Manual on Sturgeon Reproduction

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I. Introduction

In the 1800s, sturgeon often became entangled in commercial fishing nets, and the fish were discarded as a worthless nuisance. Today, the sturgeon is recognized as one of the world's most precious commercial fish, mainly prized for its caviar, but increasingly also for its meat and as an ornamental fish.

Many sturgeon species are threatened with extinction. Aquaculture, including growing, nursing and reproduction, offers the solution for a sustainable sturgeon production. Furthermore, sturgeon culture is also considered as a business commodity with great economic potential.

The first trials in sturgeon farming were already carried out in the mid 19th century, however, sturgeon culture has undergone great development and advances during the last decades. Nowadays, some sturgeon species are already successfully being reproduced and raised in captivity.

Figure 1.

Unlike their reputation as slow growers in the wild, several species have proven to have very high growth and survival rates and to be tolerant of extremely high stocking densities in culture situations. However, there are also several challenges to the production of sturgeon. Supplies of broodstock and fry are limited. There is a long maturation period before females

produce ripe eggs for reproduction. Sturgeon require moderate temperatures for ideal growth and an ample supply of well water. Building facilities for sturgeon production requires high amounts of investment and operating capital. Nevertheless, it is expected that the significance of sturgeon culture will continue to rapidly grow in the coming decade.

The basis of every fish farming operation is successful reproduction and the security of a regular supply of high quality fingerlings. Therefore, Coppens International offers this manual to assist in the set up of reliable hatchery procedures for sturgeon. This booklet contains a general description of the sturgeon biology and describes relevant items for successful reproduction and hatchery management as known for sturgeon at present. It has to be noticed that the described procedures are only meant as guidelines. All mentioned parameters need to be checked and adjusted by the hatchery manager to the specific sturgeon species, life stage and the particular hatchery conditions.

Apart from proper management, feed quality is a prerequisite for successful reproduction. SteCo is the Coppens International sturgeon feed range that is especially developed for this species and tested at the Coppens Research Centre (CRC). All Coppens International feeds are kept up-to-date according to the latest technologies and scientific knowledge. Furthermore, Coppens International works in close cooperation with its customers. The result of this combined action ensures that optimum feed quality is reached and that excellent growth is achieved. In this way Coppens International contributes to the maximum economic results for the farmers.

Biology

Sturgeon are one of the oldest groups of living vertebrates and are often described as "living fossils", with records dating back more than 150 million years. Although life-history traits vary among species, Acipenseriformes are generally long-lived fishes with a slow growth and maturation rate. Some species (e.g., *Huso huso*) can live for 100 years and exceed 2,000 kg in mass. Such specimens are no longer found but fish over 100 kg are still caught.

General morphology

Sturgeon skeletons are mostly cartilaginous (like sharks). They have spindle-shaped bodies, snouts with sensory barbells and heterocercal or top-elongated tail fins. They do not have scales, but five rows of large, bony plates called scutes on their back and sides. Paddlefish lack scales and scutes and have a large extended rostrum.

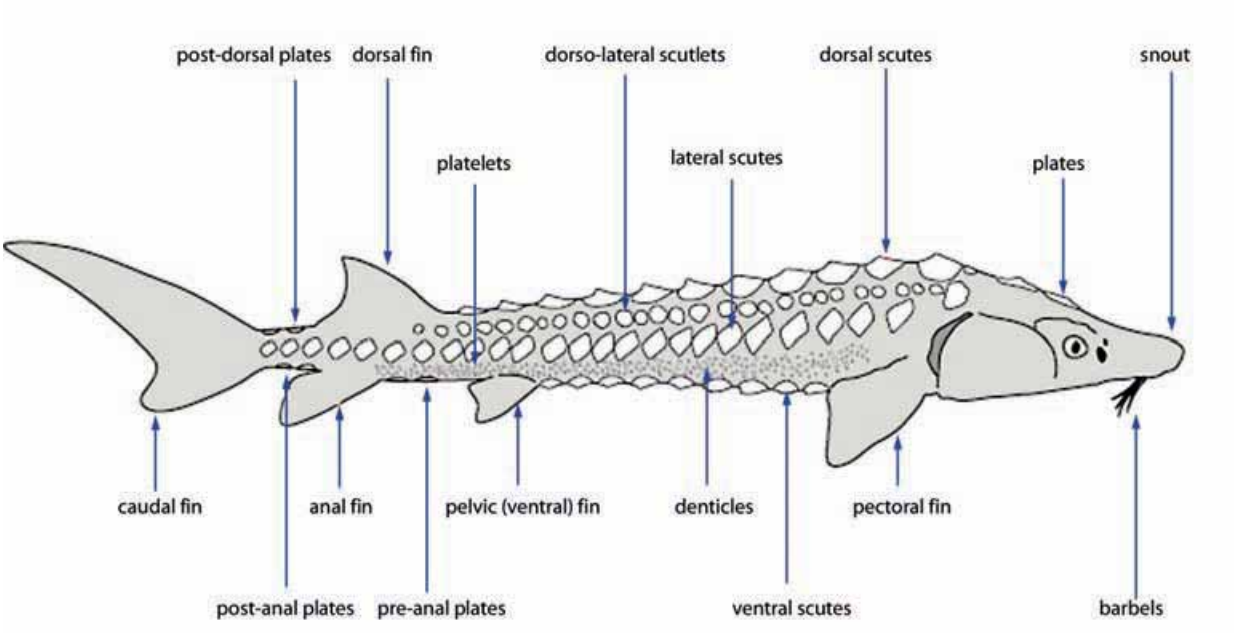

Figure 2. Major morphological characteristics of sturgeon and paddlefish.

Geography

Sturgeon populations are found primarily in cold and temperate regions of the Northern hemisphere, i.e., in North America, Europe and Asia. A few species are found in the rivers on the east and west coasts of Canada and the United States, and in the Mississippi River drainage basin. Other species occur in rivers of Europe, particularly rivers that empty into the Atlantic Ocean, the Adriatic Sea and the Baltic Sea. The most important species of sturgeon are found in the region bordering the Black Sea, the Sea of Azov, the Caspian Sea and the Aral Sea. Sturgeon are also found in watersheds of Asia in rivers emptying into the Sea of Okhotsk, the Bering Sea, the Barents Sea, The Kara Sea and the White Sea.

Habitat and natural feeding

Acipenseriformes inhabit rivers, estuaries, near-shore oceanic environments and inland seas. Some sturgeon species spend their entire life in freshwater (potamodromous). However, the majority of them are anadromous; they spend their adult life in the sea but swim upriver to freshwater spawning grounds in order to reproduce. They spawn in habitats with hard substrates (e.g. gravel, cobbles, boulders) with varying depths and water currents.

Figure 3. Protractile mouth

Most sturgeon species are bottom dwellers and feed benthically on insect larvae, small fish and occasionally on fish-related carrion. In rivers that support salmon populations, sturgeon will forage on roe, as well as the decaying salmon remnants.

Sturgeon possess tactile barbels located at the front of a thick-lipped, protractile mouth. Sturgeon also dig with their rostrum in search of food. Their eyes are very small relative to fish size and probably do not contribute much to the location and capture of prey.

