also useful for being implemented for sturgeon hatcheries intended to produce juveniles for release. Hatcheries aiming at being certified as a disease free unit undergo seasonal health inspections for target diseases and will be certified after being disease free for a two year period.

An intensification of the zoning and compartmentation concept may subsequently lead to a culture system in complete quarantine.

Fish transferred from other hatcheries or captured in natural water bodies and selected for domestication, should undergo obligatory quarantine procedures over an acclimation period accompanied by regular health inspection. The period for quarantine varies with respect to the source of the fish and the target disease for which testing is required. Intra basin transfers from health certified facilities require less strict and shorter quarantine measures than inter basin transfers or introductions from the wild.

The quarantine measures to be applied include complete separation of fish from all other units of the hatchery, utilization of distinct water supply and approved discharge (sterilization), preventing contact between transferred and resident fish, water and equipment. This requires considerable planning of facilities to accommodate the needs to safeguard the fish in a full quarantine unit. Those specimens subject to quarantine should undergo diagnosis at a competent laboratory. In case of diagnosed diseases while in quarantine, the fish treatment should be performed under control of a fish veterinarian. Transfer from these facilities should follow only after diagnostic clearance. Otherwise the fish will have to be disposed off in an approved manner. After quarantining, stepwise acclimation to common water sources to which the fish will be released is considered helpful, allowing the fish to adapt gradually to the local microbial flora (including subclinical exposure to potential pathogens over time rather than by immediate exposure.

Fish affected by virus infection should be separated, removal of virus bearing fish should only be considered if proof is available that no rare alleles are associated with the virally infested individual.

14.5 Prophylactic measures and disease treatment

Guideline 14.5

In sturgeon health management the primary objective is to employ preventive measures through adequate hygienic conditions in the hatchery and applying best environmental practices (BEPs) by using best available technology (BATs) to minimize the risk of disease outbreaks while allowing the cultured specimens to built up their natural immune system which they will have to rely on after release. Therefore, the administration of pharmaceuticals and antimicrobials should be kept to a minimum while sanitary control measures (clean operation) and prophylactic methods should be applied using specifically designed handling protocols to which all employees have to strictly adhere in their day to day work.

Ones a disease outbreak has been recognized in a system, a decision needs to be taken whether to destroy the stock or whether a treatment can be justified. Treatment of diseases in sturgeons will have to follow similar procedures as established for other fish species managed in aquaculture and must be supervised by a veterinarian (or – if available in the country- by the respective national fish health inspection service).

14.5.1 Disinfection

Justification:

Disinfection is employed as a common management tool in aquaculture to maintain proper hygienic conditions that minimize the risk of disease outbreaks within the system. They should be part of a disinfection programme designed for specific purposes. Disinfection may be applied to eradicate or exclude specific diseases agents from entering the rearing facilities, as well as a routine measure to sanitize rearing facilities.

Implementation guidance:

Disinfection of installations and equipment and transport units should be carried out using procedures that prevent the contamination of other water bodies and other aquatic animal populations with infectious material. The decision on which product to use should take into account their microbiocidal efficacy, their safety for aquatic animals, the environment while also employing safeguarding measures to protect employees.

The efficacy of disinfection is affected by various factors, including temperature, pH, and the presence of organic matter. At high temperatures, the reactivity is faster to the point that decomposition of the disinfectant does occur. At low temperatures, the biocidal efficacy of most disinfectants decreases. Also, presence of organic material and greasy substances may significantly reduce the efficacy of a disinfectant, rendering thorough cleaning of surfaces a necessity before applying disinfectants.

For most of the commercially available disinfection materials, the companies provide detailed instructions on their proper applications. These should be followed strictly.

14.5.2 Fallowing

Justification:

Intermittent utilization of facilities or production units is commonly recognised to be of value in managing and controlling the facility environment. As part of this strategy, fallowing can break re-infection cycles by removing loci of a disease from a farm. Consequently, fallowing is often carried out as a regular hygienic measure or disease management tool in aquaculture, especially prior to the introduction of new populations into a previously used site. In order to promote improved health in production the use of fallowing is recommended as a routine management strategy for keeping good hygienic conditions and reduce the risk of commonly re-occurring diseases outbreaks.

Implementation guidance:

The likely beneficial effects of fallowing in proportion to the economic costs involved should be considered, including. the level of risk to the local operation, the usefulness of previous knowledge on the severity of a disease(s) outbreak, the infective period and distribution of the disease agent(s) within the system. If fallowing is required to eradicate a disease agent or to break the cycle of likely infectious re-occurrences in relation to prevalent neighbouring farms the required period of fallowing, should be synchronized to be regionally effective.

A number of diseases are regarded as potential threats to the outcome of hatchery programmes as well as to wild stocks. The introduction of such diseases into countries recognized to be free from these diseases, may result in significant losses. In order to diminish such losses, it may be necessary to act quickly and therefore, contingency plan(s) should be developed before such events occur.

Treatment with medications must take place only after diagnosis has been carried out and an effective treatment has been identified. Legal prerequisites for treatment of fish intended as stocking material as well as restrictions with regard to the medication permitted must be observed.

14.5.3 Administration of therapeutics

Justification:

In cases where valuable stocks have to be protected from extended losses or safety of production renders treatment of fish inevitable, therapeutics are applied to prevent the spread of diseases.

Due to legal restrictions, antibiotics should only be used for fish designated for broodstock.. The application of antibiotics for treatment of fingerlings released into natural water bodies is not recommended. Because of the health risk and the limitations of application as well as disposal, hatchery staff should be properly trained in handling and application of the therapeutics as well as in safety regulations.

Implementation guidance:

Legal restrictions on the use and the approval of therapeutics vary between countries. It is strongly recommended that only those therapeutics are used that are registered for use in fish farming and their application is executed only after prescription by an authorized veterinarian service, as is obligatory in many jurisdictions to safeguard aquatic production. It is, therefore, strongly recommended that the same safety measures and quality assurance in handling valid for commercial aquafarming are also applied to fish produced for release. In countries where such regulations are not mandatory, it is strongly recommended to employ these voluntarily. Up-to-date diagnostic tools and test kits should be employed to assure the most effective and environmental friendly application. Regulatory authorities should include into the licence procedure for new hatcheries a mandatory obligation to involve public health inspection or veterinarian services to supervise the hatchery in all fish disease issues and identify the therapeutics that are registered for use in fish farming.

Different techniques for administration of medication are available.

- oral – the preparation is mixed with the feed prior the feeding (more than 6 hours storage at room temperature is highly undesirable), or using specially prepared medical feeds provided by certified companies;
- baths – preparation is dissolved in water in special tanks or containers where fish are held. It is necessary to control the preparation concentration in water, temperature regime and strictly follow the time of exposure, as exceeding it can cause fish mortality. Constant control should be provided during the process of treatment. In case of sharp decrease in the fish's state, the treatment should be stopped and the fish transferred to containers with fresh water and high rate of water exchange;
- intramuscular injections – the preparation is injected in the fish body. The dosage is calculated for each specimen based upon individual weight and physiological state.

Antibiotic treatments should preferably be accompanied by recovery therapy with the use of probiotics (bacterial preparations containing life spores of various Bacillus subtilis strains or from cultures such bifidobacteria etc. (Burlachenko, Bychkova, 2005). The special formulated feeds, containing bacterial preparation are available for recovery therapy. The duration of the recovery course is not less than a fortnight, however, this will depend on the severity of an event and should be determined in close cooperation with a veterinarian.

14.5.4 Anesthesia

Justification:

Anaesthesia (narcotization) is used to decrease the effect of external stressors deliberately imposed on the fish. Typically, anaesthesia is used in surgical interventions or upon handling (i.e. biopsy, health assessment, ichthyopatological examinations, sorting and transport, in some cases when applying certain reproduction techniques etc.).

