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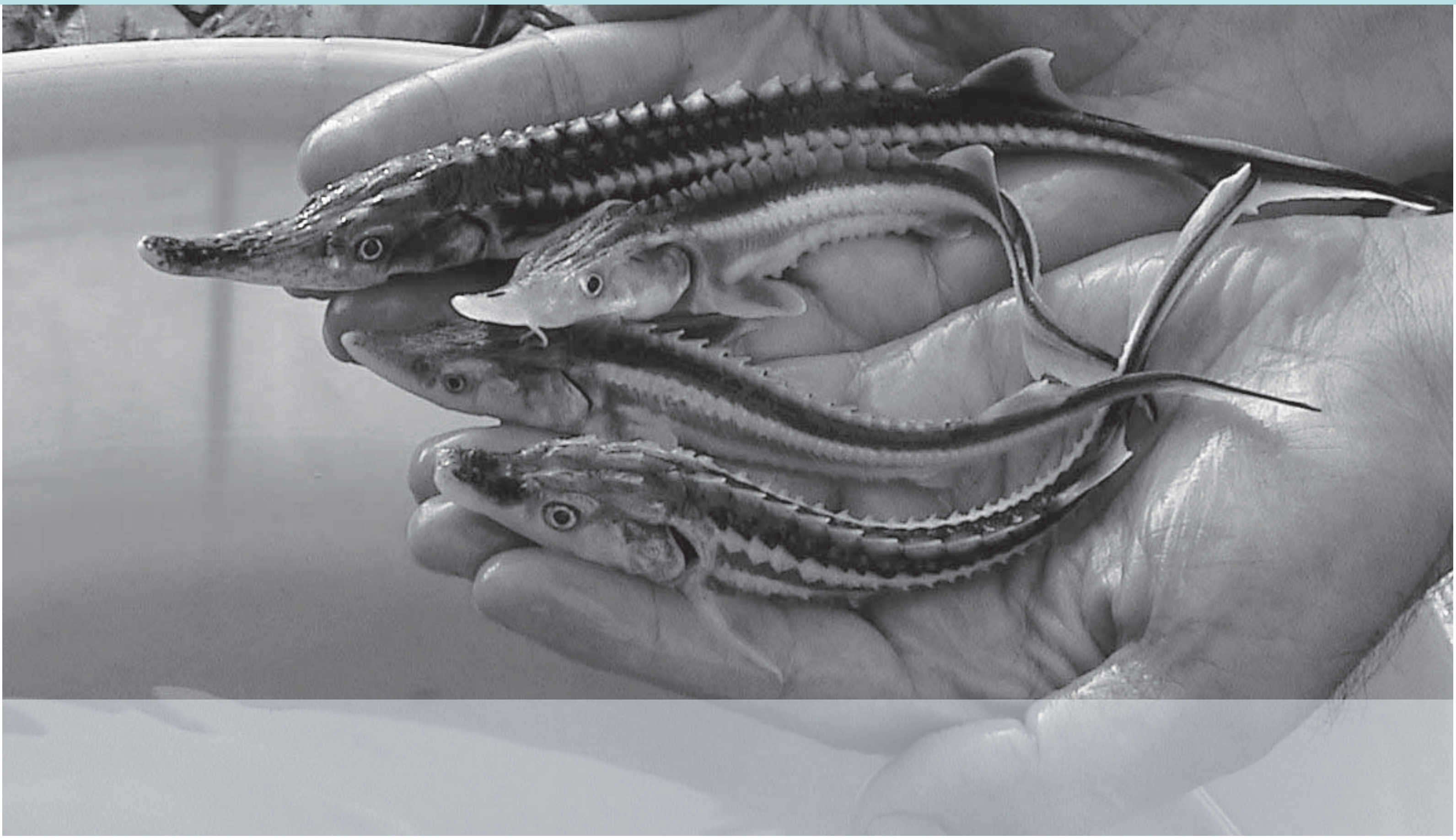
Guidelines



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Sturgeon fingerlings, International Sturgeon Research Institute, Rasht-Iran, 2011.
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Mikhail Chebanov
South Branch Federal Center of Selection and Genetics for Aquaculture
Krasnodar, Russian Federation

Harald Rosenthal
World Sturgeon Conservation Society
Neu Wulmstorf, Germany

Joern Gessner
Leibniz Institute of Freshwater Ecology and Inland Fisheries
Berlin, Germany

Raymon van Anrooy
FAO Subregional Office for Central Asia
Ankara, Turkey

Phaedra Doukakis
Sturgeon Consultant
Washington, United States of America

Mohammad Pourkazemi
International Sturgeon Research Institute
Rasht, Islamic Republic of Iran

and

Patrick Williot
Sturgeon Consultant on Aquaculture and Conservation Biology
Audenge, France

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South Branch Federal Centre of Selection and Genetics for Aquaculture

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Preparation of this document

This technical paper has been prepared by Mikhail Chebanov (South Branch Federal Center of Selection and Genetics for Aquaculture, Russian Federation) and Harald Rosenthal (World Sturgeon Conservation Society [WSCS], Germany), with the collaboration of Phaedra Doukakis (United States of America), Joern Gessner (Germany), Mohammad Pourkazemi (Islamic Republic of Iran), Raymon van Anrooy (Food and Agriculture Organization of the United Nations - FAO) and Patrick Williot (France) on the basis of comments, inputs and suggestions received on the various drafts of 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 were invited to these workshops.

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The final draft of the technical guidelines was presented to the Fourth Intergovernmental Meeting on the Establishment of the Central Asian and Caucasus Regional Fisheries and Aquaculture Commission, which was held in Cholpon Ata, on the shores of Lake Issyk Kul, Kyrgyzstan, 22–24 June 2011. Delegations from eight countries (Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Mongolia, Tajikistan, Turkey and Ukraine) and an observer from the Caspian Environment Programme (CaspEco) attended this meeting. Echoing the call from WSCS for the implementation of these technical guidelines, the Meeting adopted the technical guidelines and recommended the FAO Secretariat to submit document to the Inaugural Meeting of the Commission for official endorsement by the Central Asian and Caucasus Regional Fisheries and Aquaculture Commission and in support of region-wide implementation.

In finalizing these technical guidelines, due consideration was given to comments and suggestions received from experts in the two workshops mentioned above, as well as other international meetings, symposia and conferences, and personally to Mikhail Chebanov. The main financial contribution to these technical guidelines was received from the FAO Technical Cooperation Programme (TCP) project "Capacity building for the recovery and management of the sturgeon fisheries of the Caspian Sea (TCP/INT/3101)". In addition to this, financial and technical support to the workshops and the participation of experts in these workshops was received from the following institutions, organizations and projects: The Global Environment Facility (GEF)/United Nations Development Programme (UNDP) project Integrated Conservation of Priority Globally Significant Migratory Bird Wetlands Habitat: a Demonstration on Three Sites (UNDP Kazakhstan); the UNDP GEF project "The Caspian Sea: Restoring Depleted Fisheries and Consolidation of a Permanent Regional Environmental Governance Framework" (CaspEco); WSCS; the World Bank Trust Fund for Environmentally and Socially Sustainable Development; 6th International Symposium on Sturgeon; International Union for Conservation of Nature Sturgeon Specialist Group; and the Institute of Hydroecology (China). The finalization and publication of this technical paper was made possible with financial support from the FAO Subregional Office for Central Asia.

Abstract

Sturgeon hatcheries play an important role in the rehabilitation of the sturgeon stocks in the Caspian Sea and elsewhere. Since the demise of the Union of Soviet Socialist Republics in 1991, a large part of the experience, expertise and specific knowledge on sturgeon hatchery practices and management in the Caspian Sea Basin has disappeared, while research and knowledge on sturgeon and hatchery practices in other regions have advanced rapidly in the last few decades.

Sturgeon restocking practices that resulted in limited success have caused a critical reassessment of the entire culture process, the strategies applied for conservation culture, the design of rehabilitation programmes, the design of hatcheries and the mode of their operation. This technical paper aims to increase global awareness and to guide and build capacity about the best practices currently available by providing senior and mid-level sturgeon hatchery staff with a practical tool for modern sturgeon hatchery practices and management. The technical technical guidelines it contains focus on hatchery practices that are aimed at reproduction and growth of fry and fingerlings for restocking objectives.

The technical guidelines address a wide range of issues, including: hatchery design and location; collection and transportation of wild broodstock; selection and maintenance of broodstock; tagging of sturgeon; water quality and supply; feeding and feed quality; selection of broodstock for controlled reproduction; spawning and gamete processing; rearing of larvae and juveniles in tanks; rearing of juveniles in ponds; release of fingerlings; sanitary and hygiene measures; hatchery documentation; hatchery maintenance and repair; staff and labour issues; monitoring and research; social and environmental responsibility; international regulations and conventions on sturgeons; and implementation and updating of these technical guidelines.

This technical paper provides specific technical guidelines, justifications for these technical guidelines and suggestions to support their implementation. The guidance provided is based on the FAO Code of Conduct for Responsible Fisheries (1995) and contributes to the implementation of the Ramsar Declaration on Global Sturgeon Conservation (2006). As such, the technical guidelines in this paper are part of the capacity-building and awareness-raising efforts of the partners involved in their preparation in order to increase the success of sturgeon hatchery practices for release purposes. These partners include: FAO; World Sturgeon Conservation Society; International Union for Conservation of Nature; Caspian Environment Programme; United Nations Development Programme; 6th International Symposium on Sturgeon; World Bank; Institute of Hydroecology; and Central Asia and Caucasus Regional Fisheries and Aquaculture Commission.

Chebanov, M.; Rosenthal, H.; Gessner, J.; Van Anrooy, R.; Doukakis, P.; Pourkazemi, M.; Williot, P.

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Contents

Abbreviations and acronyms

BOD	biochemical oxygen demand
CAB	Commission on Aquatic Bioresources
CAC	Codex Alimentarius Commission
CACFish	Central Asian and Caucasus Regional Fisheries and Aquaculture Commission
CASA	computer-assisted sperm analysis
CaspEco	Caspian Environment Programme (The Caspian Sea: Restoring Depleted Fisheries and Consolidation of a Permanent Regional Environmental Governance Framework)
CBD	Convention on Biological Diversity
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMS	Convention on Migratory Species
Code	Code of Conduct for Responsible Fisheries (FAO)
CPUE	catch per unit of effort
DNA	deoxyribonucleic acid
EIFAC	European Inland Fisheries Advisory Commission
EU	European Union
GnRHa	gonadotropin-releasing hormones analogues
HIV/AIDS	human immunodeficiency virus/acquired immune deficiency syndrome
HUFA	highly unsaturated fatty acid
ICES	International Council for the Exploration of the Sea
IHE	Institute of Hydroecology (China)
ILO	International Labour Organization
ISS6	6th International Symposium on Sturgeon
IUCN	International Union for Conservation of Nature
IUCN/SSG	IUCN Sturgeon Specialist Group
IUU	illegal, unreported and unregulated (fishing)
LLTHFB	long-term low-temperature holding facilities for brood fish
mGnRH	mammalian gonadotropin-releasing hormone
OIE	World Organisation for Animal Health
P	number of pectoral fin rays
PI	(oocyte) polarization index
PIT	passive integrated transponder
PPT	parts per thousand
PUFA	polyunsaturated fatty acid
S(Lat)	number of lateral scutes
SV	number of ventral scutes
TCP	Technical Cooperation Programme (FAO)
TROs	total radical oxidants
UNDP	United Nations Development Programme
UNCLOS	United Nations Convention on the Law of the Sea
UV	Ultraviolet (light)
V	number of ventral fin rays
violet “K”	$C_{24}H_{28}N_3Cl$
WSCS	World Sturgeon Conservation Society

1. Introduction

1.1 THE NEED FOR GUIDELINES

Governments of the Caspian Sea littoral States have indicated on 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 subsector is slowly being rehabilitated after the collapse of the Union of Soviet Socialist Republics in 1991, a large part of the experience, expertise, and specific knowledge on sturgeon hatchery practices and management in the Caspian Sea Basin has disappeared. Numerous experts left the sector in the 1990s and, owing to reduced governmental funding of (State) hatcheries production declined, and technologies that were introduced elsewhere were not adopted 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 reassessed 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); and
- release for reintroduction (restocking; rebuilding of stocks either already extinct or on 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 reassess the existing hatchery methodologies in terms of their appropriateness for future sturgeon rehabilitation programmes. The pertinent issues related to this reassessment will be addressed in this document. The main emphasis of the present guidelines has been placed on the last issue (reintroduction; restocking).

As a consequence of these new considerations, the recent impetus given by the Caspian Sea countries to the sturgeon hatchery sector, in terms of funding and other support, cannot be absorbed well by the sector, in particular when addressing specifically the above-mentioned scenarios. Many managers and technicians have not been properly trained in the wide range of aspects of sturgeon reproduction, breeding, restocking and reestablishment 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 expected outputs or only achieved a small part of what was aimed at and this may have been due to 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 emphasize clearly the fundamental differences in hatchery operations for commercial aquaculture and for culture for release into natural waters.

However, in the past century, sturgeon stock rehabilitation programmes had simply adopted common commercial aquaculture technology and ignored the need for challenging the culture methods as to whether they were optimal to produce progeny that had 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 FAO to support training in sturgeon hatchery operations. In response, FAO teamed up with the World Bank and the United Nations Development Programme (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 the current guidelines in close collaboration with the World Sturgeon Conservation Society (WSCS), International Union for Conservation of Nature-Sturgeon Specialist Group (IUCN/SSG), Caspian Environment Programme (CaspEco), Institute of Hydroecology (IHE) and the 6th International Symposium on Sturgeon (ISS6).

1.2 AIM AND SCOPE OF THE GUIDELINES

The guidelines presented in this technical paper 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 guidelines do not aspire to be, and cannot function, as a sturgeon hatchery manual.

These guidelines aim to increase global awareness, and to guide and build capacity about the best practices currently available in sturgeon hatchery management by providing senior and mid-level sturgeon hatchery staff with a practical tool for modern sturgeon hatchery practices and management. The 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 (restocking), it is necessary to address specifically the major goals of both culture objectives (the fitness for performance in the two different target environments).

The objectives for aquaculture production focus on: fitness of the cultured specimens of any species in the aquaculture system and meeting consumer preferences. Therefore, the entire operation aims at:

- ensuring maximum survival (producing high numbers);
- enabling fast growth (producing a large biomass in the shortest time possible);
- producing disease-free specimens and/or specimens resistant to disease (avoid any losses);
- achieving high feed conversion efficiency (reduce production costs; no need to train to hunt for food);
- producing good meat composition/quality (provide healthy food and meet consumer expectations);
- managing all-year-round spawning (allow continuous culture, totally independent from natural life cycle);

- producing fish that are hardy for handling (allowing easy management for sorting and live transportation); and
- achieving good processing characteristics (allow easy processing for added-value products to suit the markets).

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

As briefly addressed in Section 1.1, cultivation for release may also serve different purposes that may not necessarily have a strong conservation element. This also has to be 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 a fishing community or nationally). 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. Once the ranching activity is terminated, the fishery is also terminated because the objective does not target for the establishment of self-sustaining populations (although this may happen).

Cultivation for stock enhancement (either for r- or K-selection species). Again, the main objective is to support the fishery by enlarging the otherwise self-sustained but potentially overfished population to a size beyond that which 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 a spawning ground or as a nursery area. Here, a strong conservation element has to be incorporated in the operational strategy because the released fish must have the identical fitness (genetic, 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 end-point 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 once self-sustaining populations have been established in the historical range.

The objectives of cultivation methods for re-establishment (or reintroduction, restocking) aim mainly at imitating – as closely 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 vice 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. It is important to 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. This is not the case.

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 guidelines is provided by the "Code of Conduct for Responsible Fisheries", known as the Code (FAO, 1995).

Some articles of the Code are directly associated with the objectives of these guidelines. Some examples are given below.

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

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. The production of eggs, larvae and fry will depend upon sound hatchery and grow-out management after the 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 broodstock 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 traced.

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 (or causes) of the problem (Welcomme and Barg, 1997).

> Article 9.3.5: "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."

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 populations, in a properly planned manner, in order to minimize the loss of genetic diversity (hatchery broodstock composition must be based on the diversity exhibited by wild stocks);
- by avoiding direct or induced inbreeding – that is, avoid breeding closely 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 broodstock of the native population if possible, in order to avoid outbreeding depression;
- by avoiding intra- and inter-specific hybridization; and
- 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 broodstock will be built;
- to perform an initial analysis on the natural populations of native species in order to identify the conservation units to be separately managed;
- to identify genetic 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; and
- 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 not always having 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 at imminent risk 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 under way. 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 Welcomme and Barg, 1997), these guidelines will support States to implement Article 9.3.1 of the Code: "States should conserve genetic diversity and maintain integrity of aquatic communities and ecosystems by appropriate management."

The guidelines in this technical paper 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 along the same lines.

Although it is believed that this technical paper will assist in improving common operational practices in hatchery management, it also has to be emphasized that employing best available technology and bioproduction strategies alone are not sufficient to enhance the chances of success in re-establishing or enhancing populations of highly endangered sturgeon stocks unless a much broader approach is taken by all involved. As has recently been pointed out by Lorenzen, Leber and Blankenship (2010), a responsible approach to re-establishing self-sustaining populations must include a broader integrated strategy within fisheries management systems across national borders, with emphasis on a "stakeholder participatory and scientifically informed, accountable planning process". Similarly, Bell *et al*. (2006) had already emphasized that "the preoccupation with bio-technical research at the expense of objective analysis of the need for the intervention, and failure to integrate the technology within an appropriate management scheme" 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 al*., 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 Sea Basin.

