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TECHNICAL MANUAL ON BROODSTOCK MANAGEMENT OF COMMON CARP AND CHINESE HERBIVOROUS FISH

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TECHNICAL MANUAL ON BROODSTOCK MANAGEMENT OF COMMON CARP AND CHINESE HERBIVOROUS FISH

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Preparation of this document

The Technical Advisory Committee (TAC), currently the only subsidiary body of the Fisheries and Aquaculture Commission for Central Asia and Caucasus (CACFish), has a mandate to provide scientific and technical fisheries management and conservation advice for the Commission. At its first session, organized in 2012, TAC identified fish breeding and broodstock management as one of the general key priority areas for its work. The Committees' agreed work plan for 2013 included the need for an expert consultation meeting on fish breeding and broodstock management, and this was held in Istanbul, Turkey from 10 to 12 December 2013 as a scheduled activity, in which TAC discussed the status of fish breeding and broodstock management in the CACFish area of competence. The recommendations produced by the Expert Meeting were adopted by TAC at its the second meeting organized in 2014. Preparation of these technical guidelines on cyprinid broodstock management was the one of the recommendations.

Cyprinid culture is the most widely practiced fish production method employed in Central and Eastern Europe, and within the CACFish area of competence. Consultations made within the CACFish region confirmed that there is still lack of basic knowledge on species and selection of production technologies adaptable to regional conditions. The Food and Agriculture Organization of the United Nations (FAO), via its a subsidiary body CACFish has continued to support the development of fisheries and aquaculture by issuing technical papers, books and training materials on specific subjects. Though these have been very useful, concise technical publications directly applicable to Central Asia and Caucasus regions are still missing. This document is a concise overview and inventory of basic information on the breeding and broodstock management of several cyprinid species within the CACFish region. Part one assesses common carp and part two several assesses carps commonly referred to as herbivorous fishes. It is expected that this document will support the realistic planning and successful realization of cyprinid broodstock management within the CACFish area of competence, and will enhance the more efficient use of related publications of FAO, included as a bibliography in this document.

Publication of this document was initiated by Haydar Fersoy (FAORNE). Part 1 was written in English by Zsigmond Jeney and Part 2 was written in Russian by Vitaly Bekh with subsequent English translation by Dimitry V. Ananyev. All parts in this document have undergone technical and format editing by Richard Anthony Corner. Final editing and publication was facilitated by Dr Atilla Ozdemir (FAOSEC).

All photographs used have been provided by the authors, unless otherwise stated.

Abstract

This document provides technical information on broodstock management and identifies the main problems and challenges for the application of modern techniques for breeding management of the broodstock of common carp and Chinese herbivorous fish.

Although global aquaculture production has undergone a remarkable increase over the past few decades, the use of modern genetic techniques to increase fish production and improve the quality of cultured fish has not been extensively applied in the Central Asia and the Caucuses. This is, mainly because of technical and financial restrictions, but also due to the lack of sound national policies for the exploitation, protection and conservation of aquatic genetic resources.

Countries within the CACFish area have great potential for the genetic improvement of cultured fish, and classical breeding (genomic selection) programmes should provide the bulk of the future genetic gain. Currently, limited supply of quality seed is a factor restricting the expansion of aquaculture production in Central Asia and the Caucuses, a region where classical breeding programmes were partly used for enhanced fisheries practices and the production of carp and trout.

Selective breeding is the process of captive breeding of only those fish that possess the desired phenotypic traits, such as rapid growth or disease resistance. To produce such fish requires a well-designed selection and breeding programme that is conducted over many generations. Successful fish breeding programmes depend mainly on the use of high-quality broodstock to produce quality fish seed for aquaculture and for stock enhancement into natural waters. Creating and maintaining high quality, genetically improved through improved classical breeding techniques, and doing so in a controlled environment reduces reliance on wild populations where quality is unknown and unpredictable.

The document consists of two parts; Part one evaluates breeding and broodstock management for common carp (*Cyprinus carpio*); Part two evaluates the same for three Chinese herbivorous species covering silver carp (*Hypophthalmichthys molitrix* Val.), bighead carp (*Aristichthys nobilis* Rich.) and grass (or Chinese) carp (*Ctenopharingodon idella* Val.). Parts one and two cover similar topics, but with some species-specific differences in content. General themes include broodstock selection and management, controlled reproduction and artificial propagation, nursing of fry and overwintering of broodstock.

Contents

PART I: TECHNICAL MANUAL ON BROODSTOCK MANAGEMENT OF COMMON CARP (*Cyprinus carpio*)

Prepared by Zsigmond Jeney

1. INTRODUCTION

The culture of common carp (*Cyprinus carpio*) is an important fish production practice in Central Asia and the Caucasus. Production since 2010 has amounted to approximately 140 000 tonnes to 150 000 tonnes annually (FAO, 2014), but the potential is far greater. The process of culture of common carp starts with selection, establishment and management of broodstock for the production of larvae and fry. Consultation with regional farmers within the CACFish area identified the need to provide additional support in the development and management of broodstock and this part of the guidelines is primarily about the key components needed for the broodstock management process in common carp, but also briefly outlines key requirements in natural and semi-natural propagation, and larval and fry development.

Broodstock management refers to the establishment and maintenance of a group of high quality sexually mature female and male fish, kept separately for breeding purposes. The advantage of farmers maintaining their own broodstock and undertaking artificial propagation is that eggs and fry can be produced to requirements in a controlled environment, with no reliance on uncoordinated reproduction or use of wild populations. It is, however, critical that this broodstock is selected and managed correctly, to ensure production of healthy larvae and fry, that will then develop and grow into a productive harvest.

2. REPRODUCTIVE BIOLOGY OF COMMON CARP

2.1 Natural spawning in common carp

Within its native geographical range, common carp spawns from late spring through to the early summer on repeated occasions.

Group mating occurs at dawn in shallow water areas covered with aquatic weeds or within freshly flooded grass. Fertilized eggs are sticky and stick to the spawning substrate, where they develop within 3–5 days depending on the temperature. Approximately 70 “degree-days”[1] are required for fertilized eggs to hatch. The non-feeding larval stage lasts for approximately the same period of time (70 degree-days) after which the larvae start to swim and to feed. During this early period developing young fish are extremely vulnerable to prevailing environmental conditions and to predators.

Although fully developed females release many hundreds of thousands of eggs and fertilization is usually successful, in nature the survival of developing eggs and hatched larvae, turning into fry and juveniles is relatively low with only about one percent of fertilized eggs eventually growing in to sexually mature adults.

2.2 Sexual development of carp

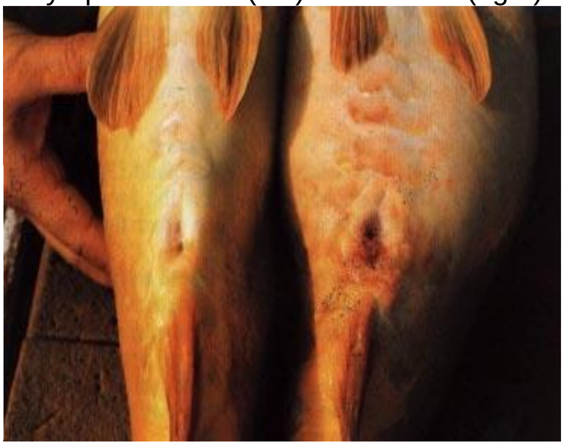
Carp will become sexually mature at different ages depending on the prevailing climatic conditions. For example, sexual maturity can occur within the first year in the tropics, where size is larger due to growth brought about by the higher water temperatures. In other regions the process is slower and can take 2–4 years in Southern Europe, 4–5 years in Central Europe, and 5 years or more in Northern Europe, where waters are much cooler and growth, and therefore maturity, is slower. There can also be difference between the sexes and males generally mature one or two years earlier than females under Central Europe's temperate-climate conditions, for example.

The point of full maturity in a breeding cycle is driven by temperature, and to avoid unnecessary natural spawning occurring in mixed ponds there is a need to undertake separation of the sexes (See 5.1).

2.3 Sexual dimorphism, differentiation of males and females

Identification of sexes is relatively simple. After capture the application of a slight pressure on the abdomen of males will induce white milt to appear from the sexual opening (Figure 1, left), while females can be identified by their medium soft bellies, which are swollen because of the developed ovaries (Figure 1, right).

FIGURE 1
Sexually ripened male (left) and female (right) carp



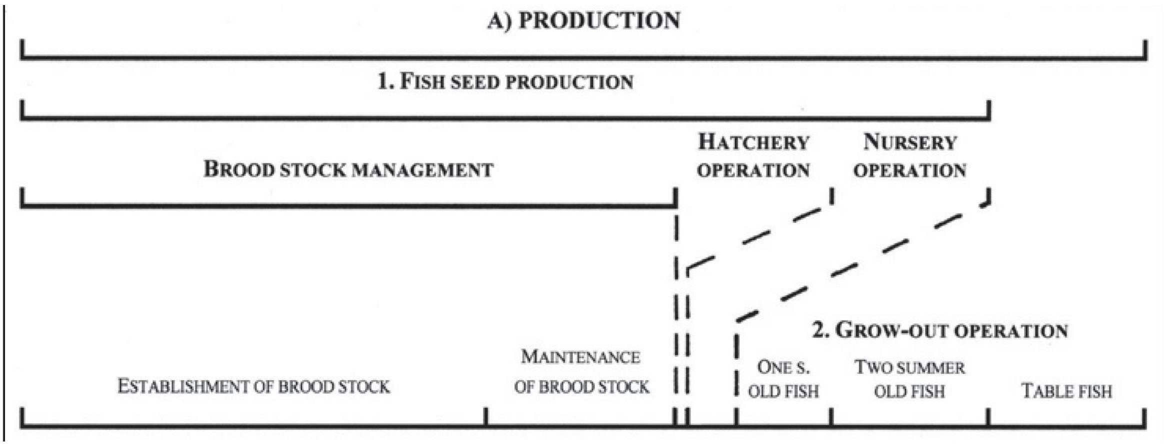
©FAO/Horvath et al., 2015

[1] Degree days = temperature of the water × days. If eggs hatch in 70 degree days and water temperature is 22 ºC then eggs will hatch after approximately 3.18 days (70/22≈3.2 days).

3. BASIS OF BROODSTOCK MANAGEMENT OF COMMON CARP

Broodstock management refers to the establishment and maintenance of a group of high quality sexually mature breeders (female and male fish), kept separately for breeding purposes. The advantage of farmers maintaining their own broodstock and undertaking artificial propagation is that eggs and fry can be produced in accordance to requirements, in a controlled environment, with no reliance on uncoordinated reproduction or use of wild populations. It is, however, critical that this broodstock is selected and managed correctly, to ensure production of healthy larvae and fry, that will then develop and grow into a productive harvest.
Broodstock management, the control of reproduction and genetic improvement are central parts of carp production (Figure 2). Broodstock management aims to provide optimal environmental factors for the broodstock to ensure maximum survival, enhance gonadal development and increase fecundity. Hormonal manipulation and acceleration of final oocyte maturation due to the economics of broodstock management is important.

FIGURE 2
Broodstock management, the control of reproduction and genetic improvement are central parts of carp production



Broodstock management covers three particular aspects of the rearing process:
(a) Establishment of the broodstock.
(b) Rearing of broodstock.
(c) Using the broodstock for quality fish seed for carp production.

3.1 Technical and biological requirements of ponds

In general, the ponds allocated for keeping broodstock should be located in the best parts of the fish farm, with good access to high quality water resources. Broodfish are relatively large in size and weight but are relatively small in number, which means they should be kept in easily manageable, smaller and deeper ponds that have an excellent water supply. In order to secure these high value fish at the farm, broodstock ponds are usually located closer to the center of the farm where guarding of the stock is easiest and general security is more effectively maintained.

Outdoor earthern ponds for broodstock should be built on productive soil in which natural food organisms will grow successfully, being the best “feed” for carp. To assist in production of natural feed organisms, the pond should be fertilized with 150 kg of carbamide and 100 kg of superphosphate per hectare. Repeated manuring with smaller amounts of organic fertilizers can also be effective. When overwintering the stock, the wintering ponds require an adequate water supply throughout the whole winter.

3.2 Sources of broodstock

Broodstock management starts with appropriate selection of the best available individuals within the population, selected on the basis of being the correct size, along with other phenotypic characteristics, which means they are suitable to provide the initial broodstock or to supplement the existing breeder population.

Selected candidates for broodstock, in an ideal case, are tagged individually by PIT-tagging and maintained in their own broodstock pond as a group in order to grow them until they reach sexual maturation. PIT-tagging[2] allows traceability (Figure 3) of individual fish performance, so that over time the farmer can assess, through detailed record keeping, which are the best performing individuals.

Maintaining genetic diversity will be important for the long-term productivity of a carp farm. Selecting individuals "from the farm" only, may decrease genetic variation on long term. This could lead to in-breeding, reduced fecundity and in bad cases malformations of produced larvae and fingerlings. It may be appropriate at times to select broodstock from other locations, such as other farms, in order the maintain the genetic diversity in the population. However, doing so, special risks related to introduction of diseases and pathogens from outside the farm, should be considered. If doing so, general principles of biological safety should be followed, e.g. only fish from farm with known disease-free history should be obtained and supported by "fresh veterinarian passport" proving their health status.

FIGURE 3
Reading of individually marked (by PIT-tag) broodfish, being checked carefully before selecting them for propagation

3.3 Selection of broodstock

Selecting high quality broodfish (Figure 4) is one of the pre-requisites of successful carp production. Ideally the fish selected for broodstock should have a known genetic background. Selection criteria is generally based on size, body shape and other physical characteristics,

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[2] For a quick review of pit-tagging methods see http://www.biomark.com/Documents%20and%20Settings/67/Site%20Documents/PDFs/Fish%20Tagging%20Methods.pdf

referred to as phenotypic features, along with consideration of health and growth performance. The selected breeders should have the following phenotypic characteristics:

1. Desired shape of body.
2. Desired scale distribution - of a good size and uniform distribution.
3. Healthy, with desirable hereditary characteristics.
4. Developed sexual organs.
5. Having no body wounds, parasites, or deformations of any type.
6. Proper amount of fat.

Once selected, the farmer needs to provide the best conditions possible, including holding fish at a lower stocking density, developing the pond to provide a high availability of natural feed, and use of highquality artificial feeding.

It is important to maintain records on the performance of individually tagged breeders, both at the hatchery and at the grow-out ponds or farms, in terms of quality of eggs and sperm, larvae produced and so on; and collected information should be monitored and analysed regularly. In general, breeders can be used up to 7–8 years for females and 8–10 years for males. Breeding performance changes as fish get older, so at some point individual broodfish will need to be deselected from the breeding group and this deselection process should be based on their performance recorded in the hatchery books/collected records. Once the broodstock management is established, the deselection and the renewing will become a continuous activity.

FIGURE 4
Healthy broodfish of common carp with good size proportions and shape, good scale distribution and having no deformities or damage

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3.4. Maintenance of broodstock

Depending on the quality of the pond available, between 100 and 200 broodfish may be stocked in one hectare. Breeders should be fed with a supplementary protein-rich feed, at a rate of 2–5 percent of the body weight per day.

In maintaining broodstock the well-balanced diet plays an important role during quantitative development of egg cells. When holding ripe broodstock (both males and females) the development of sexual products, but especially eggs, puts considerable strain upon the organism. In order to enable the fish to produce eggs and sperm efficiently, optimal environmental and nutritional conditions are required for the broodstock group.

Following the reproduction season, recovered healthy fish should be stocked into ponds where they can be kept until the late autumn harvest, or even until an early spring harvest. Fish that have already spawned should be kept in a separate post-spawning pond, and must be fed adequately to regain needed growth. If broodstock are injured during hatchery work they must be treated before being returned to the main broodstock pond. During treatment, broodstock should be held for a few days either in tanks with good water supply, or in small quarantine ponds where they can be monitored, treated and fed with specially medicated feeds rich in vitamins and proteins to improve recovery times.

Spawned fish will lose between 10 and 20 percent of their body weight during reproduction, as energy is diverted to growth of sexual organs and spawning activity, which needs to be replaced with sufficient highquality feeding. For the reproduction of new eggs, an energy-rich feed is required to promote renewed yolk formation. The best quality of animal protein for broodstock is provided by live organisms, particularly zooplankton produced in the ponds. To assist in natural feed organism production, the pond should be fertilized with 150 kg of carbamide and 100 kg of superphosphate per hectare. Repeated manuring with smaller amounts of organic fertilizers can also be effective.

Overall maintenance means in some cases small-sized carnivorous fish (e.g. European catfish) can be stocked into the broodstock ponds, in order to clean up the pond from any unwanted trash fish or fry originating from late or unwanted spawning which would then compete for food with the broodstock. New broodstock are also stocked together with old broodstock as part of the process of replacing older stock. Under proper holding conditions broodstock will have developed eggs, at rate of 10–15 percent of their total body weight, by the autumn season, ready in anticipation of the new breeding season the following spring. By the time of autumn transfer to wintering ponds, yolk production of broodstock is almost complete and the spare fat necessary for overwintering is also accumulated in the body.

In temperate zones wintering of broodstock is a part of the technological process, when broodstock are checked and selected during the autumn harvest. During the selection process spawners should be immersed in a 2-5 percent salt solution for preventive treatment against ectoparasites. Individuals showing phenotypic abnormalities are discarded. Several hundred broodstock can be kept over the winter in a wintering pond (deeper than the grow-out ponds) covering an area of 600–1 000 m^2 if there is an appropriate water supply available. In some cases (depending on the water temperature), breeders are fed at the beginning and at the end of wintering, depending on the water temperature.

4. PROPAGATION OF COMMON CARP USING NATURAL AND SEMI-ARTIFICIAL METHODS

When we propagate common carp with natural methods, we simulate the natural spawning of it.

4.1 Natural propagation

Natural propagation and semi-artificial propagation of broodstock may be done in special small ponds and associated small hatchery, depending on the facilities available on the farm. Such procedures are relatively simple and can be suitable for mass propagation. Disadvantages of the two methods are that it is less controllable and is difficult to mechanize, involves more manpower and greater loss of eggs. Under these methods, fries are collected (by principle of “from water to water”) at the age of 4 to 5 days and stocked into nursery ponds for a one-month nursing.

Two methods of natural propagation have been practised recently. Using the Dubisch Method, relatively high numbers of brood fish of both sexes are stocked into small (0.01–0.1 ha) ponds and allowed to mate and spawn as they would in the wild. After spawning, the broodfish are immediately captured to remove them as a predatory threat to the developing eggs and larvae, giving their progeny a better chance for survival. When advanced fry are sufficiently large, they are harvested and transferred to nursery ponds.