Due to their benthic feeding habits and their late puberty, which increases bio-accumulation in the various tissues (muscles, fat, gonads, etc.), sturgeon are more sensitive to habitat degradation than most other fish.

Sturgeon species

The order Acipenseriformes contains 27 species and is divided into two families, Acipenseridae (sturgeon) and Polyodontidae (paddlefish), see table. There are several hybrid species, of which the bester (*Huso huso* x *Acipenser ruthenus*) is best known.

The 27 species of sturgeon; scientific and corresponding common English names.

Scientific name	English name
Acipenser baerii	Siberian sturgeon
Acipenser brevirostrum	Shortnose sturgeon
Acipenser dabryanus	Yangtze (or Changjiang) sturgeon
Acipenser fulvescens	Lake sturgeon
Acipenser gueldenstaedtii	Russian sturgeon
Acipenser medirostris	Green sturgeon
Acipenser mikadoi	Sakhalin sturgeon

Scientific name	English name
Acipenser naccarii	Adriatic sturgeon
Acipenser nudiventris	Ship sturgeon
Acipenser oxyrinchus	Atlantic sturgeon
Acipenser persicus	Persian sturgeon
Acipenser ruthenus	Sterlet
Acipenser schrenckii	Amur sturgeon
Acipenser sinensis	Chinese sturgeon
Acipenser stellatus	Stellate sturgeon
Acipenser sturio	Common sturgeon
Acipenser transmontanus	White sturgeon
Huso dauricus	Kaluga
Huso huso	Beluga
Pseudoscaphirhynchus fedtschenkoi	Syr-Dar shovelnose sturgeon
Pseudoscaphirhynchus hermanni	Small Amu-Dar shovelnose sturgeon
Pseudoscaphirhynchus kaufmanni	Large Amu-Dar shovelnose sturgeon
Scaphirynchus albus	Pallid sturgeon
Scaphirynchus platorynchus	Shovelnose sturgeon
Scaphirynchus suttkusi	Alabama sturgeon
Polydon spathula	Paddlefish
Ploydon gladius	Chinese paddlefish

Figure 4.

Production

World sturgeon populations have tremendously declined during the last decades. After peaking in the late 1970s, sturgeon fisheries catch is currently at its lowest level ever. This decline is due to overfishing, the use of non-selective fishing gear, poaching, habitat loss (by dam construction, water pumping and dredging) and environmental degradation.

All sturgeon species have been listed in the Appendices of CITES since April 1998. Most species are listed in Appendix II and trade in them is legal if they are accompanied by the appropriate CITES export or re-export permit. Trade in protected species (CITES Appendix I) is prohibited and must be accompanied by CITES import and export permits.

Due to an increased aquaculture production, capture fisheries accounted for a decreasing percentage of total global production (capture and aquaculture combined), which levelled off in the mid-1990s. At present, aquaculture production is far more important than wild fisheries production.

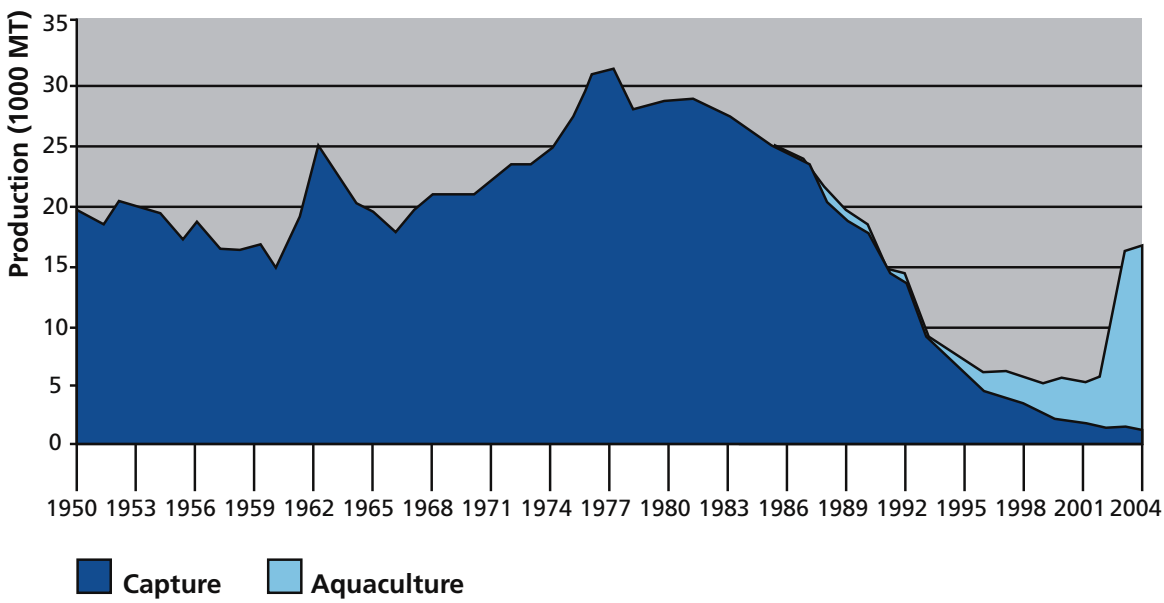

Figure 5. World catch and culture of sturgeon and paddlefish (FAO FishStat Plus, 2007).

Consequently, the amount of caviar in international trade has dropped while the amount of meat from aquaculture has increased. Aquaculture production of caviar, although increasing, has lagged behind meat production because of the time investment required for maturity and egg production.

Commercial products

Interest in sturgeon species traditionally focussed on caviar, which is still the most widely marketed product, however, other products derived from sturgeon are becoming increasingly important.

Caviar – The unfertilized eggs of mature females are, after minimal processing, transformed into caviar. Depending on the species of sturgeon, the eggs are graded according to colour, size and taste. The eggs are then salted. The designation "Malossol" on the label, which means "little salt", has become synonymous with a high quality product.

Several species of sturgeon living in the Caspian Sea account for 90% of world caviar production (CITES, 2001). Countries bordering the Caspian Sea such as Iran and Russia are the world's largest producers. Beluga *(Huso huso)* is commercially the most interesting species for caviar production.

Figure 6. Caviar

Smoked sturgeon – In recent years, smoked sturgeon has become increasingly popular. Eastern European countries are the main producers.

Fresh, frozen and dried sturgeon – These three products come primarily from the aquaculture industry.

Soup – Shark fin and sturgeon head cartilage soup is a product of Southeast Asia. It contains shark and sturgeon cartilage.

Figure 7.

Figure 8.

Live fish – Live fish are traded for sturgeon farming. Juvenile sturgeon *(A. baerii)* are also sold as ornamental fish for aquariums and garden ponds. Sturgeon are also used for recreational fisheries.

Isinglass (collagen) –The swim bladder of sturgeon is used for the clarification of wine and beer and for glue.

Sea Ivory – This new product recently appeared in the North American market at a very small scale. The scutes are sold on the market unprocessed or can be made into "sea ivory" jewelry.

Leather – Sturgeon skin is used as leather material for clothing, handbags and bookbinding.