Implementation guidance:

The concentration of any anaesthetic should be calculated on the basis of the response of the individuals subjected to narcosis under given environmental circumstances. In general, the response should leave time to interfere with the process if unfavourable conditions are unexpectedly encountered. Also the concentration must be adopted to water temperature, since lower temperatures prolong the response of the fish. The overdose of anaesthetics, excessive duration in the bath, or high water temperature can cause long-term damage and mortalities.

For sturgeon hatchery applications the following anesthetics are recommended:

- Tricaine Methanesulfonate (MS-222);
- clove oil (eugenol);
- benzocaine;
- propiscine (by gill irrigation)

Furthermore, good results have been obtained by using

- CO2 (also as a tranquilizer)
- Electricity (electromobilization after Kynard et al. (2006))

Aeration is recommended to be used in a bath/small tank while also the temperature should be the same as in the holding tank from which the fish has been taken. During handling under narcosis the fish should be supplied with water or anaesthesia-solution (pumping via a tube through the mouth and gills) to minimize oxygen deficiency in the blood (respiratory stress) and mechanical damage to the gills through desiccation.

15. DOCUMENTATION

15.1 Documentation and reporting

Guideline 15.1:

Proper management of a sturgeon hatchery should include regular monitoring, keeping good records and documentation and timely planning for the operations of the hatchery.

Justification:

Lack of necessary operational documentation or incomplete record-keeping will lead to improper monitoring objectives and result in limited control over hatchery operations; it generally will significantly affect the efficiency of the entire operation. Moreover, application of self-developed (non-standardized) record forms will hamper the analysis of the enterprise activity, and subsequently lead to restricted information exchange and eventually negatively affect the proper planning of the reproduction and release activities at national level and at Caspian Sea Basin level. Proper documentation of the various production operations is therefore one of the important elements of any sturgeon hatchery activity; particularly hatcheries that aim at achieving rehabilitation of sturgeon stocks in their natural aquatic environment. Monitoring, record keeping and documentation is important for the daily hatchery management itself, as the information collected and analyzed will allow the hatchery to improve its efficiency. Moreover, it is important for enabling adequate reporting to authorities, and to enhance collaboration between hatcheries at national and Caspian Sea Basin level.

Implementation guidance:
In the process of regular monitoring, keeping good records and documentation, reporting on hatchery operations and for the timely planning for the operations of the hatchery for subsequent culture cycles, the following issues should be considered:

- Monitoring protocols and record keeping of the hatcheries should be performed using standardized forms that are to be completed by all hatcheries active in the country and at the Caspian Sea Basin level. The standardized forms should be easy to use and available for completion on the computer, so that information can be directly inserted into a standard electronic database. The electronic database could then be shared with other hatcheries and national authorities to carry out analysis and in support of monitoring and record keeping of fisheries, restocking practices and aquaculture at the national and Caspian Sea Basin level.
- The monitoring, record-keeping and reporting activities should correspond to the standards and requirements for data, information and trend reporting of the country concerned, including its fishery and aquaculture authorities and national statistical agencies.
- The main sturgeon hatchery records, irrespective of their purpose, should contain the following information:
  - date (and time in case of working diaries) of any data records;
  - details of the personal on duty as well as identification of the person entering information into the record sheets or notebooks (name, surname and position of the employee);
  - name of the production unit;
  - species and age of the handled or monitored fish;
  - serial or individual No of fish;
  - identify code of the containers (water units) where the fish is held;
  - operation performed at each time and the reason for the record (if not self-understood).
- Appropriate financial resources, software and human resources and staff training programmes should be made available by the hatcheries to perform proper monitoring, record keeping and reporting tasks.
- In regular intervals (at the latest after one month) back-up copies of the entire monitoring data set (preferably on CD) should be maintained and be available for region-wide retrospect analysis once such programmes are initiated. For data security, back-up copies should be maintained in a building away from the location of the monitoring system.

The minimum documentation to be produced by sturgeon hatcheries includes the following:

1. Current diaries, including working records, temperature records, ichthyopathological and hydrochemical control records and others need to be carefully maintained. The working diaries should contain data on all eggs and fry and their related records, monitoring data containing the results of controls, measurements and examinations, and data on feeding frequency and daily rations as well as data that allow transparency on what background information these rations were periodically adjusted. The documentation will allow the tracking of biotechnical requirements and the compliance with established protocols and standards. The record keeping should be performed by staff directly involved in the operations (some example templates are

presented in tables of Appendix 5). Further examples of how to perform record keeping can be found in the FAO Training Series (FAO, 1992) but hatchery managers are also advised to consult the many manuals available for teleost hatcheries destined for aquaculture production. It is general practice that the documents should after use be sealed with the hatchery seal. After the hatchery season completion the documents should be kept for seven years. During this seven years period sturgeon scientists should be provided with the opportunity to scientifically analyse the data sets so as to undertake a trend analysis and improve our knowledge base not only on experimental data but on practical operational data sets from large-scale operations producing juveniles for release.

2. Specific (subject) reports, including acts of breeders capture, transfer of stocking material between production and fingerling release units (facilities), number of released post-larvae or/and fingerlings by species need to be kept; assessments and consolidated lists, such as feed registers should be maintained. These reports are generally prepared by a team of staff that are directly involved in the hatchery process and senior staff of the hatchery and are to be approved by the hatchery manager.

3. Pedigree reports. The documents of this category include diaries of pedigree activity, tagging, sampling of breeders intended for genetic certification, as well as “passports” of brood fish with data on genetics, time of sexual maturity, absolute fecundity (for females), quality of gametes, quantity and location of the progeny. The pedigree documents should be completed by the staff involved in these activities.

4. Annual hatchery report. This report should cover comprehensive and summarized information on: preparatory operations done before the hatchery season, water supply, collection and utilization of wild (hiemal and vernal races) and domestic breeders (of different species) with tags numbers including egg extraction from live females, mating, main results of rearing of juveniles (larvae, fry) at different developmental stages (issues mentioned in the tables of Appendix 6), species and production quantities of live feeds, as well as juvenile releases (species, quantities and weight, place of location in natural water bodies), number of used incubators, larval rearing tanks and ponds.

5. Hatchery managers and key staff members should be instructed by scientists how to prepare and format these above described records and protocols and how to enter them in standardized electronic spreadsheets to allow a Caspian basin-wide and cost-effective scientific analysis of data between hatchery operations in a comparative and compatible manner as briefly outlined under guideline 15.1. This is scientifically sound as the fish are released into shared waters that should be managed jointly.

16. HATCHERY MAINTANANCE AND REPAIR

Guideline 16.1:
Sturgeon hatcheries should have proper maintenance and repair plans in place and implement these plans timely in order to ensure safety of the hatchery workers, support fish welfare and long-term use of equipment and buildings and economic efficiency of the hatchery in general.

Justification:

In the Caspian Sea Basin region maintenance and repair of sturgeon hatcheries has often been neglected in the past. Insufficient funds and efforts have been dedicated to maintenance and repair.and as a consequence many buildings and equipments cannot be used anymore because of damages, missing spare-parts and safety concerns. In order to ensure wellbeing of the hatchery workers as well as good working conditions and a healthy living environment for the sturgeon broodstock it is important that maintenance and repair plans is incorporated in the overall hatchery planning. In this way the time equipment and buildings can be used will be prolonged, resulting in longer depreciation periods and timely identification of equipment that need to be replaced. A good maintenance and repair plan saves money and highly contributes to timely implementation of the main hatchery activities.