Using a more recent method, prior to the breeding season, small (1–2 ha) shallow, grassy ponds are kept dry. When the propagation season arrives, these ponds are prepared according to general principles and filled with water and 3–4 females and 2–3 males are stocked for spawning. Later in the autumn, both fingerlings and brood fish are captured. Disadvantage of this method is that it is not easy to harmonise the needs for natural spawning and for embrional development, as well as nursing and fingerling production. The results of this method will be poorer than the others.

4.2 Semi-artificial propagation

Semi-artificial propagation can also be done using two different approaches.

In the the first method, ovulating females are captured from the spawning ground and their eggs are stripped, and fertilization of eggs are done in the hatchery facility, using sperm from males that have also been captured. The fertilized eggs and hatched non-feeding larvae are incubated also under there more controlled hatchery conditions.

In the second method, induced spawning of brood fish takes place in small ponds into which artificial substrates are placed in order the capture the fertilized eggs. The eggs are released, fertilized by males and sticked onto these artificial substrates, or onto other natural substrates, which after spawning are removed and taken into the hatchery for incubation. Fertilized eggs sticked to these substrates are immersed into special tanks with good water flow. After hatching, larvae are collected and moved back to the specially prepared nursing ponds.

5. ARTIFICIAL PROPAGATION OF COMMON CARP

The aim of artificially-induced reproduction under controlled conditions, is to dramatically increase the survival of eggs and fry, so a larger percentage of the stock is available for harvest when at the correct size. While doing this, we rely on the basic biological and technological data of the propagation of common carp, shown in Table 1.

Table 1
Basic data of artificial propagation of common carp
(Horváth, Tamás and Tölg, 1984, cited from Woynarovich et al, 2011)

Description	Common carp	
	from	to
Sexual maturation of females (years)	4	5
Sexual maturation of males (years)	2	3
Size of matured female (cm)	30	40
Size of matured male (cm)	25	30
Size of matured brood fish (kg)	2.5	3
Water temperature at propagation (C°)	16	22
Sex ration at propagation (♂:♀)		2:1
Percent of ovulation in females after hormone treatment (%)	60	90
Ovulation after the decisive (2nd) dose (H°)	230	260
Number of eggs per 1 kg of female BW	100 000	200 000
Diameter of dry eggs (mm)	1	1.5
Diameter of swelled eggs (mm)	1.5	2.5
Number of eggs in 1 kg dry eggs	700 000	1 000 000
Number of eggs in 1 l swelled eggs	80 000	120000
Rate of fertilization (%)	80	95
Hatching of fertilized eggs (%)	90	95
Survival of larvae up to taking air (%)	90	95
No. of feeding larvae from 1 kg dry eggs	500 000	700 000
Length of incubation of eggs (D°)	60	70
Length of non-feeding larvae phase (D°)	60	70
Size of feeding larvae (mm)	6	7
Size of 1st feed (µm)	100	300
Size at starting species specific feeding (mm)	25	30
Amount of eggs in a 7–9 l Zuger jar[5] (gr.)	100	200
Amount of swelled eggs in a 7–9 l Zuger jar (l)	1	2.5
Amount of eggs in a 60 l jar (gr.)	100	200
Amount of swelled eggs in a 60 l Zuger jar (l)	1	2.5
Amount of larvae in a 60 l jar (No.)	80 000	120 000
Amount of larvae in a 200 l jar (No.)	250 000	400 000

5.1 Separation of sexes

Broodstock needs to be separated by sexes when water temperatures reaches 8-12 °C, coincident with the average temperature during March-April in temperate zones. Allowing mixed stock to remain together means sexual products will effectively be lost for artificial inducement as part of the programmed propagation activity. Separation of sexes is done based on typical external signs. Fish are removed from the mixed pond via netting and placed into two pre-prepared ponds.

When separating broodfish by sexes, if broodstock have not already been tagged or otherwise marked, then the separation process allows the opportunity to undertake this marking. PIT-tag methods are identified in footnote 1. If individual PIT-tags are not used, breeders still need to be marked to identify each broodfish. In this case, coloured thread inserted into the dorsal fin is an easy and rapid method for differentiation. Marking is followed by weighing the individual fish since the weight is the basis of calculations for pituitary treatment to generate artificial propagation. It is advisable to plan the operations in a way whereby the broodfish can receive the pituitary treatment when they are brought into the hatchery. Record the details on identifications and weight for future reference and to allow analysis of broodstock performance.

Once separated, feeding of respective sexes should be increased. Over this spring period, broodstock should be fed with feeds that are high in animal protein and rich in vitamins to ensure the broodstock are strong and healthy, and readied for propagation.

As an example, the following pelleted extruded feed is used in Hungary for feeding carp breeders during the "1-2 month" period before the reproduction season, for preparing them and in order to improve their general condition. An approximate content of such feed is shown in Table 2.

Table 2:
Special broodstock feed for the 1-2 month period before the reproduction season
(https://haltapkft.hu/haltap/ponty-anyatap/7/)

Component	Proportion, per cent, Quantity	Component	Proportion, per cent, Quantity
Dry matter	88.0	**Ca**	0.9
Crude protein	27.0	**P**	0.7
Crude fat	4.6	**Na**	0.1
Crude fibre	2.3	**Vitamin V** (IU)	15 000.0
Lysin	1.3	**Vitamin D3** (IU)	1 200.0
Methionin	0.7	**Vitamin E** (mg/kg)	75.0
Methionin+cystin	0.8	**Particle size** (mm)	5.0

This feeding regime is different from the standard diet of the fish. This diet, used after spawning, should provide different energy requirements used specifically for recovery and for yolk production in the further development of eggs which accumulate in the ovaries during the summer period.

5.2 Selection and handling of males and females

At the separate ponds, broodfish are caught by netting them and are examined, with developed and ripe specimens selected for artificial propagation. When checking broodstock, less developed broodstock can be returned to the pond and can be selected later after further development has occurred. There may be considerable differences in the development of individual fish that needs to be taken into account. At the point of selection the number of ripe broodstock is decisive, however, as the propagation process cannot be restricted during the short spawning season by the lack of adequate numbers of females and males. Farmers need to be able to select an appropriate number of female and male fish at the same time. Similarly, once selected for propagation, there must be sufficient hatchery facilities available to be able to handle the number of fish involved.

During artificial propagation broodstock are handled repeatedly, through netting, being moved from one holding unit to another, and during injection, suturing and stripping processes. Handling induces stress in the fish and to reduce the stress effect sedation techniques are applied, by submerging fish in small shallow containers into which aesthetic is added. Using “shallow anesthetisation” it means the fish will be easier to handle and stress will be lower.

An appropriate-sized container should be used, depending upon how many fish are being anesthetized at the same time. Anaesthetization is most typically done using a solution of tricaine methane-sulphonate or MS 222 at a dilution of 1:10 000, for example by adding ten grams of MS 222 per 100 litres of water used. Other compounds are also available for use. Clove oil can be added to water at a concentration of three millilitres per 10 litres of water, for example. Fish should remain anaesthetized for the minimum time period required and only as deep as necessary for the procedures involved. When anesthetized, gill cover movements should be monitored, and oxygen content should be optimal.

FIGURE 5
After anesthetizing and identifying individual fish, their body weight is weighed and measured and the information used to calculate the required dose of pituitary treatment

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When anesthetizing fish, a separate tank filled with fresh water should be available, with oxygen if necessary, into which the fish are placed to allow for a recovery period. The length of time required for recovery will depend on how deeply anesthetized the broodfish are.

5.3. Induced spawning by hypophisal injection

The initial doses of hormone used to induce spawning can be carried out at the pond area, before fish are transported to the hatchery. Gonadotropic hormone is prepared from the pituitary glands of carp and is the most effective agent for inducing ovulation. Pituitary glands are usually collected from mature and healthy carps and are widely available for purchase. The gonadotropic hormones in these dried glands will maintain their activity for years if kept under dry conditions, such as being kept in laboratory excicators.

To prepare the mixture, the pituitary glands are pulverized with a mortar and pestle and dissolved in a small amount of fish physiological saline solution, sufficient to ensure the mixture can be injected. The solution is taken up in a syringe and the required quantity is injected into the body cavity of fish at the bottom of the ventral fin. Propagation of female carps can be induced with 3.5–4.5 mg air-dry hypophysis per kilogram of body weight.

In females, ten percent of the calculated total dose is given as a first treatment, injected prior to transport to the hatchery (preliminary dose) to bring the eggs into the stage of pre-ovulation.

At the same time, and to prevent pre-timed depositing of eggs, the genital opening of female carps is closed with a suture (Figure 6). If not possible, suturing can occur on arrival at the hatchery, but a further anaesthetization would be required, increasing the risks of harm to the fish. Suturing must take place before the decisive and final treatment/dose. The final decisive dose is given 10–12 hours after the preliminary dose in order to induce the ovulation. The final dose must be administered under anaesthetic. In an ideal case, the second dose is applied during the evening hours, so that ovulation, which occurs 12–13 hours after the final dose depending on temperature, happens the next morning.

Male broodstock should be treated with only a single dose, with 2 mg of pituitary for 1 kg of body weight, just prior to their transport to the hatchery.

FIGURE 6
Suturing the genital opening of anaesthetized carp females

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FIGURE 7
Single dose of pituitary is given to the carp males during the transportation to the hatchery

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5.4. Stripping of eggs and sperm

In females, ovulation will be expected 240 to 260 degree-hours (approximately 12–13 hrs) after the second hypophisation treatment. Once in the hatchery, all broodfish, males and females, should not be disturbed after treatment, to allow ovulation (females) and sperm production (males) to take place. In females especially, stress can have a negative effect on ovulation. The quiet surroundings, a constant tank water temperature (22–24 ^{0}C) and high oxygen concentration in the water (5–6 mg/l) should be maintained until stripping commences.

In an undisturbed environment carp will try to spawn in the tanks. The spawning is indicated by loud splashing in the tanks. If the “signalling male” is placed with the females he will choose the ripest female and start the spawning process with her. Sometimes, in females, the spawning is simulated even when no male is present.

As the discharge of eggs is prevented by the suture, stripping should be started only after 10 to 20 minutes of vigorous spawning activity. The fish removed from the tank should be those broodfish showing most active spawning activity, thus allowing other less active fish to complete ovulation.

Female broodfish should be gently removed from the tank and anaesthetized using MS222 or other suitable product to reduce the loss of eggs during stripping and to avoid injuries. Once anaesthetized, the fish is removed from the container and dried to remove excess water and held gently in the towel (Figure 8). Care should be taken that no water is mixed with the sexual products that are stripped. Stripping of females occurs by removing the suture and gently squeezing the abdomen until eggs appear, and the eggs squeezed into a suitable sized dry container (Figure 9). The same process of anaesthetization, drying and squeezing of males, to remove milt, should be undertaken. Milt should be collected in separate containers to the eggs. Separate containers should be used for each broodfish (male and female) stripped.

After stripping, broodfish should be placed into recovery ponds without delay to prevent further injuries.

The weight of “fresh” eggs is measured after stripping and the number of eggs can be calculated. The volume (millilitres) of milt produced should also be measured. This will serve as a basis for further calculations.

FIGURE 8
Stripping of eggs by gentle squeezing, into a dry container. Note that a towel is used to dry the fish initially to remove excess water and used to hold the fish to prevent infection potential

5.5. Artificial fertilization of stripped eggs

Fertilization of the eggs occurs, when the sexual products stripped from the fish are mixed in a plastic bowl (Figure 9) in a ratio of 2 x 5 ml of milt to one litre of eggs. It is good practice to use milt from 2 male fish, 5 ml from each, added to one litre of eggs from a single female. Note and record, in hatchery workbooks, the identity of each fish used, to enable tracking of fish performance. Once milt is added to the eggs, this is gently mixed with a clean and dry plastic spoon.

Fertilization requires the addition of a fertilizing solution, which should be pre-prepared before artificial fertilization activity commences. Known as Woynarovich-solution, 40 g of salt and 30 g of carbamide should be added to ten litres of water in a container, and stirred thoroughly. The solution reduces egg stickiness by removing proteins around the eggs and allows sperm to penetrate the egg and for fertilization to take place. The fertilization solution is added to the mixed eggs and sperm at a rate of 100-200 ml per litre of eggs.

This solution is stirred thoroughly for a few minutes with a plastic spoon. During this period the egg stickiness is eliminated and sperms are activated and fertilization of eggs takes place. The eggs then begin to swell. During the swelling period the fertilization solution is added in small quantities and stirred slowly. Adding the solution too quickly can result in clogging of the eggs, so should be added in small volumes, repeatedly. Unused sperm and dissolved protein from the eggs gradually collect at the surface, which is then poured out and is replaced by fresh solution. After a few minutes the eggs no longer adhere to each other.

The process of egg swelling lasts 1–1.5 hours, during which the fertilization solution is changed 3–4 times. Each litre of dry eggs produces six to nine litres of swollen eggs. After swelling the eggs need to be hardened using a tannic acid solution, which has been pre-prepared by diluting 5 g of powdered tannic acid in 10 l of water.

One litre of tannic acid solution is added to 10–12 l of swollen eggs. The solution is stirred by hand immediately and the eggs are allowed to settle down. The “used tannic acid water” is discharged and eggs are rinsed with fresh clean water 2–3 times. The tannic acid solution removes any remaining stickiness and completes hardening of the eggs.

FIGURE 9
Mixing of eggs with sperm in order of fertilisation[3]

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5.6. Incubation of fertilized eggs

After fertilization and egg hardening the rinsed eggs should be placed into the Zuger jars immediately. One to 1.5 l of swollen carp eggs are added into a 7–9 litre Zuger jar, which is half filled with water. (Figure 10).

FIGURE 10
Incubation of the fertilized eggs in a Zugar Jar

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During the incubation period special care should be taken to maintain water flow-through the system as a means of controlling the oxygen concentration. The sensitivity of the eggs and the oxygen requirement of the eggs are changing through the incubation process. During the early stages of incubation the oxygen requirement of the eggs is low and their sensitivity to mechanical impacts is very high. This requires a slow water flow of 0.6–0.8 l/min through the system during the first 10 hours of incubation. During the second stage, the oxygen requirement of the eggs grows rapidly, so water flow must be increased up to 1.0–1.2 l/min. This flow is maintained until the tail, eyes, and colour of embryos become visible. Immediately

[3] http://www.fao.org/fishery/affris/species-profiles/common-carp/common-carp-home/en/

after this, in the third and final stage - right before hatching - the oxygen requirement is very high and the water flow should be set at 1.5–2.0 l/min. The duration of the incubation depends on water temperature (the optimum is between 22–24 °C) and usually it requires 60–70 degree-days. During this stage water flow, water temperature and development of eggs should be inspected regularly.

During the embryonic development the risk of fungal infection is high. Applying an effective fungicide, approved for use with food fish, could be necessary.

5.7. Hatching the larvae

Appearance of the first free swimming larvae is the sign of the start of the hatching activity. This process can be “provoked” or “synchronised” by reducing water flow to a minimum level to reduce the oxygen concentration, because low oxygen level will promote the process of hatching. Once the process has started the normal water flow is returned and mass hatching takes place.

6. LARVAE NURSING OF COMMON CARP

6.1. Nursing of larvae in tanks

Hatched larvae should be transferred to holding tanks when hatching is at an advanced stage. Hatched and partly hatched larvae are siphoned from Zuger jars with a rubber pipe into clean plastic bowls where, after few minutes, the hatching is completed. From here the larvae are transferred (always in water) to larger-sized (50–200 l) larvae holding jars (Figure 11) and practice shows that in a 200 l larvae holding jar approximately 500 000 larvae can be kept.

FIGURE 11
Larvae keeping tanks

Once added, the larvae attach to the surface of the jar with the help of a secretion from a gland on the head. This non-feeding stage of development lasts 3–4 days at 20–24 ^{o}C, and until the larvae can fill their swim bladders with air and can start active horizontal swimming. Once swimming has started, the digestion track of larvae will be able to receive and digest external feed. This food requirement of larvae can be fulfilled with a feed prepared by mixing boiled eggs in the form of fine suspension in water, which is added to tanks. Larvae can be fed this way a few times before they are moved to specially prepared nursing ponds.

6.2. Pond preparation

Preparation and cultivation of the nursing pond bottom is essential. During the winter dry ponds must be cleaned and hard-stem water-weeds removed. Before flooding the pond bottom, or in case of repeated use of the pond for new stocks, the pond bottom and underwater parts of the dykes should be disinfected with lime (Table 3). For treatment, lime is added at a rate of 200–300 kg per hectare spread evenly. Lime influences the structure of the soil and the chemical composition of the filling water. Aeration of the soil of the pond is accomplished by tilling, which increases plankton production once the pond is filled (Table 4).

Table 3
Application of lime at pond preparation and during the production season

(Woynarovich et al, 2010 cited form Woynarovich et al, 2011)

pH	Preparatory dose (kg/ha)	Monthly dose (kg/ha/month)
8	50–100	10–25
7.5	100–200	25–50
7	200–300	50–75
6	300–400	75–100
Less than 6	400–450	100–125

Table 4
Recommended time for filling and draining the pond

(Antalfi and Tölg, 1971 cited from Woynarovich et al, 2011)

Size of the pond (ha)	Length (days)
Smaller than 0.1	0.2–0.4
0.1–1	1–3
1–6	1–3
6–30	4–14
30–60	8–15
Larger than 60	15–30

Before being filled, the pond can also be prepared with the addition of chemicals and fertilizer that will encourage growth of plankton. These chemicals include organic phosphorus esters, as well as pyrethroids. The rules for the use of different chemicals may vary from country to country. It is always necessary to coordinate the use of chemicals with the norms and standards adopted in the country. To avoid the risk of developing unwanted plankton organisms, it is necessary to fill the ponds to half depth 5–7 days before stocking of larvae.

Artificial fertilization of the ponds should be a planned activity (Table 5 and 6). Nitrogen-containing fertilizers, such as carbamide or ammonium nitrate, should be mixed in pond water; applied at 150–200 kg/ha. Half of the quantity to be applied should be added when the pond is being filled. The remainder should be given in two applications after the first week and after the second week of nursing. Solubility of phosphorus containing fertilizers is difficult and these may be added in a semi-dissolved state to the ponds and dispersed over the greatest possible pond area. Phosphorus should be added at 100 kg/ha and may also be added when the ponds are being filled. The effect of artificial fertilizers is realized slowly through the development of algae and bacteria. Other effective fertilizers, such as organic manure can be applied also and contain their own bacteria and organic detritus content that may serve as direct nutrients for zooplankton organisms, the most important of which, for carp is *Rotatoria*. The most effective manures are stable dung produced by horses or cattle, although manure of pigs, sheep or poultry can be used together with compost, depending on local conditions. Manure is applied at 3–7 t/ha. Manure should be used, if possible, in a liquid form, mixed with water, but if this is not available or possible, it should be watered and dispersed directly over the pond from the pond embankments (Table 5).