II. Aquaculture and reproduction

Hatchery

A recirculating aquarium system is ideal for incubating eggs and rearing sturgeon larvae. Such a system could consist of two or more aquaria of 250-500 litres, a sedimentation filter, a biofilter with lava stones or a high surface open structure filter material, preferably a UV installation and well aerated clean fresh water, an aerator, a pump and a heater. The water is recycled at least 1.5 times per hour to maintain a good water quality. On a daily basis 10-20% of the total water volume is exchanged. A guideline for water quality requirements of sturgeon in the hatchery is outlined in the following table.

Recommended water quality parameters for the sturgeon hatchery.

Parameter	Recommended value
Alkalinity	100 – 400 mg/L as $CaCO_3$
Ammonium (NH_{4+})	Max. 0.05 mg/litre
Ammonia (NH_3)	< 0.01 mg/L as N
BOD	< 4
Dissolved oxygen	> 90%
Hardness	2-5 dH / 50 – 400 mg/L as $CaCO_3$
pH	6.5 – 8.0
Nitrite (NO_2)	< 0.1 mg/L as N
Nitrate (NO_3)	Max. 10 mg/litre
Carbon dioxide (CO_2)	Max. 10 mg/litre
Salinity	0 – 0.5 ppt for fry 0 – 3 ppt for juveniles 3 ppt for broodstock
Temperature*	15 – 18 °C for spawning 16 – 21 °C for grow-out

*Optimum temperature varies with species; but should be maintained constant at the optimum (like all water quality parameters).

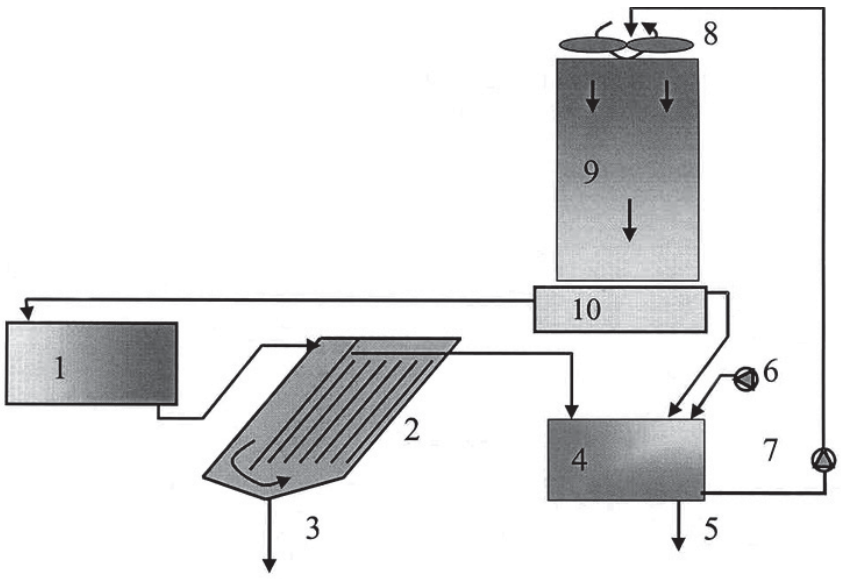

Figure 9. Simplified scheme of an aquaculture recirculation unit. 1 Growing tank; 2 Solid remover; 3 Waste discharge; 4 Pump tank; 5 Discharge; 6 Oxygen injection; 7 Water pump; 8 Spray unit; 9 Biofilter; 10 Collector tank.

Figure 10. Fluid bed filter

Figure 11. Oxygen

Figure 12. Submerged filter

Figure 13. Trickling filter

Prior to incubation of the eggs and larval rearing, the whole recirculation system should be cleaned and completely disinfected. After thorough rinsing, the system should be filled with clean fresh water (borehole or well water) to be heated to the desired temperature if necessary. The whole incubation system should be well established before egg collection could start.

Equipment

- Disinfectant solution
- Brushes
- Thermometer
- Water analysis kits
- Glassware
- Table
- Scale
- Netting
- Towels (tissue paper)
- Physiological salt solution 0,9% (9 gram of NaCl dissolved in 1 litre of boiled water)

NOTE: Good water quality and hygiene measures are essential for successful reproduction. Make sure calibrated equipment is used to monitor dissolved oxygen level, temperature and pH.

Furthermore, it is very important to maintain all water quality parameters at constant optimum levels to minimize stress.

Male and female spawners

Utilisation of wild broodstock is still a common practice in sturgeon reproduction and can not to be avoided yet. Sturgeon are a relatively slow animal to reach sexual maturity and some females may not produce eggs until they reach an age of thirty years or more in the wild. The majority of the spawners are caught during migration or on the spawning site. This practice will remain useful in specific cases like for conservational purposes or avoidance of inbreeding. However, capturing wild broodstock is often restricted by regulations of fisheries agencies and an increasing amount of broodstock is nowadays reared in captivity.

Broodstock raised in captivity

Advantages of sturgeon raised in captivity are that they adjust more easily to the tank or raceway environment and that they are more versatile with respect to the moment of reproduction. Furthermore, captive rearing of broodstock allows better control over the fish and its environment and cultured sources are less variable than those in the wild. Under proper aquaculture conditions, with good quality nutrition at the right amount and slightly increased (but constant) water temperature, sturgeon could reach maturity in ⅓ – ½ of the time span needed in the wild.

Figure 14.

Before induction of spawning it is essential that the fish undergo the seasonal temperature and daylight variations in order to trigger gonadal development. Gonad maturation requires 1-2 months at a water temperature below 10 °C. The preparation of the final ripening is mediated by an increase in water temperature to above 14 °C. This process can be supported by a slight increase in day length, however, the process is also dependant on the species, the temperature regime before the increase and the duration of the increase.

All sturgeon species develop normally when reared in freshwater only. Depending on the condition and species, males can generally spawn every year, while females every 2-4 years.

There is relatively little information available concerning the influence of environmental conditions on sexual maturation and ovulation in domestic sturgeon broodstock and it is expected that the current course of action will be optimized in the (near) future.

Feeding and feed quality

Development of gonads depends largely on feed quality, mainly on the protein supply of animal origin. The best course of action is to feed the fish with feed that is especially designed for broodstock. Fish that are starved will not undergo vitellogenesis and will not develop ripe gonads. However, it is recommended to stop feeding at very low temperatures during wintering and 1-2 month period prior to spawning.

Broodstock feed should contain a large percentage of high quality fish meal, which is especially important to facilitate qualitative and quantitative reproduction. Further requirements are a well balanced amino acid profile, vitamin level and the right Ω-3 fatty acids. An elevated level of crude fibre supports a healthy intestinal flora. High quality ingredients facilitate digestion and support the intake of nutrients. It is highly recommended to feed diets enriched with immune stimulants to train the immune system. Furthermore, the natural ingredient astaxanthin improves the health of broodstock, the development of quality eggs and the health of the fry.

To ensure that broodstock are in excellent condition and eggs develop well and carry sufficient nutrition for the embryos, specialized broodstock diets are the preferred nutrition. Coppens International Broodstock diets are formulated for this purpose and have a proven track record in many different fish species.

Handling

Broodstock can be transported in tanks of 1000 to 2000 litres with oxygenated water and preferably 0.25% NaCl (2,5 g/L; 2,5 ppt) to counter physiological stress. Approximately 250 kg of fish per m^3 of water is a safe loading rate for transport and animals should be starved for 12-24 hr before transportation starts. Obviously, consistent optimum water quality parameters should be maintained; oxygen saturation should be 70-80%. During long-distance transportation, the water may need to be changed en route.