1.4 GENERAL PRINCIPLES

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

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

- implementation of genetic monitoring programmes, including DNA technology of wild broodstock 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;
- establishment of live gene banks and sperm cryobanks of sturgeons from different river basins;
- application of optimized technologies for sturgeon reproduction to support genetic biodiversity, including different intrapopulation ecological forms (hiemal, vernal, summer-spawning) where such variety in forms exists;
- promotion of the implicit use of non-lethal methods for obtaining eggs from wild sturgeon females;
- reduction of stressors and risks during all handling, rearing and health management operations of broodstock on the basis of non-traumatic methods (e.g. ultrasound techniques) and other methods (computer-assisted video image analysis; Hufschmied, Fanghauser and Pugovkin, 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 larva and fingerling rearing to decrease the mortality rate during all stages of the life cycle and release into natural environment; and
- preparation of fingerlings for release into natural waterbodies 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 with other regions in the world. There are six species that can be found in the basin: Russian sturgeon, Persian 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.

RUSSIAN STURGEON (*Acipenser gueldensdaedtii* Brandt)

This species currently spawns in the Volga and Ural Rivers in the north Caspian Sea area and may also spawn in the Kura River and the Sefid Rud River in the south Caspian Sea area. 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 farther upstream.

Most spawning sites have been lost owing to dam construction. The Caspian Sea Basin has lost 70 percent of spawning grounds since the 1950s mainly owing to the construction of hydroelectric power stations; the Ural River 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 have decreased since the collapse of the Union of Soviet Socialist Republics (CITES, 2000).

The average age of parents of current cohorts is estimated to be 15 years under natural circumstances. However, owing 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 years and males every 2–3 years; males reproduce for the first time at 8–13 years, females at 10–16 years. 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 molluscs, crustaceans and small fish.

The wild native population of this species 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 since the early 1990s. According to FAO fisheries statistics (FAO, 2009), global catches fell from 4 250 tonnes in 1992 to 67 tonnes in 2007. Khodorevskaya, Ruban and Pavlov (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 tonnes per year in the period 2000–08. The estimated spawning stock biomass in the Volga River has also drastically declined, from 22 200 tonnes (1966–1970) to 1 000 tonnes (1998–2002). The average number of spawners (1 000 individuals) passing fishery zones to the spawning grounds in the lower Volga River (per year) has declined by 88 percent when comparing the averages of the period 1962–1975 with the period 1992–2002 (Vlasenko *et al.*, 2003).

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, the Volga and Ural Rivers and may enter the Terek and Kura Rivers 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 range 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 Sea Basin, the species spawns in the period April–September, but reproduction is interrupted in the period June–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 Sea 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 molluscs, crustaceans and small fish.

The Islamic Republic of Iran is the only country in the Caspian region that uses this species for stocking purposes. More than 80 percent of the total sturgeon stocking (and restocking) activity carried out by the Islamic Republic of Iran is with this species. In 1997, some 24.5 million fingerlings were released to the Caspian Sea, as were about 10 million fingerlings in 2008 (M. Pourkazemi, personal communication).

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 identify genetically 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 the Islamic Republic of Iran (mainly originating from hatchery-reproduced stocks). The catch data from the Islamic Republic of Iran show that in the period from 1960/65 to 2006, the annual catch declined by 75–82 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 in the wild populations owing to the long-term stocking of the species. However, it is suspected that the native wild population has declined by more than 80 percent in the past three generations (an estimated 42 years) as all the wild populations have almost disappeared, except from the populations of restocked individuals from the Islamic Republic of Iran. There are only occasional records of catches in the northern Caspian Sea Basin – in 2008, 100 immature individuals were caught in the northern Caspian Sea Basin (Mugue, personal communication).

Illegal, unreported and unregulated (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 nudiventris* 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 material surfaces. 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 (Erbulekov, 2000). 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 (M. Chebanov, personal communication). Most juveniles move to the 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, molluscs and crustaceans.

There are few catch data available on the commercial exploitation of this species. In Kazakhstan, 12 tonnes were caught in 1990 and 26 tonnes in 1999; in the Islamic Republic of Iran 1.9 tonnes were caught in 1990 and 21 tonnes in 1999 (TRAFFIC, 2000), and 1 tonne in 2005/06, with 0.5–1 percent of total sturgeon catch in the Islamic Republic of Iran belonging to this species (in past 20 years) (M. Pourkazemi, personal communication). According to the Commission on Aquatic Bioresources (CAB) of the Caspian Sea, since 2001/02, the export quota for caviar of ship sturgeon has been zero for all Caspian range States.

Illegal fishing (poaching), overharvesting, bycatch and habitat loss and destruction, along with dam construction, water abstraction and drought, have led to the loss of spawning habitats and/or grounds and have caused massive population declines.

STELLATE STURGEON (*Acipenser stellatus* Pallas)

This species ascends the 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 Caspian Sea 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 northwest 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 spawn 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 a 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 is 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, 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 sturgeons feed on a wide variety of crustaceans, molluscs and benthic organisms as well as pelagic fish. Currently, the greatest number of stellate sturgeons migrate to the Ural River (Peseridi, Mitrofanov and Dukravets, 1986; Dovgopol *et al*., 1992). Stellate sturgeons stop eating after the beginning of the spawning migration. After spawning, the fish move directly downstream to the sea, where they begin feeding actively.

In the early 1990s, it was estimated that almost 30 percent of the Caspian Sea's stellate sturgeon population originated from stocking (or restocking). Recent estimates are that more than 50 percent of the Caspian Sea populations are from stocking (M. Pourkazemi, personal communication). Khodorevskaya, Ruban and Pavlov (2009) show that the average number of spawners entering the lower Volga River per year has fallen from a peak of 230 000 (between 1986 and 1990) to just 50 000 (between 1998 and 2002), a decline of 78 percent. The decrease in the catch per unit of effort (CPUE) has been more apparent in the southern part of the Caspian Sea. The stellate sturgeon population 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 Sea Basin, the catch of stellate sturgeon peaked in 1977 at 13 700 tonnes. Since then, an almost continuous decline has taken place, down to 305 tonnes in 2003 (most recent data), a decline of more than 97 percent 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 (M. Pourkazemi, personal communication).

Similarly to other sturgeon species in the Caspian Sea, the main threats to the survival of this valuable species are IUU fishing, poaching, overfishing and habitat destruction (e.g. dam construction), water removal, and industrial, oil and agricultural pollution. 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, Ruban and Pavlov, 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 Sea, at least three beluga populations have been identified by microsatellite technique (Pourkazemi, 2008). In the past, it was the largest fish species in the Caspian Sea, reaching lengths of more than 5 m 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. The average length of females and males is 240 cm and 220 cm, respectively, and their respective weights are 130 kg 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 the sea just before spawning. Individuals migrating in the autumn 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 a current of 0.8–1.2 m/s exists. 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 the sea during their first summer and remain there until maturity. Spawning numbers for the Volga River declined from 26 000 (annual number of spawners) in 1961–65 to 1 800 in 1996/97 (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, almost 100 percent of beluga in the Volga are hatchery reared, but there is some evidence of spawning at the remaining spawning grounds (Khodorevskaya, Ruban and Pavlov, 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). The annual number of fingerlings released into the Volga River was

0.4 million in 1951, 13.1 million between 1966 and 1970 (average per year), 19.4 million between 1981 and 1985, 11.3 million between 1996 and 2000, and 3 million between 2001 and 2005 (Khodorevskaya, Ruban and Pavlov, 2009). The report of the 28th Session of the Commission on Aquatic Bioresources of the Caspian Sea, which took place in December 2008, states that the total fingerling release of beluga in 2008 was 2.93 million from the Islamic Republic of Iran, Kazakhstan and the Russian Federation.

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 fallen drastically. Average annual catches in the Caspian Sea were 1 380 tonnes in 1945–1955, 1 283 tonnes in 1956–1965, 1 623 tonnes in 1966–1975, 849 tonnes in 1976–1985, 506 tonnes in 1986–1995, and 61 tonnes in 1996–2003, according to Doukakis *et al*. (2010), which shows a decline of 95 percent. The official catch statistics support this trend, as they show that the species was abundant in 1938 and then stable to the late 1980s, with the major decline starting from 1990 to the present, showing a decrease of 90 percent in the past 60 years (see Khodorevskaya, Ruban and Pavlov, 2009). The agreed beluga catch quota for all the Caspian Sea (2007/08 – 28th Session of the CAB) was 99.8 tonnes, but 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 that coincides with the highest densities of food organisms. Mature individuals of beluga are less sensitive than immature ones to low temperature, 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, belugas narrow down the range of depths at which they feed. In spring and autumn, immature individuals 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 Sea occur during the migration of its main prey organisms (herring, kilka, goby, roach, etc.).

Global fisheries statistics show that there has been a 93 percent decline in catch of beluga, down 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 River dropped from 26 000 (1961–1965) to 2 800 (1998–2002), a decline of 89 percent in 33 years (Khodorevskaya, Ruban and Pavlov, 2009). Only 2 500 beluga migrated upstream in the Ural River 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, bycatch 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 the 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. Owing to the longevity of the species, there is evidence of pesticide contamination, leading to many fish health problems, including a reduction in reproductive success (J. Gessner, personal communication).

STERLET (*Acipenser ruthenus* Linnaeus)

This freshwater species inhabits the Volga River and the Ural River. Its population in these rivers decreased by almost 40 percent between 1990 (116 tonnes) and 1996 (80.6 tonnes) (CITES, 2000).

This species is 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 bottoms, rarely on gravel-sand bottoms. 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 the temperature rises above 10 °C. There was a migratory population with large-growing individuals in the Volga River until the end of the nineteenth 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 molluscs. Overfishing, poaching, habitat destruction and pollution are the most important threats to the conservation of the species. Pollution, including oil products, phenols, polychlorinated biphenyls (PCBs) 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 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 nearby 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. on local biodiversity).

Justification

Inappropriate siting and location of hatcheries has caused production failures, loss of investment, conflicts with other resource users and reduced efficiency in restocking. Planning for a hatchery site should take into consideration the local area plans, and foresee land- and water-use practices of neighbours that may affect positively or negatively the hatchery functioning. It should be recognized by the respective licensing authorities that other resource users (e.g. the 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 coexistence 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), and groundwater availability;
- distance from the capture sites of parental fish (preferably less than 25–30 km), to avoid long and stressful transportation of wild broodstock;
- distance from the infrastructure (e.g. nearest settlements and transport systems, road, railway or airport);
- distance from an energy supply source (possible powerline 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 groundwaters, which should not influence full seepage and drainage of pond beds;

- level of rivers and/or Caspian Sea must be considered (as must as the surges to prevent flooding);
- protection against overflow;
- on-site or nearby availability of impermeable pond-construction materials (dykes, bottom sediments). There are many construction guidelines available for conventional aquacultural ponds as pond aquaculture has been practised 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; and
- 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 selection of the site).

3.2 DESIGN OF THE HATCHERY

Guideline 3.2

In the past few decades, hatcheries for teleosts and sturgeons have seen design improvements with regard to optimized system layout and 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 and fingerling nursing, but also the system layouts and materials that permit the efficient (worker friendly) operation and management of the hatchery, while also integrating 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 and heating systems, the water quality monitoring systems and the feeding systems are 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 labour, electricity and water than modern hatcheries. Similarly, hatcheries that were built in the past for other species and are being modified for sturgeon reproduction often need adjustments to the design of their essential units in order to meet the performance output required 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 good 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 on-site 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 their perimeters to prevent the entrance of wildlife and unauthorized persons.

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 separation, separate handling of males and females]).
- Incorporate modern technical equipment that permits modifying 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 bypass options in the recycle flow can help solve the problem.
- Consider daily cleaning and maintenance work requirements 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 enable observance of proper hygienic conditions in all "corners" of the system (all parts such as pipes and fittings) can be obtained from European Union (EU) regulations for certified aquaculture hatcheries.
- Select adequate construction materials (e.g. durability and toxicological aspects) also for the equipment and facilities.
- Allow for future increases in the 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). These considerations are essential as they will have a noticeable impact 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 constant monitoring and control of all production procedures throughout the process. Without a comprehensive monitoring programme, performance cannot be reliably assessed. Moreover, 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 reestablishment 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 above 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 intergovernmental working groups on environmental interactions of aquaculture (e.g. the Working Group on Environmental Interactions of Mariculture of the International Council for the Exploration of the Sea [ICES]; the European Inland Fisheries Advisory Commission [EIFAC] Working Party on Fish Farm Effluents), and extensive guidance can be obtained from numerous publications by these organizations and by professional aquaculture societies and intergovernmental as well as regional organizations.

- 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. In addition, much 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 that would allow for a suitable living environment and conditions for the fish as well as a suitable working environment for the hatchery workers. Again, for many of the detailed requirements, much can be learned from commercial aquaculture practices, with specific adjustments being made in line with the specific requirements for culture systems designed to produce fish for release (see Sections 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 the 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 well-being 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 recirculated water systems (also called recirculation aquaculture systems). It has to be emphasized that the design of such recirculation systems is different from that for commercial aquaculture systems. They must allow water quality to be managed in a predictable manner so as to imitate the natural conditions of waterbodies into which the produced progeny are 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; and
- broodstock holding unit with feed preparation farming house.

The following structural design aspects should also be considered:

- System layout should allow incubation facilities and tanks to be operated either as flow-through or recirculation units. The latter should be designed as multiloop 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 (precalculated based on metabolic rates and mass transfer theory models).
- Units for disinfection using ultraviolet (UV) light or ozonation should always be installed in bypasses and never in the direct flow.
- A low-temperature holding facility for brood fish will enable operation with broodstock on a continuous basis with water thermoregulation. However, the biofilter design for such cold-water systems requires extensive dimensions that must be precalculated while the rate of recycling will have to be reduced (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 escape 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 pretreatment, 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 unintentional invasion by 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 should be 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 pond, regardless of 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 cases where modernization 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 that ensure the optimal hydrological conditions for pseudo-migration of broodstock and include the possibility of annual clearance of spawning ground and rearing areas (Appendix, Figure A1.4).

3.4 ECONOMIC ASPECTS OF HATCHERY CONSTRUCTION AND OPERATIONS

Guideline 3.4

In the planning for design and construction of sturgeon hatcheries, whose main purpose is the supply of fingerlings for release and rehabilitation of populations in waterbodies 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 and intergovernmental donors as well as from licensing agencies. In cases where hatcheries are destined for ranching or stock enhancement (supporting specific clients such as commercial fisheries and also providing aquaculture with stocking material), a financial/business plan presenting total investment and running costs as well as amortization 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 in part) the running costs from beneficiaries once natural populations have recovered to the extent that a limited and tightly regulated fishery can be reopened 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 waterbodies serving a fishery specifically being supplied with recruits. Therefore, the economic feasibility of the hatchery should be taken into consideration from its planning and design phase. However, hatcheries that are constructed and operated with the sole purpose of supplying fry and fingerlings for restocking purposes have other goals than making profit and serving society at large. Moreover, some hatcheries, particularly in the Caspian Sea Basin, are state-owned and have as a main objective the supporting of rehabilitation of the sturgeon stocks 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 once the fishery on recovered stocks is reopened.