Implementation guidance:
In terms of proper maintenance and repair of a sturgeon hatchery the following issues should be considered:

- During the re-productive season at the hatchery the daily cleaning, maintenance and repair should be conducted following a strict maintenance and repair plan.
- At the end of the hatchery season it is necessary to drain residual water from the water inlet- and outlet systems in all hatchery units and undertake maintenance of the water control and water supply devices. The incubation unit sections of incubation apparatus should be dismantled; in the tank unit the water supply devices and fish protection devices should be dismantled in order to avoid damage and/or destruction of these in the winter season. Following the dismantling and conservation of the equipment, a thorough cleaning and maintenance of production facilities should be carried out.
- Preparation for the hatchery (re-productive) season consists of installation of the necessary equipment, disinfection and washing of the water supply system and the fish tanks, preparation of the necessary production equipment, appliances and devices.
- All major repairs (and reconstruction) of the hatchery should be conducted in accordance with established designs as detailed in the hatchery's work plan.

In case it is decided at some point that the hatchery should be closed it is important that it is attempted to bring the hatchery terrain as much as possible back in the same situation as before hatchery establishment; meaning that the hatchery buildings are not just abandoned, but that they are destroyed and debris removed from the site. Alternatively another use should be found for the buildings and ponds.

Following the polluter pays principle, it is recommended that all waste is removed from the hatchery site before handing over of the site to a new owner or before abandoning it.

17. STAFF AND LABOUR ISSUES

17.1 Hatchery working conditions

Guideline 17.1:
The sturgeon hatchery should provide a safe, healthy and fair working environment for its staff.

Justification:
The success or failure of a sturgeon hatchery depends on its management and staff. Recognizing the limited availability of experts trained in sturgeon hatchery design and

operations, it is essential that selection of staff is done with care and that (once selected) staff is trained properly in all aspects of hatchery work.

It is important that sturgeon hatcheries, as well as the hatchery related activities (e.g. capture of broodstock, release of fingerlings), are carried out in a safe, healthy and fair working environment and that the living conditions of the hatchery staff meet internationally agreed standards adopted by relevant national and international organizations.

Implementation guidance:
The adoption of hatchery practices that reduce health and safety risks and hazards for workers (e.g. the use of protective clothes –when necessary - and equipment) is a priority. This could include education and training programs to build hatchery workers' knowledge and skills.

It is recommended to:

- Regulate working time while maintaining the flexibility required to manage seasonal and market related fluctuations in the demand for (agricultural) labour (ILO Convention 184).
- Ensure that the conditions for hatchery workers are consistent with national and international labour standards and legislation.
- Establish and enforce minimum wages for hatchery workers and equal pay for men and women.
- Apply social protection policies such as pensions and employment and ensure that hatchery workers are covered by insurance against death, injury and disease (ILO Convention 184).
- Use codes of conduct, contracts and collective-bargaining agreements to improve working conditions.

Although maybe not considered a problem at present it is advisable to consult hatchery workers in developing workplace policies to prevent the spread of HIV/AIDS and related discrimination (ILO Code of Practice on HIV/AIDS).

17. Training and education of hatchery staff

Guideline 17.2:
Hatchery staff should be properly trained in the activities they perform and receive updates or advanced training whenever new developments in terms of technology, standards or protocols are introduced.

Justification:
Modern sturgeon hatcheries make use of advanced technologies and equipment that require in-depth knowledge of the workers on how to use and maintain these technologies and equipment. Hatchery workers should master the traditional as well as innovative methods and technologies. Training and education (capacity building) of all specialist workers at the hatcheries, and particularly those involved in reproduction operations, is an essential part of the hatchery functioning. Developments in fish health management, feed management, and sanitary and hygiene requirements demand continuous update of knowledge of hatchery staff.

Implementation guidance:
Recognizing the fact that staff capabilities and knowledge are the key factor to success of the hatchery management and operations, the following aspects should be considered:

- Capacity building of sturgeon hatchery staff, through training, can have various forms and could include amongst others on-the-job training, formal education, short-courses and study visits to other hatcheries.
- Staff involved in operations at any production unit of the hatchery, should be trained regularly to follow best practices on safety and sanitary standards. Training and other guidance provided should be based on industry- and regional standards is generally promoted and supported by competent authorities.
- Appropriate training at all levels (management, biologists, technical personnel) is required for handling of endangered fish species and in particular sturgeons, in order to ensure professional handling of brood stock specimens, appropriate technical management of culture systems, re-production (including e.g. cryopreservation of sperm), adequate care of behavioural needs of the species concerned and care of nutritional requirements as well as best handling practices during the entire maturation and spawning phase.
- Regional and national level training and education centres and dedicated programmes should be used to ensure professional training and education; by collaboration with such institutes and other hatcheries the costs of training per hatchery worker can be significantly decreased.

18. Monitoring and research

Guideline 18.1:

Monitoring and research activities on sturgeon hatchery management and practices for restocking should be made mandatory for basic information and highly encouraged for specific operational and research aspects. The monitoring and research should support sturgeon restocking and general rehabilitation activities, greatly enhance our understanding of the performance capacity of released sturgeons in light of potential increase of hatchery efficiency, survival and growth in nature, and number of returns per recruit, so as to better assist policy decisions with regard to sturgeon rehabilitation programme needs for successful population recovery in the aquatic environment, and to improve sturgeon stock management in the Caspian Sea basin in general.

Justification:

Although hatcheries and research institutions in the Caspian Sea basin have dedicated decades of monitoring and research to sturgeon rehabilitation and management, there are still many gaps and uncertainties in the knowledge available on sturgeon reproduction and restocking. There are large scale uncertainties associated with the details of the biotechnology for fish reproduction and rearing, and with regard to the achieved output not only in terms of volumes (numbers of fish) but also in terms of the fitness of the produced fish to cope with the environmental settings in the habitat of destination.

Any research requires coherent and largely standardized format of information collection through comprehensive monitoring of the local situation, current and past practices and new trends (or modifications made in any of the hatcheries).

The monitoring requirements should be comprehensive, permitting to outline at any time the main targets for future research needs. The factors impacting the performance of released fish under natural conditions are largely unknown. Here, the data from the monitoring programme should help to identify adaptations in the rearing procedures as well as defining criteria for the characterisation of the fitness of a fish designated for release.

Firstly, monitoring steps should include a posterior analysis of the reproductive efficacy of the broodstock, helping to identify the shortcomings of the methods applied and the susceptibility of different (individuals or groups thereof.

Secondly, the performance of juvenile fish must be monitored throughout the rearing process in captivity. Data acquisition and record keeping must be sufficiently detailed to allow posterior analysis of the rearing conditions, their differences between hatcheries and their subsequent outcome. Again, such monitoring is required to avoid the development of rearing conditions that adversely affect parts of the populations and result unintentional and unwanted genetic selection while at the same time to derive gradually at commonly agreed standard procedures which will be adopted by all hatcheries to improve performance.

Thirdly, in order to determine the efficacy of the stocking attempts, a detailed monitoring of the performance of the stocked fish has to be carried out, following strict concepts to be able to verify the hypothesis behind the anticipated performance of the subsequent life stages of the released fish. Sampling should be non detrimental and should apply methods that ensure the survival of the fish upon sampling.