Figure 15.

Special broodstock nets may facilitate handling of the large animals. A stretcher, that supports the entire body to prevent injury, is useful when handling the fish for sex determination, sampling of ova, tagging or short distance transport. Cranes or forklifts may be useful when lifting large spawners in the stretcher. Leather gloves are recommended for the workers because the strong armoured sturgeon may cause severe injuries.

Tagging of spawners is recommended to easily separate the males from the females during the reproductive period. Individual recognition is especially important in breeding programmes. The tagging of spawners with passive integrated transponder (PIT) tags has proven to be the best choice for persistence, performance, user-friendliness and with the least risks of injury.

Figure 16.

NOTE: Sturgeon spawners are highly susceptible to stress which could lead to reduced gonadal development. Any handling should be reduced to a minimum and carried out with the greatest caution and as effective as possible. Furthermore, egg quality is dependant on broodstock feed, feeding management and water temperature.

Anaesthetics

MS222 (20-25 mg/litre) or clove oil (25-30 mg/litre) might be used for anaesthetics. However, in all cases dosing should be examined carefully in order to obtain the optimum level for the specific sturgeon species and size. In all cases, the fish should be carefully observed during the whole period to check if the dose was not too high or the effect of the anaesthetic has not ceased off (see table).

Response levels of sturgeon to anaesthetics.

	Observation	
Level	**Respiration**	**Behaviour**
1	Increased ventilation frequency	Abnormal swimming behaviour may occur
2	Movement of operculae drops below normal frequency	Reduced swimming speed, temporary loss of equilibrium which is rapidly adjusted to normal
3	Irregular operculae motion	Complete loss of equilibrium, no compensation when turned on its back
4	No operculae motion; supply fish with fresh water immediately!	
5	Wide spread operculae, fish is shocked; flush gills immediately!	

Fish continuously kept in waters at temperatures below 6 °C or above 15-18 °C will not mature and/or resorb their eggs.
If fish with ripe eggs don't get the opportunity to spawn for several consecutive times, they will not survive.

The spawners can be kept in maturation tanks (e.g. round tanks with 4-8 m in diameter are very suitable) until they are ready to spawn; then they are placed in spawning tanks. It is very important to keep a constant cool water temperature and a high enough oxygen level. Keeping the fish in a small recirculation system offers the benefits of maintaining constant optimum conditions and of better disease control.
In cooler climates, small ponds (at least 2 meter deep) can be used for long-term holding and maturation of broodstock. It is important that the ponds do not contain much plants; sturgeon can only swim in forward direction.

Maintenance of good water quality is essential to minimize stress. Avoid any rapid changes in salinity, temperature or any other water quality parameter, (e.g. caused by any difference between pond or river water and water in the hatchery).

Broodstock reared in captivity should be held at lower densities in order to increase individual fitness, reduce pathogen pressure and avoid density-related stress and risk of disease.

Males and females should be disinfected with a 1% salt or a 100 ppm formalin bath for max. 1 hour before they are brought into the hatchery to prevent pathogens being transmitted to the eggs and larvae. This procedure is usually omitted if the broodstock is kept in an adjacent recirculation system.

Stock the disinfected females in the hatchery at least for 36-48 hours without any feeding so that the alimentary tract is empty at the time of stripping. It is very important that the collected eggs don't get contaminated.

When fish are kept outdoors, the basin should be (partially) covered to avoid direct sunlight or intensive radiation.

Broodstock selection

After the wintering period the temperature increases and the reproductive period may commence. For some sturgeon species, the ripe females have a full, soft belly while the males are slimmer. Red colouration of the swollen genital papilla is sometimes visible in ripe fish and might be more intense in females. In some species, the urogenital opening of the males is in the shape of the letter Y, while that of the females is more circular. However, external sexual dimorphism is often not obvious in sturgeon or external sexual indicators may not be developed well. An important aspect in sturgeon reproduction is the effective and reliable determination of sex and maturity stages without killing or harming the fish.

Currently the most certain method of sexing the fish is by visual examination of a tissue sample of the gonads. For this aim the fish is anaesthetized and placed on a stretcher. After having made a small incision in the abdomen, the oocytes or a piece of testicular tissue are obtained by a sterile canule, catheter or forceps. Afterwards, the incision is stitched, disinfected and sealed. The general appearance of the gonads will differentiate males from females. Other less invasive methods such as blood screening and ultrasound are currently becoming more utilized. The latter is recommended as this is less stressful for the fish, however, these methods need further development for an optimal reliable result.

Male and female broodstock are selected for spawning by determining the stage of gonadal maturity. When male are ripe for spawning, semen can often easily be collected by stripping or collection through canule from the urogenital opening behind the anus.

The colour, size and stage of maturity of the oocytes determine whether a female is ready for spawning or not. Mature oocytes of most sturgeon species are dark brown to grey-black and 2.5 to 4.0 mm in diameter. Females that can be induced to release eggs will have oocytes that are undergoing final maturation.

During the ripening process, the egg nucleus, called the germinal vesicle (GV), is moving from its initial position in the centre of the oocyte to the animal pole where the micropyles are located (Figure 17).

Figure 17. The animal polar area is located by the presence of a number of funnel-shaped holes (micropyles) in the egg membrane.

A reliable method of female selection is by determining the position of the GV, in relation to the egg diameter. This can be estimated either visually or more accurately by calculating the oocyte polarization index (PI). Oocytes with the GV close to the edge, or near the surface of the outer membranes of the eggs, are the most likely to ovulate.

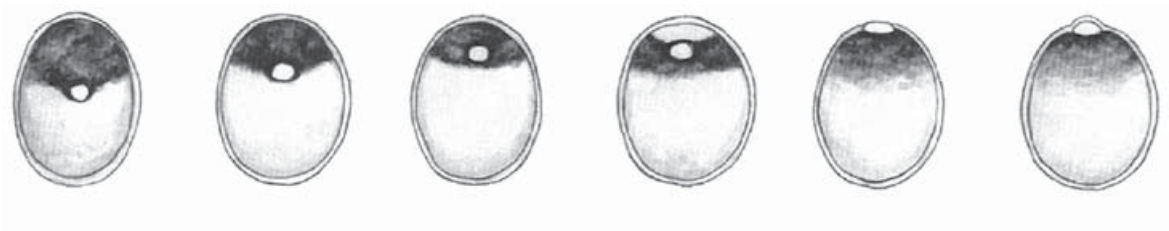

Figure 18. Bisected sturgeon oocytes showing the stages according to the germinal vesicle (GV) positions after migration from the centre towards the animal pole. Stage VI shows an ovulated egg.

Accurate method for female selection

- Insert gently a (disinfected) canule in the papilla, 4-6 cm inside the ovary
- Put the other end of the canule in your mouth and suck carefully
- Withdraw the canule and gently blow out about 30 eggs onto a glass slide
- Boil the eggs for 2-4 minutes and let them cool down
- Cut the eggs in half through the polar axis*
- Measure the distance between the GV and the cell membrane
- Measure the diameter of the oocyte
- Calculate the oocyte polarization index (PI**)
- If more than 75% of the PI's is smaller than 0.07, the female is suitable for artificial induced reproduction

If the PI's are larger, the female is not a good candidate at that time. Resample oocytes at a later stage to determine when the female is ready.