Table 5
Recommended quantities of manure and fertilizers
(Horváth and Pékh, 1984; Horváth et al, 1985 cited from Woynarovich et al, 2011)

Name	Total quantity (tonne/ha)	% of total quantity	
		Start	Later
Production of advanced fry			
Manure	1.5–2.5	100	0
Carbamide (urea)	0.15	100	0
Superphosphate	0.1	100	0
Production of elder age groups of fish			
Manure	3–5	25	75
Carbamide (urea)	0.4–0.5	25	75
Superphosphate	0.3–0.4	25	75

Table 6
Frequently used fertilizers (Xinhua, 2011 cited from Woynarovich et al, 2011)

Type of fertilizer	Description
Nitrogenous fertilizers	**Liquid ammonia (NH_4OH) or (NH_3 x H_2O) with a nitrogen content of 12–16%** It is a water solution of ammonia which is an important product of small-scaled nitrogenous fertilizer factories with simple synthesizing procedure and low cost. Ammonia is in an unsteady state when it is in water, easy to volatilize, so it could almost lose its effect through the volatilization if it is exposed to the air for a long period of time.
Nitrogenous fertilizers	**Ammonium sulphate ($(NH_4)_2SO_4$) with a nitrogen content of 20–21%** It is produced from the liquid ammonia directly neutralized with diluted sulphuric acid. It is white crystal when pure, apt to dissolve in water. 100 kg of water can dissolve 75 kg of ammonium sulphate. With a little absorption of moisture it is convenient to preserve and apply.
Nitrogenous fertilizers	**Urea ($CO(NH_2)_2$) with a nitrogen content of 44–46%** Ammonia and carbon dioxide are interacted and synthesized into urea under high heat & pressure. It is white crystals with a strong absorption of moisture. After dissolution in water, urea does not turn out ions and is unable to be absorbed directly by plants. It can be utilized by plants only after it is decomposed by urease excreted by urea-decomposing bacteria and transformed into ammonium carbonate. The conversion rate of urea is related with the temperature. It can be totally transformed into ammonium carbonate in 4---5 days at 20°C and in 2 days at 30°C.
Phosphoric fertilizers	**Calcium superphosphate ($Ca(H_2PO_4)_2$) H_2O with 12–18% of P_2O_5** It has a subsidiary content of $CaSO_4$, $2H_2O$, (about 50%). Usually, it is a white powder. It is corrosive and is apt to absorb moisture, smelling acidic since there is some free acid in the product.

Insecticides can also be used to minimise the overall concentration of aquatic insects and large size zooplankton, so that other more appropriate species of smaller size can develop. Insecticides should be dissolved in water (e.g. in a bucket) and poured into the pond from around the embankment. For larger ponds a boat can be used for this purpose. The amount of chemicals to be used depends on the volume of water present in the half-filled pond, with the aim to yield a concentration of 1 part per million (ppm).

Following the proper pond preparation, *Rotatoria* introduced into the pond with the flooding water will reproduce rapidly and after 5–7 days will be present in large quantities in the pond water. 5-10 ml of *Rotatoria* biomass per 100 litres of pond water providing the best nourishment for common carp.

After treatments have been undertaken the pond can be filled to its full extent.

Properly prepared ponds can be stocked with larvae. Larvae should be transported in plastic bags held in the dark in boxes (Figure 12) or in bulk transport tanks. Special care should be paid to temperature differences during transport of fry from the hatchery to the pond. If there is a water temperature difference of more than 1–2 ^{0}C, between transport bags and the pond water, then the transport bags should be placed in the pond for 10–20 min to equalize. Similarly, if there is a difference between the bulk tank and pond water, pond water should be added gradually to the transport tank for slow equalization of the difference in temperature. Sudden changes in water temperature have detrimental effects on larvae and may lead to high mortality. The fry should not be introduced into the pond at one place but should be input at different points along the wind protected side (embankment) of the pond (Figure 13)

FIGURE 12
Transportation of nursed fries in plastic bags which are placed in boxes

FIGURE 13
Release of nursed fries from plastic bags into the recipient nursing pond along the wind-sheltered side of the pond, occurring after equalization of water temperature in the bag and the pond

Stocking density of larvae is always dependent on the abundance of feed organisms and productivity of the pond. Generally, 1-2 million larvae are stocked per hectare of nursing area, but with a proper chemical treatment for *Rotatoria* sp. propagation, this number may be raised to as high as 5–6 million larvae/ha.

6.3 Pond growth of larvae

In well-prepared ponds the rich and plentiful zooplankton population that develops will meet the initial feed demand of larvae, even when the stocking densities of larvae are high. Larvae grow quickly, however, and as the larvae/fry's grow the amount of feed consumed increases, and after this initial period the zooplankton population will not provide sufficient feed to cover the fry's daily feed requirement. If there is not enough food, the initial rapid growth may stop and can weaken the larvae, which means they will be more susceptible to diseases. This condition may be prevented by proper supplemental feeding.

6.3.1 Feeding of carp larvae/fry in ponds

Feeding with artificial feed should start on the first day after the stocking, in small quantities initially, so larvae/fry will become accustomed to the flavour or taste of the artificial feed. The nutrients in the feed, as it disintegrates and become soluble, will also serve as nutrients for zooplankton as well.

Starter feed of the required nutritive value is available for Cyprinid larvae. Due to economics, live feed organisms present a better solution. The feed must provide the energy and protein nutrients required for growth and larval feed should contain 40–50 percent protein. Feed composition is similar to that during nursing (Table 7), although the protein content is generally higher in the second half of the three to four-week growing period. The size of feed particles is most important. Initial feeding larvae can consume feed of only a few hundred microns in size. After approximately 2 weeks common carp consume feed of approximately 1 mm size.

Table 7

Simple mixture of supplementary feeds for rearing advanced fry of carps in pond (Horváth and Tamás, 1981 cited from Woynarovich et al, 2011)

Ingredients	%
Wheat or barley flour	25
Soya	25
Fish meal	25
Meat or blood meal	25

Theoretically all feed crumbles must contain all nutrient components and consequently must be thoroughly mixed in meal form and afterward sieved to the desired size. The quantity of artificial feed to be applied during the nursing period depends on the abundance of zooplankton, the survival rate and the age of the fry. Nursing fry in ponds may require 1–1.5 kg artificial feed/100 000 larvae/day. The feed should be given every day in two portions. The amount also depending upon the appetite of the fry, and this may be gradually doubled during the last days of rearing. Overfeeding should be avoided.

6.3.2 Monitoring growth and health status of fry in ponds

As a consequence of high stocking density, intensive metabolic processes, and unstable biological equilibrium within the pond during the nursing period, there is an increased (high) risk of disease outbreak, including infectious diseases. It is imperative that farmers assess the behavior of their stock as an indication of health status. Strange swimming behavior, larvae/fry resting on the water surface or gathering at the inflow or outflow, plus other indicators, could suggest the fish are under stress or have an abnormal health status. For closer investigation, specimens that have the strange behavior should be chosen for investigation. Water quality of the pond, especially oxygen concentration should also be monitored systematically.

Fish farmers may recognize some of the most typical diseases. However, it is always useful to have an experienced veterinarian for more complex cases.

During the nursing period parasitic diseases, like Trichodinosis, Costiosis, Chilodonellosis and Ichtyophtiriosis can also cause problems. The appearance of these parasites is a sign for the fish farmer that something is wrong with the fry; which may be associated with inappropriate feed, contaminated water supply, stocking density, and so on. Poor appetite, poor feed conversion and slow growth are also characteristic of parasite infestation.

6.3.3 Harvest of fry

After 3–4 weeks of nursing, advanced fry of 20–30 mm and 0.1–0.3 g in weight must be harvested and transferred to rearing ponds for fingerling production. Harvesting and transportation of nursed fries must be executed with the greatest care. One day before removal, feeding should be stopped to prepare the nursed fry for transportation. For larger ponds, requiring collection over several days, feeding should be reduced gradually then stopped. The first step in collecting fish is the partial drainage of the pond through the main outlet, over which a fine-mesh net should be placed to capture the nursed fry (Table 8). The flow of water should be controlled and should not press the fry into the net. When harvesting larger ponds, water flow should be stopped and the net should be emptied and cleaned of debris regularly otherwise the net will clog and drainage may take too long.

Table 8
Recommended mesh size of screen at filling and drainage of ponds

(Horváth and Pékh, 1984 cited from Woynarovich et al, 2011)

Age of stocked or harvested fish	Mesh size (cm)	
	Stocking	Harvest
Fish larvae	0.2	-
Advanced fry	1	2–3
One-summer-old fish	2-3	6
Two-summer-old fish	6	15
Table fish	15	20

Small ponds of a few hundred square metres, and which are not too wide, can be fished with a sieve-cloth net pulled across the pond once the water level is lowered (Table 9). Most of the nursed fry can be harvested, but some may escape/remain in the pond. In larger ponds one should wait until the nursed fry accumulate in a few hundred cubic-metres of water in front of the drainage structure. At that time the pond may be fished with a pull-net several times.

Table 9
Recommended mesh size for catching carp

(Horváth and Pékh, 1984 cited from Woynarovich et al, 2011)

Age groups	Full mesh (mm)
Advanced fry	2–3
One-summer-old fish	5–10
Two-summer-old fish	15–30
Table fish	30–50

Nursed fry generally need to be stored for a few hours before transportation. For this purpose rectangular nets prepared from fine material with a volume of 5-10 m^3 can be used. These holders are placed near the outflow, and can be used to hold 200 000–500 000 nursed fry for a few hours. The nursed fries are less endangered if placed directly into the transportation vehicle, however. Nursed fries are counted volumetrically. A unit volume (e.g. 0.5 l) of nursed fry is removed from the holding boxes and counted (with the principle “from water to water”). This is repeated several times and the average number in the unit volume may be calculated. Then desired numbers of nursed fry may be volumetrically transferred to a transport container. Nursed fry can be stocked for transport at a rate of 70 000 to 150 000 per m^3 and may be transported for several hours. During transportation adequate oxygen must be provided with the help of air pumps. The stocking process of nursed fry into rearing ponds is similar to that of feeding larvae described previously, except that they are less sensitive to minor differences (1–2 ^{0}C) in water temperature changes so acclimation may not be necessary when input into the rearing pond.

Whether the ponds are large or small, remaining nursed fry may be captured by complete drainage, through capture boxes or drainage nets. From here the nursed fry can be removed with small hand nets before transport.

7. WINTERING

7.1 Wintering of common carp

Wintering is a critical phase in freshwater fish culture in the continental climate prevalent in Central and Eastern Europe, and within the CACFish area of competence, where winters can be harsh and cold. There is a need to protect fish as much as possible and to undertake mitigation measures to prevent big losses during winter. Live fish should be stored safely and the best option is to collect fish in autumn and move them into special wintering ponds.

Table 10
Stocking densities of common carp depending on size of fish
(Antalfi and Tölg, 1971, cited from Woynarovich *et al*, 2011).

Fish size (g)	Stocking density (kg/m^2)	Quantity of water (l/min/100 kg fish)
10–20	8–10	6–12
20–50	10–12	6–12
200–600	15–20	6–12
1 000–3 000	18–22	6–12

During the autumn fish should be harvested, sorted and separated by species and size and transferred to specific wintering ponds based on this size to increase uniformity within the ponds. Health status should be monitored. Certain prevention treatment should be applied. Wintering ponds are usually smaller and deeper ponds with a good water supply. The stocking densities of common carp, depending on fish size, are shown in Table 10.

Wintering ponds should be well prepared in advance. They should be dried, cleaned and disinfected well with quick lime. Once re-filled they should be provided with a continuous supply of good quality water, through good setting of supply and drainage structures from storage ponds, for example. During the winter months, oxygen content of water of wintering ponds should be checked daily. When water is covered by ice then snow should be cleaned regularly for better light conditions and ice holes need to be cut for better aeration.

In case, fish stays in production ponds where they were grown, they will be harvested only at spring. Another possibility is to harvest the fish during the autumn and move them to the production pond of the following year. In these non-ideal cases the production ponds have to be deep enough in order to provide ideal water temperature conditions for wintering of carp.

7.2 Wintering of broodstock

Broodstock are selected from the table-size fish population in autumn and the best specimens stocked into wintering ponds. Several hundred broodstock can be kept over the winter in a wintering pond measuring approximately 600 to 1 000 m^2, providing there is sufficient water supply available. During the selection process, fish selected should be immersed in a 2–5 percent salt solution for a few minutes to remove external parasites. In case of necessity (special diagnosis by veterinarian) other treatments could be applied against other external parasites, according to the local regulations. At the start of wintering period and again at its end in early spring, health status of broodstock has to be checked by fish veterinarians. If needed, fish should be treated accordingly.

Wintering ponds require a regular water flow that operates throughout the entire winter, in order to replace water, add additional water that may be lost due to ice formation and more generally to maintain good oxygen concentration in the water column. Adequate water supply is necessary and especially so for broodstock, being larger fish and requiring proportionately more oxygen to undertake normal maintenance functions, such as respiration. Over winter broodstock will not be disturbed by string flows, but these may cause difficulties, especially during early spring warm-up periods when flows may increase.

Towards the end of winter it is important for farmers to ensure their broodstock have sufficient food available, and may need to add feed to make sure. If the fish farmer does not feed the broodstock when the water temperature increases in the spring, the fish will lose weight and become more susceptible to various diseases. In the larger winter ponds, the warmer water in spring may induce growth of pond organisms, that may provide optimal feed for the fish when they start feeding early in the spring.

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PART II. TECHNICAL MANUAL ON CHINESE HERBIVOROUS FISH BROODSTOCK MANAGEMENT

Prepared by Vitaly Bekh

1. INTRODUCTION

Three Chinese herbivorous fish species; silver carp (*Hypophthalmichthys molitrix* Val.), bighead carp (*Aristichthys nobilis* Rich.) and grass carp (*Ctenopharingodon idella* Val.) (hereafter referred to as herbivorous fish): occupy the most important place in aquaculture for the countries of Eastern Europe, Central Asia and the Caucasus. In China, the herbivorous fish industry provides the main commercial output in the aquaculture sector of the country (FAO, 2014). The demand for stocking material (fish seed) of these species increases every year, with many region facing shortages of seed. In the last decades herbivorous fish have become the most important object of aquaculture on the territory of the countries of the former USSR, since the more traditional production of common carp in ponds has suffered, due to high costs of artificial feeds or their poor quality (Dmitriev, Bekh, and Kucherenko, 2000).

Acclimatization activities related to the introduction of herbivorous fish to the countries of Eastern Europe, Central Asia and the Caucasus were initiated in the 1950s. These fish originated from the Far East, particularly the rivers of China and the Amur River running through Russia and China (Vinogradov and Erokhina, 1977).

The herbivorous species, at a practical level, do not reproduce naturally in the countries of Eastern Europe, Central Asia and the Caucasus. There exist some occasional reports of natural spawning of silver, bighead and grass carp in the lower reaches of the Danube River, or in a number of water bodies in Central Asia (the Kara Kum canal, the Amu Darya River), but the effectiveness of that reproduction was low and their fry practically did not survive (Chertikhin, 1989). Importantly, there does not appear to be a threat of biological invasion of herbivorous fish into the natural ecosystems of the CACFish region, and this provides fish farmers with a huge, almost unlimited potential for these species to be introduced into various natural and industrial water bodies and reservoirs (including canals, multipurpose water reservoirs, cooling reservoirs of power facilities).

2. BASIS OF HERBIVOROUS FISH BROODSTOCK MANAGEMENT

2.1. Biological characteristics of herbivorous fish

The bighead carp has the fastest growth rate among the fish of the Far Eastern Central belt. In water bodies of China, Central Asia countries and the South of Ukraine it can reach 40–55 kg body weight. Its highest annual weight gain is up to 5–6 kg, reported in cooling reservoirs of power facilities, for example. This partially herbivorous species naturally feeds on zooplankton, but in case of low abundance it is able to switch partly to phytoplankton and detritus.

The silver carp can reach a weight of 16 kg in general, and 20–25 kg in some cases. Its average annual weight gains have been assessed at 2–2.5 kg per year. This fish feeds on phytoplankton, the microscopic algae which are sometimes the cause of "blooming" in water. It is also a detritus feeder on the decomposed remains of plant and animal origin, which also plays a significant role in the nutrition of the silver carp (Borutskiy, 1973, Danchenko, 1974).

The grass carp stands apart among the herbivorous fishes from the Central and Far Eastern region. In physical features it differs significantly from the silver and bighead carp. Its prime food is higher aquatic plants and submerged terrestrial vegetation (e.g. during flooding). The grass carp prefers soft vegetation, and can reach 50 kg in weight, with an annual weight gain between 2.5 and 3 kg per year, and has a feed conversion rate of 30–40. In nature, these fish typically spawn in riverbeds with strong currents. The herbivorous fish shed bathypelagic (semi-floating) eggs that remain suspended in the water column and buoyant due to river turbulence. The spawning period is extended, with duration depending on the climatic conditions within the habitat area. The spawning and egg-laying of all three species in natural conditions commences during the period of summer flooding, typical for areas with a monsoon-based climate. The minimum temperature for the onset of spawning is 18–20 ºC, while the maximum temperature ranges from 26 to 30 ºC. After fertilization, eggs swell considerably; egg diameter increases 4–5 times and its volume up to 100 times. Depending on the water temperature, the spawning period lasts from 18-20 hours (at temperature of 27–29 °C) to 60 hours (at 17–18 °C). Passively drifting in the current embryos begin to concentrate in the coastal area, then fry are entrained or actively migrate into adjacent floodplain reaches or lakes, where they spend the rest of the summer feeding period (Nikolskiy, 1971).

2.2. Rearing of broodstock

Herbivorous fish are more heat-loving (thermophilic) than the common carp. Rearing of herbivorous species broodstock can be carried out in ponds, floating cages, suspended in cooling water reservoirs of power facilities, directly in these cooling reservoirs, and as well in other large multi-purpose water bodies (water reservoirs).