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 Sea Basin countries) must have sufficient working capital to carry out the necessary operations. Before deciding to build a hatchery, one needs to examine carefully 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 past experience in commercial aquaculture development on how to prepare a feasibility study, develop a business plan and establish operational procedures. However, while the overall principles apply, specific adjustments for sturgeon hatcheries will be needed. The following actions are recommended:

- 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, so 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 depending solely on state subsidies and grants for economic sustainability but gradually seek accompanying measures that provide some income to defray a certain portion of the running costs (e.g. training programmes, entrance fees for guided visits, a small proportion of fry production for sale to aquaculture operations). Planning such activities may have some small design implications for the construction of a hatchery (e.g. a visitor corridor alongside, 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 person responsible (i.e. the hatchery manager) for control over the budget.
- Obtain life and accident insurance to cover the main risks involved in sturgeon hatchery activities. Worldwide, there are only a few 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 the non-economic value of the hatchery and its produce, including the social, cultural and environmental values that are associated with the hatchery. In particular, 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 broodstock

4.1 SITES AND PERIODS OF BROODSTOCK 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 predetermined period. As a principle, it is advisable to catch as many brood fish as necessary to maintain the genetic integrity of the species without ignoring the need to keep the impact to a minimum on those natural populations that are highly endangered or on 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. However, the national and local laws and regulations on the catch of sturgeons and sturgeon broodstock that are in place are often enforced only to a limited extent or not at all. In general, 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 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 the 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. These conditions should be monitored continuously in order to update and adjust regularly the estimates on the time window for collecting mature brood fish, thereby improving the 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 the number of broodstock is very low, it is advisable 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 harvesting, 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. It is highly beneficial to the hatchery and the fish to do all that is 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 Organisation for Animal Health (OIE, 2010) provides key guidance on how to guarantee the welfare of fish during transport. However, adult sturgeons are exceptionally large specimens (much larger than most of the teleost fish species handled in common commercial aquaculture). Therefore, adjustments to equipment to handle fish at capture and during transport must be made, and specific handling skills will be 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 for which freshly captured broodstock are handled and transported should be as short as possible. Good planning and organization of the transport by competent (trained) persons is essential in order 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, preferably using splashless tanks of 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 in which they were caught. This water must be temperature controlled, either to maintain the temperature from which the fish originate (water should not warm up while the fish are in transport) or cool the water to reduce metabolic activity.
- Cradles/slings, tanks and containers for broodstock transportation should be rinsed with freshwater before use and subjected to approved disinfection procedures. Stressless (e.g. splashless) tanks should be used, to avoid mechanical damage to fish and reduce “seasickness”. Moreover, to avoid fish damage, the inner surface of containers should be smooth (no rough surfaces, and also easier to disinfect).
- Broodstock 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 may be necessary to transport live sturgeon from one country to another. In such circumstances, the recommendation is 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 the Code as well as the ICES Code of Practice on the Introduction and Transfer of Marine Organisms (ICES, 2004), which is also endorsed by FAO, incorporating several of its principles in the FAO technical paper *Assessment of freshwater seed resources for sustainable aquaculture* (Bondad-Reantaso, 2007). These guidelines give advice on how to reduce the risk of introduction and spread of serious transboundary aquatic animal diseases. Although they deal primarily with safe transboundary movement at the international level, they are also applicable to domestic movements between different provinces, basins, sub-basins, geographical areas or zones of differing disease status. As such, the Technical Guidelines on Health Management for Responsible Movement of Live Aquatic Animals can form a valuable addition to the guidelines outlined in this document. In additionally, the ICES Code of Practice on the Introduction and Transfer of Marine Organisms 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 broodstock, 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 intraspecific groups (spatial, seasonal) must be ensured in the established hatchery broodstocks intended for release either to re-establish a sturgeon species in its previous natural range or to replenish natural stocks that 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 specimens 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 the 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 Section 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 broodstock through rearing and releasing. Coordination within and among river systems and countries and establishment of a centralized entity for overseeing and coordinating hatchery practices are recommended. Research into the genetic diversity of wild sturgeons is needed to manage hatchery practices more accurately.

Justification

Genetic diversity is essential to the long-term survival of a species. Individual populations of sturgeons may have unique adaptations and genotypes (Ireland, Anders and Siple, 2002). 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 attribute an individual genetically 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 brood fish of the target species available. This is essential to avoid genetic pollution of the original populations. If the currently 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 it is impossible to obtain sufficient broodstock from an individual river (i.e. the local population is extinct), animals from the closest river system and/or with close genetic relatedness may 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 such 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 be non-lethally stripped of their sperm for the purposes of cryopreservation. Captured individuals should be retained in captivity for future use when a suitable mate (i.e. 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 fewer 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 the Appendix (Table A1.1). 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 brood fish 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 Table A1.1) be achieved each year and that different individuals be bred each year (St. Pierre, 1999). If fewer 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 (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 be understood of each cross to the resulting population that will be released.

It is preferable 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 (preferably not fully domesticated) females and wild males can be used, and vice versa in the case of a lack of males. It is desirable to also use a rotational breeding scheme in these scenarios, incorporating at least 5–10 percent of wild brood fish into the broodstock (Bartley, Kent and Drawbridge, 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. There is a 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 retrospective analysis on the success and failure of release programmes.

There will probably be several hatcheries 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 located closer to release sites. However, in order to safeguard the broodstock and protect against unforeseen risks, 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 hatcheries in the Caspian Sea Basin. Such information should be linked to a database. The body could further support additional genetic research on the 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 in order 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, individuals with typical exterior characteristics without anomalies and with 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 facilitate 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 intrapopulation groups;
- age of sexual maturity and interspawning intervals; and
- productive capacities of the hatchery (at the first stage of broodstock establishment).

Criteria for fish selection for broodstock development include:

- Consideration should be given to the 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 minutes) when subjected to water, transparent ovarian fluid, sperm with spermatozoa concentration not less than 3 billion/ml and their duration motility.
- It is advisable to start selection of fish intended for stock rehabilitation from fry of 5 g weight upwards.
- Prior to selection of juvenile fish for use as an evaluation of the fitness indices of the juveniles should be conducted with the use of specific techniques.
- Within each genetically homogeneous group, weak specimens with different abnormalities and irregular body coloration should be culled (Piskunova *et al.*, 2001; Shevchenko *et al.*, 2003). 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, intraspecific 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 terms of 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

Owing to a lack of space or proper facilities, and as a consequence of limited knowledge of how to hold sturgeon broodstock, many hatcheries currently hold sturgeon broodstock under suboptimal conditions (Popova *et al.*, 2001). 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 is important to hold the broodstock at low stocking density levels or in individual hatchery units. Holding of the brood fish 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 brood fish, 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 waterbodies should be preferably used for broodstock holding.
- Pond stocking densities 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 acclimation unit for at least 2–3 months, providing a low-stress environment (low stocking density, natural photoperiod, minimum ambient-factor 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 (vernalization of 2–4 months at a temperature 4–6 °C) is necessary before final maturation of breeders (Chebanov and Savelyeva, 1999; Chebanov *et al*., 2002) for successful spawning.
- The use of non-invasive methods (e.g. ultrasound diagnostics) when handling the broodstock is much preferred to 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

Guideline 5.4

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

Justification

Recognizing the dramatic decline in wild captured sturgeon broodstock in the Caspian Sea Basin, their adaptation to artificial conditions of holding is important. Adaptation allows for building productive broodstocks with a genetic structure that is similar to that of the wild population. In fact, adaptation is required to keep the broodstock alive, ensure 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 °C), at a high level 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 broodstock using standard protocols in terms of handling and hygiene;
- gradual transition of caught fish from live food to composed diets;
- holding at the hatchery until (repeated) maturation has been achieved; and
- attempts to repeat utilization of mature brood fish.
- At the beginning of the process of acclimation, wild fish should be trained to feed on natural food (fish, molluscs, 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 feed 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); and
- forced feeding of fish with pasture-type feeds using catheters.
- The use of non-lethal, specific ultrasound methods for monitoring the alimentary system is recommended (Chebanov and Galich, 2009).
- Wild fish that do not accept formulated feeds within 90 days after the adaptation has started should be released to natural waterbodies 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 all 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 broodstock 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 specific ultrasound techniques of sexing and staging. The maturity stage is reached at different body weights and different ages depending on the sturgeon species (Table 1).

TABLE 1
Age at first maturity and corresponding weight by sturgeon species (based on practical experience on-site on farms)

Species	Rearing at seasonal temperature	
	Body weight (kg)	Age (years)
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;
- interspawning intervals for females and males; and
- 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.

Table 2 shows the number of juvenile fish that will, on average, have to be raised in order to rear one brood fish to maturity.

TABLE 2
Number of juvenile fish of each age group to be held at the hatchery for each brood fish to be produced for the reproduction programme

Age	Number of individuals
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 that is preferably composed of 30 percent mature females and 10 percent males of the required number of brood fish.

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 its quality and physiological condition.

Justification

Frequent physiological monitoring of the broodstock (at 2–3-month intervals; 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 brood fish quality and good fish health conditions (Geraskin *et al.*, 2000).

Implementation guidance

Monitoring and control of the hatchery broodstock is an essential requirement for each sturgeon hatchery. Recommended actions for hatcheries in the Caspian Sea Basin are:

- Broodstock monitoring should be performed during spring and autumn assessments. It is important that the spring assessment is completed before spawning temperature is reached.
- In the course of the assessment, morphological and biological indices, as well as the physiological and ichthyopathological status of the fish, should be evaluated and recorded, and any fish with evident developmental malformations should be culled.
- 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 should be performed of the individuals 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 concerning the identification (Barmintseva *et al*., 2003) and the standardized individual brood fish passports (Safronov and Krylova, 2004) should be recorded in the hatchery database and preferably also in a regional hatchery database (register of sturgeon brood fish) 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 developing breeding programmes and in terms of effective assembling of brood fish mating pairs.
- Each fish should preferably be marked with individual tags of two different types (external and passive integrated transponder [PIT] tags) 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, this strategy takes care only of the paternal gene pool – it is yet not possible to cryopreserve oocytes.

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

Justification

Sperm cryopreservation is being applied in some countries, and it has proved 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 could 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 editions of the International Workshop on Fish Sperm Biology, summarizing the global expertise on the subject (Alavi, Linhart and Rosenthal, 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.
- Where broodstocks are collected from coastal areas, 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. Where 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. 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.
- The sperm of each species should be kept separate and stored independently. Therefore, systematic labelling that allows long-term and logical following of breeding lines for decades can be possible (see specific instruction below).
- All morphometric and genetic characteristics of broodstock should be recorded and registered.
- The collected broodstock should be held in the best possible conditions (in terms of oxygen, temperature, pH, light), and exposure to any kind of stress should be limited.
- Only very high-quality spermatozoa should be used regardless of whether for direct fertilization, short-term storage or cryopreservation (based on predetermined 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 requires well-prepared logistics to minimize the variability of results by uncontrolled experimental handling times.
- For short-term storage (hours), a suitable temperature should be determined (about 4 °C). The samples should remain in contact with air but dehydration should be avoided. The design of proper 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 vials, plates) depends on sperm volumes. Moreover, to collect "clean" spermatozoa, catheterization is recommended. Furthermore, using catheters, sperm from the same testis should be collected repeatedly at intervals of several minutes.
- Sperm quality evaluation (both for fresh sperm and post-thawed sperm) should include sperm volume and density of spermatozoa. Once the proper cryopreservation media have been designed (ionic composition, percentage

of cryoprotectants 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.
- The code for each tube and its numbering for storage should be registered and a computer databank should be established, 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, Suquet and Cosson (2010). To make a precise evaluation of sperm performance, computer-assisted sperm analysis (CASA) software should be used as a more reliable methodology, although the method is relatively expensive (involving the use of software, a microscope and a video camera). However, the species in question are rare, valuable and highly endangered. The use of CASA also needs a standardization of effort (times, dilutions, frames captured per second, etc.) but this can be employed to standardize all other aspects of sperm handling and use (see Hatef *et al*., 2010). Basic parameters to measure using CASA are percentage spermatozoa and spermatozoa velocity (curvilinear velocity and average path velocity), but also flagellum beat frequency is increasingly used.
- In addition, assisted sperm morphometry analysis (ASMA) software (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.
- Furthermore, 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 performance (Linhart *et al.*, 2006). The eggs fertilized with cryopreserved sperm should be observed at certain time intervals, e.g. 24, 48, 72 or 96 hours, to count fertilization rate, abnormalities in ontogenetic development and mortality of early stages.
- The cryopreserved sperm should be stored in the appropriate storage tank and the liquid nitrogen changed as per predefined appropriate standards. Modern equipment allows the level of liquid nitrogen in storage tanks to be monitored automatically and either give an alarm at low levels or automatically refill from a general liquid nitrogen storage tank.
- Frequent opening of the lid of liquid nitrogen containers should be avoided. 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.
- Hatchery staff working on the cryopreservation of sturgeon sperm should be well-trained in the handling of the equipment and in monitoring cryopreserved samples.

At the level of the Caspian Sea Basin, it would be important to undertake the following 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 at regular intervals the methods and technology on sperm cryopreservation and gene banking once such a bank has been established.

- Develop a Caspian Sea Basin sturgeon sperm cryopreservation strategic plan, supported by a regional-level plan that is strongly supported by national institutions of the Caspian Sea area.

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 to and coordinated with national plans. The objective of the plan should be clearly determined by the end users. It is important, for example, 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 (Grunina *et al.,* 2009).

As 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 could exchange their experiences and achievements and benefit from the knowledge of international centres and scientific institutions.

Sperm cryopreservation is an important component but only a part of preserving the gene pool, while the maternal component will have to be taken from live broodstocks. Research should also be encouraged on cryopreservation techniques 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 release practices, it is mandatory to collect information on the origin of wild broodstock and other individuals, including precise river or coastal locations where spawning does occur naturally.

Record-keeping by tagging should include documentation on the reproductive state and genetics. Numbers returning into the fishable stock can play an important role in management decisions to achieve sustainable stock sizes.

A sound reference number for monitoring and tracking fish should be provided by tagging, using a variety of methods as may be appropriate for the specific situation under study. The tags used should be fixed to fish in a manner that may allow tracking over many years. Selection of the best and most appropriate tag will depend on many factors (e.g. fish size) with a view to causing minimum damage and injury to the fish as well as ensuring that long-term and high retention rates are achieved for these tags.

Implementation guidance

The following procedures are recommended for tagging sturgeons:

- Use appropriate tags (preferably internal tags, PIT tags and/or external tags).
- Register all tag information in a database, including: full species name (common and scientific names), sex, state of maturity, catch date and location, total weight, total length, fin tissue sample (coded and stored appropriately for genetic analysis), and the time of the broodstock's migration.
- Record all information related to reproductive performance of females, such as total egg biomass obtained per female (kilograms), number of eggs per gram of egg mass, fertilization rates and hatching rates achieved, while also registering the fish tag code of the respective male (males) used to fertilize the various egg batches.
- Besides external and internal tag registration, obtain genetic tagging or DNA fingerprinting for each parental pair and cross-breed (e.g. using microsatellite analysis.

Because of the dramatic decline in wild-breeding sturgeons in the Caspian Sea, conservation of broodstock is highly recommended. Egg collection for fertilization should be undertaken without killing the females used, employing microcaesarean techniques. Brood fish may be used repeatedly for several years and, therefore, the internal tag (e.g. a PIT) may provide a suitable option for tracking and monitoring the performance of individual fish broodstock members kept at the hatchery.

In order to prevent inbreeding and also to increase genetic variability and avoid any crosses between close relatives, it is highly recommended that a genetic identification system should be designed for each specimen using not only an appropriate tag but also developing an analytical matrix for precise selection of mates, as has been tested and proposed by Congiu *et al*. (2011) and Mugue and Barmintseva (2011). These authors propose a standardized and simple protocol based on mitochondrial and microsatellite information specifically designed for polyploid species that can facilitate the management of genetic diversity, as exemplified by the *ex situ* conservation for the tetraploid Adriatic sturgeon *Acipenser naccarii* (Congiu *et al*., 2011) and *A. gueldenstaedtii* (Mugue and Barmintseva, 2011).

Tagging of wild broodstock from which tissue samples have been taken will assist in the identification of the genetic origin as well as providing opportunities to monitor the behavioural performance of broodstock fish as well as their offspring.

6.2 STURGEON TAGGING PROCEDURES

Guideline 6.2

Tagging of sturgeon should be practised following clearly defined procedures that minimize handling stress and avoid negative effects on fish welfare while also ensuring high retention of tags over a long period.

Justification

It is important to determine the proper tagging procedures and criteria for monitoring fish inside the hatchery but also to use adequate techniques that the tags can endure on fish for time periods after release. The tagging methods as well as the type of information that is to be recovered from the tags should be kept up-to-date and follow 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:

- collection of broodstock, aiming at separation of brood fish for the period of the spawning run;
- collection of fish intended for broodstock replacement, aiming at cohort identification and tracking in future mating schedules; and
- release of fingerlings into natural waterbodies, aiming at monitoring growth and survival rates, as well as control and evaluation of production efficiency

Regardless of the ultimate aim of the operation, the materials and methods used for tagging and marking operations should match the following objectives:

- achieve the lowest possible level of fish injury during the tagging procedure;
- ensure a minimal effect on the hydrodynamical properties (swimming capabilities, etc.) of the tagged fish and fish survival rate after tagging and throughout the 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 (minimizing total handling to shorten the stress on fish; procedures may be designed to handle small batches of fish during a given time window to achieve this goal);
- allow easy tag detection in and on the fish concerned;
- provide non-lethal techniques to read the information from any tag at any stage of the monitoring programme; and
- provide the possibility to perform *ex situ* tagging.

Recognizing that there are many types of tags available, it is important to select a type of tag that suits the purpose and objectives of the tagging activity, while meeting the specific fish characteristics (mainly size).

The following tag types can be distinguished:

- internal tags:

- PIT tags,
- magnetic tags (coded wire tags)
- external tags;
- dye marks and tattoos; and
- resection of fin parts and scutes.

Small fish (juveniles) should be marked (mostly by bath). Use of different markers should be tested before any general conclusion is drawn and decision taken. A control group should be kept under farm/hatchery conditions. Tagging should be used for larger fish only. It is anticipated that tagging and marking methodologies will advance rapidly in the near future, allowing a widening of the scope of regularly or continuously recorded data on fish behaviour and the environment in which they are thriving. Hatchery managers are advised to update their knowledge in order to apply the latest technology available.

7. Water quality and supply

7.1 ACCESS TO AND AVAILABILITY OF WATER

Guideline 7.1

Hatcheries should be provided with water of good quality. Water quality considerations should already receive a high priority when selecting a site for a new hatchery. There may be a need to include pre-treatment of intake water to achieve the quality criteria for a safe and healthy hatchery operation.

In particular, water sources should have a high oxygen content (close to saturation), an acceptable pH range (preferably between 6.5 and 7.5), and a suitable temperature range (species and season dependent).

The supply should be of sufficient volume to guarantee adequate water exchange rates for all units and at all times throughout the production period so that the water quality will remain adequate (within acceptable limits) in all tanks and holding facilities. The water quality has to be monitored continuously to ensure fish health and welfare.