** The polar area is recognised by the presence of several funnel-shaped holes (micropyles) in the egg membrane.*

$$**PI = \frac{\textit{distance of the GV to the outer membrane}}{\textit{oocyte diameter}}$$

Equipment for selection

- Canule or catheter
- Disinfectant
- Sterile scalpel (sharp knife)
- Forceps
- Surgical needle and suture
- Glass slide
- Ruler
- Microscope or binoculars

Only use sterile equipment for surgical procedures!

NOTE: A proper breeding program should be set up to avoid inbreeding problems. Furthermore, selecting female and male spawners is more than choosing the fastest growers. Other important criteria are feed conversion ratio, disease resistance, fillet yield and behaviour. Fast-growing fish spend most of their energy for weight increase, while less energy is then available for their health.

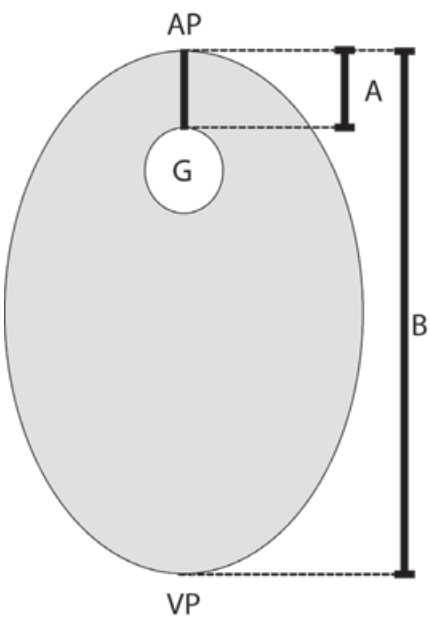

Figure 19. Diagram of a sturgeon oocyte showing the position of the germinal vesicle (GV) at the animal pole. The polarization index (PI) is determined by A/B. AP, Animal pole; VP, Vegetal pole; GV, Germinal vesicle; A, Distance between GV and cell membrane; B, Diameter of oocyte along animal-vegetal axis.

Hormonal injection

Following gonadal maturation by the environmental factors, the fish are ready for hormonal injection to induce spermiation and final egg maturation and ovulation. Different types of hormonal treatment are possible to induce final maturation. Common carp (*Cyprinus carpio*) pituitary (CCP) gland material is widely used in aquaculture. However, using sturgeon pituitaries (SP) is also very well feasible. Optimum dosing is critical and depends on species and body weight, but in general males should receive half of the dose that the females will get. Problems are encountered with variable concentration of the active ingredients of the material to inject, which also depends on the state of the donor fish.

Method for hormone injection

- Put the pituitary material into a mortar
- Add 10-20 ml physiological salt solution

Using 30% glycerine in the physiological salt solution will prevent leaking of injected solution

- Grind the pituitaries thoroughly
- Fill the syringe with the material to inject
- Insert the needle on the syringe
- Empty the syringe again into the mortar

Checking if the needle is not blocked is important because the pituitary material might not completely been crushed, giving lower results

- Point the syringe upwards and eliminate the air
- Keep the fish in the broodstock net or in the water
- Insert the needle 2-2.5 cm at an angle of 30-45° in the dorsal muscle between the lateral and dorsal scutes
- Slowly inject the suspension while retracting the syringe a few millimeter
- Finger-rub the injected area to evenly spread the injected suspension throughout the muscles

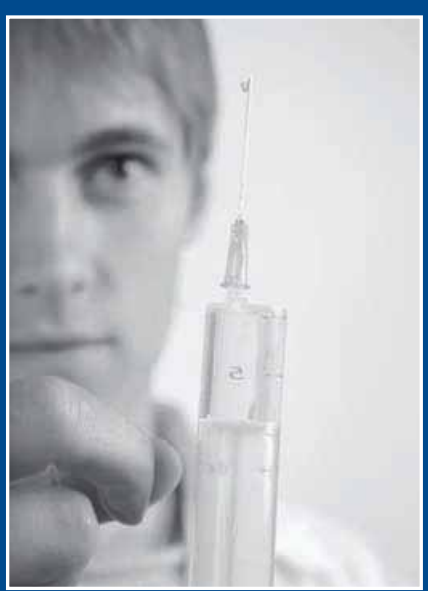

Figure 20.

The hormone solution should be prepared fresh for each treatment

For females

- Administer a priming injection (10% of total dose)
- Administer the resolving injection (90% of total dose) 12 hours later

If responsive, females will ovulate within 18-30 hours after the second injection at a water temperature range from 13-17 °C

For males

- Administer one injection with half of the total dose that females receive

If responsive, males will spermiate within 18-24 hours after the injection at a water temperature range from 13-17 °C

Equipment

- Hormones of pituitary gland
- Porcelain mortar
- Towel
- Physiological salt solution (NaCl 0.9%)
- Syringe (1-3 ml)
- 23-G needle
- (Glycerine)

Keep the injected fish separated in a holding facility. Of major importance is that the fish can be caught easily for semen collection to minimize stress.

Semen collection

Method for semen collection

- Place the male ventral side up or hold the fish by the tail
- Thoroughly dry your hands
- Thoroughly dry the male's body surface in the urogenital area
- (Attach a catheter to the syringe)
- Insert the tubing/syringe into the urogential opening and gently rub the belly
- Slowly withdraw the plunger and collect the semen

Small fish may also be stripped for semen collection

Semen can be stored for several hours in sealed containers at 4°C; with aeration it could be stored for a few days

Equipment

- Towel
- 60-ml syringe
- Catheter
- Microscope slide
- Pipette
- Microscope
- Fresh water for checking motility

Figure 21.

To be sure that the semen solution is suitable for fertilization, motility should be checked by using a microscope.

Method for checking semen quality

- Put a smear of semen solution on a microscope slide
- Bring the quiet cells into focus
- Add a small droplet of clean fresh water (1:100)
- Check immediately at a magnification of 50-100x the motility of the sperma-tozoids

Only semen that exhibits progressive motility is considered to be motile (semen that vibrates in place is not counted as motile)

Highest motility is observed within 15 seconds after addition of water - if more than 75% of the sperm cells are actively moving for a period of 60 seconds, the semen is of good quality; if motility is less than 75% the semen should be discarded

NOTE: Most of the semen will loose all its activity within 60 seconds after contact with fresh water. One drop of water in the bottle with semen will destroy the semen completely (while one drop of water in the bowl of eggs will only destroy some eggs).

Figure 22.

Figure 23.

Female egg collection

Normally 80-90% of the injected females respond to the hormone injection with ovulation. They start to swim at the walls of the tank and sometimes rubbing along the walls. The exact timing of egg extraction depends on temperature, weight of the fish, species and physiological state of the animal. At 16 °C it takes 18-24 hours before final ripening has occurred.

Check the females every 2 hours, starting at least a few hours before the predicted time of ovulation. If free adhesive eggs are observed in the tank, the female is ready for egg removal.

NOTE: If fish require a longer ovulation time or if the eggs are not sticky, the fertilization rate is usually low.

Figure 24.