The monitoring and research on sturgeon hatchery issues should comprise a variety of points necessary to allow full evaluation of the efficacy of the measures carried out to identify future improvements of hatchery practices:

- Identify magnitude of natural reproduction
- Differentiate performance of both groups (eventually making it necessary to apply genetic or physical tagging to be able to do so with sufficient precision) for the following objectives:
    - Monitoring of survival of released juveniles at different life stages;
    - Identifying the main causes of mortality (starvation, predation, disease);
    - Monitoring of habitat utilization;
    - Identifying the habitat preferences of the released fish, compare to the fish from wild reproduction;
    - Identify feeding preferences and feeding rates (requires not only studying gut content of fish but also abundance and composition of food organisms in the respective habitat);
    - Assess growth performance;
    - Determine behavioural differences between both groups (naturally produced and releasted fish)with regard to swimming activity, migration patterns, predator avoidance;
    - Determine proportions, ages and composition of breeders originating from stocking attempts;
    - Determine homing rate of released fish;
    - Identify reproductive success of released fish in the wild and in the hatchery upon catch;
    - Determine the vulnerability of released fish to fishing compared to offsprings derived from the natural population (also as part of the objective to reduce such effects through appropriate mitigation measures).
    - Determine the contribution of the released fish to the natural population;
- In case habitat improvement are implemented anywhere within the natural distribution of the released fish (e.g. through waste-water purification, improvements of juvenile feeding grounds through reduced utilization for other purposes, artificial spawning grounds, etc.) the efficacy of such measures must be verified in detail by selecting

reference areas to be compared with regard to the critical parameters mentioned above.

Implementation guidance:

In terms of monitoring and research by sturgeon hatcheries it is important to consider the following general guidance:

- Multidisciplinary, interdisciplinary and transdisciplinary approaches should be applied to problem solving at the hatchery, as traditionally focus has been on biology/ichthyology research and possible improvements in other fields have been ignored.
- Research programmes on sturgeon hatchery issues should work across hatcheries and multi-level governance systems at local, regional, national, and international levels, as well as involving various bodies with sturgeon rehabilitation and management and research responsibilities, such as universities, private sector organizations, national research institutes, and international organizations such as the Commission on Aquatic Bioresources of the Caspian Sea and the Central Asian and Caucasus Regional Fisheries and Aquaculture Commission.
- Adequate resources, including research facilities and trained staff should be provided for sturgeon hatchery research programs. These programmes should be provided financial support from public sources and from a variety of self-sustaining funding mechanisms, such as user-pay initiatives and cost-recovery mechanisms.
- Sturgeon hatchery research must use robust and accurate data collection, monitoring and analysis strategies that incorporate appropriate standardized methods. Introduction of proper record keeping and documentation at hatcheries is therefore an essential starting point.
- Research results should be shared with other hatcheries in the Caspian Sea Basin and used to establish rehabilitation and management objectives, reference points, performance criteria and to formulate and update rehabilitation and management plans.
- Research activities and findings should be communicated to the wider public, including fishers organizations, fisheries managers and researchers in order to create awareness and seek their assistance in the implementation of rehabilitation, restocking and management programmes of sturgeon.

Development of frameworks to identify meaningful sturgeon hatchery research questions is important for successful rehabilitation and management of the sturgeon in the Caspian Sea Basin. Researchers should take final responsibility for development of appropriate research proposals and approaches to answer these questions.

Aspects that have been identified and which require urgent monitoring and research include the following:

Rearing of broodstock

In order to be able to rear fish successfully to maturation and to allow them to contribute to the population through reproduction, the key factors affecting performance are facility design, alimentation and management. Nutrient requirements have to be identified on a species specific base including the inter-annual variations in requirements imposed by climatic conditions. Understanding the effects of hormonal cycles and the triggers for the onset and continuation of vitellogenesis would allow to optimize the rearing conditions to provide secure recruitment into the reproductive guild in time.

Reproduction

Currently, despite the progress made in the last 10 years we are far from understanding the impact and the resulting regulation for current, salinity, temperature, photoperiod and physiological state. If so, hormonal manipulation would not be necessary and therefore, reproduction would be deprived of the selection aspect currently prevailing thorough this impact.

In the mean time the establishment of sound and easy to use predictors for ovulatory success, identify species specific minimal dosage for hormonal induction, identify the effect of the induction on the fitness of the progeny, improve the fertilization technique to match the conditions (and the selective pressure in natural conditions), as well as to determine the genetic differences indicating the functional differences of biological groups might readily improve the outcome of the controlled reproduction.

Early life stages

Again, in early life stages the most critical aspect for the survival and well being of the fish is associated with lack of knowledge with regard to the nutritional requirements of the early life stages. The attempt to provide live or frozen feed is associated with poor performance and selection imposed during the adaptation process of the juveniles to formulated diets. Neither the taste nor the formulation is appropriate to trigger the feeding response quantitatively. Detailed knowledge on this matter could provide also valuable assistance in formulating diets for the broodstock to ensure the presence of essential feed components in sufficient amounts.

One further field of research deals with the behavior of the individuals. In order to ensure maximum fitness upon release, the rearing conditions have to provide environmental clues that enable the fish to be trained for its life in the wild. Not static environmental conditions are the key target, but rather environments that reveal comparable fluctuations in accordance to the river systems utilized. The first question therefore is, which parameters have to fluctuate? Secondly, what are the amplitude and the interval of fluctuations?

Also related to the behavior of the animal but focusing on the sensual prerequisites, it is critical to determine the species specific point when olfactorial imprinting sets in. In salmonids, this stage is determined by hormonal changes, resulting in morphological alterations that are providing the ability to determine and memorize the olfactorial stimulus. This point is not been determined in sturgeons with sufficient reliability up to now. Also the verification of the results in a field study, to determine differences in return rate upon maturation is necessary.

Handling

Sturgeons are reported to be extremely hardy when compared with for instance to higher teleost fishes. Nevertheless, handling as well as crowding imposes stress on the animals which in return reduces the fitness and affects the immune response, increasing the risk for disease outbreaks. Several studies describe the stress response in fish in relation to rearing density (e.g. Würtz et al. 2006), the effect of confinement and handling (Webb at al. 2007), and the effect of feed depriviation (Deng et al. 2009).

Nevertheless, guidelines for optimal rearing densities and rearing conditions with regard to tank design and light climate based upon physiological data are lacking. Also, no systematical study has been carried out to determine the optimal rearing densities in Caspian sturgeons, the amount of feed organisms per volume of rearing tank or means of efficient and stress free handling (for instance during grading).

Genetics
Genetic sampling is mentioned earlier in the Chapter on characterization of breeders. If done properly, each batch of offspring can be characterized through their individual genetic properties. This approach opens opportunities for the differentiated analysis of the performance of the released fish. As a prerequisite, genetic sampling according to a uniform protocol is required to allow posterior analysis of the genetic makeup of the population if the means are not readily available.

Also in mid to long term, selection mechanisms impacting the fish stocked can be identified including the identification of vulnerability of genetic groups, potentially making it necessary to alter the hatchery practices to increase survival of such individuals by altered rearing argents.

Diseases
One main topic for future research is associated with disease interactions. The knowledge about specific or ubiquitous diseases in sturgeons under controlled culture conditions is still very incomplete. Viral diseases for instance have been reported for years, a standard technique for identification is still lacking. Also, therapeutics for sturgeons are largely obtained by adopting products and procedures used in commercial aquaculture for numerous other aquatic species. Specific recommendations for treating sturgeons are absent or rudimentary. In contrast to many commercial farms, designating their production to markets for human consumption, especially brood stock rearing allows and requires adapted, safe, and effective treatments in order to gain the long-term safeguarding effects of the ex situ stocks that are in some cases the only remaining representatives of certain stocks and need to be maintained for many years if not decades.
Determination of horizontal and vertical transmission pathways of viral diseases also across species boundaries are urgently required to allow adapted transfer protocols to be developed also in international trade.