Justification

Good water quality determines to a great extent the success of any fish culture and hatchery operation and this holds also for sturgeons. Sturgeons have been cultured in wells and surface waters of varying water quality. Although precise optima for various water quality parameters have not yet been fully assessed for all life cycle stages of most species, general ranges of water quality parameters for hatchery operations can be identified and these are listed in the Appendix (Table A1.2).

Implementation guidance

Failing to supply good water quality and sufficient volumes can result in severe mortality in hatcheries in both short-term and long-term operations. To improve intake water quality characteristics, it may be necessary to employ the following pre-treatment procedures:

- The water supplied to any hatchery must be free from contaminants. Pre-filtration may be required to eliminate large particles (suspended solids of either inorganic or organic nature). Removal of large particles (larger than 30 µm in diameter) is essential before any disinfection procedure can be applied with a reasonable effectiveness. For disinfection purposes, it would be better to capture particles down to 20 µm in diameter or less; however, this requires specifically designed filtration methods that may often not be cost-effective).
- The effectiveness of suspended solid removal has a great effect on the required size and capacity (and subsequently on the running costs) of any of the available disinfection procedures.
- For the purpose of this guideline, a clear distinction must be made between disinfection and sterilization. While disinfection tries to eliminate target species (e.g. key pathogens), sterilization attempts to remove all microbial organisms, a condition requiring a high technical and operational input. However, culturing fish for release would also require the early exposure of specimens to the natural bacterial community in order to stimulate the immune system. Thus, sterilization is not a target objective for hatcheries producing fish for release. Therefore, disinfection procedures should strive for a healthy environment in which the microbial community does not exceed the natural level in terms of species and numbers.

- In general, two well-established disinfection procedures can be employed today to eliminate to a great extent target pathogens while also reducing the overall bacterial load in the hatchery system: ozonation and UV-light disinfection.
- There are some important considerations when applying ozonation (Rosenthal, 1981; Summerfelt and Hochheimer, 1997). Usually, available ozone generators produce a number of different total radical oxidants (TROs), the mix of which is influenced by the use of high-frequency or low-frequency ozonizers. The air supply to ozonizers must be absolutely dry to prevent the production of nitric acid (HNO_3), which can lead to aggressive acidification of the piping system and treatment water. Moreover, the reaction of TROs (including ozone) with organics leads to intermediate products (such as primary ozonides) that are highly electrostatically loaded and should be removed as quickly as possible. This can be achieved by combining the contacting process of ozone-containing air with counter-current foam stripping ("foam fractionation"); a technique that greatly helps to assist in the removal of fine particles via their aggregation through electrostatic loading and by capturing these in the foam. Ozonation should always be performed in a bypass with about 10 percent of the total water flow rate of the system so that the residual ozone (or TROs) is greatly diluted. In order to allow dissipation of residuals to safeguard fish health, the ozone-treated water should also pass through a retention tank (for about 10–15 minutes) before entering any fish tank again. The main concepts on ozone application can be found in Rosenthal (1974, 1981), Rosenthal and Wilson (1987), and Summerfelt and Hochheimer (1997). Disinfection via ozone and other accompanying TROs produced in the electrical discharge of an ozonizer is mainly achieved by cracking double bonds of organic compounds in the surface of a bacterial cell membrane, thereby destroying the capacity of the cell to osmoregulate. It has also been demonstrated that ozonation in recirculation systems has a positive effect on the stable composition of microbial biofilms in aquaculture systems (Wietz, Hall and Høj, 2009). Human health aspects need to be considered when using ozone in indoor aquaculture facilities. Off-gas from the contact chamber should be collected and not allowed to escape into the culture hall but be released outside (e.g. via an off-gas pipe through the roof). Although ozone is usually self-controlling (specific smell below health threshold values), all equipment used should be regularly checked for air tightness. Exposure standards for residual ozone of various international occupational health and safety administrations range between 0.05 and 0.1 ppm for an 8-hour work period and a maximum single dosage of 0.3 ppm for less than 10 minutes.
- Ultraviolet light has frequently being used in water disinfection and sterilization. In fish hatcheries (as in most aquaculture facilities), UV light can achieve good disinfection results for a number of bacterial pathogens, the effectiveness of which will depend greatly on the dosage, the particle load of the water and the contact time. Even very small particles can provide attachment space for bacteria, thereby acting as "hiding places" so that target pathogens are not fully "hit" and may survive. More importantly, survivors only partly hit by UV light may show mutations and build massive populations after having passed through the UV unit while most of the competitors have been eliminated (so-called "after growth"). However, modern contact equipment allows high turbulences in the contact tubes so that the likelihood of hiding is greatly reduced, but still not zero. Therefore, UV light application may be most effectively used in reducing the overall bacterial load of intake water, while special maintenance and monitoring of effectiveness remains necessary in a recirculation setting. Ultraviolet treatment does not leave any residues in terms of chemically modified compounds but only "dead bodies".

The only human health hazard is the possibility of direct exposure to the UV lamp when in operation, which should be avoided. Any handling (or service) near the lamps should be undertaken only when these units are switched off. Ultraviolet lamps emit a wide spectrum of wavelengths (also dependent on the type of lamps); however, the most effective germicidal wavelength is 254 nm, which all low-pressure UV lamps produce. Usually, the penetration of the effective dose of UV light into the water is not very deep (a few centimetres) and the treatment of large volumes per time unit may require the operation of multiple lamp units in parallel. The effectiveness will also depend on water flow rate, dosage and the transmission depth of the water (influenced by salinity and dissolved organics). Moreover, there is an initial loss of dosage (up to 10 percent) usually after the first 100 operational hours (product dependent). In addition, there is a limited lifetime for such lamps. Monitoring the full functionality in certain time intervals (several months) may be advisable.

- Besides the two above-mentioned water treatment methods, one may consider using other pre-treatment techniques in cases of poor water quality in the water supply. Most frequently, such measures include temperature control (usually heating), acidity neutralization (e.g. liming), suspended solid removal (e.g. sediment traps, settling basins, drum filters) and iron oxidation with subsequent settling of the oxides.
- Protection against incidental contamination of inlet waters (e.g. leaf and litter or dust and sand input through strong wind action in outdoor facilities) should be provided. Such potential contaminations are often neglected but methods to avoid them should be included in the system design. Monitoring of inlet water quality should be mandatory and performed at regular intervals (automated), and continuous recording of some parameters is highly recommended.
- Sturgeon fry are susceptible to gas supersaturation, entering gas-bubble trauma and finally may develop gas-bubble disease. The threshold for total gas supersaturation (oxygen, nitrogen, carbon dioxide and noble gases) may be in the range of 102.5 percent (maximum 105 percent). Thus, there is a very limited safety margin, and adequate degassing technology to drive off any excess of dissolved gases should be incorporated into the system design.
- Internationally agreed standards for water quality should be employed also for sturgeon hatcheries. There are many published and nationally agreed values for a number of parameters. The appropriate sources should be consulted (e.g. EU Water Framework Directive; water quality standards set out in various national regulations for aquaculture, FAO guidelines, and other published tolerance limits compiled in various codes of conducts, guidelines and manuals for aquaculture).
- The application of recirculating aquaculture systems, particularly for LLTHFB, should be promoted in order to provide controlled environments meeting target values for key water quality criteria. Recirculation also allows the unit to be largely independent from external water sources, thereby avoiding accidental contamination while also minimizing the demand for the overall water supply volume. In addition, recirculation may (under certain circumstances) reduce water costs and waste discharges.

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 in a hatchery should match or be superior to those of con-specifics growing under natural conditions. Characteristics to describe the required criteria for fitness have to be developed on a species-specific basis, allowing differentiation between the performance of fish of reared and wild origin. The fitness may be reached by step-by-step adjustments of the methodology to the respective life cycle stages (e.g. changing mouth gape size to feed on various food items offered, feed consistency and nutrient requirements of each life cycle stage).

Justification

Together with water quality and facility design, feeding is among the most important elements in determining the success of the rearing process.

Optimal feed quality improves the condition and hence the suitability of the fish for release. Cost only plays a secondary role in this process, while also the ease of supply must not be a driving force for feed selection. It should be recognized that rearing 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 for raising 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 and seasonal temperature).

Implementation guidance

Timely preparation of the ponds prior to production is necessary. Drying, wintering, ploughing and fertilizing the ponds as well as inducing of plankton blooms should be carried out based upon the methods well described and established for both ponds in subtropical 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 managed depending on the productivity of the pond, 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 percent 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 (Mikhailova and Mamedov, 2000). In this case, the administration of natural diets is based on 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 not economically feasible to have a large-scale cultivation of food organisms continuously in operation. Food shortages in ponds often occur unexpectedly 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; and 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 ensure the balance between metabolite dilution and good retention of life food organisms (or micropellets). At low exchange rates, oxygen is often 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. Nauplii of *Artemia salina* are usually used for this purpose owing 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. The quality of commercially available cysts varies greatly with year, and origin and advice from professional services (such as the Laboratory of Aquaculture and Artemia Reference Center, Ghent, Belgium) should be sought. 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 should be recognized through precautionary checks of plankton samples prior to application. In addition, for very small larvae, the use of *Brachionus* grown in captivity has proved to be a good starter diet in many marine fish cultures. Feed administration of live feed is carried out by providing a density of 2–4 *Artemia* per millilitre of culture water. In order to maintain a fairly constant density of food organisms in the culture tank, high-density *Artemia* nauplii stock cultures should be supplied almost continuously to replace the uptake and flushout of nauplii through the drain as the larvae are highly susceptible to starvation in cases where food density becomes too low. Moreover, dead food organisms and leftover 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.

In addition to being easy to produce, both *Artemia* and *Brachionus* 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* cyst origin (see also Section 8.1.1) as well as suitable culture technology (incubation jars, culture tanks, aeration systems, and harvesting and cleaning methods).

FAO has published a manual on the production and use of live food for aquaculture (Lavens and Sorgeloos, 1996) that 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 (Spektrova, 1988).

The timing of the first phase of the feed supply largely depends upon the feed organisms used in the second phase. Usually, 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. The 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 by a gradual increase in the proportion of new feed items in the diet over an extended period (up to 14 days depending on species).

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 earthworms can be used as foods. For beluga, the use of eggs and larvae of cyprinids or anchovy is recommended.

The methods of live food rearing were initially elaborated and widely used in the aquaculture industry, and many recommended procedures are practised (Bogatova, Tagirova and Ovchinnikova, 1975). Besides the many reports in the scientific literature, a detailed description of live food production is presented in *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 two hours. The size of feed particles depends on the fish species and the actual wet weight.

Some constraints of live food utilization may be 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 undergo 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 used to ensure that the change of the feed source is not associated with a selection in the weaned fish. More intensive genetic monitoring trials should be applied to identify the genetic background of mortalities and growth suppression upon weaning.

For formulated diets, the protein and lipid concentrations in larval feeds should be 48–60 percent and 8–16 percent, respectively. This composition is altered with increasing fish size and age. In larger fish, a protein content of 42 percent is sufficient, while optimal lipid concentrations are below 15 percent. Digestible carbohydrates are kept close to zero, as 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 is considered effective. These rations are reduced with size to reach 3 percent at 10–20 g 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 200 g.

8.3 FEEDING AT ACCLIMATION OF WILD FISH

Justification

The integration of adults or juvenile fish caught in the wild into the broodstock has to be undertaken during a transitional feeding period when fish are initially offered live food and gradually transferred to formulated diets.

Implementation guidance

Upon transfer into controlled rearing units, the fish should be maintained at optimal temperatures for feeding. Initially, the fish will have to be offered a variety of natural prey organisms to stimulate the restart of feed uptake in captivity. Subsequently, after feeding has been resumed, pasture-like feed mixtures prepared from live fish and formulated diets are commonly used to complete the transition period. In some species, it has been beneficial to acclimate the fish to accept dry diets by filling small fish or squids with the semi-moist diet to increase acceptance. In addition, the use of different attractants has been useful in some cases to ease the acclimation of fish to culture conditions and complete weaning with formulated diets. Attractants reported useful include anise, vanillin, hironomid extracts and Tubifex water.

During the final transition stage, the water contents in the semi-moist diets can be gradually reduced. This can be carried out step by step until all fish readily accept the pelleted diets. Feed composition for grow-out is reported to vary between seasons with regard to optimal fatty acid requirements. The scientific literature on the subject is growing and should be consulted before preparing respective diets. In addition, improved feed composition may include palatability issues while species-specific adaptations of diet preparations are still warranted.

8.4 FEEDING OF BROODSTOCK

Justification:

Diets and feeding regimes for broodstock 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

Broodstock 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. 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 and 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 be used to 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 and 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, and noise should be reduced to a minimum. 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 and Gamygin, 2006).

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

Therefore, special attention should be paid to the safety of feed, particularly to its microbiological characteristics and the terms and conditions of its storage. Moreover, in locally produced feeds, the use of antioxidants 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 the OIE to provide for relevant aquatic animal health measures. This 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 has 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), *Good Practices for the Feed Industry – Implementing the Codex Alimentarius Code of Practice on Good Animal Feeding* (FAO and IFIF, 2010), 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 in order 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, in regard to the potential risk of disease radiation. The considerations include the following:

- Concentration of aquaculture establishments heightens the risk of disease transmission, whether the pathogen enters the culture system via feed or other means.
- Historically, animal proteins used in feed were mainly sourced from the marine environment, owing 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 are used.
- The use 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 owing to the processing applied to the feed.
- 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 condition, for which there exist several manuals, particularly in salmonid farming systems (from which many insights can be gained for sturgeon hatcheries).
- 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.
- The expression of disease may be facilitated by culturing species under intensive and novel conditions. Moreover, it is necessary to conduct research and develop new feeds (and feed ingredients) that are appropriate to the species and its culture system.

9. Selection of broodstock for controlled reproduction

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

9.1 CONTROL OF SEASONAL PROPAGATION

Guideline 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.

Traditional hatchery biotechnologies imply the use of broodstock within a short period of time. Such operational strategies do not allow for breeding all the intraspecific groups. While this might be appropriate for common commercial culture to serve the market for human consumption, it is less suitable for sturgeon cultivation reproduced for restocking of natural populations. The long-term holding in brood-fish facilitates solving these problems and provides for a more efficient use of the production capacity of the hatcheries, in order to conserve the genetic and ecological structure of natural sturgeon populations. (Kazansky and Molodtsov, 1974; Kazansky, 1975).

Implementation guidance

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

- Long-term holding in brood fish facilities of sturgeons at a pre-spawning temperature regime depending on the species or race.
- Transition of the mature fish to spawning temperature regimes, based on the natural system of temperature variations and duration corresponding to holding of sturgeons of different species and races (Chebanov, Galich and Chmyr, 2004).
- Shifting the sexual cycle of wild “hiemal” sturgeon brood fish to an earlier season. Obtaining winter and early spring progeny from spring-run migrants with the use of thermoregulated systems and a recirculated water supply.
- Basing selection of brood fish (for long-term holding in brood fish facilities to initiate reproduction) mainly on the oocyte polarization index (PI).
- Bringing fish of both sexes 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 (Trusov, 1964; Bruch, Dick and Choudhury, 2001), laparoscopy (Matsche, Bakal and Rosemary, 2011), endoscopy, etc. (Conte *et al*., 1988; Williot and Brun, 1998); and
- non-invasive (do not require surgical intervention) – ultrasound diagnostic technique (Chebanov and Galich, 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); and
- delaying egg maturation at completion of the reproductive cycle (delaying by long-term holding of broodstock at somewhat lower temperatures).

9.1.2 Criteria of ripe-female selection and pre-spawn holding of broodstock

Prior to spawning initiation, the readiness of broodstock to produce ripe and fertile gametes should be evaluated using the basic prediction criteria described in Kazansky, Feklov and Molodtsov (1978) and Williot (2002):

- overall appearance of gonads (normal drawing, absence of marble appearance may illustrate overmaturation);
- absence of lipids in the sample (presence might illustrate lack of full maturation of ovaries);
- homogenous size of oocytes;
- PI value should be lower than 0.1, better if lower than 0.05; and
- *in vitro* maturation competence, measured by counting the boiled ovarian follicles of which the envelopes of the nucleus break down (germinal vesicle breakdown) at a rate of over 90 percent.

The efficiency of controlled preproduction depends on several environmental cues that trigger maturation, most importantly the temperature regime applied prior to hormonal stimulation of the maturation process (Chebanov and Savelyeva, 1996, 1999; Doroshov, Moberg and Van Eenennaam, 1997; Williot *et al*., 1991; Williot, Kopeika and Goncharov, 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 produce their gametes naturally 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 spawning have been successfully applied, it is strongly recommended that mammalian gonadotropin-releasing hormone (mGnRH) be used:

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

The reasons for which mGnRH or GnRHa should be preferably applied include:

- The reliability of a constant content of active substance is higher.
- The absence of any other non-specific organic molecules is certain.
- The action of the applied substance through the own hypophysis of the treated fish is more certain.
- Easy availability is a management factor.
- Easy longer-term storage without loss of activity is possible.
- Overdosage 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 a 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 a 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 most important tasks is to collect ovulated eggs and sperm properly. In the absence of any means to check and control fully the start of ovulation and spermiation, it is difficult to decide when to collect the best-quality gametes. 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 (Dettlaff, Ginsburg and Schmalhausen, 1993) (see Appendix, Figures A1.2 and A1.3).
- 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 the process of ovulation to be evaluated without stress caused by (Chebanov and 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 brood fish to be maintained for future reproduction cycles.