Egg removal is relatively complicated in sturgeon; the female has a valve in the oviduct that is not to be opened mechanically without injury. There are some techniques that allow quick egg removal without sacrificing the female and are ideal for maintaining female broodstock for use in subsequent years.

Figure 25.

Caesarean section is a relatively quick surgical method (30 minutes), however, suturing is time consuming and muscular stress on the incision may result in weak suture retention or even

mortality of broodstock. The minimally invasive surgical technique (MIST) permits quicker and safer removal of ovulated eggs (about 10 minutes), requires less handling than caesarean section and no suturing. This method permits ovulated eggs to pass from the body cavity through the incision without going through the oviducts. The ovulated eggs could also be stripped which involves less stress, but is more time consuming and may not always succeed.

Methods for egg collection

- Catch the female carefully with a net
- Anaesthetize (if needed) the female and place it on a stretcher
- Gently dry the body surface
- Disinfect the belly

1. Caesarean section

- Carefully make an incision of 5-15 cm at the posterior part of the body cavity
- Gently remove the eggs and collect the eggs in a dry plastic bowl
- Stitch the tissue
- Disinfect the wound
- Place the fish in a tank with well aerated clean water

2. Minimally invasive surgical technique *(Figure 27)*

- Carefully make a small internal incision of 1-3 cm into the oviduct, just inside the urogenital opening
- Directly strip the eggs through the incision and collect the eggs in a dry plastic bowl
- Repeat stripping after 30-60 min
- Disinfect the wound
- Place the fish in a tank with well aerated clean water

3. Stripping

- Hold with 2 persons the female tightly with leather gloves or wet towels
- Insert gently a clean (disinfected) canule in the papilla, 4-6 cm inside the ovary
- Strip the fish by gently pressing its abdomen with a thumb from the pectoral fin towards the genital papilla
- Collect the eggs in a dry plastic bowl
- Stop for 2 hours and repeat stripping up to 5 times

Prevent any mingling of the eggs with water, blood or faeces

Continuously observe the state of the anaesthetized fish and add water or anaesthetics if needed

Equipment

- Broodstock net
- Stretcher
- Towel
- Anaesthetics
- Disinfectant
- Sterile scalpel
- Dry plastic bowl
- Surgical needle and suture

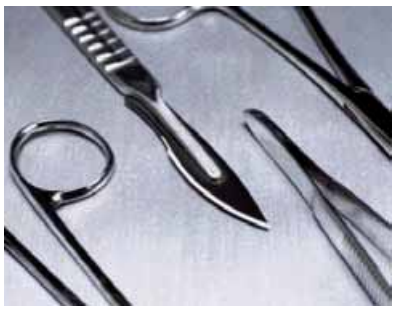

Figure 26.

After care of broodstock and equipment

After stripping the female broodstock, it should be checked if all eggs are released to prevent internal contamination and inflammation.

For optimum hygiene measures, females are disinfected with a 100 ppm formalin bath for one hour before they are brought back into the broodstock pond or tank.

All equipment, tanks and containers should be cleaned and properly disinfected and afterwards rinsed with fresh water.

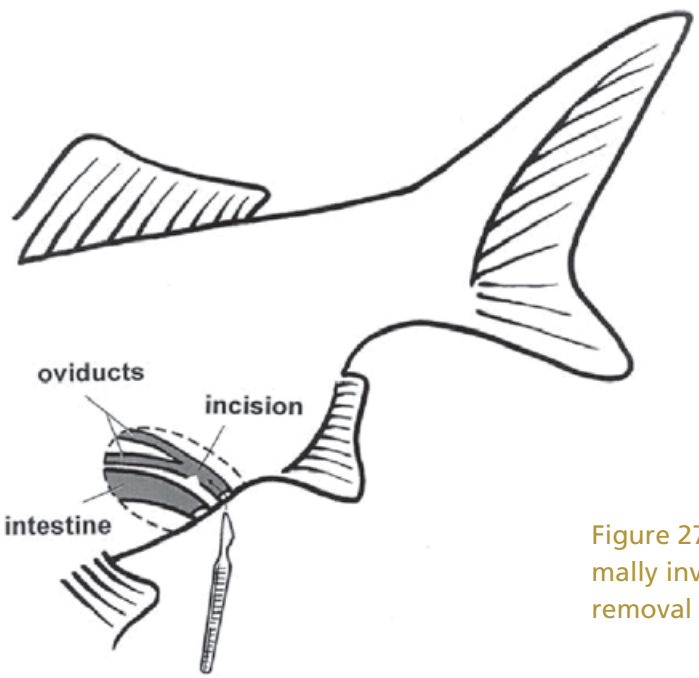

Figure 27. Schematic illustration of the minimally invasive surgical technique (MIST) for removal of ovulated eggs from sturgeon.

Artificial fertilization and egg incubation

Method for egg fertilization

Fertilize the eggs within 1 hour after collection

- Weigh or estimate the weight of the egg mass
- Divide the eggs in portions of (at the most) 200 gram in a dry tray or dish
- Distribute 2 ml of sperm on top of the egg mass
- Gently shake to mix the eggs with sperm while slowly adding 250 ml of clean fresh water
- Gently shake the mixture for at least 1-3 minutes until the eggs become adhesive
- Put the fertilized eggs in the (incubation) jars, add a de-adhesion suspension and mix through aeration (from the bottom) for 30-60 minutes (temperature dependant) until stickiness disappeared

If necessary the de-adhesive suspension is added several times to provide sufficient material to cover the cells

- Stop aeration and wash the eggs several times with clean fresh water until the water is clear
- Incubate the washed eggs in running clean fresh water, flowing from beneath, until hatching
- Determine total amount of incubated eggs and record survival
- Regularly remove dead eggs and clumps of eggs by syphoning

Before fertilization, make sure that all important water quality parameters of the incubation unit are appropriate for incubation!

Equipment

- Scale
- Trays or dishes
- Good quality sperm
- Clean fresh water
- Egg incubation cylinders/cones

NOTE: It is very important to keep the eggs in only a few layers thick while mixing them with the sperm and water. In this way the sperm can immediately reach all the eggs. If it takes more than 60 seconds before all eggs are brought into contact with the sperm, the sperm is inactivated and fertilization cannot be completed.

The development process from fertilized egg to hatching varies with different batches and is dependant upon species and water temperature. The hatching rate is, next to egg quality, dependant on the water quality, oxygen content and load of the incubation system, etc. Generally, hatching occurs after 90-130 day-degrees (°C).

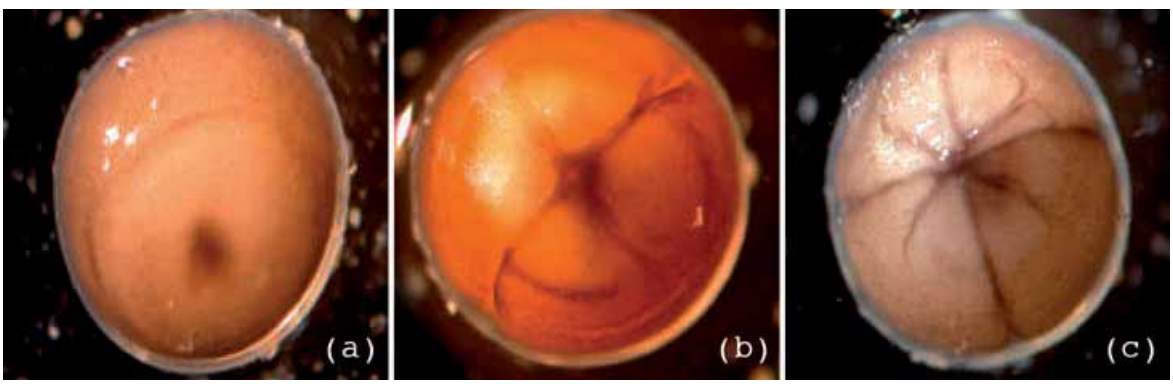

Figure 28. Sturgeon eggs at 6 hr after incubation at 16 °C. Unfertilized egg which shows no signs of division (a). Fertilized eggs at the 4-cell stage (b) and at the 8-cell stage (c).