2.3. Establishment of broodstock and rearing brood fish in ponds, cages and water reservoirs

2.3.1. Rearing of broodstock in ponds.

The pedigree material of herbivorous fish can be reared in conventional carp ponds, provided they are designed and developed correctly, have area of 2–3 ha on average; and draining and filling should not take more than 3–4 days. Co-cultivation of different age cohorts of fish of the same species in one pond is not recommended in order to avoid retarded growth of older fish, which are more sensitive to feeding conditions. Replacement and broodfish of silver and bighead carp can be reared in the same ponds with pedigree material of common carp. In this case the stocking density standards for common carp culture are the same as when growing it in monoculture. Grass carp can be grown in the same ponds as common carp if the stocking density of the common carp is set where the natural productivity of the pond is sufficient without the need to add supplementary feeding using compound feeds, since it is highly undesirable to have grass carp broodfish transitioning on to compound feeds. Intensive feeding of grass carp with mixed feeds, including compound) feeds due to lack of green vegetation, can cause serious functional disturbances in grass carp, that delay growth and maturation, but can also cause massive fish mortality (Vinogradov, 1966).

When growing pedigree herbivorous fish, it is necessary to consider the species natural age specific requirements and try to comply with them to the maximum extent. Grass carp, and especially its older age groups, is capable of quickly eliminating all aquatic vegetation in a pond. Therefore, in case of its shortage, it is necessary to introduce green vegetation (of aquatic or terrestrial origin) at a rate of 30–50 kg per 1 kg of weight gain.

As the silver carp and bighead carp consume primarily phytoplankton and zooplankton as their main food source, it is necessary to satisfy their food requirements (Borutskiy, 1973) from within the pond itself. If the biomass of zooplankton decreases below 3–5 mg/l, bighead carp growth rate can sharply slow down. To avoid this, measures to stimulate the development of its natural forage base should be undertaken. Mineral fertilizers are introduced depending on the requirements for specific biogenic elements. For instance, nitrogen and phosphorus concentrations should be raised to a level of 2 mg/l and 0.5 mg/l respectively. Whenever possible, organic fertilizers should be also applied. Additional nutrients can reduce oxygen concentration, so in case of a worsening of the oxygen regime in the ponds, a constant water flow should be arranged, aerators installed if needed and lime introduced. Routinely, 1-2 times a month, a hydrochemical analysis is conducted and development of the natural food organisms is evaluated.

When growing pedigree material of herbivorous fish in ponds, it is necessary to adhere to strict standards (Table 1). In the course of grass carp feeding with terrestrial vegetation, the productivity of this species can be increased up to 200–300 kg/ha. With an equal supply of food, silver carp shows the slowest growth rate of the three species, with bighead and grass carp being higher the latter two species exhibiting similar growth.

Stocking density of the broodfish for summer holding periods should be based on a maximum number of individuals per hectare and for silver carp and grass carp should not be more than 100 ind./ha and for bighead carp not higher than 50 ind./ha; For grass carp, and throughout that period, there should be obligatory regular feeding with terrestrial plants in periods when macrophytes are lacking in the water body.

The mean weight gain during the summer feeding should be not less than 1.0 kg for silver carp, and 1.0–1.5 kg for bighead and grass carp. On this basis, the carrying capacity of broodfish (i.e. equilibrium weight of fish) in ponds during the summer holding period can be planned for, and set at 225–250 kg/ha overall, including 100 kg/ha, 50–75 kg/ha and 100–150 kg/ha for silver, bighead and grass carp respectively.

TABLE 1
Basic standards of rearing of pedigree replacement material of herbivorous fish in ponds
Weight figures for fingerlings and two-summer-old fish are given as mean weight before and after the selection respectively.

Age of fish	**Survival rate per cent**	**silver carp**		**bighead carp**		**grass carp**	
		weight (kg)	**carrying capacity of ponds kg/ha**	**weight (kg)**	**carrying capacity of ponds kg/ha**	**weight (kg)**	**carrying capacity of ponds kg/ha**
fingerlings	70*	0.04 (0.055)	300 - 400	0.08 (0.1)	200-300	0.08 (0.1)	100
two-summer-old	90	0.85 (1.0)	300	1.35 (1.5)	200	1.35 (1.5)	100
three-summer-old	95 - 98	2.0	200 - 300	3.0	150	3.0	100
four-summer-old	~ 100	3.0	200	5.0	150	5.0	100
five-summer-old	~ 100	4.0	200	7.0	100	7.0	100

*** -** at stocking with nursed fry

Similar to larval and fry production, there is a need to determine the size of the broodstock population, required to meet the overall production objectives, which requires an understanding of likely fecundity and survival rates for different stages in production (Table 2).

TABLE 2
Average productivity of herbivorous female fish in terms of fish stocking material or marketable fish

Mean indices for all three species	Units	Indices of productivity
Working fecundity of young (non-first maturing) females	thous. psc.	500
Survival rate of the successive age group:		
- larvae (from hatch to onset of mixed feeding), 50 per cent	thous. psc.	250
- fingerlings (from mixed feeding of larvae), 40 per cent	thous. psc.	100
- of one-year-old fish (from fingerlings), 80 per cent	thous. psc.	80
- of two-summer-old fish (from one-year-old), 80 per cent	thous. psc.	64
Mean weight of two-summer-old fish	g	500 – 1 000
Total potential yield of marketable-sized fish from one female during the season	kg	32 000 – 64 000

When determining the population size of the broodstock, it is essential to consider that for various reasons, some of the females subjected to hormonal injections do not reach maturation or produce eggs that are not of sufficient good quality. Therefore, it is wise to have a stock of female reserves in the broodstock population, which should be a minimum of 50 percent, and preferably 100 percent above that required if all fish spawned. It is not obligatory to have a reserve of males, however, because the requirement for males is lower overall, given it is sufficient to have 3–4 males (in case of bighead and silver carp) and 2–3 males (grass carp) for every 5 females in the broodstock; though some level of reserve may be useful.

During spawning activity there is a likelihood that a certain level of stock will be damaged in some way through stress or physical damage. During the spawning period the loss of herbivorous broodfish can amount to 20 percent and in silver carp up to 50 percent. These rates of "lost" broodfish should be considered while determining the size of the annual broodstock replenishment.

Knowing the requirements to rear broodfish and being aware of the standards for selecting different age groups for replacement and the carrying capacity of the water body, the number and area of ponds required for a given fish farm, can be determined (Table 3). When performing calculations, differences in the species-dependent timing of sexual maturity of males and females should be taken into account. Table 3 presents an estimate of the surface area of ponds for rearing and wintering of the broodstock replacement of silver carp as a species with a high demand for broodfish.

TABLE 3
Calculation of the area of ponds for rearing and wintering the broodstock replacement of silver carp (100 females and 100 males).

Psc = pieces

Selection strategy	**Age and sex**	**Rearing**			**Wintering**		**pond area (ha)**	
		number (psc)	**mean weight (g)**	**stocking density (psc/ha)**	**number, (psc)**	**mean weight (g)**	**summer**	**winter**
without selection	15-20 day larvae	not less than 2 000	0.03	not higher than 15 000	-	-	1.4	-
without selection	fingerlings (autumn)	-	-	-	14 200	40.0	-	0.07
spring selection of one-year-old fish – up to 50per cent	one-year-old fish after selection	5 750	50.0	not higher than 500	-	-	11.5	-
autumn selection of two-summer-old fish – up to 10per cent	two-summer-old fish after selection	-	--	-	460	1 000	-	0.05
without selection	two-year-old fish	440	1 000	220	-	-	2.0	-
autumn selection – about 95per cent	three-summer-old fish	-	-	-	420	2 000	-	0.10
without selection	three-year-old fish	420	2 000	200	-	-	2.1	-
autumn selection – about 95per cent	four-summer-old fish females males	- -	- -	- -	200 200	3 000	-	0.20
spring selection of males – up to 50per cent	four-year-old males	100 four-year-old males transferred to the broodstock, while 200 four-year-old females left for rearing						
without selection	four-year-old females five-summer-old females	200 -	3 000 -	200 -	- 200	- 4 000	1.0 -	- 1.0
spring selection – up to 50per cent	five-year-old females	100 five-year-old females transferred to the broodstock						
Total:							18.0	0.52

2.3.2. Rearing of broodstock in floating cages, set up in cooling reservoirs of power facilities.

For rearing broodstock of silver and bighead carp in floating cages, it is recommended to use cages of 12 or 24 m^2 area and 30 or 60 m^3 volume that has netting of 20 or 30 mm mesh size. The cages are installed in pontoon sections, sited at the boundary between warm and cool currents, in places near the discharge of warm water channels from power facilities into the cooling pond. The depth of water from the bottom of the cages to bottom of the water body should be not less than three metres. The optimal flow velocity in the cage placement is 0.2 m/s. Periodical rearrangement of lined cages is recommended depending on hydrological conditions (Baltaji, 1996).

An important parameter for the building of broodstock is the sum of effective temperatures (at temperature above 15 ºC), and all year round it should exceed 5 000 degree-days. At this, the mean seasonal biomass of phytoplankton and zooplankton should amount to 2-3 and 3-5 g/m^3 respectively.

Routinely, stocking of cages is carried out in the autumn with two-summer-old specimens of silver and bighead carp, weighing 200–300 g. If the cages are stocked with fingerlings or one-year-old fish, their weight should be 100 g or above. The ratio of both species at stocking is 1 to 1 (Table 4).

TABLE 4

The main fish-breeding and biological standards for the cultivation of the pedigree material of herbivorous fish grown in cages established in the cooling ponds of power generation facilities.

Age	**Stocking density, psc./m^2**	**Individual Weight g**
bighead carp		
two-summer-old	up to 30	400-700
three-summer-old	20-25	1 100-1 700
four-summer-old	10-20	1 700-2 700
five-summer-old	5-10	2 200-3 400
six-summer-old	5-10	3 000-5 000
silver carp		
two-summer-old	up to 30	300-400
three-summer-old	15-20	600-800
four-summer-old	10-15	900-1 300
five-summer-old	5-10	1 200-2 400

Annually in the spring, an assessment is conducted that results in the culling of ill and/or injured fish and removal of fish with delayed growth.

Throughout the year broodfish of silver and bighead carp are held in floating cages at a stocking density of 20 or 8 psc./m^3. In spring, in order to avoid over-ripening of broodfish, it is advisable to transfer some of them to wintering ponds, where they will be held till the start of the spawning campaign. It is sufficient to have a sex ratio 7M:10F in the broodstock, noting the reserve requirements mentioned previously.

It is not expedient to culture grass carp in cages.

2.3.3. Rearing of brood fish directly in cooling reservoirs of power facilities and large multi-purpose water reservoirs.

As a rule, cooling reservoirs of power generation facilities have a large fishery potential with a high level of development of the natural food base for herbivorous fishes. In ideal conditions the water temperature of the reservoir will not be above 40 °C, and ideally within the range 35–36 °C. The stocking of cooling reservoirs is typically carried out with two-year-old fish of 150–300 g wet weight, or sometimes with large fingerlings or one-year-old fish with a weight not less than 80–100 g.

It is critically important to ensure the stocking of fish is done exclusively with the use of pure species, and not any hybrids, which should be inadmissible in these water bodies, because it will not be possible, or at the least very difficult, to identify hybrids in the process of broodfish selection for the broodstock, which requires pure strains.

The rates of stocking of cooling water reservoirs are calculated on the basis of their natural forage potential. Refer to a more detailed discussion of this issue in section 9 of the present manual.

Stocking material should be evenly distributed throughout the reservoir, while injured fish are subjected to culling. Before the stocking, it is necessary to equalize the water temperature in the live fish transport containers with that of in the reservoir, by placing the containers in reservoir water for a period of time.

Herbivorous fish in the cooling reservoirs have a high rate of growth, and annual weight gain can reach 1.3–2.0 kg/yr for silver carp, 1.5–4.0 kg/yr for bighead carp and 1.5–2.5 kg/yr for grass carp. The maximum weights of these species amounts to 20–25 kg, 40–55 kg and 30–40 kg respectively. Harvest of broodfish and replacement of older broodfish is performed from March to November with the use of straining fishing gear, such as seine or stationary (fixed) nets. With the help of special sleeves, the fish are transferred to canvas or PVC vats, where healthy fish weighing 3–5 kg for grass and silver carp or 10–15 kg for bighead carp are selected. Then, the selected fish are held in small ponds with an area up to 0.5 ha for 2–3 weeks. During this period, fish with injuries or fungal infection (such as Saprolignia) are culled, the remaining healthy fish are transferred to wintering, summer broodstock or pre-spawning ponds, depending on the season.

The stocking density in summer broodstock ponds for reserve and brood fish are 200 ind./ha for silver carp, 80 ind./ha for bighead carp and up to 20 ind./ha for grass carp (with feed deprivation).

Rearing of herbivorous broodfish in other multi-purpose water reservoirs is also of great economic importance and allows rearing of fish at low production costs. In this case, special attention should be paid to the temperature regime within these reservoirs, and the water should not be too cool, as this can significantly slow down the maturation of herbivorous fish and reduce their mean annual weight gain. At the same time, in the regions of Central Asia, Transcaucasia, southern Russia and Ukraine, herbivorous fish can produce significant gross weight gain, especially in lowland, well-warmed water reservoirs.

3. CONTROLLED REPRODUCTION OF HERBIVOROUS FISH WITH TRADITIONAL HATCHERY METHOD

3.1. Sexual development of herbivorous fish

Controlled reproduction of herbivorous fish should be based on precise indicators, considering the influence of environmental conditions on the development of gonads. Such conditions characterize the changes likely to occur in the fish gonads during the maturation and annual cycles. The timing of sexual maturity onset in herbivorous fish depends primarily on the annual amount of heating, expressed in degree-days. In regions where the annual sum of heat exceeds 5 500 to 6 000 degree-days (e.g. South of Central Asia, cooling water reservoirs) herbivorous fish are capable of spawning twice during the vegetative period, whereas in tropical regions up to 3–4 times a year with the sum of heat being 8 000 to 11 000 degree-days. It is believed that in a temperate climate, 2 500 to 2 800 degrees-days (being the sum of effective temperatures above 15 °C) will be sufficient for the maturation of females between two adjacent years (Bagrov, 1993).

It is also important to take into account, that under controlled reproduction, some percentage of females do not participate in spawning and in summer period they have an intensive resorption (atresia) of oocytes. At the same time, under favorable feeding conditions the resorption of unshed eggs does not, on its own reduce fecundity or violate the subsequent oogenesis, nor the timing of readiness for the next spawning. Conducted trials proved that by the mid-August, in temperate climatic zones, the maturity indices of fish participating and not participating in spawning are equalized.

Fecundity of females maturing and spawning for the first time is low, and is typically two times lower than females spawning for the second time. Eggs and larvae obtained from the first maturing females of all three species are also much smaller than those from older breeders. It is necessary to avoid the use of first maturing females for breeding purposes. Good breeding results can be gained using females of 6–8 years in age (i.e. in their 2nd–4th years of utilization as broodstock) and fecundity falls slightly after that, though it is variable (Table 5). Females older than this have more demanding feeding conditions, however, and need a better food supply than younger specimens.

TABLE 5
Working fecundity of females of herbivorous fish at different ages. Values are in 000's of eggs.

Age (years)	silver carp	bighead carp	grass carp
3	167	-	-
4	332	293	302
5	486	620	434
6	488	780	560
7	805	730	561
8	546	605	911
9	631	850	834
10	666	900	646
11	744	796	916
12	1 000	840	740
13	912	1 244	700
14	786	903	720
15	1 033	1 000	775

In the broodstock of herbivorous fish it is not expedient to hold the fish older than 10-12 year. In the South of Ukraine, North Caucasus, Moldova, Central Asia and Transcaucasia, females of silver, bighead and grass carp generally reach maturity at age of 3–4 years, 4–5 years and 4 years respectively. Males reach sexual maturity one year earlier than females.

3.2. Sexual dimorphism and differences between males and females

As water warms in spring to above 17–18 °C it is necessary to carry out an assessment of the herbivorous fish broodstock. Indicators used to assess whether or not to include fish in the breeding programme are fish species, sex, age, whether or not they are tagged (individually or as a group), the degree of expression of the sex and readiness to spawn, and weight. Conducting broodstock assessments at an earlier time is meaningless, since before the onset water temperatures suitable for spawning, the broodfish do not externally show differences between males and females. The main characteristic indicating readiness of females to spawn, is the presence of a well-round, bulging and soft abdomen. This feature is especially clear evident in silver and bighead carp, but to a lesser extent in grass carp. In young females with a low fecundity, this sign will not be expressed clearly.

A physical characteristic feature that distinguishes males of herbivorous fish from females (besides spermiation) is the presence of peculiar horny denticles on the rays of the inner side of the pectoral fins. These are most evident in males of silver carp, having large and sharp denticles, most usually on the second and third rays. In the bighead carp they are less sharp and resemble the tubercles. In the male of grass carp, the denticles are tiny, but most apparent on the first hard ray, and the internal surface of the pectoral fins feels like an abrasive paper to the touch.

Denticles on the pectoral fins of the silver and bighead carp males can be found throughout the year. In males of grass carp, denticles are presented on the pectoral fins only during the feeding period; in the autumn, when the temperature decreases they disappear, and reappear in the spring after the water has warmed up.

In the process of pedigree material breeding, it is wise to tag (mark) the fish. Group marking is performed by cutting the pectoral, ventral and caudal fins. For individual marking, water-soluble Procyon dyes can be applied by subcutaneous injection. The use of different color combinations and various sites of injections allows for the individual marking of an almost unlimited number of fish. Moreover, tagging of pedigree material can be made using anchor tags, for example, Floy-Tag (http://www.floytag.com/) or electronic chips (referred to as PIT Tags) available from various producers. The use of PIT tags allows fish farmers to easily and efficiently trace and individually differentiate the active breeders and reserve group within the population of herbivorous fish.

3.3. Control of reproduction

Rearing of herbivorous fish in the countries of Eastern Europe, Central Asia and the Caucasus has been based exclusively on controlled reproduction in hatcheries (or incubation units) and with the use of hormonal stimulation.

During the assessment of females, at the start of the spawning campaign, readiness to spawn is checked and, depending on the degree of readiness, they are divided into three groups:

- The 1st group comprises the most mature females. Their abdomen is soft to the touch and saggy. Sometimes swelling is noticeable near the genital opening. This group of females is used on a first priority basis.
- The 2nd group – females with similar, but less evident characteristics.

- The 3rd group – females, which are almost indistinguishable from the males in appearance. Immediately after the assessment, they are culled or transferred for summer feeding.

For more accurate evaluation of females, if deemed necessary, it is essential to use the biopsy technique (see 3.4).