Justification

In the past, when there was a high abundance of wild brood fish, conventional practice included the collection of ovulated eggs after slaughtering the females. Under the current conditions, extraction of ovulated eggs should be performed using modern minimally invasive techniques of mature gamete collection, which keep the fish alive, consider the fish welfare and its reuse in the production (and reproduction) cycle.

The use of minimally invasive techniques for mature gamete collection ensures minimal influence of stressors and survival of the broodstock, including the possibility to release the fish into natural waterbodies. Moreover, the reuse of the broodstock is generally a cost-effective investment for the hatchery in times when not many wild brood fish 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 proved 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 until 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 takes between 2 and 20 minutes; use of anaesthetics is particularly recommended for large females (> 30 kg), as they are otherwise difficult to hold (Mohler, 2003). In the case of even larger females (> 150 kg), it is advisable to use a 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 in order to enable the brood fish to breathe normally, which is a prerequisite for 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 become sticky post-water exposition (stellate sturgeon, 6–12 minutes, Russian sturgeon, 8–19 minutes after fertilization) may also be used as maturity assessment criteria.
- A longer time interval from insemination to adhesion is generally an indication of delay in ovulation, while a shorter one shows overmaturation of females (Gorbacheva, 1977).

10.2 SPERM COLLECTION AND 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 proved essential to keep stress levels minimal when collecting sperm from mature sturgeon males. Experience has 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 the necessary quantity to be estimated without additional measuring equipment.

Neglecting preliminary evaluation of sperm quality (ratio of 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 considered 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 point, ejaculate with evident, 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 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 that is not higher than that applied for holding the males. In cases of mid-term (a few days) preservation, the hypothermic method should be used.

Criteria for sperm quality determination include:

- Motility of spermatozoa by five-point grade scale. In this scale, sperm assessed as less than three 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, Suquet and Cosson, 2010; Hatef *et al*., 2010; Cabrita *et al*., 2010), and some details are also given in Section 5.7 (cryopreservation).
- Density of spermatozoa per ejaculate volume unit. This characteristic is evaluated visually. The sperm of appropriate quality should not have fewer than 1 billion spermatozoa per millilitre.
- Test of absence of motility without adding water should be used to control the quality of the sample.

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

10.3 EGG INSEMINATION

Guideline 10.3

Egg 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 in order to elevate the genetic heterogeneity of populations produced through controlled reproduction.

In order to obtain genetically different graded sturgeon progeny, it is highly recommended that eggs collected from one female be divided into 3–5 portions and each portion be inseminated 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 (Dettlaff, Ginsburg and Schmalhausen, 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 for 2 minutes, taking into account the fact that only 10–20 percent of spermatozoa will be active after 2 minutes (Dettlaff, Ginsburg and Schmalhausen, 1993; Billard, 2000).
- The limiting factor in insemination is loss of fertility of eggs after adding water. An additional 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, replacing the added sperm solution after one minute with clear and clean water.

10.4 EGG 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 procedure deviates from the natural embryonic development of sturgeon 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 impacts 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, sufficient oxygen should be provided and freshwater added to the mixture applied, although the oxygen consumption rates of early cell stages are relatively low. Egg 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 of their adhesive coating should be carefully rinsed in freshwater 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:

- Mineral mud, blue clay, talcum, milk and tannin may be used as de-adhesive substances.
- In cases 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.
- To reduce egg/embryo mortality, it is necessary to add fresh aerated water (for 20 minutes) to the system during 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 a 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 have 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, fibreglass or metal (aluminium). Because of corrosion risks and subsequent heavy metal ion release after long-term employment (years), 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 in order to facilitate optimum gas exchange (e.g. oxygen into the eggs, carbon dioxide release from the eggs). This can be achieved in different ways: (i) holding the eggs in a monolayer over which water flows in a well-controlled current (e.g. Heath tray); (ii) eggs are continuously moved and held in suspension by the water flow so they do not stick together (e.g. Zuger jars); and (iii) moving eggs at intervals (mechanically, e.g. Yushchenko incubators) while water exchange is kept at constant (excess) rates throughout the incubation period (see also Crespi and Coche, 2008). The rate of the incubation system water supply depends on the type of the system and the egg developmental stage.

The light level of incubation units is generally provided at a low level in accordance with the environmental requirements of the reproduced species in order to provide maximum survival rate of the embryos and to diminish the number of abnormal eggs and larvae. High light intensity is detrimental to most demersal fish embryos and retards 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 that may also enter 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 preferable that incubation units have a separate water supply to minimize transfer risks. However, if incubators are in the same room, transfer of spores can also easily occur via the humid atmosphere and may accumulate at wet points in the incubation room, acting as reservoirs. For all incubation units, the ratio of fertilized eggs to dead eggs must be calculated in order to assess the quality of the incubated eggs.

In general, 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, all system components should be checked, 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, as should 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 freshwater once again. The water supply should be adjusted depending on the incubation system type. Surface water should be avoided as much as possible. Preferably, groundwater should be used as this is bacteria-free and helps to avoid the use of antimicrobials from the start.
- For small quantities of eggs (sterlet for example), MacDonald jars could be very efficient incubation systems. Moreover, 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) and has to be combined with UV sterilization of water in the incubation unit.
- The light level of the incubation system should correspond to the environmental requirements of the fish species (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 assessment of the quantity of incubated eggs should be performed at loading of the eggs 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 practised in marine fish hatcheries where individual fish release several million eggs, a quick estimate without damaging the eggs is determined by 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 each day, depending on the daily mortality records (increase in frequency needed immediately when increased mortality occurs).
- If the UV bactericidal disinfection and thermoregulation are 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 the incubation period has proved most effective) (Mamedov, 2000). In cases where UV disinfection is repeatedly insufficient, a system design error has to be assumed and a reassessment of the UV unit and its capacity must be undertaken as soon as possible, while the capacity will have to be adjusted accordingly.
- In cases where groundwater 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, and backwash filters).
- The duration of the incubation period depends on the thermal regime and the specific sturgeon species; it should range from 1 000 to 1 500 degree-hours.
- The quality of water in the incubation system should meet the general hatchery requirements (FAO, 2007) as also flagged in the regulations for EU hatchery 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 or the hatched larvae.

10.6 HATCHING

Guideline 10.6

The hatchery should ensure proper conditions in the incubators (see above), conditions that 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 effort.

Justification

Hatching usually extends 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. Moreover, during and immediately after hatching, oxygen demand increases owing to increased activity. In order to prevent pre-larval mortality associated with a lack of oxygen, it is necessary to collect the pre-larvae in a timely manner from the incubation containers and larval collectors. Illumination has proved an important factor in determining the speed of the hatching of pre-larvae as well as their directed movement (in accordance with species-specific peculiarities of phototaxis).

Implementation guidance

The following aspects should be considered at this stage in the reproduction 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 follows a Gauss 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 an increase in the number of abnormalities and yield of larvae with lower yolk deposit. At temperature close to the lower limit, a prolonged incubation is observed, hence, more prophylactic work is needed. The duration of hatch of embryos from eggs takes 24–48 hours on average; however, efforts should be made to try to optimize incubation conditions so that hatching time is shortest 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 in order to assess the quality of progeny. Therefore, for quality assessment of eggs and larvae derived from controlled broodstock incubation, it is necessary to perform three replicate small batches (minimum of 150 eggs per replicate) from each female with precise counting of fertilized and hatched eggs. 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 cases of separated incubation) or for each sturgeon group (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 YOLK-SAC RESORPTION

Guideline 11.1

In the pre-larval stages, the hatchery should provide adequate environmental conditions, which could be groundwater in the beginning (after transfer of hatched larvae to rearing tanks) but should gradually become a supply of clean (pre-filtered) water from the river to which they will eventually be released (a reason why hatcheries should be built close to the release site).

Justification

Taking into account that homing (a return of mature broodstock to a spawning site in their "native" river) is an objective, then hatchery stock rehabilitation (and eventually enhancement) programmes should consider it highly desirable to hold pre-larvae and specifically post-larvae not in groundwater (artesian water) or any other foreign water source but use the intended home river as the water source. Despite economic and other operational considerations (e.g. favourable and pathogen-free groundwater and constant temperature, identical with the incubation temperature), the prime source should be river water from the release site. Despite the fact that there is no strong evidence about a strong homing behaviour in sturgeons, it is desirable to apply the precautionary approach on this subject, which is promoted by the Code (FAO, 1995). The need for early imprinting is also expressed in the "Wuhan recommendations" of the 6th International Symposium on Sturgeons (ISS6; Rosenthal *et al*., 2011).

As a consequence of possible olfactory imprinting in pre-larvae and most expressively at the transition to active feeding (Boyko, 2008; Boyko and Korniyenko, 2001) and in early juvenile stages, it should be obligatory for the holding and feeding of early life history stages to take place in waters taken from the rivers to which these fish will be released after rearing.

A gradual transfer from groundwater to river water is recommended as groundwater is the preferred medium for egg incubation. From studies with teleosts (e.g. salmonids), it has been well established that imprinting is particularly linked to a specific physiological state of juveniles and occurs in a fairly fixed time window. This subject needs further attention in sturgeon research dealing with rearing various sturgeon species for release.

Implementation guidance

The following aspects should be considered at this stage of the rearing process:

- Appropriate design of the pre-larval and larval holding units is important in order to allow prompt removal of dead larvae (which deteriorate quickly, altering the natural smell of the culture water).
- Constant temperature and degassing (in cases of supersaturation of river water) or aeration (in cases of initial groundwater blending) should be provided along with an appropriate (gentle) water exchange rate that permits sufficient flow between larvae for optimum gas exchange. Low water depth (20 cm) is considered appropriate.

- Holding of pre-larvae and ongrowing of larvae are generally performed in circular tanks of various materials (e.g. concrete, plastic or fibreglass) or in trays with a bottom area of 1–4 m^2 and a water depth of 17–20 cm. To avoid skin abrasion, the inner surface of these tanks or trays should be smooth and not rough.
- Prior to larval stocking, the inlet and outlet systems should be rinsed and their functionality checked; tank bottom and walls should also be disinfected and rinsed with freshwater.
- If the traditional technology is applied, the minimum water flow rate (1–3 litres/minute) should be set during the first 24 hours of pre-larval holding (approximately equivalent to a full water volume exchange in three hours). Subsequently, this flow rate should be increased to 8–14 litres/minute (equivalent to no less than a two-times tank volume exchange per hour).
- During the period of yolk-sac resorption, when pigmentation of pre-larvae has not yet developed, illumination should be kept low (as in egg incubation) in order to assure adequate development of the light-sensitive early stages (there may be species-specific differences; Kasimov, 1987; Chebanov, Galich and Chmyr, 2004).
- During the first 24 hours of pre-larvae holding, hatching may still continue and it is necessary to remove empty eggshells and unhatched dead embryos. Removal of dead larvae should be performed at regular intervals, at least 1–2 times per day. Dead embryos and larvae as well as larvae obviously infected by *Saprolegnia* should be frequently collected using a siphon. Removed specimens should be treated with a disinfectant (e.g. chloramine). Similarly, the equipment used (e.g. siphon) should be disinfected before reuse (when checking the next tank).
- To detect potential microbial infections, a sample of larvae should be checked by appropriate methods for anticipated target pathogens. Such check-up exercises should be performed every 3–4 days or more frequently if initial signs of an outbreak appear. In addition, parasitological examinations are also necessary employing the same schedule.
- The time of the transitional period to active feeding should be monitored visually by observing the behaviour of the pre-larvae (Dettlaff, Ginsburg and Schmalhausen, 1993). It should be noted that pre-larvae being initially in the quiescent state (swarming) will disperse along the bottom of the tank in search of food after starting active feeding.
- Optimal thermoregulation results not only in good survival and reduced occurrences of morphological and functional anomalies, but also enhances the performance of the controlled production of juveniles, especially at successive culture stages in ponds when rearing fingerlings to release size.
- The importance of evaluating the yolk-sac size and shape of pre-larvae in the course of rearing can hardly be overstated. The consequences of small yolk-sac reserves may affect larval growth to the point of onset of feeding, resultingin higher mortalities. A too-large yolk sac can be observed when osmoregulatory disorders have occurred during embryogenesis, and this may negatively affect the transition to exogenous feeding. Such disorders also have a negative effect on excretory functions during development. One of the indices to assess yolk-sac deformation in pre-larvae is the height-to-length ratio, which should normally range from 0.55 to 0.69, while for malformed (pear-like or oval) yolk-sacs this ratio can be as low as 0.29 to 0.44 (Belayeva, 1984).
- The duration of the yolk-sac resorption period depends on water temperature and ranges on average between seven and ten days. At start-feeding, the exchange rate of tank water should be increased, providing a high oxygen supply while also flushing the metabolic by-products out of the system.

- The preferred stocking density of larvae at the start of exogenous feeding varies with species. Past experience shows that a lower stocking density (700–1 000 individuals/m^2) supports good growth and survival, especially when prepared feeds are being used.
- Initial growth should be monitored by weighing a representative sample of fish at intervals of about 3 days. Based on the average weight gain, the daily feed ration should be recalculated.

11.2 REARING OF LARVAE FOR BROODSTOCK REPLACEMENT

Guideline 11.2

The hatchery should, in cases where 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 behaviour to formulated feeds, maintaining of optimal stocking densities and timely grading (sorting) of fry are important factors in determining success of the development of the broodstock.

Implementation guidance

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

- After a short period (1–2 days of feeding with *Artemia* nauplii), the recommendation is to use starter dry feed, with low stocking densities, following the existing methods of commercial sturgeon aquaculture (Ponomarev *et al*., 2002; Chebanov, Galich and Chmyr, 2004; Nekrasova, 2006).
- It is necessary to conduct size grading of larvae and fry at regular intervals. The resulting different cohorts should be reared further to avoid culture-based selection pressure and inbreeding effects. Regular sorting is mainly for good management practice to control the growth process, reduce behavioural stress and adjust feeding regimes according to size. Therefore, a sufficient number of tanks of various sizes should be available in the hatchery to allow operation of flexible logistic schemes to permit rearing in parallel various cohorts of the same origin.
- 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 and Williot (2002) reported that it is possible not to use nauplii, but to start with compound diets directly.
- Observation of the behaviour of larvae and small juveniles is useful for detecting unusual situations and stress factors in the tank.

11.3 REARING OF JUVENILES FOR RELEASE INTO NATURAL WATERBODIES

Guideline 11.3

Hatcheries aiming at restocking of natural waterbodies should prepare the juvenile sturgeons through training and adaptation to natural conditions in order to increase the rate of survival after release.

Justification

Experience from sturgeon restocking and rehabilitation programmes in the last few decades has shown that training and acclimation of juvenile sturgeon in tanks and ponds to the challenging conditions they will face in nature after release can help to increase survival rates of the released fish.

For the purpose of acclimation to more natural conditions, hatchery-reared juveniles should be ongrown in ponds where a variety of natural food organisms can be raised to allow this fish to learn to feed on various food items. In addition, small predators may be introduced into such ponds in small numbers that would challenge (attack) the fish but not be able to prey on the juvenile sturgeons. This would help to train fingerlings to learn to distinguish between friend and foe. Such fingerling raising should be done in compliance with modern (combined tanks/ponds) hatchery technology.

It should be further noted that feeding different types of live food organisms, especially during the onset of active feeding (at the end of the yolk-sac stage), supports the natural metabolism and physiological functions, including enzyme and thyroid hormone functions in tissues, partially because of the supply of these metabolic key components via the live food supply (Boyko, Grigoryan and Chikhachev, 1993; Boyko and Grigoryan, 2002; Boyko, 2008).

Such a feeding strategy is also beneficial for minimizing the frequency of morphological anomalies and supports olfactory imprinting towards chemical stimuli, thereby determining homing fidelity (return to native rivers) when reaching maturity and commencing spawning migration.

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 waterbodies, it is necessary to take the following aspects into consideration:

- A natural photoperiod (Ruchin, 2007) should be provided at the same or preferably at a higher level of illumination, corresponding to species-specific peculiarities of sturgeons (Kasimov, 1987). In general, at high illumination, an increase in the swimming speed of fingerlings can be observed. 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. Sturgeons do not perceive light of this frequency (Sbikin, 1974).
- An astatic thermal regime should be maintained with a daily amplitude of 4–5 °C or thermogradient field; this stimulates more intensive energy exchange and favours a higher survival rate of the fry (Konstantinov *et al*., 2005).
- Creation of a water flow in the tanks enables the fry to train their swimming capabilities (Shcheglov, Mineyev and Vitvitskaya, 2000) and improve the adaptive performance of their central nervous systems (Nikonorov and Vitvitskaya, 1993; Kozlov, Nikonorov and Vitvitskaya, 1989).
- The use of live feeds (nauplii *Artemia*, rotifers) enriched with omega-3 HUFAs will improve survival and growth of fry and enhance stress resistance.