In contrast to eggs of most other fishes, sturgeon eggs contain several micropyles, therefore, the sperm should be diluted to avoid polyspermy.

Although very little sperm is needed, it is recommended to use semen of different males for fertilization of 1 batch of eggs.

The number of motile sperm cells decreases to 10-20% after 2 minutes.

Within a few minutes after fertilization the eggs will absorb water and become sticky. During this process the eggs can easily stick together and therefore they should be treated to eliminate stickiness under aeration. In this way all the eggs also get sufficient oxygen. River silt suspension, Fuller's earth, milkpowder, talcum or starch are all used as de-adhesive agent.

The water is continuously renewed in order to provide oxygen and remove metabolites. Maintain an oxygen level equal or higher than 90% saturation. Keep eggs slightly rotating by a moderate flow rate for the first 2-3 days, afterwards the water flow is increased so that the eggs are rotating well. The water current should be regularly checked to avoid heavy movement.

Newly fertilized eggs are very sensitive and should be handled with the greatest care. Keep appropriate temperature and avoid any temperature or other shocks since they are detrimental to embryo development. Also avoid any hand contact with the eggs.

Monitor the incubated eggs regularly. Healthily developing eggs are transparent grey-black in colour. White eggs can be present amongst developing ones and should be removed by siphoning to avoid the development of fungi. If all eggs turn white, the batch should be discarded.

Fry will hatch in 7 to 9 days and the larvae are swimming with the current so it is very easy to trap them with the effluent water. In this way the healthy larvae are easily separated from the dead eggs, egg shells and deformed larvae. This is of utmost importance in order to avoid fungal infections of larvae and consequent larval mortalities.

Larval rearing

Larval rearing is probably the most difficult part of the hatchery process. Fry survival depends on having a proper culture system and a complete nutritional program with attention to diet formulation, feeding schedule, food presentation and preference.

Figure 29.

Figure 30.

Figure 31.

The newly hatched larvae do not need to be fed as they rely on the food resource within their yolk-sac for the first 7-9 days (temperature dependant). During this time the mouth and foregut develop, the residual yolk is absorbed, and the body becomes darkly pigmented. It is recommended to start with external feeding just before the yolk-sac is fully absorbed.

First feeding with *Artemia* nauplii

The success of the intensive production of fingerlings of the sturgeon is greatly dependant on the use of *Artemia* nauplii as first feeding. See the Coppens International *Artemia* manual (Appendix) for more information on the *Artemia* hatching procedure.

> *NOTE: Follow the standard operating procedures for hatching and storage of* Artemia *nauplii to derive the maximum benefit from* Artemia *feeding.*

The feeding response of the larvae is stimulated by the movement of the nauplii in the water. *Artemia* is administered throughout the day and night at regular intervals. Check larval gut content after every feeding and adjust feeding level if necessary.

> *NOTE: It is advisable to have some frozen* Artemia *nauplii in stock in case an* Artemia *hatch is of poor quality. It is not recommended to feed decapsulated unhatched* Artemia *cysts because of their inferior nutritional profile.*

First dry feeding

Start with an appropriate co-feeding diet, next to the *Artemia* nauplii, 3 days after first feeding. High quality *Artemia* replacing diets such as Coppens International sturgeon larval diets are especially developed for the co-feeding phase where a dry diet is introduced early. The advantage of automatic feeders is that they provide consistent presentation and uniform delivery over a 24-hour period. However, feed uptake should always be checked and adjusted when needed.

Initially *Artemia* is given followed after a few minutes by the dry feed. After 2-3 days first the dry feed is given followed after a few minutes by *Artemia*. Every day the amount of *Artemia* is reduced and the amount of dry feed increased. Coppens International provides the ideal follow-up hatchery feed. Larvae are fully weaned after about 10-14 days of co-feeding.

NOTE: Research on nutrition of larvae and early juveniles, particularly with regard to their ability to utilize certain dry feeds has greatly enhanced the production of fry for stocking. However, weaning must still be undertaken using the correct strategy which takes into consideration feed demand, larval condition, water quality, attention and patience!

The survival of post-weaned fish can be increased through the use of appropriate diets. It is important to note that the quality difference between different feed products will obviously become more significant when substituting *Artemia* at an earlier stage. The selection of any nutrient product should therefore be dominated by the diet quality over any other parameter.

Coppens International offers a selection of high quality sturgeon diets from first feeding until harvest. These diets are aimed at healthy development of the fish and fast and efficient growth. All diets are based on scientific research at the Coppens Research Centre (CRC). Research is continued to be able to offer you state-of-the-art sturgeon feeds, not only now but also in the future. For further information on the complete sturgeon feeding program or other information about Coppens International's diets see: **www.coppens.eu**.

NOTE: It is important to use only clean and disinfected equipment for all hatchery procedures. This is in particular important during Artemia *hatching, because the use of* Artemia *involves introduction of microbes into the system.*

Health management

Hygiene and disinfection

In aquaculture diseases are rarely the resultant of the contact between the fish and a potential pathogen alone. Aquatic pathogens are often opportunistic; they only cause disease outbreak when a stressor like poor water quality, reduced oxygen level or sub-optimal density is present.

Figure 32.

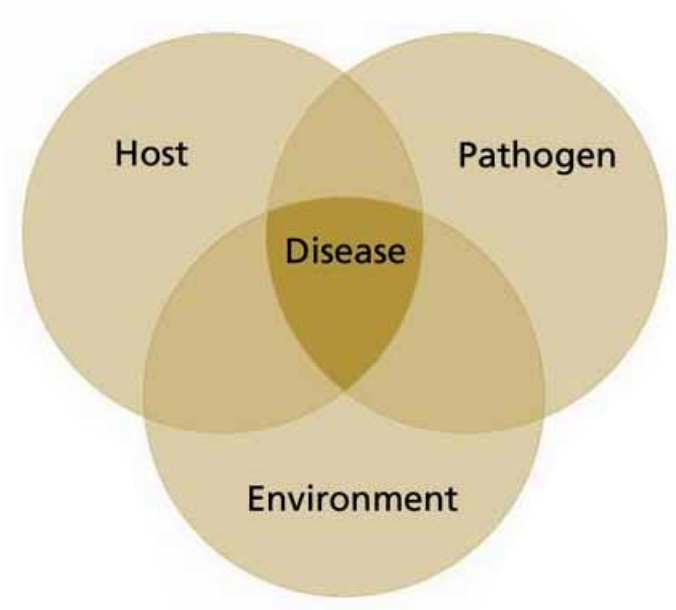

Figure 33. The relation between the host, pathogen and environment in regards to occurrence of fish diseases (Schnieszko, 1974).