In the course of assessment of males, they are divided into two groups:

- The 1st group comprises males that give milt easily. They have a well-defined nuptial dress.
- The 2nd group are males that give off very little milt or do not flow. Such males are used as a reserve, and subjected to culling or stocking for feeding.

Fish selected as spawners (to produce progeny) are sorted by species, sex and groups, and stocked into ponds for pre-maturation holding. These ponds should have a small area, typically 0.05-0.2 ha and depth up to 2 m, be well designed, and have an independent water supply that provides for fast filling and draining of water, that should take less than 2–3 hours. The flow of water allows for constant water exchange and temperature control. It is desirable to have a water temperature for the Group I females below the 20 ºC, so as to prevent untimely overripening.

Holding mature females in ponds that have unsuitable conditions leads to degenerative changes in the ovaries. High temperatures and proper feeding conditions are factors that accelerate the process. At the first stages of over ripening, functional changes occur, that significantly reduce the viability of the offspring. As this process worsens, the quality of eggs deteriorates sharply, and at a certain stage it may not be possible to stimulate the maturation of such females by pituitary injections. The administration of gonadotropic hormones to such females generally causes their death, especially if traditional acetone-dried pituitaries of bream or carp have been used. During the pre-spawning period unripe females would be better placed in well-heated fattening ponds that ensures the most intensive development of the vitellogenesis process (yolk formation) and accelerates their maturation.

Males of herbivorous fish reach their maturity when the water arms up during spring coincident with the start of milt production, and generally occurs 10-15 days prior to female egg production. The holding of males during the spawning campaign in fattening ponds can significantly prolong the period of their functional maturity.

Overall, based on the aforementioned activity, hatchery reproduction should be conducted within a relatively short period, that does not exceed 30 days.

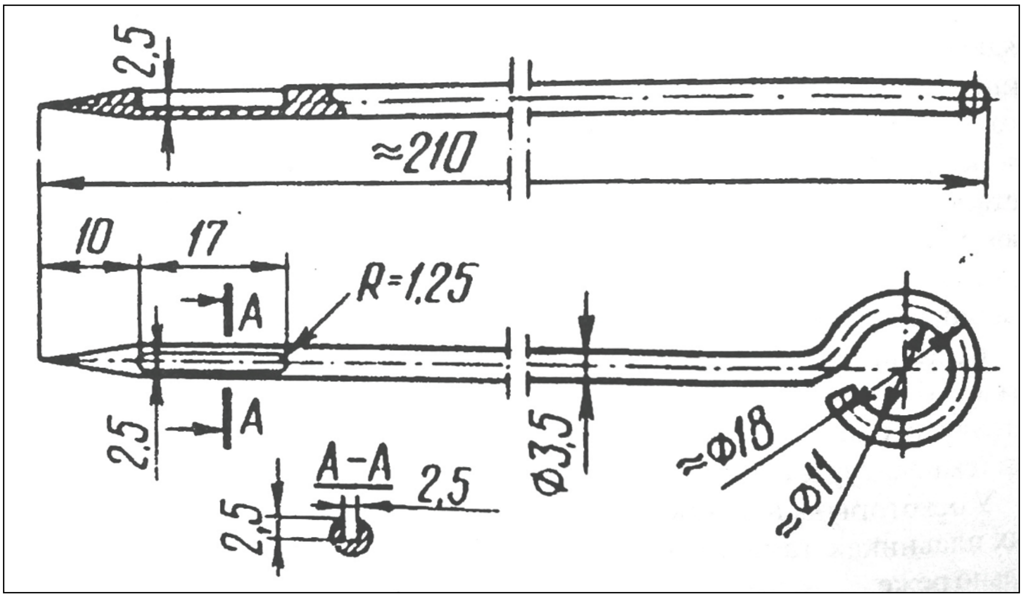
3.4. Hormonal stimulation of spawning

Work on production of herbivorous fish offspring should be started when temperatures are stable and range between of 18 ºC and 20 ºC. Hatchery reproduction begins with grass and silver carp, then 10–15 days after this with bighead carp. In areas with a hot climate mans in some years, all three species reach their maturation at the same time.

Timing is usually determined with the help of a "trial" batch of breeders. If the females from group I easily give off their sexual products, operations for the full loading of the apparatus in the hatchery should be initiated. If not ready it is possible to postpone the work for a week, and the ponds holding the broodfish should be filled with water at a temperature of 20–22 °C to improve the situation. An important indirect sign of female maturity is a good "fluidity" of males of this species.

A second, more complicated but more reliable method to assess readiness, is the biopsy. Biopsy techniques imply sampling of several eggs from the ovaries of a few properly prepared females of one species with the use of a special probe (Figure 1) or a syringe with a thick needle. In this a puncture is made in front of and above the genital opening at an angle of 30–40 degrees and the probe pushed in and pulled out to remove eggs. The sample is placed in to a solution, made from six parts rectified alcohol, three parts pure 40 percent formalin and one part of acetic glacial acid. Then the shape of an egg and the position of the nucleus are determined under a low magnification microscope. If an egg has an oval shape and a nucleus displaced toward the periphery the female is ready to spawn and can be used. If an egg is round and the nucleus is sited in the center, the female is not ready to spawn.

FIGURE 1
Probe (needle) used to extract oocytes from ovaries of herbivorous fish.

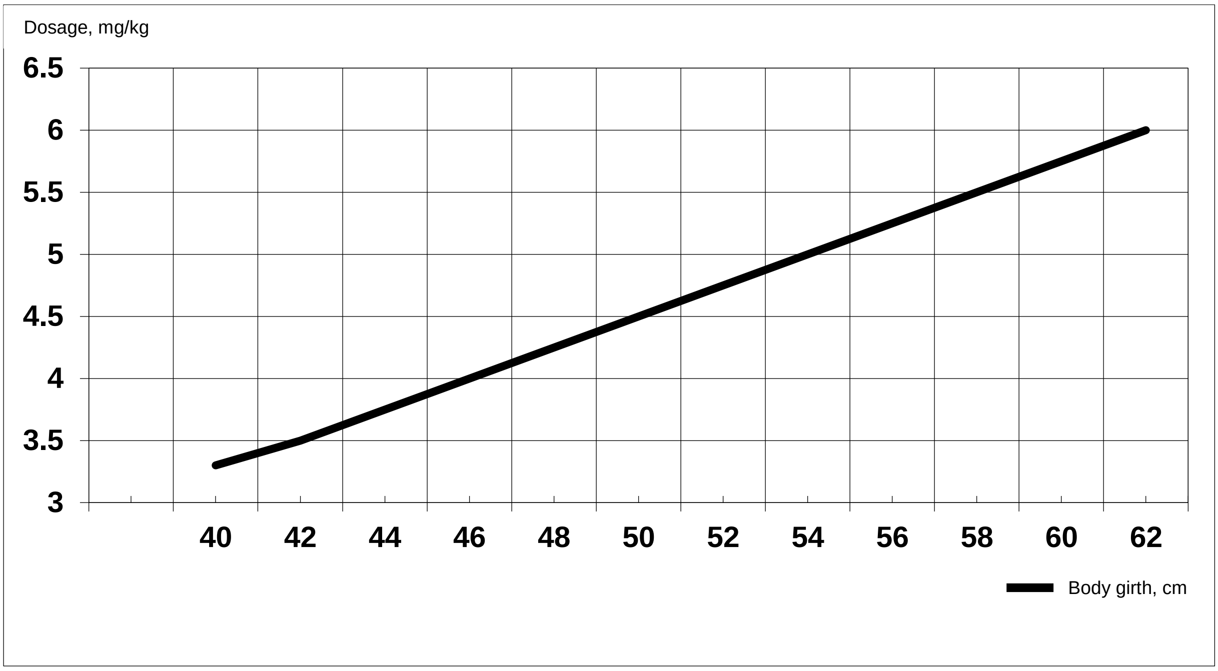


Pituitary injections are carried to replicate the normal hormonal changes that occur in herbivorous fish female fish to stimulate the maturation to the final phase of maturity, stage IV. Prior to spawning changes in the ovaries take place in two stages, that requires two separate injections of pituitary. For the first relates to pre-evolutionary changes in oocytes that turn eggs into mature eggs. This stage occurs under the influence of a small amount of the hormone. Ovulation is the second stage, related to extrusion of oocytes from the follicular membrane, which surround them in the ovary. This stage occurs under the influence of a large dose of the hormone. For this reason pituitary hormone injection occurs in two stages and is the basic principle of the fractional stimulation of breeders. The first injection (the so-called preliminary injection) contains 1/8–1/10 of the total dose required followed 12–24 hours later by a second "resolving injection" in which the remaining portion of the dose of the pituitary hormone is injected.

To obtain sexual products of proper quality from herbivorous fish, it is important to determine the correct dosage of pituitary hormone required. Routinely, it amounts to 3–6 mg per 1 kg of female weight but depends on the period of procedure, fish species, the degree of female readiness to spawn and the activity of the pituitary gland. It is recommended to use acetone-dried (and defatted in chemically pure acetone) pituitary of carp, bream or crucian carp pituitary. At the beginning of the spawning campaign, the proper dose is determined on the basis of a "trial" batch of fish, and corrected as the activity continues. Doing for females often relates to body girth and in females that have a large body girth, the dose of the pituitary should be increased by 10–20 percent, or dosed according to the relationship defined in Figure 2.

At the beginning of the incubation campaign, the interval between the preliminary and resolving injections typically amounts to 24 hours, although checks can be made using a small trial batch of fish after approximately 12 hours, and extended to 24 hours if required.

FIGURE 2
A nomogram of the relationship between dosages of pituitary and body girth in herbivorous fishes.



The active substance in the pituitary is administered to breeders as an aqueous suspension. It should be prepared immediately prior the injection, because it loses its activity when stored. Typically the suspension is prepared for a batch of fish of the same weight and age, which are then treated at the same time.

To prepare the suspension for injection, a pre-weighed pituitary is poured into a porcelain mortar and powdered thoroughly with a pestle. Further, a few drops of physiological solution (6.5 g NaCl per 1 l of distilled water) are added to the mortar and ground into a dough-like mass. After that, the necessary amount of physiological solution is drawn into a syringe (at a rate of 1-2 ml per fish to be injected) and gradually added to the pulverized pituitary, until a uniform suspension is formed. This suspension is taken up into a syringe using a small opening needle to preventing "lumps" from being taken up.

To inject herbivorous fish, it is recommended to use 5 ml or 10 ml or 20 ml syringes with replacement needles of 4-6 cm length. Administration should be best done in a special stretcher fabricated of PVC fabric on high legs. Routinely, one person holds the fish in the sleeve, while the second person makes the injection. The needle is inserted under a scale into dorsal muscles, in front of the dorsal fin, above the lateral line, at an angle of 30–40 degrees. The syringe piston is pressed smoothly, without jerking. The injection site, after removing the syringe, is pressed with a finger and gently massaged to prevent the solution from escaping. Before each injection, the syringe should be shaken, as the suspension particles may settle on the syringe walls that means some fish do not receive the necessary amount of pituitary.

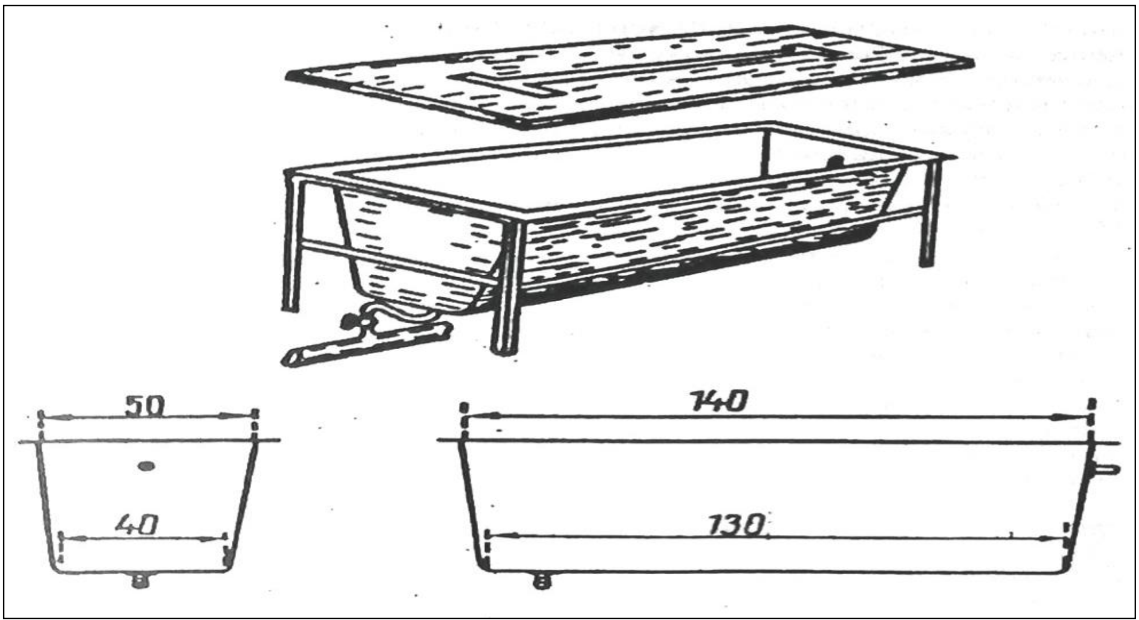
Other currently popular synthetic drugs can be used for the hormonal stimulation instead of fish pituitaries. Typically these are endogenous stimulants of gonadotropin, produced in the hypothalamus of vertebrates, so-called gonadotropin-releasing hormones (GnRHs). These preparations exist under different trade names in different producing countries (Ovopel in Hungary, Nerestin in Russia, and Dagin in Israel, for example) and as a rule they are used in combination with dopamine-receptor antagonists (antidopaminenergics). The technology for administering the drugs are similar to that used for fish pituitary application. Although there may be certain advantages to using synthetic drugs (e.g. a similar stimulatory effect but with the absence of foreign proteins), more generally the quality and quantity of fry obtained is more dependent on the skill and execution of the injections by the fish breeder, rather than on the type of stimulant used.

Timing on injections is critical so that the production of sexual products falls in daylight hours when the farmer and staff can manage the process more effectively. Although in farms where temperature regulation of water, supplied to the incubation unit, is not provided, it is somehow advisable to arrange a daytime holding of broodfish after the final resolving injection when there would be a rise in water temperature.

After injection, females and males should be held separately in two earthen ponds with a surface area of just 20 m^2 or less, and a depth up to one metres. Once in the ponds it is necessary to ensure a constant water exchange through good water flow, with discharge water running through a bottom outlet pipe, which is also protected by a grid. The bottom of the pond should be firm, while the slopes of the dam should be covered with soil and dense vegetation. Filling and discharge of water from these small ponds should take no more than 15–20 minutes. 10–12 females and 12–18 males can be stocked into the separate ponds.

During the injection period broodfish can also be held in plastic tanks or PVC vats of elongated shape that are numbered, divided into sections using a knotless netting and equipped with safe lids. A very simple bath container for holding one brood fish is shown in Figure 3. A regular water exchange and temperature kept at optimal range (21–25 ºC) is an obligatory condition in all cases. The temperature should not fall below 18 ºC nor rise above 28 ºC.

FIGURE 3
Bath container used to hold one breeding herbivorous fish



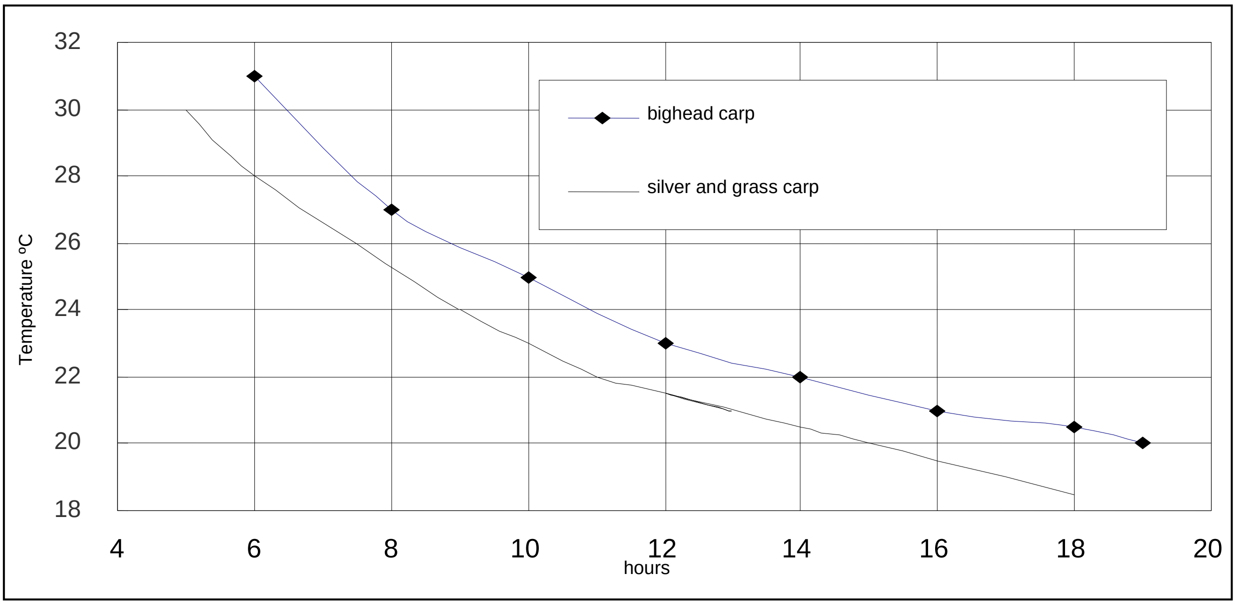
The eggs are obtained routinely every second day, which allows the operation of the incubation unit (hatchery) to be run most efficiently.

3.5. Stripping of eggs and sperm

The timing of females maturation after the 2nd resolving injection strongly depends on the water temperature (Figure 4). Typically, at a temperature of 23–25 ºC, ovulation occurs after 8–10 hours.

To catch mature females in ponds the water should be released to a level that allows the farmer to catch fish using special sleeves. Routinely, fish are caught by two person - one carefully pulls the sleeve on the female (over the head) and lifts it from the water, while the second person – holds the tail stalk, using a gauze or a towel and clamps the hand over the genital opening to avoid egg leakage. Then, the female is transferred to a place for egg extraction, which should be protected from direct sunlight.

FIGURE 4
Relationship between duration of female maturation and water temperature in the case of herbivorous fish.