11.4. FRY AND FINGERLING MONITORING AND QUALITY CONTROL

Guideline 11.4

Monitoring of the fry and fingerlings to ensure a high quality at the time of release of the juveniles into the natural aquatic environment should be carried out frequently by use of standardized methods. The use of fitness indices and environmental stressor 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 the quality of hatchery-produced progeny. Monitoring should be conducted not only prior to release into the natural environment but also throughout all the technological cycle of rearing. In the course of monitoring, it is necessary to check 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, Kasimov and Kokoza, 1984) and malformations of fish reproductive systems, can lead to a reduction in the number of broodstock that will be suitable for reproduction (Zaplavnaya, Yakubov and Kychanov, 2001). 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 the effects of stressors), rearing, training and evaluation of fitness indices of fry and fingerlings, as well as proper release of juveniles into natural waterbodies (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 hatching on the basis of species-specific behavioural responses to changes in water depth (only viable larvae are capable of "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 evaluation of their physical endurance, the general formation of their body, gills and swimming capacity and water current resistance (Khodorevskaya, Ruban and Pavlov, 2009).
- Assessment of the physiological state of larvae and fry based on the melanophores (pigment cells) background reactions reflects the state of the neurohormonal 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 enables evaluation of the frequency of morphological anomalies in progeny obtained at hatcheries from wild and domestic broodstock (Galich, 2000 Levin *et al.*, 2001, Chebanov, Galich and Chmyr, 2004; Goryunova, Shagaeva and Shevchenko, 2000).
- Evaluation of adaptive abilities of the fingerlings on the basis of their 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 and Vitvitskaya, 1993).
- Express analysis of the physiological development stage of larvae, fry and fingerlings enables assessment of tolerance to extreme values of basic abiotic stressors, such as high water temperature, salinity and oxygen deficiency.

Evaluation of 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 intraspecific variability and possible decrease in heterozygosity of built population, as well as the occurrence of environmental stressors (Valentine, Soule and Samollow, 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/left, and some other indices.

12. Rearing of juveniles in ponds

12.1 POND 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. However, costs 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 (Coche, 1986; FAO, 1994; Coche, Muir and Laughlin, 1995).

Implementation guidance

The recommendation is 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 programme. In the ponds, the fry and fingerlings, besides learning to find and feed on live food, learn how to prey on 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 disease-free.

12.2 DEVELOPMENT OF FEED POTENTIAL AND PHYLLOPOD 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. Therefore, the hatchery manager needs to make a detailed plan for pond preparation and management in accordance with regional climatic conditions and considering past experience.

Phyllopods occurring in the ponds suppress the development of sturgeon fry, resulting in a sharp decrease in their abundance owing to feeding competition. In some cases, fry can be eaten by phyllopods. Traditionally, various toxic preparations have been used to exterminate phyllopods, such as the tadpole shrimp (*Lepidurus apus*) and *Leptesteria* sp. (Chebanov and Galich, 2011).

Implementation guidance

In addition to the guidance provided in Section 8, the following aspects should be considered:

- When developing the natural live-food feed base in the pond, it is necessary 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.
- Besides conventional feeding activities, additional measures to enhance feed organism biomass (zooplankton and benthos) and establish a proper species composition should be applied, including:
- gradual (step-by-step) filling of ponds with application of organic fertilizers and Daphnia culture;
- introduction of nectobenthos feed organisms (mysids and gammarids, captured in the coastal areas of the sea and at future locations of fingerling release), and extermination of phyllopods.
- There are several methods of exterminating phyllopods:
- 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);
- application of biological ameliorators;
- repeated pond flushing (with rapid water drainage and refilling of ponds);
- 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 fry 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 juvenile rearing is 2 ha; at this size, the pond can still be properly managed in terms of marinating and improving its hydrobiological and hydrochemical conditions.
- Fry density in the pond is highly dependent on the species reared and live-food availability in the pond. Generally, 50 000–100 000 fry/larvae of 100 mg are

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

- In order to conserve the intraspecies population structure and create the necessary environmental conditions (thermal regime, photoperiod and feed availability) for all sturgeon groups, it is advisable 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-day shift from the traditional dates) is possible using thermoregulation of fertilized eggs to obtain an early rearing of the fry (Kokoza, 2004. 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 juveniles;
  - stocking during the traditional period (late April–May);
  - stocking during the second production cycle (July–August) by larger fry (1–2 g) at a lower stocking density (10 000 individuals per hectare and grow them to a weight of 7–10 g) (Grigoryeva *et al*., 2003; Kokoza *et al*., 2006)
- 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 the growth and health of the fish.
- During fingerling rearing, it is necessary to maintain an optimal level of water in the ponds. To limit development of filamentous algae, the constant control of hydrochemical and hydrobiological regimes should be performed throughout the whole period of rearing.
- The duration of fry/fingerling rearing in ponds depends on feed availability, but the average number of days is 30–35 (in some cases up to 45 days). During this period, the fingerlings reach a weight and fitness that make them 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), preventive and protective measures should be applied, such as:
  - nets and bands (shading) to prevent birds from reaching the water surface;
  - ultrasound scaring systems to scare away larger fish and aquatic mammals (through an acoustic signal);
  - gas-noise guns – highly effective at scaring away predator birds (such as cormorants), one gun can scare away all the birds in an area of 20–30 ha;
  - watch dogs and appropriate devices (e.g. traps and electric fences).
- To prevent damage to certain types of (endangered) predators (e.g. animal species listed as endangered), the preventive measures applied should be approved by regional and/or national conservation authorities.

13. Fingerling release

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

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, offspring 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 waterbodies, 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 wild, have suitable tagging procedures in place, select the best sites for release and arrange for suitable transportation to the release sites.

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. In an analysis of seven release experiments (including 53 stocking events), Lorenzen (2000) considered survival based on linear length growth with the objective of assessing the applicability of alternative release sizes. Although the analysis is based on freshwater teleosts, the principal usefulness of this approach should also be tested with sturgeons as current practices are mostly based on a rule-of-thumb approach 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 into 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 deprivation of reared specimens as compared with open-pond reared individuals. Moreover, with specimens developed in the wild, it affects the protective behaviour of the juveniles and their capability to form required reflexes (Kasimov, 1980). Therefore, it is highly advisable to provide a set of measures at the hatchery to improve basic fitness characteristics of the fingerlings in order to enhance the stamina and survival of the juveniles in the wild (see above).

In addition, it might be useful to develop further, through training, the necessary reflexes that make the juveniles better adapted to natural conditions. This “training” can include varying current velocity to gain higher sustained swimming speed and maximum escape burst. Moreover, small predators could be introduced 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 be unable to swallow them, thereby teaching the juveniles to distinguish between friend and foe. Such "training" to environmental cues may be at different levels corresponding to the intended size and/or age of release (e.g. larvae, fry, fingerlings and juveniles).

Implementation guidance

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

- Operational means should be provided 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 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 the 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 for 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 release into natural waterbodies. 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, Ruban and Pavlov, 2009).
- When planning for release, the availability of the required relevant human resources, equipment, maps and records has to be checked, as does the functional state of the needed special equipment. This is necessary to ensure 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 the fingerlings released should be conducted under the supervision of this "commission", which cross-links with similar bodies within the region to ensure good guidance, exchange of experiences and guaranteeing comparability of procedures.
- The release of fish produced in hatcheries should be performed only when a valid ichthyopathological certificate has been issued (FAO, 2007) and when approval has been obtained from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).
- Juveniles should be tagged before release in support of restocking-impact monitoring and sturgeon research and management purposes. The tagging methods need to be predetermined, as does 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 the biological peculiarities of the sturgeon species under consideration. Therefore, in selecting sites for release, consideration should be given to habitats and locations where, normally, the natural cohorts of the population thrive. Thus, it is essential to choose 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 process of selecting 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 at different times of the day (e.g. interim stress-free holding capacity on site).

A hydrobiological and hydrochemical study should be conducted at prospective sites for fingerling 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) in order 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 for ongrowing and negatively affecting the state of fingerlings (Levin, 1989).

Preferably, identification of potential sites and final selection of a release site 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 interspecific 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 the 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 of being 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 in 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 rate of released juveniles, it may be necessary to enclose a certain area at the release site temporarily and eliminate any predators prior to release. This will enable the released juveniles to acclimatize to the new environment without being hunted directly. This approach is 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, a step-by-step acclimation approach should be preferred at all stages while gradually transferring the juveniles from the culture facilities to the receiving waters.

13.3 TRANSPORT OF FINGERLINGS TO RELEASE SITES

Guideline 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 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 release sites, it is essential to consider the following aspects:

- During the loading and transport, the fingerlings should be constantly held in water.
- Loading of fish during the daily peak in temperature should be avoided.
- Juvenile densities in the holding facilities during transport should depend on the type of transport means, species, size of fingerlings and the conditions provided during transport (duration, temperature, use of oxygenation, etc.) and have to be carefully calculated by hatchery personnel.
- A sufficient number of means of transport have 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 the state, behaviour and feeding of fingerlings and broodstock, as well as periodically carrying out a complete external ichthyopathological examination.

Justification

Fish health monitoring is of critical importance for identifying at an early stage any potential problems that may arise during the culture period. Generally, diseases occur naturally, but they may become an over-riding problem under culture conditions. They often result from suboptimal rearing conditions, which adversely affect the physiology of the fish and reduce its ability to combat pathogens effectively. Moreover, rearing under controlled conditions often favours the development of bacterial strains well adapted to the rearing system, which makes it necessary to monitor the hygienic conditions in addition to the regular water quality monitoring of key parameters. This would allow either development of system-specific prophylactic strategies or recognition of early warning signs of unexpected outbreaks and the implementation of measures to counteract them in a timely fashion.

Furthermore, 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 from gaining sufficient immunocompetence so as to cope with common exposures in natural waters after release. Therefore, fish health status is considered vital as a prerequisite for release. Moreover, disease outbreaks with pathogens typical for culture systems can potentially result in: (i) a lack of fitness of the immune system against disease unavoidable in the receiving waters; and (ii) converting the cultured juvenile into a carrier, spreading pathogens to natural populations.

Implementation guidance

To prevent outbreaks of fish diseases in hatcheries, sanitary guidance standards should be strictly implemented, including those outlined in the Aquatic Animal Health Code (OIE, 2010). Specific references should also be made to several other guidelines and protocols, such as those developed by FAO (2007). These standards cover a variety of issues relevant to 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.

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 include:

- Grow-out tanks, ponds and other hatchery units should be cleaned regularly; periodic 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 with its own hatchery equipment, which should be disinfected prior and after use and kept in a dry and clean place.
- Rearing and feeding conditions should be controlled regularly; all observations and operations should be recorded in special diaries.
- All the entrances at the incubation unit and nursery facility should be equipped with a disinfection barrier that is 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.
- Transfers of fish from hatchery to hatchery should be planned carefully. Transfers during or immediately after incidences of disease should be strictly prohibited (considering the EU regulations for temporary closure).

Moreover, the following issues should be considered:

- In cases of transfers between facilities, fish health monitoring and appropriate quarantine measures are recommended in order 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 by a variety of critical indications, sometimes at early infestation stages. Therefore, regular control of the status of the fish reared allows a prompt response to such challenges at an early stage when simple metaprophylactic methods can still 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 in order to allow sufficient adaptation of the immune system.

Relevant protocols should be established. The assessment of 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. Therefore, it is necessary that: (i) treatments use standardized protocols; (ii) treatments are conducted according to good manufacturing practices; and (iii) no step in the treatment and subsequent handling of the fish jeopardizes 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 spread 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 of identifying at an early stage any problems that may emerge in terms of fish welfare, health and sanitary conditions.

Implementation guidance

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

- fish swimming performance and feeding activity (in comparison with 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 with observations during previous days);
- abnormal (unusual) production of slime on the skin of the fish;
- abnormalities in pigmentation around the mouth (potential indicator of yersiniosis);

- abrasive or irregular shape of pectoral fins (potentially indicating cannibalism and/or fungal infections);
- presence of sores, white spots, injuries, blisters, inflammation of the anal area;
- presence of ectoparasites and/or their cysts on the skin of the fish;
- gill malformation (hyperplasia, necrosis, erosion), extravasation (haemorrhage), necrotic nidus.

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

14.3 COMPLETE ICHTHYOPATHOLOGICAL EXAMINATION

Guideline 14.3

The overall ichthyopathological and physiological state of the fish should be examined 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 aetiology and development of disease outbreaks or will allow prophylactic measures to be employed at an early date 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 diseased individuals discovered early on in order to save the rest of the broodstock).

Implementation guidance

The ichthyopathological 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 advice. The routine measures may include:

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

The results of visual and complete ichthyopathological examination of fingerlings should be 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, 2010).

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, 2010; Arthur, Bondad-Reantaso and Subasinghe, 2008; FAO, 2008; ICES, 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 compartmentalization are procedures to define subpopulations of distinct aquatic animal health status for the purpose of disease control. Compartmentalization 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 as 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 compartmentalization 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 compartmentalization allows functional separation through biosecurity measures. Following an outbreak of disease, compartmentalization may help 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 also be useful 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 compartmentalization concept may subsequently lead to a culture system in complete quarantine.

Fish transferred from other hatcheries or captured in natural waterbodies and selected for domestication should undergo obligatory quarantine procedures over an acclimation period accompanied by regular health inspections. The period for quarantine varies with respect to the source of the fish and the target disease for which testing is required. Intrabasin transfers from health-certified facilities require less strict and shorter quarantine measures than interbasin 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 in order 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 cases of diagnosed diseases while in quarantine, treatment of the fish should be performed under the control of a fish veterinarian. Transfer from these facilities should follow only after diagnostic clearance. Otherwise, the fish will have to be disposed of in an approved manner. After quarantining, step-by-step 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 viral infections 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 apply best environmental practices by using the best available technology to minimize the risk of disease outbreaks while allowing the cultured specimens to build 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 adhere strictly in their day-to-day work.

Once 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 hygiene 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 disease 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 waterbodies 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 and the environment, while also employing safeguard 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, reactivity is faster to the point that decomposition of the disinfectant does occur. At low temperatures, the biocidal efficacy of most disinfectants decreases. Moreover, the 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 application. These should be followed strictly.

14.5.2 Fallowing

Justification

Intermittent utilization of facilities or production units is commonly recognized to be of value in managing and controlling the facility environment. As part of this strategy, fallowing can break reinfection cycles by removing loci of a disease from a farm. Consequently, fallowing is often carried out as a regular hygiene 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 reducing the risk of commonly reoccurring disease 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 outbreak, the infective period and distribution of the disease agent (or agents) within the system. If fallowing is required in order to eradicate a disease agent or to break the cycle of likely infectious reoccurrences 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 plans should be developed before such events occur.

Treatment with medications must take place only after diagnosis has been carried out and an effective treatment 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.

Antibiotics should only be used for fish designated for broodstock and used in line with national legislation. The application of antibiotics for treatment of fingerlings released into natural waterbodies is not recommended. Because of the health risk and the limitations of application as well as the issue of 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 among countries. It is strongly recommended that only those therapeutics registered for use in fish farming be used and that their application be executed only after prescription by an authorized veterinarian service, as is obligatory in many jurisdictions to safeguard aquatic production.Therefore, it is strongly recommended that the same safety measures and quality assurance protocols as used for handling of fish in commercial aquaculture be applied also to fish produced for release. In countries where such regulations are not mandatory, it is strongly recommended that they be employed voluntarily. Up-to-date diagnostic tools and test kits should be employed in order to ensure the most effective and environmentally friendly application. Regulatory authorities should include in 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 to feeding (more than six hours storage at room temperature is highly undesirable), or using specially prepared medical feeds provided by certified companies.
- Baths – the 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 cases of a sharp deterioration in the state of the fish, the treatment should be stopped and the fish transferred to containers with freshwater and a high rate of water exchange.

- Intramuscular injections – the preparation is injected into the body of the fish. 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 as bifidobacteria (Burlachenko and Bychkova, 2005). Special formulated feeds containing bacterial preparation are available for recovery therapy. The duration of the recovery course is not less than two weeks; however, this will depend on the severity of the event and should be determined in close cooperation with a veterinarian.

14.5.4 Anaesthesia

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, ichthyopathological 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, there should be time to interfere with the process if unfavourable conditions are unexpectedly encountered. Moreover, the concentration must be adjusted to the water temperature, as lower temperatures prolong the response of the fish. An 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 anaesthetics are recommended:

- tricaine methanesulphonate (MS-222);
- clove oil (eugenol);
- benzocaine; and
- propiscine (by gill irrigation).

Furthermore, good results have been obtained by using:

- CO_2 (also as a tranquilizer); and
- electricity (electromobilization) (Henyey, Kynard and Zhuang, 2002).

Aeration should 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 will generally significantly affect the efficiency of the entire operation. Moreover, application of self-developed (non-standardized) record forms will hamper the analysis of the enterprise's activity, and subsequently lead to restricted information exchange and, eventually, negatively affect the proper planning of the reproduction and release activities at the national level and at Caspian Sea Basin level. Therefore, proper documentation of the various production operations is 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 are important for the daily hatchery management itself, as the information collected and analysed 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 the national and Caspian Sea Basin levels.

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 levels.
- 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 the case of working diaries) of any data records;
  - details of the personnel 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 number of fish;
- identification code of the containers (water units) where the fish is held;
- operation performed at each time and the reason for the record (where not self-evident).