One major research question is associated to disease susceptibility and the current standards of regulating releases of infected fish. It seems necessary to identify rate and specificity of such infections to certain genetic groups. In turn, the question can also be put if disease prevention imposes selection mechanisms on a population that is driven by culture environment rather than fitness for survival.

The above list, being far from comprehensive or complete, is intended to identify urgent research topics that will require ample attention, to improve the rearing practices not only in the Caspian Sea range but worldwide.

19. Social and environmental responsibility

Guideline 19.1
Sturgeon hatchery management and practices should be performed in a socially responsible and ecologically sustainable manner.

Justification:
Society demands that aquaculture practices are performed in a socially responsible and ecologically sustainable manner. The same principles should be applied for sturgeon hatchery development and its operations while being designed for producing progeny for

release.. The hatcheries should benefit not only the hatchery business itself, but also the local communities and the country (or region), and are required to support aquatic biodiversity goals (through release) without compromising the environment and to contribute effectively to rural development and employment generation.

Every sturgeon hatchery should aim at producing fry and fingerlings of high quality for release. Sturgeon hatcheries should also be managed and operated to be respected and valued as an active and positive contribution to societal values.

Large international reputed companies are often obliged to produce corporate social and environmental responsibility reports. In many cases these are undertaken voluntarily as a public relations measure. In these reports companies record their actions to engage with society and address concerns expressed and issues raised. In these reports also the environmental performance in terms of the resources used and the environmental impact of the activities are discussed. While for sturgeon hatcheries this may be a bridge to far at present, it is recommended that step-wise action towards reporting on these fields are undertaken and regulatory agencies should make such reporting mandatory when granting the permit. Moreover, sturgeon hatcheries that play an important role in the release of an endangered species into their natural aquatic environment, may be an attractive partner for national companies and multinationals demonstrating social and environmental responsibility.

Implementation guidance:
With respect to socially responsible and ecologically sustainable sturgeon hatchery practices it is recommended that the hatchery should aim at least to:

- Minimize conflicts with local communities that may result from use of shared resources (e.g. water), hatchery development and operations and ensure that hatchery activities are mutually beneficial.
- Take measures to ensure that the sturgeon hatchery benefits the local communities it is active in, by for instance purchasing local products where this is possible and incorporate local people among its staff.
- Avoid social dumping is in this context particularly important. Specifically in areas where unemployment is low and alternative employment is scarce the risk of social dumping is relatively high. It is well known in many parts of the developing world that such behaviour by companies is a reality. It should therefore be made imperative that transparency of employment policies is guaranteed in particular in hatcheries which have received investment support from public sources or international or inter-governmental donors.
- Wherever new hatcheries for sturgeons are constructed, the respective licensing authorities should demand that minimum worker welfare and fair working conditions are identified and are linked with the hatchery licence while also being enforced through regular control measures.
- Promote application of good practices (BAPs), standards (BATs), guidelines and codes of conduct on hatchery aspects as well as practices for sturgeon release to natural waters through appropriate advice and training of its workers. Awareness should also be raised among others that the hatchery always applies these practices, measures and tools on the basis of the latest advanced state of know-how.

20. INTERNATIONAL REGULATIONS AND CONVENTIONS ON STURGEONS

Guideline 20.1
Sturgeon hatcheries should be aware of and implement international regulations and conventions on sturgeon rehabilitation and trade that are relevant to their activities.

Justification:
A number of international regulations and conventions, as well as voluntary instruments, are in place that guide the rehabilitation, management and trade of sturgeon species.

Although few of these relate directly to sturgeon hatchery operations, they do affect, among others, restocking strategies and accompanying measures such as the availability of sturgeon broodstock, monitoring restocking efforts, studying factors triggering habitat choice (e.g. eco-hydraulics) and national and transboundary movement of live sturgeon.

Relevant global instruments include the following:

- Convention on International Trade in Endangered Species (CITES, Washington Convention, 1973). The CITES convention governs trade in endangered species, including sturgeon species, with the aim to conserve the endangered species. Sturgeon species were listed either in Appendix I (1975) or Appendix II (1997) of CITES, which meant that all international trade in sturgeon products has come under CITES control as of April 1998.
- Convention on Biological Diversity (CBD, 1992). The CBD aims to conserve biological diversity in general, as well as its use in fostering ecosystems sustainably and regulating access to genetic resources.
- Convention on Migratory species (CMS, Bonn Convention, 1979). This conference promotes international cooperation in support of conservation measures for species that include straddling stocks, crossing national borders.
- United Nations Convention on the Law of the Sea (UNCLOS, 1982). Important features of the UNCLOS relate to the following issues that are covered in the convention: Navigational rights, territorial sea limits, economic jurisdiction, legal status of resources on the seabed beyond the limits of national jurisdiction, passage of ships through narrow straits, conservation and management of living marine resources, protection of the marine environment, a marine research regime and, a more unique feature, a binding procedure for settlement of disputes between States. As such it is generally supportive of conservation culture but does not provide specifics in regard to hatchery management.
- Code of Conduct for Responsible Fisheries (FAO CCRF, 1995)[1]. The FAO CCRF is a voluntary code that sets out principles and international standards of behaviour for responsible practices with a view to ensuring the effective conservation, management and development of living aquatic resources, with due respect for the ecosystem and biodiversity. The Articles of the Code cover all major issues and practices in fisheries, including fisheries management, fishing operations, aquaculture development, integration of fisheries into coastal area management, post-harvest practices, trade, and fisheries research; general principles; and provisions related to its implementation, monitoring, updating and the special requirements of developing countries. Again, this Code is of principle assistance but – by design – does not contain specific recommendation as to the operation of sturgeon hatcheries.

- Ramsar Declaration on Global Sturgeon Conservation is another voluntary instrument in the "Strategy of sturgeon conservation" as presented in the recommendations of the "Ramsar Declaration on Global Sturgeon Conservation" (2005). The declaration was an outcome of the 5th International Symposium on Sturgeons, May 9 to 13, 2005,

[1] The full-text of the Code of Conduct for Responsible Fisheries and technical guidelines under it is available at: http://www.fao.org/fishery/ccrf/en

Ramsar, Iran, and subscribed to by the many of the key sturgeon experts in the world.[2] The declaration has also been published in the Proceedings of the 5th ISS (Rosenthal et al., 2006) and contains highly pertinent recommendations with major importance to sturgeon broodstock and hatchery management.

Relevant regional agreements and instruments in the Caspian Sea Basin include:

- The Tehran Convention (Tehran, 2003). This Convention on the Protection of the Marine Environment of the Caspian Sea was agreed in Tehran on 4 November 2003 and came into force on 12 August 2006 after ratification by all five Caspian littoral States. While this convention does not specifically mention sturgeon, it's implementation includes activities on sturgeon stocks conservation and rehabilitation.[3]

- The Agreement on the Central Asian and Caucasus Regional Fisheries and Aquaculture Commission (Rome, 2010). This Agreement was approved by the FAO Council at its Hundred and Thirty Seventh Session on 1 October 2009 through Resolution No 1/137 under Article XIV, paragraph 2 of the FAO Constitution. The Agreement on the Central Asian and Caucasus Regional Fisheries and Aquaculture Commission came into force on 3 December 2010. The Commission aims to promote the development, conservation, rational management and best utilization of living aquatic resources, as well as the sustainable development of aquaculture in the wider Central Asia and the Caucasus region.

- Commission on Aquatic Bioresources (CAB). The CAB is a so-called interagency body. The chairmanship of CAB rotates every two years among the five countries Caspian Sea riparian countries. During the two-year period, the chairing country also acts as the CAB Secretariat. CAB aims to coordinate among range states on conservation and exploitation of Caspian aquatic bioresources; promote scientific collaboration and data exchange, including conducting joint research (stock assessment); Regulate fishing based on scientific data; and determine Total allowable Catch and export quotas of shared stocks.