Considering there is an increased susceptibility to stress in herbivorous fish undergoing stripping, the use of anesthesia is highly advisable. The most widely used anesthetic drugs in fish culture are MS-222 (Tricaine methane-sulfonate), Propiscine and clove oil (Eugenol). The most affordable one is clove oil, in a form that is used in dentistry. In practice, before egg stripping and milt stripping fish are placed for 30-50 seconds in a non-flowing container with Eugenol at concentration of 0.05 ml/l. A prolonged stay in the anesthetic solution is not advisable, and fish should be removed quickly, especially if the frequency of movement in the gill covers has decreased. Since Eugenol is insoluble in water, it is highly advisable to mix it well to get a homogeneous emulsion.

After removing the female from the anesthetic solution, water and mucus around the genital opening should be gently wiped off using a towel. It is convenient if a fish culturist uses special aprons with a pocket to hold the fish. The pocket size should afford free accommodation of the head and most of the body of the fish. The use of an apron (Figure 5) allows the farmer to optimize the process of sexual products extraction, with one fish farmer holding the fish and squeezing to extract the eggs, while the second person handles a container for collecting the eggs and sperm.

Eggs are stripped into a clean and dry bowl by pressing and massaging the belly (Figure 5). Prior to use, the bowl is weighed and its number and weight are written onto the container using a marker pen. Ingress of water or other foreign objects into the bowl should be avoided. It is necessary to control the flow of eggs so it is slow and consistent down the bowl walls and to avoid too much movement of eggs through a strong stream. It is desirable to strip eggs from each female into a separate identified containers.

Mature eggs can easily flow from the genital opening of the female and will contain a small amount of ovarian fluid, the colour of which can vary. Overripe eggs contain a lot of ovarian fluid, and individual eggs have a cloudy white colouration. The timely stripping of eggs is very important. After the onset of ovulation, eggs can stay in the female body for no more than 20–30 minutes, after which eggs rapidly lose their quality that results in decreasing fertility. Therefore, determination of the female "fluidity" becomes a very crucial moment that contributes to the success of the entire procedure.

FIGURE 5
Stripping of ovulated eggs from anaesthetized female grass carp, by two operatives, and using an apron to hold the head and tail

Counting of obtained eggs is performed separately for each female by weighing each bowl of eggs, and deducting the weight of the bowl, to give egg mass. One kilogram of unfertilized eggs contains:

- grass carp – 700 000 to 1 million eggs
- silver carp – 800 000 to 1.2 million eggs
- bighead carp – 550 000 to 800 000 eggs.

The working fecundity of females ranges from 20 000 to 2 million eggs and depends on the size of the fish, its age and degree of its readiness to spawn.

As distinct from females, males can be used 2-3 times during the spawning campaign, if necessary. Sperm from males can be collected 30 minutes to 1 hour before eggs are extract or immediately after egg stripping. The collection of sperm is carried out into a clean, preferably sterile dish. Before stripping, the abdominal part of the male is dried with a clean dry towel. The first portions of the sperm are discarded, and the remainder is collected It is necessary keep the sperm from being contaminated by mucus, excrement and scales. If blood appears in the sperm, collection should be ceased, since the spermatozoa in the blood serum quickly stick together and die. Dishes with collected sperm should be covered and stored in a cool place.

When collected spermatozoa in the seminal fluid are in a static, non-mobile state and activation occurs only after they contact water. Once contact with water occurs they quickly lose nutrients and die in 1–2 minutes. It is therefore critical that the milt extraction procedures does not come into contact with water.

Temperature is an important factor that affects the vitality of spermatozoa. When temperature is within the range 0–2 ºC spermatozoa are not active and retain their motility in water for several days. To store the sperm at this temperature, a thermos with a wide neck is used. Ice should be put on the thermos bottom and covered with several layers of gauze to prevent direct contact between sperm and ice (i.e. water that should be avoided). To avoid shock the temperature should be reduced gradually at a rate of one to two degrees Celsius per minute.

Short-term storage of sperm at a room temperature of 22–23 ºC is feasible when collected immediately before use. In this case, the viability of spermatozoa can be retained for several hours.

Sperm of good quality has the appearance of condensed milk, one of medium quality is similar to whole milk (i.e. more liquid), while one of poor quality looks like diluted milk. The latter consists of low or non-active spermatozoa with low fertility, and is not suitable for fish breeding. For more accurate estimation of sperm motility a microscope should be used.

Careful handling of breeders is very important for a successful spawning campaign. In no case should they be allowed to fall to the floor. Blows, injuries, scrapes and other damages affect their physiological state and will lead to fish mortality. The mortality rate of breeders in ponds after the incubation campaign can amount to 10 percent for grass carp, 15–30 percent for bighead carp, and 25–50 percent for silver carp. If broodfish grown in cages or cooling reservoirs have been used, their mortality can reach up to 100 percent.

The main reasons for the high mortality broodfish relate to injury during the collection process, injections and stripping of gametes, infection contamination and the emergence of an acute inflammatory process, which is especially active at high water temperatures (25–28 ºC). In addition, possible causes of cloudy eyes and appearance of thrombi after the first injection may result from failure to comply with elementary rules of hygiene during injections, introduction of foreign protein in the form of a pituitary suspension, or the use of immature or overripe females to produce offspring.

In order to reduce the exposure of fish to stressors, it is recommended to use anesthesia at all stages of the technological process related to eggs production, but especially during stripping, and to follow strict sanitary and hygienic standards. Sometimes, in order to prevent the inflammatory process, breeders do administer antibiotics available in chemist's penicillin for example, at a dose of 50 000 MU per fish. Penicillin should be dissolved in water, which is used to prepare a pituitary suspension. When working with females of herbivorous fish, fractional (double) injections are administrated with a total dosage of 100 000 MU penicillin. Males are subjected to single injection, receiving 50 000 MU of antibiotic. Penicillin use produces no significant effect on the quality of the offspring. It should be noted, that penicillin does not prevent the mortality of females that do not ripen after injection (i.e. unripe or overripe females), but only halts the inflammatory process in potentially healthy fish and prevents complications caused by its progression.

3.6. Artificial insemination of extracted eggs

Insemination of eggs in herbivorous fish is carried out by a dry method (Figure 6). Regardless of the number of eggs, sperm from two to three males is used. For insemination of 1 kg of eggs, 2–3 ml of milt (semen) is sufficient. The fresh milt should be carefully and evenly spread over the eggs with a cock feather. Only then, pure filtered water is added to the bowl to barely cover the upper part of the eggs and are thoroughly mixed with the feather. At this time, the fertilization takes place. Then, after 1–2 minutes, it is necessary to add fresh water and mix it again, breaking lumps of eggs with the feather and removing mucus and blood clots. This operation is carried out several times until the eggs begin to swell. After this, the eggs are transferred to the incubation (hatching) apparatus.

FIGURE 6
Insemination of eggs using the "dry" method

3.7. Hatchery – Incubation unit

Incubation units (hatchery), intended for breeding of herbivorous fish, should be located near the ponds for where injection and pre-spawning activity occurs, and it is desirable to have gravity flow water supply into the unit. The unit should include an area for allocation of systems for eggs incubation, a table for eggs insemination and washing and an area for placing trays (Figure 7, 8).

FIGURE 7
Layout of the incubation unit for herbivorous fish: 1 - container area; 2 – hatchery room (for egg fertilization); 3 - tables for eggs washing and de-adhesion; 4 - shipping ground; 5 - laboratory; 6 - warehouse; 7 and 8 – incubation systems "Amur" or IVL-2; 9 – revolving system for larvae packing; 10 – tank with larvae receiving cage; 11 - containers; 12 - earthen cages

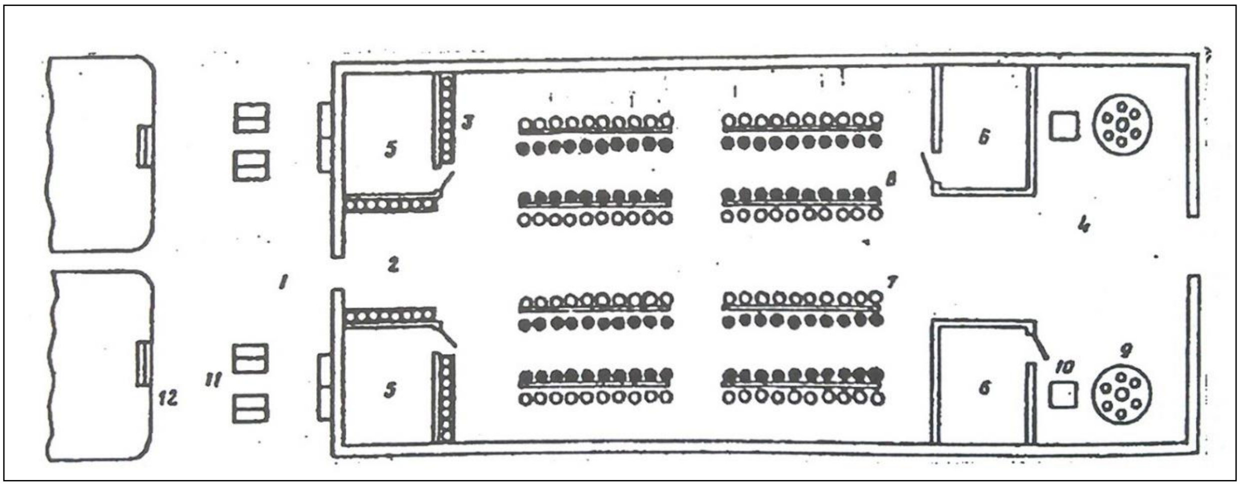


FIGURE 8
General view of the incubation unit with “Amur” systems used in incubation of eggs

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The incubation unit should have a well-equipped laboratory where the necessary tests can be performed and pituitary suspension prepared and so on. Water in the incubation unit should be supplied from a settling pond of sufficient capacity to provide a reserve water flow for at least 12 hours. The difference between the water level in the settling pond and the incubation systems should be at least 1.5 m. This ensures normal water pressure in the apparatus and does not lead to depositing of the incubating eggs near the bottom. If possible, the water in the settling pond should be fed from both warm and cold (an artesian well) sources.

The water supply of the incubation unit should pass through a special filter that collects litter, larvae of trash fish, plankton and detritus. Especially dangerous are predatory cyclops, which can completely exterminate eggs and larvae of herbivorous fish. In order to prevent foreign objects, a floating frame-like filter is used, which has a cube or a parallel-piped form with dimensions of 1 x 1 x 1 m or 1.5 x 1.5 x 2m, and covered with a kapron sieve (mill gas – no less than # 60) and a protective metal mesh with a hole diameter of 2 mm. These interchangeable filters are installed in the water column, and should be constantly monitored, cleaned, and in case of damage replaced by spare ones.

If the incubation unit is located in a site with a normal temperature regime (i.e. not in water cooling reservoirs), it is wise to equip the unit with an automatic water heating system with temperature regulation. Electric heating elements are placed in a separate container at the water inflow with obligatory installation of an air outlet, that prevents the release of air bubbles directly in the apparatus and is associated with cases of gas bubble disease in larvae, or release of eggs from the apparatus by air bubbles.

At present, “Amur” (Figures 9) and IVL-2 (Figure 10) apparatus with a capacity of 200 liters have been widely used for eggs incubation and for holding newly hatched free embryos of herbivorous fish. It is possible to load approximately 1.5 million. eggs in each type of system with water flows from 8 to 14 l/s. The incubation systems "Amur" and IVL-2 can be mounted both independently and sectionally in sequence. In the incubators a vortex water flow is formed to simulate the water flow. All systems should be equipped with special extension filters, made from kapron sieves # 18–25 and with height of 15–17 cm. This prevents the escape of larvae.

FIGURE 9

Diagram (left) and photograph (right) of Amur apparatus consisting of 1 - container of cylindrical shape; 2 – rubber washer; 3 - wing stud; 4 - level tube; 5 - filtration grid; 6 - spacer; 7 - spillway; 8 – outlet pipes; 9 - height-adjustable rack; 10 - nozzle swirl; 11 - cone; 12 - water distribution unit.

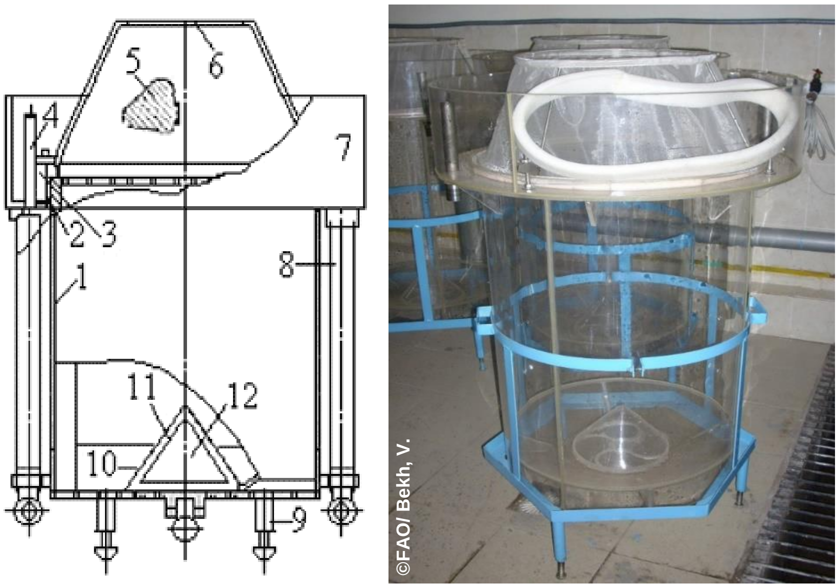
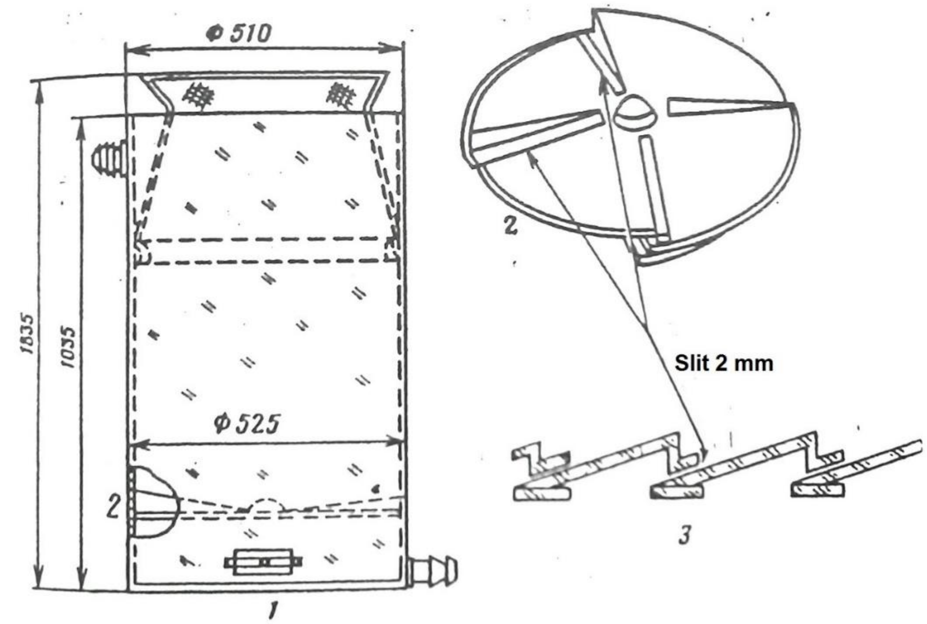


FIGURE 10
IVL-2 apparatus showing 1 - the general layout of apparatus; 2 - disk, water splitter; 3 – guide bars



Compared with the IVL-2 system, the “Amur” apparatus is easier and simple for operation and maintenance; it ensures lower mortality of larvae; has a lower water consumption; and higher capacity and yield of larvae. The "Amur" apparatus can also be used for incubation of common carp and channel catfish eggs. The use of incubation systems for holding of pre-larvae allows farmers to skip their holding in trays and thereby simplify the entire production technology and significantly reduces the area required for the incubation unit.

Before the start of the spawning campaign, a thorough flushing of the incubation systems should be performed, as well as disinfection of the inventory, fish equipment and pipelines.

3.8. Egg incubation and hatching

Each incubation system, if possible, should be loaded with eggs from one female to maintain the ability to assess performance of individual fish. If eggs are of proper quality, but its quantity is insufficient, it is possible to load eggs from several females in one apparatus. The loading of the apparatus should be carried out prior to full swelling of eggs, no later than 5-10 minutes after the fertilization. Before loading eggs into the incubation system, the water supply should be shut off and water level reduced by 20–30 cm. It is recommended to load eggs with polyethylene measuring containers or directly pour them from the containers used for fertilization, but should be poured carefully and slowly to avoid the pour being a powerful jet from a height.

After loading of eggs in the apparatus, water flow should start to begin exchanging water so the eggs are in a constant slight movement. The more eggs swell and increase in volume the stronger the water exchange should be, but not so strong that eggs are in danger of being washed out from the apparatus or so slow that stagnant zones can form. Flow therefore needs to be assessed regularly and adjusted accordingly.

To assess the quality of eggs, development from 4-8 blastomere cleavage stages to the early morula should be determined, and a percentage fertilization success calculated. A sample of eggs from the apparatus is taken with a large pipette, and eggs placed in Petri dishes and submitted to the laboratory. Typically, at least 100 eggs are scanned under a binocular microscope, and the numbers of eggs with normal and abnormal cleavage are counted.

Typically, in eggs of proper quality the percentage of fertilization amounts to 90 percent or above.

Eight to ten hours after the onset of incubation, the unfertilized eggs and those of poor-quality become cloudy, acquire a whitish shade and are concentrated in the upper water layer in the apparatus. If this layer is significant, the dead eggs should be removed using a siphon.

The length of the incubation period depends on the water temperature. The optimum water temperature lies within the range of 21–25 °C. At this temperature egg incubation takes between 23 and 33 hours. At a water temperature of 27–29 °C, the incubation period can be reduced to 17–19 hours (Vinogradov and Erokhina, 1967)

As a rule, mass hatching of embryos occurs within a one to three hour period. At low water temperatures, it may take up to 10–12 hours or above, but this greatly complicates the operation of the incubation unit. If low temperature prevail, it is recommended to resort to artificial stimulation of the hatching process. For this, after the onset of mass hatching, the supply of water to the incubation system is sharply reduced, which leads to unfavorable conditions for egg respiration, which stimulates the functioning of the hatching glands and egg shell dissolution by the egg shell enzyme. If the hatching process does not increase within half an hour, the water exchange should be restored.