- 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.
- At regular intervals (at most after one month), backup copies of the entire monitoring dataset (preferably on a CD) should be maintained and be available for region-wide retrospective analysis once such programmes are initiated. For data security, backup 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:

- 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. The documentation will allow the tracking of biotechnical requirements and compliance with established protocols and standards. The record-keeping should be performed by staff directly involved in the operations (an example template is presented in the Appendix, Table A1.4). Further examples of how to perform record-keeping can be found in the FAO Training Series (Williams, 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 completion of the hatchery season, the documents should be kept for seven years. During this seven-year period, sturgeon scientists should be provided with the opportunity to analyse the data sets scientifically so as to undertake a trend analysis and improve the knowledge base not only on experimental data but also on practical operational data sets from large-scale operations producing juveniles for release.
- Specific (subject) reports, including acts of broodstock capture, transfer of stocking material between production and fingerling release units (facilities), and number of released post-larvae and/or 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.
- Pedigree reports. The documents of this category include diaries of pedigree activity, tagging, sampling of broodstock intended for genetic certification, as well as "passports" of brood fish with data on genetics (Rastorguyev *et al*., 2008), time of sexual maturity, absolute fecundity (for females), quality of gametes, and quantity and location of the progeny. The pedigree documents should be completed by the staff involved in these activities.
- 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 broodstock (of different species) with tag numbers including egg extraction from live females, mating, main results of rearing of juveniles (larvae, fry) at different developmental stages (issues mentioned in the Appendix, Table A1.4), species and production quantities of live feeds, as well as juvenile releases (species,

quantities and weight, place of location in natural waterbodies), number of incubators, larval rearing tanks and ponds used.

- 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 cost-effective scientific analysis at the level of the Caspian Sea Basin of data of hatchery operations in a comparative and compatible manner as briefly outlined under the above guideline. This is scientifically sound as the fish are released into shared waters that should be managed jointly.

16. Hatchery maintenance and repair

Guideline 16.1

As is standard in any modern aquaculture facility, sturgeon hatcheries should have proper maintenance and repair plans in place and implement these plans in a firm and timely manner in order to ensure continued safety of hatchery workers as well as the entire operation, thereby ensuring good support of fish performance (e.g. health and welfare). It will be only then that reliable long-term use of installations and equipment can be guaranteed while at the same time being cost-effective. Failures because of lack of maintenance or delayed repair often lead to substantial losses in both fish growth and survival and will, in the end, prove much more costly.

Justification

Past experience in the Caspian Sea Basin has been that proper maintenance, repair and timely renovation of hatchery facilities have often been neglected and delayed. Insufficient funding and limited operational budgets have frequently forced operators to manage on short-term strategies in the hope that severe system failures will not occur under these conditions. As a consequence, many hatchery buildings and quite a large part of the equipment can no longer be used or at least does not function properly. This situation is of particular concern as it also relates to electrical devices that can become a hazard to humans when working in a high humidity environment. Not only the safety of workers is at risk, but also the survival of the cultured specimens.

The well-being of the hatchery workers as well as the provision of a good and safe work environment for all parts of the operation (e.g. broodstock management, incubation, larval rearing and weaning) is a prerequisite that should not be compromised, and it needs to be addressed forcefully in the overall hatchery management and financial plans. The operational logistics must consider the lifetime of equipment and the time scale for proper maintenance and repair schedules. Carefully designed and realistic logistical maintenance plans will enable the useful life of all system components and equipment to be prolonged, resulting also in a longer depreciation period and timely identification of replacement plans for critical parts.

A proper maintenance and repair plan saves money and contributes significantly to the operational success expected to be achieved for predetermined objectives.

Implementation guidance

Proper maintenance and repair work in a sturgeon hatchery needs to follow some basic guiding rules that have to be incorporated into the overall management plan:

- During the operational season (mainly during the reproductive phase as well as larval rearing and weaning of juveniles), the daily cleaning should follow a fixed routine procedure that follows a given sequence of handling all tanks and equipment. This should also apply to the maintenance routine, replacement of those parts with a limited lifetime, and repair of system components that have been identified as defective or not functioning properly. For some parts, the replacement may be on a routine basis at set time intervals.

- At the end of the annual hatchery season, the entire system should be completely drained, ensuring that residual water from all system components (including intake and outflow pipelines and all valves) is removed and all parts are completely dried. Thorough cleaning and disinfection of all components commonly in touch with water during the operational period is highly advisable.
- The incubation units and all the installations associated with these units should be completely dismantled and disinfected. In outdoor facilities and also in indoor hatcheries that are not heated during winter, all parts should be dismantled and stored safely so as to avoid winter damage by frost. It should be self-evident that, after dismantling all the equipment, all the parts are to be thoroughly cleaned while also the hatchery rooms should be carefully cleaned in order to be ready for the next season.
- Handling equipment and system parts in this manner also extends the lifetime of all components while also allowing damaged and/or not well-functioning parts to be identified at an early stage, and so being able to replace them in time (before the next season starts).
- Reinstalling all system components well in advance of the next upcoming hatchery season should also include another round of careful washing and disinfection of all parts (e.g. water supply system, tanks, incubators, piping system) while checking the functionality of appliances and other operational devices such as monitoring systems, electrodes, aeration and oxygenation devices.
- All major repairs (and installations and replacements) within the hatchery should be conducted in accordance with established designs as detailed in the hatchery workplan.

In cases where the management decides at some point to close down the hatchery operation completely, all installations (buildings, indoor and outdoor facilities, tanks, equipment, etc.) are to be dismantled and disposed of in an approved manner, leaving no remains and debris behind but returning the terrain to its pre-hatchery state. Simply abandoning a site should not be permitted and a proper cleanup should be a legal condition to be incorporated into the contract or official hatchery permit.

The licensing agency may consider allowing some elements to be left in place, provided that a subsequent use of buildings or ponds is already identified and legally licensed before the hatchery operation is finally closed down.

Following the "polluter pays" principle, it is recommended that all waste is removed from the hatchery site before handing over the site to a new owner or before abandoning it. Again, this should be an obligatory undertaking that is already built into the licensing process.

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 largely on its professional management and well-trained, dedicated and motivated staff. Recognizing the limited availability of experts trained in sturgeon hatchery design and operations, it is essential that selection of staff is undertaken in such a manner that: (i) the selected team includes the necessary expertise for the key operational functions (e.g. broodstock capture and handling, mass incubation of eggs, rearing of larvae and weaning of juveniles for release); and (ii) continued training (upgrading) is provided for staff members in all fields of hatchery work.

It is essential that sturgeon hatcheries and all activities related to their operation are carried out in a safe and healthy work environment where also fair conditions for workers are met for all hatchery staff members in line with national legislation (e.g. often laid out by the respective workers compensation board) while also meeting standards adopted by the respective international or intergovernmental organizations supporting hatchery operations at the international level.

Implementation guidance

The adoption of hatchery practices that reduce health and safety risks and hazards for workers (e.g. the use of protective clothing – where necessary – and equipment) is a priority. This could include education and training programmes to build hatchery workers' knowledge and skills.

The following measures are recommended:

- Regulate working time while maintaining the flexibility required to manage seasonal and market-related fluctuations in the demand for (agricultural) labour (Convention 184 of the International Labour Organization [ILO, 2001]).
- 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 those on 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 perhaps 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).

17.2 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 that workers possess in-depth knowledge of 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 updating of knowledge among hatchery staff.

Implementation guidance

Recognizing the fact that staff capabilities and knowledge are the key factors in the success of hatchery management and operations, the following aspects should be considered:

- Capacity building of sturgeon hatchery staff, through training, can take various forms and could include *inter alia* 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 and be generally promoted and supported by competent authorities.
- Appropriate training at all levels (management, biologists and technical personnel) is required for handling endangered fish species and in particular sturgeons and in order to ensure professional handling of broodstock specimens, appropriate technical management of culture systems, reproduction (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-level and national-level training and education centres and dedicated programmes should be used to ensure professional training and education; through 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 understanding of the performance capacity of released sturgeons (in the light of potential increase of hatchery efficiency, survival and growth in nature, and number of returns per recruit); better assist policy decisions with regard to sturgeon rehabilitation programme needs for successful population recovery in the aquatic environment; and improve sturgeon stock management in the Caspian Sea Basin in general.

Justification

Although hatcheries and research institutions around the Caspian Sea Basin have dedicated decades of research and monitoring to sturgeon rehabilitation and stock management, there are still many gaps and uncertainties in the current knowledge base available on natural and controlled reproduction and the effects of culture on performance of fish after release (Secor *et al*., 2000). There is considerable uncertainty associated with the biotechnology employed for reproduction and rearing, including the standardization of large-scale production (millions per season), in particular as it relates to fitness of the fish for meeting the physiological and behavioural performance requirements when released into nature.

Any research on culture for release needs a coherent study design with clearly formulated scientific working hypotheses that include a paradigm shift from employing conventional aquaculture strategies towards close-to-nature cultivation approaches that effectively avoid any performance deviations of the cultured specimens from the natural population. For documenting the performance of fish, a strong system of adequate and comprehensive data collection through monitoring of the biological and environmental parameters is equally important as monitoring the past and current culture practices and the trend in performance they produce.

Monitoring should not stop at the release point. A coherent and adequate monitoring programme should also enable investigation of the factors affecting the performance of the released fish under given natural conditions that vary over seasons and years. The impacts of the changing environmental and ecosystem conditions are largely unknown, have so far received little attention and urgently require extensive study. Here, the data from a sound monitoring programme should greatly assist in identifying the necessary adjustments to be made in future rearing procedures so as to help improve the fitness of cultured fish designated for release.

First, monitoring steps should include *a posteriori* 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).

Second, 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 *a posteriori* analysis of the rearing conditions, of differences between hatcheries and of their subsequent outcome. Again, such monitoring is required to avoid the development of rearing conditions that adversely affect parts of the population and result in unintentional and unwanted genetic selection. At the same time, it is also needed in order to arrive gradually at commonly agreed standard procedures that will be adopted by all hatcheries to improve performance.

Third, 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, in order 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 in order to identify future improvements in hatchery practices:

- Identify magnitude of natural reproduction.
- Differentiate performance of both groups – naturally produced and released fish – (eventually making it necessary to be able to apply genetic or physical tagging 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, compared with fish from wild reproduction;
- identifying 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);
- assessing growth performance;
- determining behavioural differences between both groups (naturally produced and released fish) with regard to swimming activity, migration patterns, predator avoidance;
- determining proportions, ages and composition of broodstock originating from stocking attempts;
- determining homing rate of released fish;
- identifying reproductive success of released fish in the wild and in the hatchery upon catch;
- determining the vulnerability of released fish to fishing compared with offspring derived from the natural population (also as part of the objective to reduce such effects through appropriate mitigation measures);
- determining the contribution of the released fish to the natural population.
- In cases where habitat improvements are implemented anywhere within the natural distribution of the released fish (through wastewater purification, improvements in juvenile feeding grounds through reduced utilization for other purposes, creation of 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 hatcheries, as, traditionally, the focus has been on biology/ichthyology research while possible improvements in other fields have been ignored.
- Research programmes on sturgeon hatchery issues should work across hatcheries and multilevel governance systems at the 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 CAB and the CACFish.
- Adequate resources, including research facilities and trained staff should be provided for sturgeon hatchery research programmes. These programmes should receive 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. Therefore, the introduction of proper record-keeping and documentation at hatcheries is 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 and 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 sturgeon rehabilitation, restocking and management programmes.

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

Aspects that have been identified and require urgent monitoring and research include those outlined below.

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 basis including the interannual 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 the rearing conditions to be optimized to provide secure recruitment into the reproductive guild in time.

Reproduction

Currently, despite the progress made in the last ten years, understanding remains far from complete as concerns the impact and the resulting regulation for current, salinity, temperature, photoperiod and physiological state. With a more complete understanding, hormonal manipulation would not be necessary and, hence, reproduction would be without the selection aspect currently prevailing through this impact.

In the meantime, the outcome of controlled reproduction might readily be improved by: the establishment of sound and easy-to-use predictors for ovulatory success; the identification of species-specific minimal dosage for hormonal induction; the identification of the effect of the induction on progeny fitness; improvements in fertilization techniques to match conditions (and the selective pressure in natural conditions); and the determining of the genetic differences indicating the functional differences of biological groups.

Early life stages

Again, in early life stages, the most critical aspect for the survival and well-being of the fish is associated with a 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 behaviour 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. Static environmental conditions are not the key target, but rather environments that reveal comparable fluctuations in accordance with the river systems utilized. Therefore, the first question is which parameters have to fluctuate? Second, what are the amplitude and the interval of fluctuations?

Also related to the behaviour 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 provide the ability to determine and memorize the olfactorial stimulus. This point has not yet been determined in sturgeons with sufficient reliability. Moreover, it is necessary to verify the results in a field study in order to determine differences in return rate upon maturation.

Handling

Sturgeons are reported to be extremely hardy when compared with higher teleost fishes. Nevertheless, handling, as well as crowding, imposes stress on the animals, which in turn reduces fitness and affects immune response, increasing the risk of disease outbreaks. Several studies describe the stress response in fish in relation to rearing density (e.g. Wuertz *et al*., 2006), the effect of confinement and handling (Webb *et al*., 2007), and the effect of feed deprivation (Deng *et al*., 2003).

There is a lack of guidelines for optimal rearing densities and rearing conditions with regard to tank design and light climate based upon physiological data. Moreover, no comprehensive 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 example, during grading).

Genetics

Genetic sampling was mentioned above in Section 5. 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 in order to allow *a posteriori* analysis of the genetic makeup of the population where the means are not readily available.

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

Diseases

A major topic for future research concerns disease interactions. The knowledge about specific or ubiquitous diseases in sturgeons under controlled culture conditions is still very incomplete. For example, although viral diseases have been reported for years, a standard technique for identification is still lacking. Moreover, 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 (whose production is destined for markets for human consumption), broodstock rearing in particular allows and requires adapted, safe and effective treatments in order to gain the long-term safeguarding effects for 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, is urgently required in order to enable adapted transfer protocols to be developed, also in international trade.

One major research question concerns disease susceptibility and the current standards of regulating releases of infected fish. It seems necessary to identify the rate and specificity of such infections to certain genetic groups. In turn, the question can also be put as to whether 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 help identify urgent research topics that will require ample attention in order to improve 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 surrounding local communities and the country (or region) they are located in, and they 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 to produce 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 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. These reports also discuss environmental performance in terms of the resources used and the environmental impact of the activities. While for sturgeon hatcheries this may be too onerous a task at present, it is recommended that step-wise action towards reporting on these fields is 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 its natural aquatic environment may be an attractive partner for national companies and transnationals 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), possible pollution (e.g. smell), 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 example, purchasing local products where this is possible and incorporating local people among its staff.
- Avoid social dumping – particularly important in this context. Specifically, in areas where employment 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. Therefore, it should be imperative that transparency of employment policies is guaranteed,

in particular in hatcheries that have received investment support from public sources or international or intergovernmental donors.

- Provide a safe and fair working environment. Wherever new hatcheries for sturgeons are constructed, the respective licensing authorities should demand that minimum worker welfare and fair working conditions are identified. These should then be linked with the hatchery licence while also being enforced through regular control measures.
- Promote application of good practices (best available practices), standards (best available technologies), guidelines and codes of conduct on hatchery aspects as well as practices for sturgeon release to natural waters through appropriate advice to and training of workers. Awareness should also be raised among others that the hatchery always applies these practices, measures and tools on the basis of up-to-date knowledge.

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. ecohydraulics) and national and transboundary movement of live sturgeon.

Relevant global instruments include:

- Convention on International Trade in Endangered Species of Wild Fauna and Flora (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 came 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 (the Code [FAO, 1995]).[1] The Code 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; fisheries research; general principles; and provisions related to its implementation, monitoring, updating; and the special requirements

1 The full text of the Code and related technical guidelines are available at: www.fao.org/fishery/ccrf/en

of developing countries. Again, this Code is of considerable assistance but – by design – does not contain specific recommendations as to the operation of sturgeon hatcheries.

- The Ramsar Declaration on Global Sturgeon Conservation is another voluntary instrument in the "sturgeon conservation strategy" 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, 9–13 May 2005, Ramsar, Iran (Islamic Republic of), and subscribed to by many of the key sturgeon experts in the world.[2] The declaration has also been published in the Proceedings of the 5th International Symposium on Sturgeons (Rosenthal *et al*., 2006) and contains highly pertinent recommendations of major importance for sturgeon broodstock and hatchery management.

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

- The Tehran Convention (Tehran, 2003). The Framework 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 Sea littoral States. While this convention does not specifically mention sturgeon, its implementation includes activities on the conservation and rehabilitation of sturgeon stocks.[3]
- The Agreement on the Central Asian and Caucasus Regional Fisheries and Aquaculture Commission (CACFish) (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 came into force on 3 December 2010. CACFish 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 presidency of the CAB rotates every two years among the five Caspian Sea riparian countries. During the two-year period, the presiding country also acts as the CAB Secretariat. The CAB aims to: coordinate among range States on conservation and exploitation of aquatic bioresources of the Caspian Sea; 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 evident that sturgeon hatcheries will also be bound by their provisions. The implementation of most of the conventions, agreements and other instruments is supported by the secretariats and organizations that host these conventions and agreements. Through the respective authorities for sturgeon conservation and management at the national level, sturgeon hatcheries can seek specific guidance and support on the implementation of the relevant conventions and agreements.