Implementation guidance:

Considering the importance of the international and regional regulations, voluntary instruments, agreements and conventions on sturgeon rehabilitation and trade, and the fact that the authorities of the countries in the Caspian Sea basin have ratified and agreed to implement most of these, it is obvious that sturgeon hatcheries will be bound as well by the provisions provided. The implementation of most of the conventions, agreements and other instruments is supported by the secretariats and organizations that host these conventions and agreements. Sturgeon hatcheries can, through the respective authorities for sturgeon conservation and management at national level, seek specific guidance and support on the implementation of the relevant conventions and agreements.

[2] http://www.wscs.info/publications/iss/RamsarDeclarationEnglish.pdf

[3] http://www.tehranconvention.org/spip.php?article4

21. Implementation and updating

- All agencies, institutions and experts involved in sturgeon production and reproduction should collaborate in the promotion and implementation of the objectives, principles and guidelines contained in these Technical Guidelines.

- Relevant international and national agencies, including non-governmental organizations, should promote these Technical Guidelines among those involved in Sturgeon production, reproduction and restocking.

- The FAO, WSCS and IUCN SSG, as appropriate, will promote the endorsement of and support the implementation of these Technical Guidelines via regional Commissions, meetings and conferences. Together they will also (try to) monitor the application and implementation of these Technical Guidelines and the effects on sturgeon hatchery management and practices in the Caspian Sea basin and elsewhere.

- The FAO, WSCS and IUCN SSG, as appropriate may revise these Technical Guidelines, taking into account the developments in sturgeon hatchery management, in consultation with the relevant stakeholders.

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Glossary

(unless otherwise stated the below terms are derived from the FAO Glossary of Aquaculture, FAO, Rome, 2008)

Best practice: planning, organization, managerial and/or operational practices that have proven successful in particular circumstances in one or more regions in the field and which can have both specific and universal applicability (EIFAC Code of Practice, FAO, 2008) .

Broodstock: Specimen or species, either as eggs, juveniles, or adults, from which a first or subsequent generation may be produced in captivity, whether for growing as aquaculture or for release to the wild for stock enhancement.

Cryopreservation: The freezing and storage of gametes (usually sperm) so they can be used at a later date.

Domestication: In a broader sense: process by which plants, animals or microbes selected from the wild adapt to a special habitat created for them by humans, bringing a wild species under human management. In a genetic context: process in which changes in gene frequencies and performance arise from a new set of selection pressures exerted on a population.

Fingerling: Related to any fish from advanced fry to the age of one year from date of hatching regardless of size, usually applied to trout of about 10-70 g in weight, or 8-15 cm fork length. The term is, however, not rigidly defined.

Gamete: Mature sex cell (egg or sperm), haploid, that unites with another gamete of the opposite sex to form a diploid zygote; such a union is essential for true sexual reproduction.

Hatchery: Place for artificial breeding, hatching and rearing through the early life stages of animals, finfish and shellfish in particular. Generally, in pisciculture, hatchery and nursery are closely associated. ~~On the contrary, in conchyliculture, specific nurseries are common, where larvae produced in hatcheries are grown until ready for stocking in fattening areas.~~

Homing: The regular return of migrating fish species to their native spawning grounds; or the return of fish to their last juvenile home waters after being transferred from their native river to another and having become well acclimatized to the latter.

Inbreeding: Mating or crossing of individuals more closely related than average pairs in the population

Incubation (period): The time during which eggs (embryos) develop, for example, in a hatchery. Usually the period between fertilization and hatching of the last embryo of a given egg population

Juvenile: Young stage of animals, usually up to the time they first become sexually mature. For fish usually between the postlarval stages up to the time they first become sexually mature. They are generally hardy at this stage.

Larvae: An organism from the beginning of exogenous feeding to metamorphosis into juvenile. At the larval stage the animal differs greatly in appearance and behaviour from a juvenile or an adult.

Nursery: In aquaculture: culture facility where a farming system intermediate between the hatchery and grow-out stages is applied. In conchyliculture: transitional culture facility where post-larvae 1-2 mm long produced in a hatchery are grown out until reaching a size (about 20 mm) suitable for their transfer to marine rearing facilities, using technologies, which are simpler and cheaper than those used in hatcheries.

Progeny: The offspring of a particular pair of fish.

Appendices

Appendix 1

Table 1.Effective population number for a given year class (breeding event) based upon the actual number of males and females used in breeding. Adapted from St. Pierre 1996.

Number of Male Parent	Number of Female Parents 1	2	3	4	5	6	7	8	9	10	11	12
1	2.0	2.7	3.0	3.2	3.3	3.4	3.5	3.6	3.6	3.6	3.7	3.7
2	2.7	4.0	4.8	5.3	5.7	6.0	6.2	6.4	6.5	6.7	6.8	6.9
3	3.0	4.8	6.0	6.9	7.5	8.0	8.4	8.7	9.0	9.2	9.4	9.5
4	3.2	5.3	6.9	8.0	8.9	9.6	10.2	10.7	11.1	11.4	11.7	12.0
5	3.3	5.7	7.5	8.9	10.0	10.9	11.7	12.3	12.9	13.3	13.8	14.1
6	3.4	6.0	8.0	9.6	10.9	12.0	12.9	13.7	14.4	15.0	15..5	16.0
7	3.5	6.2	8.4	10.2	11.7	12.9	14.0	14.9	15.7	16.5	17.1	17.7
8	3.6	6.4	8.7	10.7	12.3	13.7	14.9	16.0	16.9	17.8	18.5	19.1
9	3.6	6.5	9.0	11.1	12.9	14.4	15.7	16.9	18.0	19.0	19.8	20.5
10	3.6	6.7	9.2	11.4	13.3	15.0	16.5	17.8	19.0	20.0	21.0	21.8
11	3.7	6.8	9.4	11.7	13.8	15.5	17.1	18.5	19.8	20.6	22.0	23.0
12	3.7	6.9	9.6	12.0	14.1	16.0	17.7	19.1	20.6	21.8`	23.0	24.0

Appendix 2.

Table 1.Recommended water quality parameters for a sturgeon hatchery (modified after Conte et.al., 1988)

Parameter	**Value**
Alkalinity, mg/L as $CaCO_3$	50-400
Ammonia (un-ionized), mg/L	< 0,01
BOD_5, O_2 mg/L	< 2,5
Cadmium (soft water 100 ppm alkalinity), mg/L (hard water 100 ppm alkalinity), mg/L	0,004 0,003
Carbon dioxide, mg/L	0-10,0
Copper, mg/L in soft water	0,006
Dissolved oxygen, mg/L	5,0 to saturation
Gas saturation	< 105%
Hydrogen sulfide, mg/L	0,002
Iron, mg/L	<0,01
Lead, mg/L	0,03
Nitrite, mg/L as N in soft water in hard water	0,1 0,2
Oxidability permanganate, O_2 mg/L	$\leq$ 10
Ozone, mg/L	0,005
pH	6,5-8,5
Salinity, ppt for fry for juveniles for adults	0-0,5 0-3 3
Total hardness, mg/L as $CaCO_3$	10-400
Total suspended and settleable solids, mg/L	80 or less
Zinc, mg/L	0,03

Appendix 3.