Soon after hatching free embryos become active and make "candle" movements (up and down movements), rising to the upper layers of the water. With good egg quality and normal incubation conditions the yield of free embryos amounts to at least 70–80 percent of the number of loaded eggs.

The most convenient approach after hatching is to hold the larvae in the “Amur” apparatus for a period (Table 6) before transition to mixed nutrition, at a stage that coincides morphologically with the filling of the swim bladder.

TABLE 6
Duration of larvae holding in the incubation systems

Water temperature	Duration of holding, (h)
18-20	90-100
20-23	80-85
26-27	48

The length of normal pre-larvae is 4.0–5.2 mm. Their body is transparent, but with evident small dark spots in the eyes. On the second day (at 23–25 ºC), pre-larvae have a "stage of rest", when they concentrate in the lower part of the apparatus and are inactive. Through this first period (the first 2–2.5 days) they absorb nutrients from the yolk sac and do not need additional feeds. On the third day they darken, begin to move actively, initially in the bottom layers, then rise into the water column, and then begin to float horizontally and gradually switch to external feeding on small planktonic organisms.

If there is a lack of “Amur” systems in the farm, different plastic containers can be used for pre-larvae holding. These containers should be equipped with filters from kapron sieves at the water inlet (# 35-70) and outlet (# 18-25). The water level in these trays should be set from 4–5 cm (during the laying of pre-larvae) to 10–12 cm in other periods. In one tray measuring 4.5 x 0.7 x 0.5 m up to 2 million pre-larvae can be held.

The survival rate between fertilized egg to 3–4 day larvae under normal conditions should amount to a minimum of 50 percent. The larvae are counted using a standard method, i.e. by determining a certain standard amount of larvae (ranging from 10 000 to 100 000 larvae) in one bowl through either counting or concentration, and then visually reproducing this in the other bowls.

Time for transportation of larvae to ponds depends on water temperature (Table 7). Transportation of larvae of herbivorous fish is carried out in polyethylene bags filled with water and oxygen. In a plastic bag of 40 litres capacity filled to 1/3 with water and 2/3 with oxygen, it is recommended to place 100 000 larvae per bag if transportation takes up to 5 hours or 50 000 larvae when more than 5 hours. The larvae that have switched to mixed nutrition are suitable for transportation. The mortality rate of larvae during transportation should not exceed 3–5 percent. Within the limits of the farm, at a distance of less than 5–10 km, the larvae can be transported in milk cans or another plastic containers, held at a density of up to 1–2 thousand larvae per liter.

3.9. Hybridization between bighead carp and silver carp

Often, under temperate climatic conditions, especially in areas with a cool summer, there is a need to obtain and rear a hybrid between silver and bighead carp, that has a higher survival rate, and as well a faster growth due to heterosis and the intermediate structure of the gill apparatus.

As a rule, the hybrid is obtained when a female of bighead carp is crossed with a male of silver carp.

When building the broodstock, it is necessary to cull specimens of the hybrids between bighead and silver carp, which have the following distinguishing features:

- in hybrids, unlike its paternal and maternal species, the keel does not reach the throat, but it is located from the anus to the middle of the distance between the throat and the ventral fins;
- in hybrids the gill apparatus is intermediate between the similar organs of its paternal and maternal species. In addition, experienced fish farmers can distinguish hybrids according to the body color – hybrids have a steel coloration.

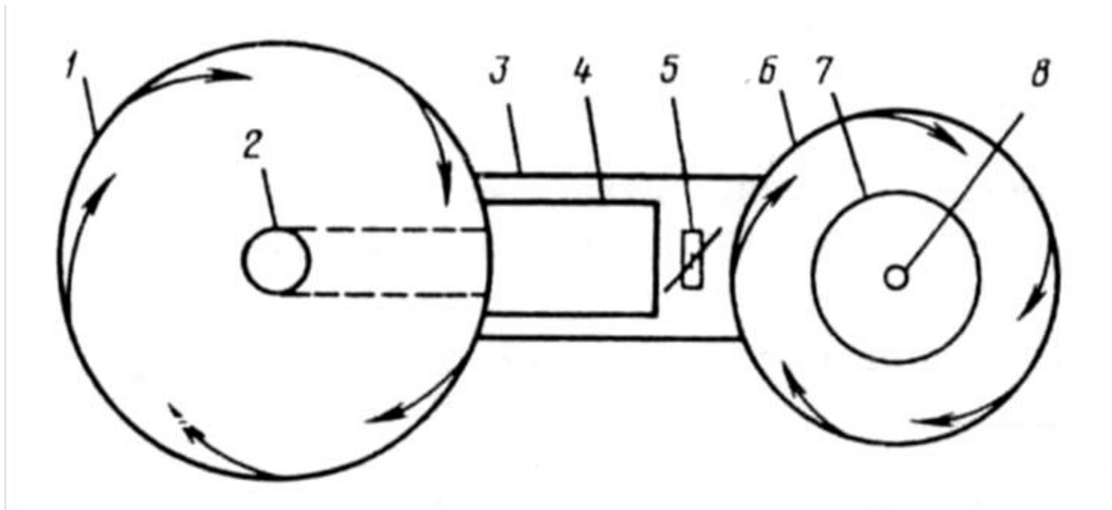
Despite the high growth rate of more carnivorous hybrids, their use under controlled reproduction is challenging, often the sexual products they produce have low biological qualities and increased mortality rate at all stages of rearing.

In the southern regions of a temperate climate zone, with a high level of photosynthesis and development of phytoplankton, preference in rearing should be given to silver carp, as the production of hybrids and their rearing in these regions is not expedient.

4. CONTROLLED REPRODUCTION OF HERBIVOROUS FISH IN ROUND TANKS

An alternative to traditional hatchery technology is the production of progeny of herbivorous fish in round tanks. Round tank culture technology (Figure 11) is based on a combination of physiological and environmental factors, notably that the circular current of water created in the tank simulates natural river conditions. This “circulation” technology has been used in China for a long period.

FIGURE 11
Schematic outline of circular spawning-incubation complex: comprising of 1 - spawning tank; 2 - bottom drain (pipe); 3 - concrete tank for installation of egg collector; 4 – egg collector; 5 - discharge pipe with a valve; 6 - incubation tank; 7 - filter; 8 - discharge pipe



The prime advantage of round tank culture use is higher reliability and lower labour costs for egg extraction from females, and is useful during the periods when weather conditions worsen, that systematically complicates the breeding activity related to herbivorous fish. It is also useful when there is a shortage of qualified personnel, as the overall system is easier to operate.

It is advisable to use tanks of 10.5 m diameter, made of monolithic reinforced concrete or metal. Their height should be approximately 2 m, with a water depth of 1.4–1.5 m. The rotation of the water is provided by 6 branch pipes that supply water at an angle of 45°. The outflow of water is carried through a funnel-shaped outlet at the bottom of the tank, measuring 25–30 cm diameter. The outlet is closed by a grid preventing the ingress of fish. The pipe goes into a concrete well, where the egg catcher is located. It is a cage of mill gas, connected by a sleeve of the same material with a pipe emerging from the basin into the well. During spawning, the flow velocity near the wall of the tank should be kept at 0.12–0.15 m/s, although the speed in the tank centre will be above this.

After hormone treatment, herbivorous fish are capable of spawning in this current and to completely release sexual products within 3–5 hours after the onset of spawning. In hatchery conditions up to 25 females and the same number of males can be introduced in to a tank of 10 m diameter. The number of males may be higher, but should not be less than that of females, as the percentage of fertilized eggs will decrease. Typically, a double pituitary injection is used, similarly to the hatchery method, but the pituitary dose can be significantly reduced using this method. For 15–60 minutes before spawning, fish undertake mating activity in the upper layers of water.

After mating the eggs accumulating in the tank should be periodically removed with a large net, made from bolting cloth, deposited in to a container filled with water and periodically transferred to other hatchery systems for incubation. In China, eggs are typically incubated in other tanks, for example.

At the completion of spawning, the tank should be partially drained, the breeders caught, making sure the famer counts the number of spawned-out females.

Using this tank culture technology for production provides a range of advantages over the traditional method:

- The percentage of females producing eggs increases to 80–85 percent, against 50 percent according to average data for a number of years with conventional technology.
- The working fecundity increases by 10–15 percent due to a more complete yield of eggs.
- The quality of eggs improves, that results in a 10–15 percent increase in 3–4 day old larvae yield.
- Mortality of the broodfish is eliminated, provided operations are conducted carefully.
- The technology is less labour consuming; and
- the technology ensures the greatest advantages during periods of unstable weather, in terms of heterogeneity in the broodstock size and readiness of broodfish (especially females) to spawn.

5. LARVAE NURSING IN MONOCULTURE

The need to rear the larvae of herbivorous fish until they are fully viable is associated with some peculiarities in the biology of these species. The growing larvae require enhanced conditions in relation to temperature, species composition and quantity of food organisms. The larvae in the open, suffer from various predators and pests, including other fish, but also invertebrates such as predatory species of cyclops and insects. Therefore, the growth of larvae, as a biotechnical phase, becomes urgent, but is a technologically complicated and labour consuming process. It is associated with increased expense, but this will be recouped by reducing the loss of fish stocking material. During the larval period (10–15 days) in herbivorous fish, fundamental morpho-ecological and physiological changes occur in the animal. In the course of this period all three species of herbivorous fish feed on zooplankton.

5.1. Nursing of herbivorous fish in tanks

Containers of various shapes made from modern materials may be used to rear the larvae of herbivorous fish. The containers should be equipped with systems to maintain the required level of water and provide for its discharge to maintain water flows, as well as having filters to prevent the removal of larvae. These filters are made from a kapron sieve # 18-25 and installed on the spillway. Water supply to the trays is carried out through pipelines, which are equipped with more dense filters (sieve # 35-70), that prevent the entry of various undesirable fauna and litter to the containers in which growing fish ae maintained.

Tank fish farms should be located near water bodies rich in zooplankton. Typically, the water flow should amount to 8–11 l/min with a working volume for the tank or tray of 1 m^3. Before stocking of larvae takes place, the flow rate should be reduced to a minimum, so there is less disturbance in the water. The water temperature in the container with larvae, and the tank in which they are to be placed need to be equalized.

The larvae are reared in a monoculture with a stocking density of 60–65 ind./l and the length of the maturation period should take up to 15 days. Every 5 days, controlled catches should be conducted, in which at least 50 larvae are collected and their mean weight determined.

For all three species of herbivorous fish, the prime food items are small zooplanktonic organisms, such as rotifers, infusoria, invertebrate eggs and so on. Larvae, particularly of bighead and silver carp, are especially sensitive to zooplankton unavailability, so zooplankton numbers need to be maintained at a high level in the tank.

Zooplankton can be caught in natural water bodies, but this is time-consuming and may not guarantee supply, so it is wise to master the technological cultivation of the nauplii of brine shrimp (*Artemia salina*), infusorians, rotifers, and branching crustaceans. The technology required to achieve zooplankton growth is described in detail in specialized literature. In case of zooplankton harvesting` from the water bodies, it should be filtered through kapron sieve # 25 in the early days of larval growth and # 10 at the end of that, in order to prevent the entry of litter, large zooplankton and insects.

During feeding with zooplankton the water flow is stopped and the fine zooplankton is introduced evenly along the walls of the tank. The recommended feeding frequency is 4–5 times per day, during daylight hours. The level of live food required should amount to 55–60 percent of the estimated weight of the larvae and (if previous guidelines followed) amounts to 250 g, 400 g and 550 g per 1.5 m^3 tank over the first day, second day and third to fifth days respectively and increase thereafter as the larvae grow in weight.

The use of artificial feeds for nursing of herbivorous fish is possible, but ideally this should be combined with live feeds (especially brine shrimp), rather than being the only food source. When using artificial feeds it is assumed that 50 percent of the weight gain will be provided by artificial feeds, and 50 percent from live zooplankton. When using artificial feeds, size is critical, and should be no more than 0.25 mm in diameter. The feed can be routinely introduced by scattering on the surface of the tray during the intervals between feeding with zooplankton.

During the whole nursery period:

- water temperature should be kept at an optimal level of 25–30 ºC;
- oxygen concentration in the rearing tanks should be at least 5 mg/l;
- the bottom and walls of the tanks should be thoroughly cleaned on a daily basis, using a siphon to remove old food and debris. Larvae will be sufficiently active, and will actively move away from the siphon when being used. Some modern designs of containers for nursing larvae enable pumping of sediment, debris and other wastes that collect in the conical bottom of the tank.
- the water flow rate and discharge for nursing up to one million larvae is approximately 12 m^3/h, so it is sometimes advisable to use modern circulating systems that are able to cope with this level of flow, especially if water heating is applied.
- it is not expedient to nurse larvae for more than 15 days. Cannibalism is possible in grass carp, for example, after this. The yield of the grow-out fry from 3-4 day up to 15 days larvae should be within the range of 50 percent to 60 percent; and after 15 days the average weight of individual larvae should range from 15 to 25 mg.

5.2. Nursing of herbivorous fish in ponds

Rearing of larvae of herbivorous fish in fry ponds has proven to be the simplest technological approach for these species.

Ponds with an area of 0.5–1 ha area are the most suitable sizes for larval nursing. Sometimes, with the help of small dams, shallow sections of large water bodies can be separated, and so-called "satellite ponds" created out of the larger pond. To prevent predators, invertebrates and other non-required fish species entering into the pond water, the ponds should be equipped with skimming filter systems. These are available in different designs, made from kapron sieves # 18-32 (Figure 12). In the simplest approach, a tray measuring 200 x 60 x 50 cm or a sleeve from a sieve can be made and used for this purpose.

FIGURE 12
Examples of skimming filters used to remove predators, invertebrates and other non-required fish species from the pond inlet water when nursing larvae in ponds.

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In ponds stimulation and development of a zooplankton population, to feed larvae, is important and can be encouraged through the introduction of organic fertilizers into the ponds, including manure, compost, wilted vegetation, and grass mowed from the pond dams. The application rate for manure varies from 3 to 10 t/ha dependent upon the productivity of the soils in which the ponds are located. Rotted manure can be applied to the pond just 7–10 days before it is filled whilst fresh manure should be added much earlier - 1–1.5 months before the filling - to enable the manure to breakdown before the pond is filled. Manure needs to be is applied evenly over the whole pond and ploughed into the pond bottom with a harrow.

To enhance the productivity of ponds, and also to neutralize the negative effect of organic fertilizers on the oxygen regime, it is recommended to apply mineral fertilizers at a rate of 120-140 kg/ha, 50 percent each, of both ammonium nitrate and superphosphate.

In order to prevent the development of predatory fauna, the filling of fry ponds with water should be conducted just 2–3 days before the stocking of larvae. The water sources used for filling ponds should contain relatively high concentrations of small zooplankton (200–300 ind/l and above) to provide the initial source of food. Although live food is present in the pond, it is recommended to arrange supplementary feeding, using soybean meal (soy milk) or a starter compound feed for carp fish, introduced from the first day of stocking. Starter feed should be applied twice a day – early in the morning and in the afternoon at the rate of 2–3 kg per 100 000 larvae. During a strong winds or rain, the moist feed should be distributed over the whole area of the pond in small portions. For grass carp, feeding can be further enhanced by introducing duckweed or chopped lucerne from day 20.

To suppress the development of insect and insect larvae and their larvae in ponds, substances that form a film on the water's surface are used, referred to as higher fatty alcohols (HFAs). HFA is a dark amber liquid obtained by processing paraffin. When applied to the water, HFA forms a film over the surface that is one molecule thick that is not broken up by weak winds. HFAs are inert, do not react with water and are non-toxic. Between the molecules in the film there are pores, ensuring that gas exchange in to the pond is not stopped. The film can retain the water molecules and reduce evaporation, and thus contributes to the accumulation of heat in the pond, increasing overall temperature by up to 2 ºC compared against untreated ponds. The film is formed when temperature in the pond exceeds 17 ºC, whilst at a lower temperatures the HFAs freeze down. Typically they are introduced in to ponds three times: in the first days after filling the pond, in the middle of the nursing period and at the end of nursing. the amount of HFAs required to form the film is so small that water does not change in taste or smell. The treatment of 1 ha of ponds requires 0.7–1.0 kg of HFA. Use of this treatment reduced the ability of insect larvae to develop and yield of larvae can increase by 15–20 percent. The application of other chemicals, for formation of zooplankton and other forage in the fry ponds has not been practiced.

Nursery rearing of larvae of herbivorous fish is carried out in monoculture. Duration of nursery period is dependent on the target weight required and may amount to 8–14 or 25–30 days to reach the weight of 25–30 mg and 0.8–1.2 g respectively. The stocking density, in the case of rearing to a weight of 25-30 mg (without artificial feeds application) in the southern regions of Ukraine and Russia, Central Asia and Transcaucasia can amount to 5–6, 6–8 and 7–10 million individual per hectare for grass carp, bighead and silver carp respectively. At a target fry weight of 0.8–1.2 g the stocking density should range from 0.5 to 3 million individuals per hectare .In both cases the yield should be 40–60 percent.

Drainage of ponds and removal of fry should be carried out at night or in rainy weather. The catching of larvae is done using a fry collector, which consists of a concrete tank or a waterproof wooden harvest box of 3.5–4.0 m length and 1.2–1.5 m width. The height is variable and selected to ensure there is a difference of not higher than 10 cm between the level of water in the pond and that in the collector at the start of harvesting. The back wall of the tank should contain a sluice gate. Inserted into the tank should be a container made of kapron sieve # 7-12 (depending on the size of fingerlings), which should be 15-20 cm smaller in width and 50 cm smaller in length than the tank. Such a difference in size ensures better filtration of water through the walls of kapron sieves. The container should be equipped with a canvas sleeve to be fixed at the outlet pipe, which should be 50 cm (or above) in diameter. The kapron collector is secured with ropes behind the staples built into the concrete tank. A longitudinal incomplete partition is installed in the collector, behind which a quiet region is created, where the larvae can escape from the rapid flow.

The larvae are caught from the collector with a net made of nylon sieve with a size of # 20-23, and larvae transferred to bowls or other containers. Larvae should be carried along with water, for which a bowl with water is brought under the net. The count of larvae is performed using the standard method.

During the whole period of nursing in ponds, the water temperature and oxygen regime are monitored, measured in during the morning hours, when they would expect to be at their lowest. Regular sampling of larvae should be undertaken determine the weight and health status of the fry.