2 The Ramsar Declaration on Global Sturgeon Conservation is available at: www.wscs.info/publications/iss/RamsarDeclarationEnglish.pdf

3 The test of the convention is available at: www.tehranconvention.org/spip.php?article4

21. Implementation and updating

In order to facilitate implementation and updating of the guidelines set out in this technical paper:

- 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 this technical paper.
- Relevant international and national agencies, including non-governmental organizations, should promote the guidelines among those involved in sturgeon production, reproduction and restocking.
- FAO, the WSCS and the IUCN/SSG, as appropriate, will promote the endorsement of and support the implementation of the guidelines via regional commissions, meetings and conferences.
- CACFish will monitor the implementation of the guidelines among its members on a biennial base, through a survey.
- FAO, the WSCS and the IUCN/SSG, as appropriate, may revise the guidelines, taking into account 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 [CRESPI AND COCHE, 2008])

Acclimation: Adjustment of organisms to conditions in the laboratory.
Aquaculture: The farming of aquatic organisms in inland and coastal areas, involving intervention in the rearing process to enhance production and the individual or corporate ownership of the stock being cultivated.
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 for Recreational Fisheries* [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.
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.
Restocking: The release of cultured or wild caught aquatic species (usually juveniles) into the wild to restore the spawning biomass of severely overfished stocks to levels at which they can once again provide sustainable yields.
Stocking: Process of moving live organisms to a rearing unit so that ongrowing (e.g. in nursery ponds, fattening ponds) or reproduction (e.g. in spawning ponds) may take place.

Appendix

Tables and figures

TABLE A1.1
Effective population number for a given year class (breeding event) based upon the actual number of males and females used in breeding

	Number of female parents											
Number of male 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

Source: Adapted from St. Pierre (1996).

TABLE A1.2
Recommended water quality parameters for a sturgeon hatchery

Parameter	Value
Alkalinity, mg/litre as $CaCO_3$	50–400
Ammonia (unionized), mg/litre	< 0.01
BOD_5, O_2 mg/litre	< 2.5
Cadmium (soft water 100 ppm alkalinity), mg/litre	0.004
(hard water 100 ppm alkalinity), mg/litre	0.003
Carbon dioxide, mg/litre	0–10.0
Copper, mg/litre in soft water	0.006
Dissolved oxygen, mg/litre	5.0 to saturation
Gas saturation	< 105%
Hydrogen sulphide, mg/litre	0.002
Iron, mg/litre	< 0,01
Lead, mg/litre	0.03
Nitrite, mg/litre as N in soft water	0.1
in hard water	0.2
Oxidability permanganate, O_2 mg/litre	≤ 10
Ozone, mg/litre	0.005
pH	6.5–8.5
Salinity, ppt for fry for juveniles for adults	0–0.5 0–3 3
Total hardness, mg/litre as $CaCO_3$	10–400
Total suspended and settleable solids, mg/litre	80 or less
Zinc, mg/litre	0.03

Source: Modified after Conte *et al*. (1988).

Figure A1.1

Layout of a Yushchenko incubation system

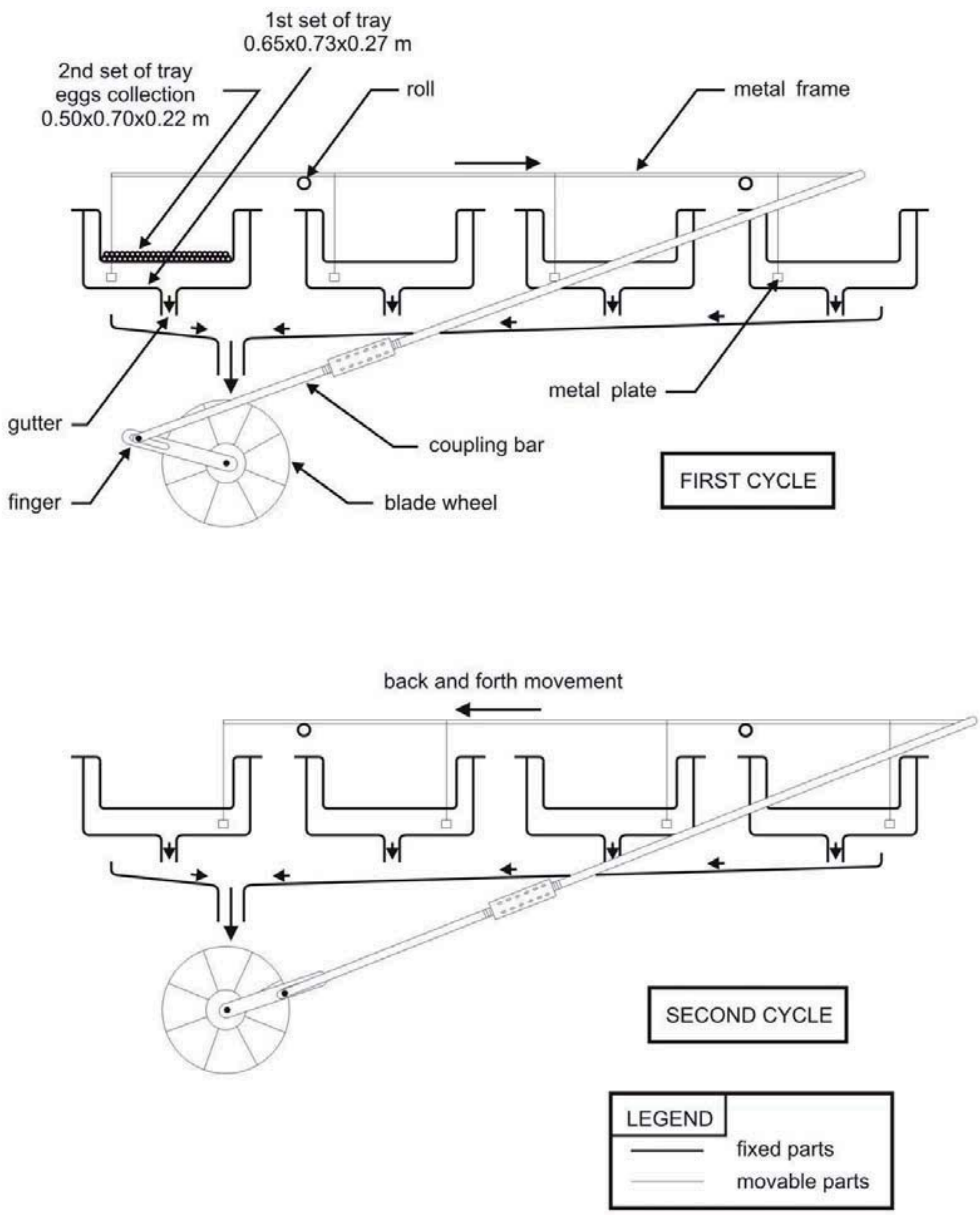
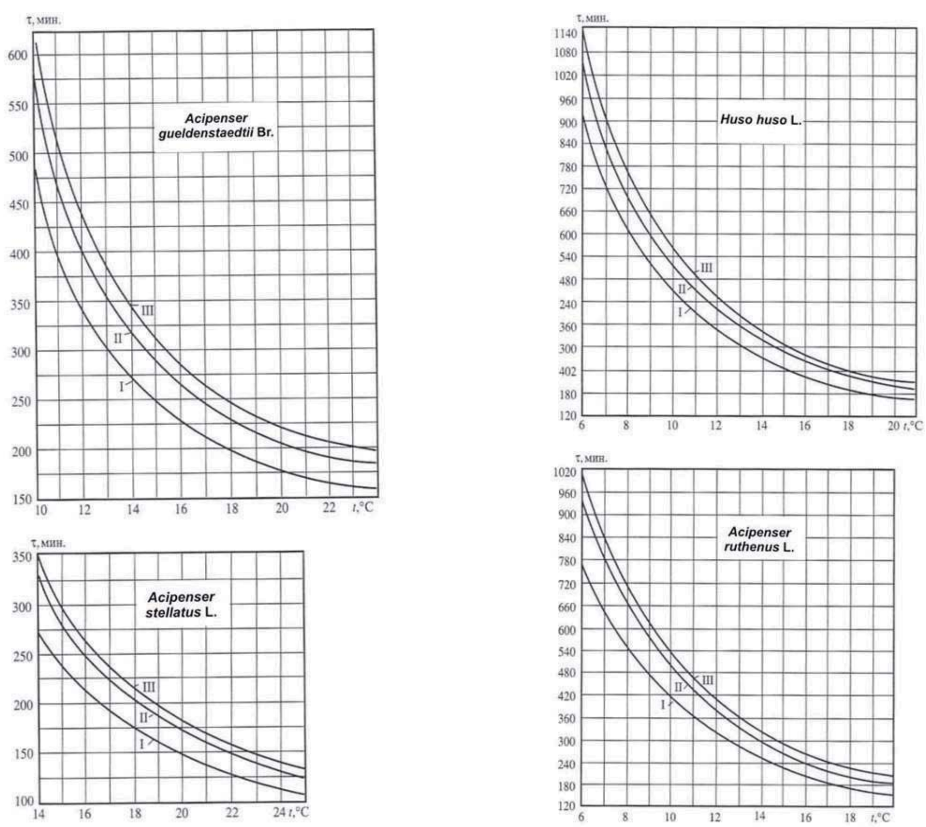


TABLE A1.3
Recommended egg load for incubation systems (different sturgeon species)

Species	**Incubation system**					
	Yushchenko(for 1 section) *	**"Osetr" (for incubation box)**	**MacDonald**			**Weiss (8 litres)**
			5 litres	6.5 litres	10 litres	
	(thousand individuals)					
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.
Notes: Standard egg load for "Osetr" incubation system is 2 kg per box. It is equal to approximately 100 000 eggs but not 150 000 as in table (50 eggs in 1 g). For beluga, this parameter is equal to 70 000–80 000 eggs (35–40 eggs in 1 g). For stellate sturgeon, the values in the table are correct.

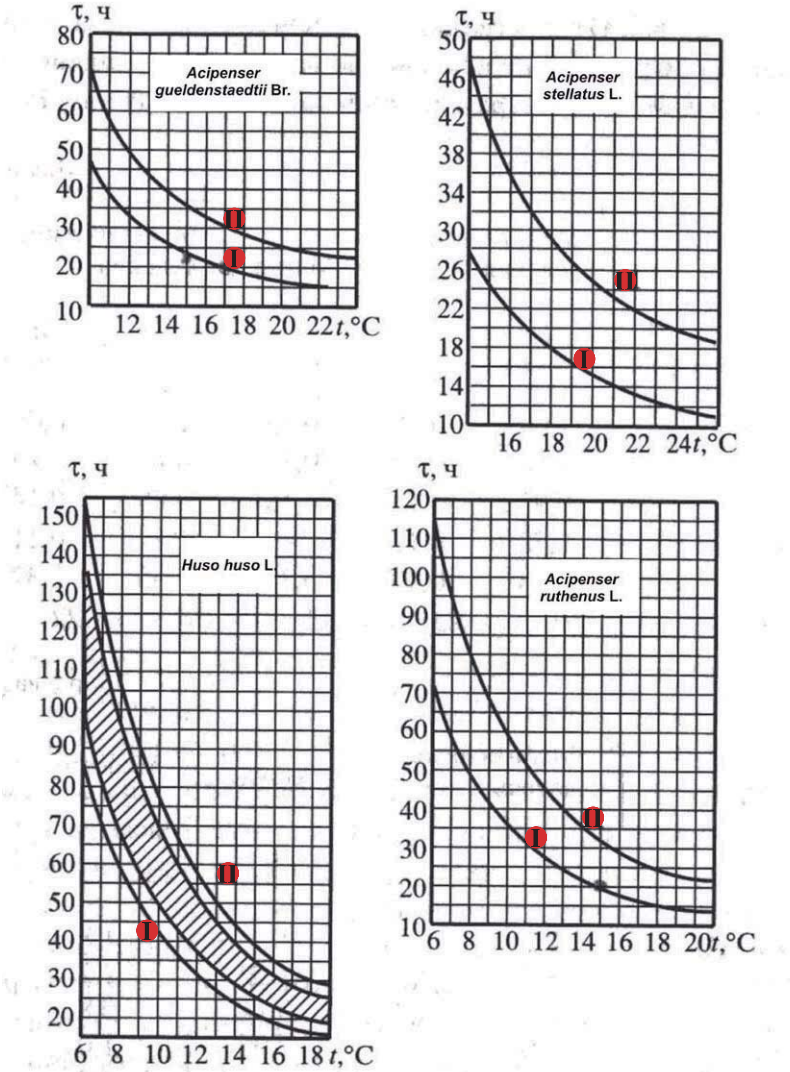
Figure A1.2
Diagrams enabling evaluation of time of the second and third blastomere cleavage divisions and time of completion of the second division on the surface of the egg in Russian, giant and sterlet sturgeons



Source: Dettlaff, Ginsburg and Schmalhausen (1993).

FIGURE A1.3

Duration of maturation in Russian, stellate and sterlet sturgeons females after hypophyseal injections at different temperatures



Note: Curve I shows the time of first female maturation; curve II – time after which the females do not mature or produce eggs of poor quality.

Sources: Igumnova (1985); Dettlaff, Ginsburg and Schmalhausen (1993).

TABLE A1.4

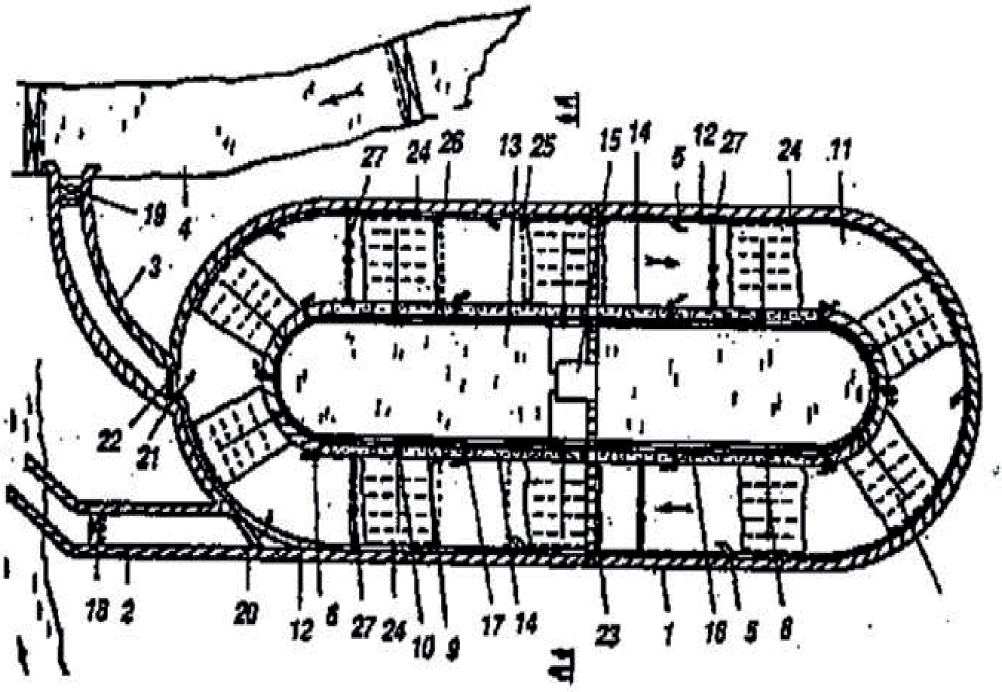
Species, seasonal race (intrapopulation group) strain. Individual tag, personal DNA fingerprint. Year of capture or year of culture

No.	Date	Size/weight characteristics			No. of generation or maturation order number ♀	Eggs, quantity				Used males (no. tags)	Egg fertility (%)	Yield of larvae	Survival of 3–5 g fingerlings	Comments, progeny location
		Length (cm)		Weight (kg)		Eggs weight, (kg)	% of total body weight	In 1 g	Thousand individuals			(%)	(%)	
		L	l_1	W										
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Note: L, cm – total body length; l_1, cm – distance between the end of the snout to the end of caudal fin mid-rays.

FIGURE A1.4

Artificial spawning complex for natural propagation of sturgeon with controlled regime and ensuring conditions of "pseudomigration" of brood fish



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.

Sturgeon hatcheries play an important role in the rehabilitation of the sturgeon stocks in the Caspian Sea and elsewhere. The guidelines presented in this technical paper aim to increase global awareness and to guide and build capacity about the best practices currently available by providing senior and mid-level sturgeon hatchery staff a practical tool for modern sturgeon hatchery practices and management. The guidelines focus on hatchery practices that are aimed at reproduction and growth of fry and fingerlings for restocking objectives.

The guidelines address a wide range of issues, including: hatchery design and location; collection and transportation of wild broodstock; selection and maintenance of broodstock; tagging of sturgeon; water quality and supply; feeding and feed quality; selection of broodstock for controlled reproduction; spawning and gamete processing; rearing of larvae and juveniles in tanks; rearing of juveniles in ponds; release of fingerlings; sanitary and hygiene measures; hatchery documentation; hatchery maintenance and repair; staff and labour issues; monitoring and research; social and environmental responsibility; international regulations and conventions on sturgeons; and implementation and updating of these guidelines. The guidelines provide specific guidance, background and justifications, and make suggestions to support their implementation.

The guidance provided is based on the FAO Code of Conduct for Responsible Fisheries (1995) and contributes to the implementation of the Ramsar Declaration on Global Sturgeon Conservation (2006).

South Branch Federal Centre of Selection and Genetics for Aquaculture

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