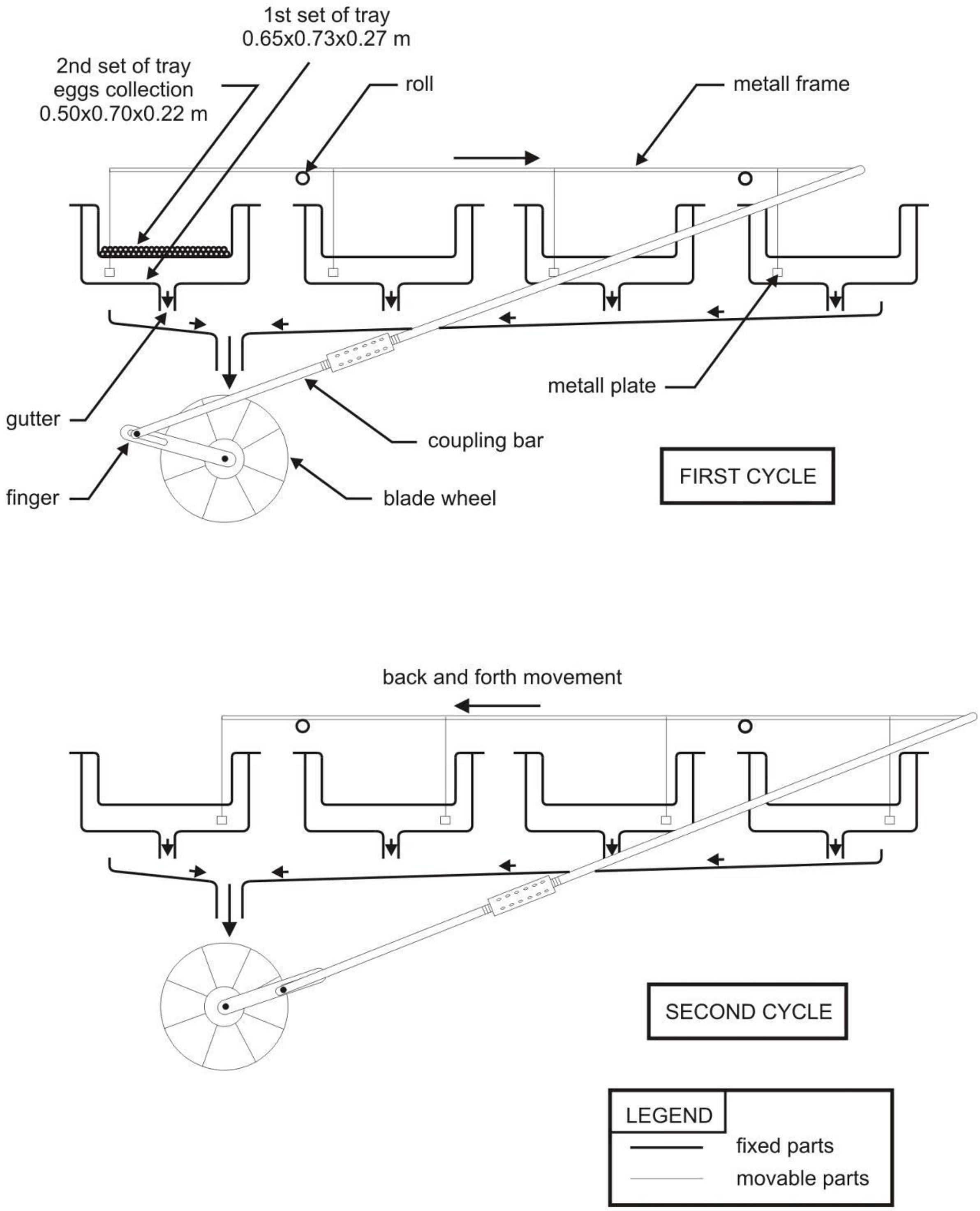

Fig.1 Principle layout of Yushenko incubation system.

Table 1.Recommended egg load for incubation systems (different sturgeon species), ths. ind.

Species	**Incubation system**					
	Yuschenko (for 1 section)	**“Osetr” (for incubation box)**	**Mac Donalds**			**Weiss (8 L)**
			5 L	6.5 L	10 L	
Russian sturgeon, ship	100-120	150	8	10	20	10
Stellate sturgeon	120-150	180-200	13	20	40	15
Beluga	120-150	120	8	10	20	12
Sterlet	200	200-250	20	25	50	18

*the blade speed is 3-4 times per minute at mentioned egg loads

Standard eggs load for “Osetr” incubation system is 2 kg per one box. It is equal approximately to 100000 eggs but not 150000 as in table (50 eggs in 1 g). For Beluga this parameter is equal to 70-80 thousand eggs (35-40 eggs in 1 g). For stellate sturgeon the values in table are correct.

Appendix 4.

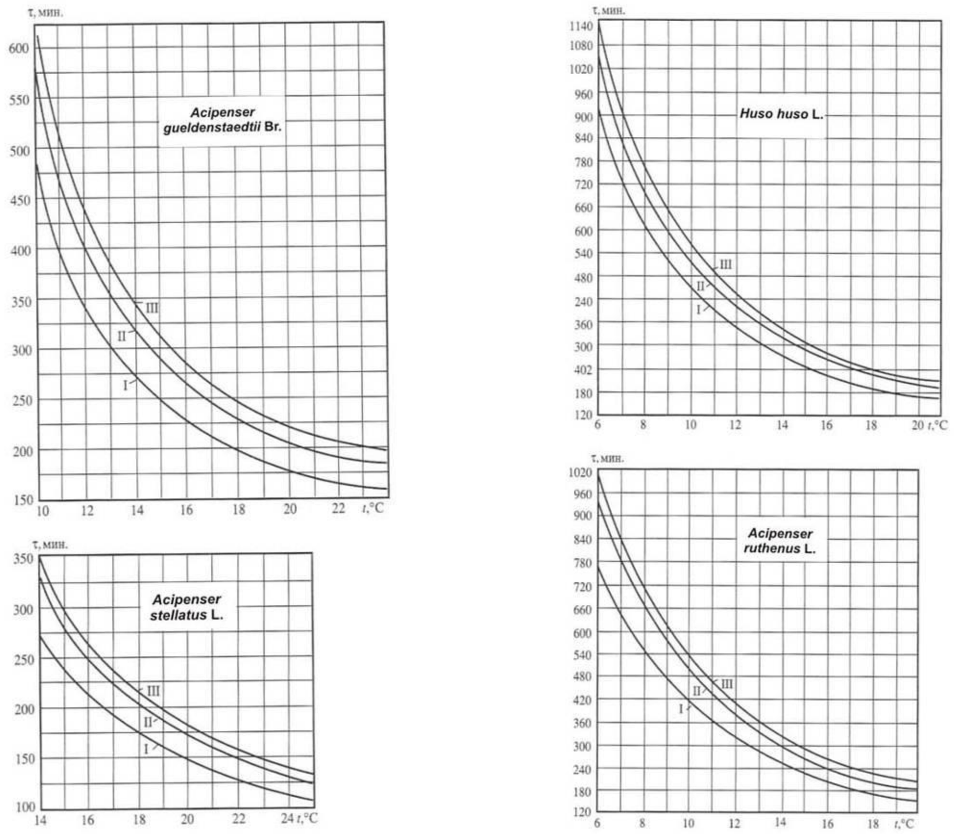

Fig 1. Diagrams enabling evaluation of time of the second and third blastomere cleavage division and time of the second division completion on the surface of the egg in Russian, giant and sterlet sturgeons (Detlaff et al., 1993)