Sturgeon is relatively resistant to diseases, in particular when food supply is of good quality and quantity. However, fingerling production in the hatchery is often accompanied by infection risk. Cleaning, feeding and prophylaxis are of utmost importance to reduce the risk of diseases. Spilled feed and faeces should be siphoned out to prevent deteriorating rearing conditions. Survival and growth of fish primarily depends on management, mainly on maintenance of good water quality, feed quality and frequency of feeding.

NOTE: Though sturgeon is relatively hardy, good water quality, sufficient oxygen supply, quality feed and adequate hygiene measures are very important for successful culture of this species.

Grading

Larval rearing occurs at relatively high densities. When the early juveniles are transferred to the nursery system they are recaptured and size graded. The same applies when they are transferred to the production ponds or high-density tank culture units. In addition, regular grading takes place when needed, which may be every 6 weeks especially in smaller fish. Generally, size differences in a group should not exceed 20% from the average value. Grading is very important to reduce the rate of cannibalism and facilitate proper (feeding) management. When fry are smaller than 2 gram grading should be carried by a grading mesh without handling. Above 2 gram, they can be graded by using a bar grader.

It is recommended to starve the fish 1 day prior to grading in order to reduce susceptibility to stress. It is essential to record weight of the graded fish being the basis for future calculations on feed supply.

Feed and feeding

The quantity of feed distributed is calculated on a day to day basis and adjusted if necessary. Overfeeding should be prevented at all times since it leads to adverse environmental conditions, including low oxygen, high ammonia and high suspended solids, and is expensive.

NOTE: Sturgeon, like other fish and animals, do not have a direct requirement for high protein content alone but rather for a well-balanced mixture of essential and non-essential amino acids from which to construct their own tissue proteins. The protein digestibility and the balance of essential amino acids in the diet are, therefore, more important than crude protein levels.

The future of feeding

In comparison with other fish species, nutritional requirements and feeding characteristics of sturgeon have not extensively been studied and documented yet. Present knowledge about nutritional requirements, however, is combined with the latest know-how on aquaculture feed technology. This combination has provided the foundation for the formulation of efficient, economical diets and for the development of feeding strategies by Coppens International and will prove instrumental in the success of the sturgeon industry.

Coppens International commercial available products are a result of continuous research. Coppens International aims at transferring the present existing applied technologies to assist the development of the aquaculture industry. This continuous research will undoubtedly further contribute towards the enhancement of sturgeon production as well.

Figure 34.


Figure 35.

References

This manual is compiled according to the present day knowledge and techniques by the well-experienced staff of Coppens International and after consulting the following publications:

Agh, N., Rosenthal, H. and Sorgeloos, P. (2007). International workshop on advanced techniques in sturgeon fish larviculture, Proceedings of the workshop held on 12-14 March 2007 in Urmia, Iran.

CITES Identification Guide - Sturgeon and Paddlefish (2001). Guide to the Identification of Sturgeon and Paddlefish Species Controlled under the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Hochleithner, M. and Gessner, J. (1999). The sturgeon and paddlefishes of the world. Biology and aquaculture. AquaTech Publications, Kitzbuehel, Austria, 207 pp.

Mims, S.D., Lazur, A., Shelton, W.L., Gomelsky, B. and Chapman, F. (2002). Species Profile. Production of Sturgeon. Southern Regional Aquaculture Center. SRAC Fact Sheet, Publication No. 7200.

Mims, S.D. (2001). Aquaculture of paddlefish in the United States. Aquat. Living Resour.
14: 391–398.

Wayman, W. R. (2003). From gamete collection to database development: Development of a model cryopreserved germplasm repository for aquatic species with emphasis on sturgeon. Dissertation of the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College, 300 pp.

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Copyright Photography:

Figure 15:	AFP Photo/José Luis Roca
Figure 22, 23, 24, 25:	AFP Photo/Henghameh Fahimi
Figure 36:	AFP Photo/Danièle Schneider

Figure 36.

Notes

Notes

Appendix

Artemia manual

Artemia are tiny salt water shrimps that are an ideal live food for many fish species due to their size, nutritional value and movements. In several cases fish larvae can not be grown without *Artemia* as with sea bass, sea bream, catfish and sturgeon. For several ornamental fish species, too, the results are much better with *Artemia* than with dry feed only.

Artemia are sold as dry encapsulated cysts. These need to be hatched in salt water. When the correct procedure is used a very high hatching percentage is possible.

Equipment

- Cone shaped container
- Sea salt, *Artemia* salt or agricultural salt (NaCl without Iodine, anti-caking agent etc.)
- Aerator
- Heating (heating element)
- Light (2000 lux)
- Sieve (100 µm)
- Siphon
- Sodium bicarbonate (NaH2CO3), if pH is lower than 7.5

Procedure:

1) In the container a solution is made with salt with a concentration of 15 – 35 gram/litre. Normally 15 gram/l is sufficient. The pH needs to be 7.5 – 8.5. If it is too low it can be increased using sodium bicarbonate.
2) The water temperature needs to be between 27 and up to 30 °C maximum. The water column has to be aerated, rather strongly, from the bottom. At the same time there needs to be enough light (2000 lux) above the container.
3) The cysts are added at 2 - 6 gram/litre. The water with cysts has to whirl and bubble due to the aeration over 24 hours. Due to the turbulence a layer of cysts may attach above the water level. This should be rinsed back occasionally.
4) After 24 hours hatching should be complete and aeration can be stopped. The red *Artemia* sink to the bottom of the container while the brown shells float to the surface. This separation happens even better when the light above the container is switched off and another lamp under the container is switched on. The *Artemia* are attracted by light.

5) After 10 minutes the separation is complete and the *Artemia* can be harvested. Ideally this can be done with a tap at the bottom of the container. However, with a siphon the *Artemia* can also be hatched perfectly. Harvesting should stop before the brown shells are siphoned out.
6) The *Artemia* can be collected in a sieve of 100 µm. After collection the *Artemia* have to be rinsed with tap water to clean them from the remaining shells, proteins etc. to help retain a high quality.
7) The *Artemia* can now be fed or stored in portions in a freezer for later use. Another option is to keep the *Artemia* in a new clean salt water solution at 5 °C with some aeration and light above the container. The *Artemia* can be kept this way for up to 2 days. The advantage is that every time live *Artemia* can be fed.

Practical tips:

- *Artemia* cysts should always be stored in a cool, dark and dry place if possible in the original, vacuum packaging.
- Opened up packaging should be thoroughly closed and not be taken into and out of the warm and humid hatchery. The cysts would be negatively influenced by the moisture they attract.
- Always buy small quantities of *Artemia* cysts since the nutritional value may decrease in time.
- For above reasons Coppens International packs its *Artemia* cysts in vacuum packs of 500 gram.
- Always feed *Artemia* in small portions that can be finished within a few minutes. Check if the fish consumed enough, if so they will have red bellies.

With great passion and care, we develop and supply a wide range of high-quality fish feed programs. By focusing solely on aquatic feeds, we are a reliable expert in the field, fully dedicated to the performance of our customers.

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