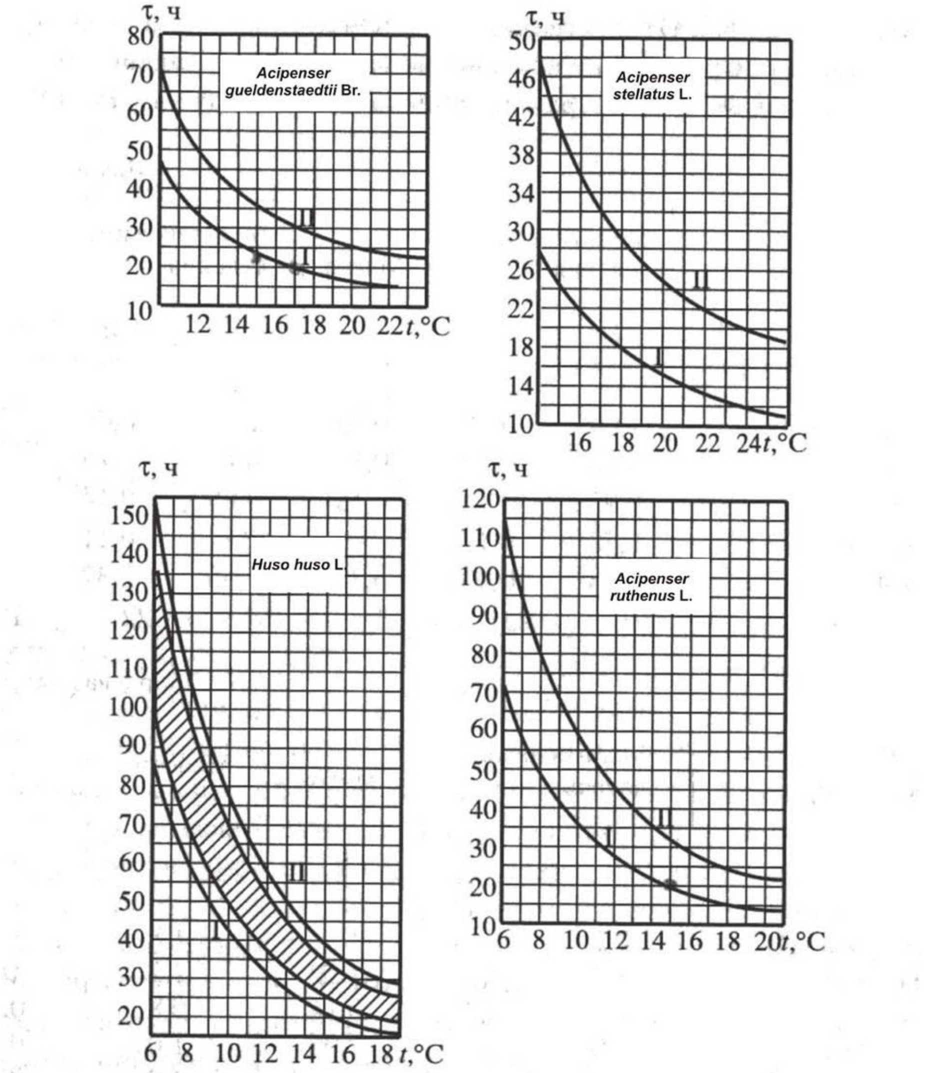

Fig 2. Duration of Russian, stellate and sterlet sturgeons females maturation after hypophysial injections at different temperatures. Curves I show the time of first females maturation, curves II – time after which the females do not mature or produce eggs of poor quality (Dettlaff et al., Igumnova, 1975, 1985; Dettlaff et al., 1993).

Appendix 5. Sample passport of a breeder

Species, seasonal race (intra population group) strain. Individual tag, personal DNA-fingerprint. Year of capture or year of birth (obtaining)

No	Date	Size-weight characteristics			No of generation or maturation order number ♀	Eggs quantity				Used males No tags	Egg fertility percent	Yield of larvae	Survival of 3-5 g fingerlings	Comments, the progeny location
		Length, cm		Weigh, kg		Eggs weight, kg	percent of total body weight	in 1 g	Thous. ind.			percent	percent	
		L	l_1	W										
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

L, cm – total body length

l_1, cm – distance between the end of the snout to the end of caudal fin mid rays

Appendix 6.

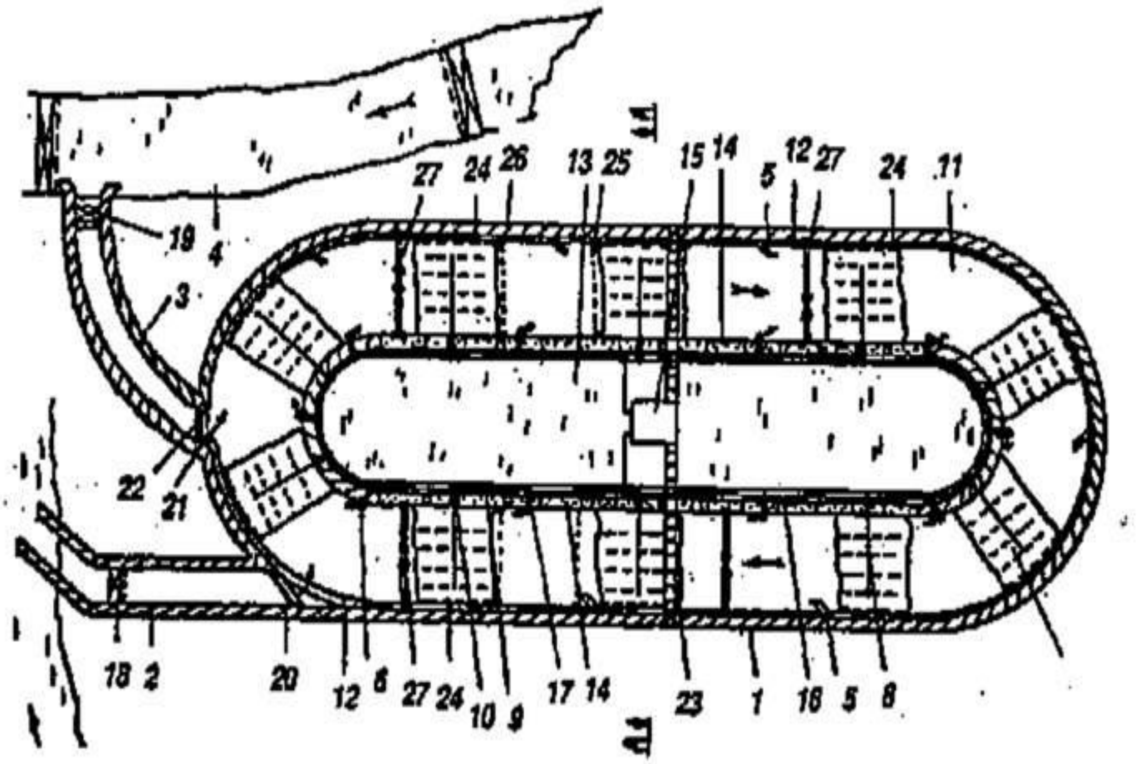

Fig. 1. Artificial spawning complex for natural propagation of sturgeon with controlled regime and ensuring conditions of "pseudomigration" of breeders

1 - Circle spawning channel; 2 - Channel for letting broodstock to pass and runoff; 3 - Channel for prelarvae runoff, 4 - Water body for rearing of larvae, 5 - Canopies for regulating current velocity; 6 - Injectors, 7 - Spawning grounds; 8 - Rinsing flute; 9,16 - Circle water pipelines; 10,17 - Turn off valves; 11 - Pool; 12 - Larvae collection tray; 13 -Internal water body; 14 - Drainage filters; 15 - Pump station; 18,19 - Sluice gates regulators; 20,21 - Protective meshes and turn off dampers; 22 - Removable large-sized fish protective net; 23 - Crossing gangways; 24 - Gauze screens; 25 - Grooves for removable gauze blocking gratings; 26 - Blocking gratings; 27 - Mobile surface rinsing flutes.