6. POLYCULTURE AND REARING OF FINGERLINGS OF HERBIVOROUS FISH IN PONDS

6.1. Polyculture practices

Polyculture is the most important means to intensify fish farming, and is the joint cultivation of various fish species within the same pond, each having different nutritional requirements and therefore able to make full use of the available natural forage base. Polyculture using Far Eastern herbivorous fish is undertaken in many countries. Depending on the climatic conditions, the composition of local ichthyofauna, the level of fish farming development, the traditions and preferences of the local population, herbivorous fish can be considered either as a source of increased fish production or as biological meliorators that allow increased production of affordable protein.

The advantages of fish farming in polyculture include the following:

- Most omnivorous fish are not able to use the overall natural forage base of the water body and polyculture allows increased use of that food base.
- No two fish species have exactly the same food composition requirements, and do not fully compete with each other in that consumption. These small differences in the range of nutrition needed, makes it possible to co-cultivate even closely-related fish.
- Under conditions of polyculture, some species can promote the reproduction of feeds for other fish species.
- Some fish species can use the excrement of other species as a food source.
- In monoculture cultivation of some species with a narrow food spectrum, can result in development of poor environment conditions in a pond, that can be overcome by polyculture if the different species used have a wide food spectrum.
- Intensive consumption of one or another food item by one species can indirectly contribute to the excessive development of non-consumed hydrobionts in the water column, whereas polyculture of species that have a variable diet means the overall hydrobiont concentrations are maintained in equilibrium.

Traditionally, introduction of multiple species of fish in polyculture has been developed takin account of the feed niches of each herbivorous, and can be done without a radical change from the technology applied carp monoculture. The main difference is that intensification through polyculture requires the boosting of natural feeds through the partial application of organic fertilizers and/or adding artificial feed. In modern pond polyculture the share of silver and bighead carp depends primarily on climatic conditions and can range from 20–30 percent in areas such as Central Russia, North of Ukraine and Belarus, to 60–80 percent in Southern areas of Central Asia and Transcaucasia. The main factor that determines the differences between locations relates to the sum of heating during the vegetation period. The share of grass carp typically does not exceed 10–20 precent and is more dependent on the food supply.

6.2. Fingerling rearing techniques

The area on ponds intended for co-cultivation of common carp and herbivorous fish fingerlings can range from 1 to 50 ha. The pond bed should be well planned and fully drained during the pond discharge. The water supply into the pond should be through collecting filters with a mesh of no more than 1 mm size, to prevent predatory fauna entering into the ponds. Another important requirement is to ensure that the pond is watertight, so it is not lost through drainage. The proper time for filling the pond is 7–10 days prior to stocking. If larvae of herbivores fish are stocked in the pond, it is desirable to shorten this period to 2-3 days.

In the spring, liming of ponds should be conducted, in order to stimulate development of the natural food organisms, with 1–5 t/ha of rotted manure introduced into the ponds, laying it out in small piles along the pond bed and the water's edge.

Before stocking, it is necessary to equalize the water temperatures in the transport container and the pond. The proportion for various herbivorous species can vary, but as a general rule, the share of bighead and grass carp should not exceed 25–30 percent and 10–20 percent of the total number of herbivorous fish respectively.

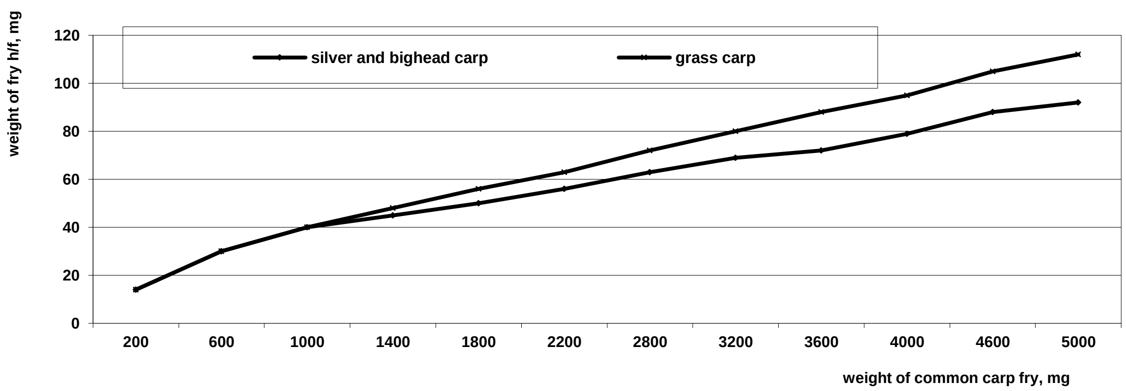
There are several ways to stock fry in ponds in polyculture, as follows:

- With larvae of common carp and herbivorous species for a short period (not more than in a week);
- With fry of common carp and herbivorous fish (never mind the timescales);
- With larvae of carp and fry of herbivorous species (never mind the timescales);
- With larvae of herbivorous species and fry of carp. In this case, stocking of common carp fry is possible only once the herbivorous fish have reached a weight of 0.5 g, so after 25–30 days.

Larvae stocking of herbivorous fish into fry ponds, should not be undertaken where common carp fry have been already stocked (Panov *et al*. 1973) (Figure 13).

The stocking density per hectare of newly hatched larvae in to grow-out ponds varies considerably and can amount to: 60 000 to 110 000 for silver carp, 20 000 to 40 000 for bighead carp and 30 000 to 125 000 for grass carp, depending on the feeding regime employed (larger where artificial feeds are added for example). When larvae are older or fry are being added then the stocking density falls to 30 000 to 50 000 for silver carp, 10 000 to 20 000 for bighead carp and 15 000 to 65 000 for grass carp. It is important to understand that in the absence of or having inadequate application of intensification measures (such as increased feeding and fertilization being added), ponds should preferably be understocked than overstocked. If overstocked for example fish will not subsequently reach their target weights due to insufficient food supply. If fingerlings are of a low weight, they may not be capable to pass through wintering successfully and will not give sufficient growth in the second year of rearing, and thus will become difficult to sell.

FIGURE 13
The weight at which fry of bighead, grass and silver carp can be eaten by common carp fry



Regardless of the polyculture type, stocking occurs when the pond is 1/3 full and after the stocking the water level in the ponds should be raised to the desired pond design level and kept at this level until the end of the vegetation season. Over the course of the fingerlings rearing period water lost through natural drainage and evaporation should be replaced. In case of a drop in dissolved oxygen concentration to a level at or below 2 mg/l during production, then this should be improved through maintaining a water flow through the pond, but ensuring this is operated through a grid with an appropriate mesh size at the pond spillway to prevent escape of fry from the pond.

During the rearing of fingerlings, the temperature and the level of dissolved oxygen in the water should be measured daily (at dawn), and the water flows implemented to maintain sufficient oxygen in the pond water. The concentration of nutrients (nitrogen and phosphorus), and the abundance and biomass of phyto- and zooplankton, should be determined every 10 days. To increase biological productivity, it is desirable to apply mineral fertilizers, including superphosphate and ammonium nitrate, to the nursery ponds. The amount of these fertilizers to be added is calculated each time according to measured nitrogen and phosphorus concentrations in the water column and sufficient added to bring the nitrogen and phosphorus level up to 2 mg/l and 0.5 g/l, respectively. A general hydrochemical analysis should be carried out once a month. Every 10 days, control catches are made, in which a minimum of 30–50 fingerlings should be taken from each pond, and their length and weight measured to determine an overall average. Health of the collected samples should also be checked to assess for parasites, fungal infection and other ichthyopathological characteristics.

In ponds where common carp is reared in polyculture with herbivorous fish, the amount of artificial feed that needs to be added above standard levels increases as the proportion of herbivorous fish increases (Table 7).

TABLE 7
Increase in amount of granulated feeds required for different percentages of herbivorous fish in polyculture with carp fingerlings.

Percentage of herbivorous fish in polyculture (per cent)	**Amount of additional feeds (per cent)**
20	5
30	8
40	13
50	15
60	20
70	25

Fish pond harvest begins when the water temperature drops to 10 ºC. Ponds should be gradually emptied, avoiding sharp fluctuations in the water level. Once water levels are sufficiently low, removal of fingerling is performed with a thick net to remove the bulk of the stock, and remaining fish are collected through a fish trap behind the dam at the water outlet. As a general rule, when water is drained from the ponds, bighead and silver carp are the first to move towards the trap, followed by grass carp, and finally by common carp.

The count of fingerlings is performed by volumetric-weight method. This method suppose that a certain amount (volume) of fish is caught with nets into containers (buckets, creels) with water; then weighed, counted and its amount and average weight is calculated. Subsequently, when buckets are filled to look visually similar tot eh measured bucket then an overall estimate of stock can be made.

Under polyculture conditions within Central Asia, Transcaucasia, South of Ukraine and Russia, the total fish productivity of the fry ponds can vary from 1 730 to 2 330 kg/ha, made up from 1 050–1 260 kg/ha for common carp, 360–830 kg/ha for silver carp, 150–240 kg/ha for bighead and 80-90 kg/ha for grass carp, but is dependent on the starting stocking density. The average weight of each species grown in polyculture should amount to not less than 27 –

30 g for common carp, 20–25 g for silver and bighead carp, and 25–30 g for grass carp when they are collected. The yield in the ponds from the on-grown larvae of common carp and herbivorous fish amounts to 33–35 percent and 30 percent respectively, while that grown from grown larvae amounts to 65 percent and 60–65 percent respectively.

7. WINTERING OF HERBIVOROUS FISH IN PONDS

Wintering of herbivorous fish is carried out in a similar way to common carp. In warmer regions where a stable ice cover is not observed, it is possible to use ponds of other categories. As for survival rate in the winter period, herbivorous fish are similar to common carp.

Typical wintering ponds should have an area of 0.2–1.0 ha, a minimum depth of water that does not freeze 1.2 m (so deeper than this if the surface does freeze) and total water exchange period of 15–20 days. The duration of filling and discharge of pond water should be 0.5–1 days.

The stocking density of carp and herbivorous fish fingerlings, destined for wintering should not exceed 450 000 to 550 000 per hectare (or 10 tonnes per hectare). At the end of wintering the yield of fingerlings from wintering ponds should amounts to 75–85 percent. As feeding is lower over winter the overall weight loss of fish over the winter period should not be more than 10–12 percent.

During the period of winter holding of stocking material, the temperature and oxygen concentration should be monitored daily (samples are taken at the outlet) and, in case of their worsening, appropriate measures should be undertaken to improve the situation. In ponds that freeze this would include punching holes in the ice, or more generally increasing water flow, or using aerators.

8. USE OF GRASS CARP FOR WEED CONTROL

Grass carp has a large potential as a fish-meliorator and can be used effectively to combat the overgrowth of weeds and algae in water bodies (Zolotova, 1971). The optimum temperature of this fish is slightly higher than that of common carp and ranges from 20 to 30 ºC. Its growth depends primarily on the temperature of the water body and the provision of food. The transition of fry from feeding by zooplankton to consumption of its preferable food occurs at the age of 30–60 days. In water bodies rich in zooplankton, this transition may be extended in time and occurs at a later period.

After transition to the consumption of plant feeds, the grass carp is a macrophytophage with a wide food spectrum, which includes almost all types of aquatic flora and most of terrestrial plants. However, in conditions of abundance of feeds, grass carp show a fairly selective attitude to food. In general, the grass carp prefers soft submerged vegetation represented by various species of pondweeds (*Potamogeton* sp.) and duckweed (*Lemna* sp.), as well *Elodea canadensis*, stonewort (*Chara fragilis*), reed mace (*Typha latifolia*) and reed (*Phragmites communis*) in a young form. Plant species to avoid include rush (*Schoenoplectus* sp.), water soldier (*Stratiotes aloides*), water mint (*Mentha aquatica*), buckwheat (*Polygonum amphibium*), frogbit (*Hydrocharis morsus-ranae*), and sedge cane (*Acorus calamus*).

In optimal temperature and feeding conditions, in heavily overgrown natural water bodies and with abundant feeding with aquatic vegetation in ponds, the feeding intensity of grass carp can be very high. The amount of food consumed per day can significantly exceed the weight of fish by 1.5–2 times. The exceptional gluttony of the grass carp is shown by the high feed conversion ratio, consuming up to 30–70 kg of grass per 1 kg of weight gain. Moreover, it is important to understand that grass carp is able to undermine its own forage base quickly, while elder fish can effectively influence the extent of the powerful rhizome grass (e.g. reed, cattail), control of which is ineffective with conventional means.

When choosing the stocking density of grass carp for an ameliorative purpose, the following factors should be considered:

- the site climatic conditions;
- the composition and biomass of phytocenoses;
- fish weight and age at stocking; and
- the economic value of the water bodies.

In southern regions, where the temperature conditions most closely correspond to the biological requirements of the grass carp, the ameliorative (cleaning and filtering) abilities will be fully developed, but there is a risk of overstocking. The release of grass carp should always be preceded by assessment of the food potential of the water body. Stocking of grass carp should be carried out with due account for the floristic composition of the vegetation and the nature of its subsequent alterations in the process of fish rearing. The extermination of submerged vegetation can cause a decline in yield of phytophilic faunal biomass, for example, which is consumed by many commercial fish species (Vinogradov and Bolotova, 1974).

The efficiency of the ameliorative effect of grass carp is directly dependent on the total number of fish released into the pond. However, the age-related food selectivity of the grass carp should be considered and its weight at stocking compared with the species composition of algae. Aero-aquatic plants, including reeds, cattails, and others, occupy a significant place in the diet of grass carp of older ages (weight of 500 g and above) with the expansion of its nutritional spectrum. Therefore, in water bodies overgrown with powerful hard grass, the greatest ameliorative effect should be expected when older groups of fish (3 year-olds and older) is used.

Let us briefly consider the peculiarities of biomanipulation (biomelioration) of water bodies with the help of grass carp, depending on specific types and their economic purpose:

Pond culture

The grass carp has limited application in pond culture. Actually, the development of soft submerged vegetation is considered permissible, if it cover up 10–25 percent of the pond surface. Experience shows that stocking of grass carp with high densities leads to the almost complete elimination of heavy vegetation in fish ponds. When co-cultivated with common carp, grass carp easily switches to the consumption of carp compound feeds, but due to its low value (digestibility) this transition is unacceptable. Moreover, the feeding of grass carp with unusual feeds causes various metabolic disorders and, as a consequence, leads to its poor productive qualities.

Hence, the role of the grass carp in pond culture should be restricted to its use as a supplementary species to the primary species being grown, particularly for the purpose of biological reclamation of ponds suffering excessive weed over-growing, where its productivity should be 70–120 kg/ha. In this respect, the norm for stocking of overgrown carp ponds with one-year-old specimens of grass carp should not exceed 50-150 ind./ha in southern regions (i.e. South of Russia and Ukraine, the Caucasus, Central Asia) and up to 300 ind./ha in northern regions (i.e. North of Ukraine - Central Russia). The standard weight of fingerling used for stocking should be 20–30 g. In ponds with hard aquatic vegetation it is desirable to stock larger specimens weighing 80–100 g or to use two-summer-old fish weighing 250–300 g. Stocking of silver carp in these ponds is carried out in the earliest possible period to allow the fish to actively consume young shoots of wetland vegetation.

It should also be considered that, in addition to stocking of grass carp for softer aquatic species of plants, stiff aquatic vegetation should be removed using alternative mechanical melioration methods (elimination of shallow water, two-time mowing of vegetation: routinely from the third decade of May and June) and using agrotechnical measures, such as the application of herbicides in autumn or early spring and sowing of cereal or legume mixtures along the pond bed with their subsequent filling.

Industrial water reservoirs, irrigation canals and cooling water reservoirs

The non-agricultural economic importance these water reservoirs means it is important to completely remove weed vegetation. In this case, high stocking densities of grass carp can be applied, to use this species high meliorative potential to its full extent. In the course of trials conducted in Central Asia, South of Russia and Ukraine on irrigation canals, it has been established that a significant meliorative effect can be gained at a stocking density of 100–500 kg/ha, depending on the development of plant communities. The greatest effect can be achieved by stocking in early spring with two-year-old fish weighing 300–500 g.

Cooling water reservoirs of various power generation facilities also have a significant productive capacity. It is expedient to carry out stocking using one or two-year old specimens of grass carp at a rate of 100-250 ind./ha, that subsequently allows an annual harvest of not less than 100 kg/ha of valuable fish products.

Natural water bodies and reservoirs

Stocking of grass carp into water reservoirs, delta sections of rivers and deltaic lakes, should be dependent on addressing the melioration objectives required and should be conducted with extreme caution. The uncontrolled release of a highly effective phytophagus into natural water bodies with limited plant resources can inflict irreparable damage to them, expressed in the degradation of spawning grounds of phytophilic fishes that ultimately will lead to alteration of the whole ecosystem.

Since spontaneous spawning of grass carp has not been observed in the vast majority of the water bodies of South-Eastern Europe, Central Asia and the Caucasus, and in other sites where its effectiveness was reported to be insignificant, the use of grass carp for melioration is highly advisable. In the USA, triploid sterile grass carp has been widely used for this purpose, not posing a threat to the environment and effectively combating the excessive overgrowing of water bodies. In any case, any activity should proceed from the need to conserve the ecological balance in relation to grass carp, the highest aquatic vegetation and non-migratory fish. In this case, even at a moderate stocking, water bodies with well-formed and productive communities should be selected. Systematic monitoring over the population size of grass carp should be an obligatory measure, and increases in commercial harvest instigated at the first sign there is degradation of the aquatic phytocenoses.

9. STOCKING OF SILVER AND BIGHEAD CARP TO LARGE WATER BODIES

The river flow regulation and creation of large lowland water bodies has caused significant changes in the structure and functioning of global aquatic ecosystems. This has necessitated activities related to the artificial formation of ichthyofauna in these water bodies, which has subsequently formed the basis for their exploitation. One of the most important approaches to increase the productivity of water bodies in the countries of Eastern Europe and Central Asia was the introduction of herbivorous fish, especially the silver and bighead carp (Vinogradov, 1976, Vovk, 1976, Magomayev, 1980), with other species of secondary importance. At present, vast relevant experience has been gained, which shows that even with mass introduction, the silver and bighead carp do not cause a negative effect on the ecosystem, and it is therefore possible they can form a significant part of the overall commercial fish production. In this case, the introduction of herbivorous fish fry into water bodies should be carried out with an understanding of likely predators and the composition of ichthyofauna in the target water body.

The stocking of large water bodies by silver and bighead carp is based, primarily, on their productivity of phytoplankton and zooplankton. Let us briefly consider the basic principles of calculating the norms of introduction of these species:

- At the first stage, it is necessary to determine experimentally the average seasonal values of the phytoplankton (Ph, g/m^3) and zooplankton (Z, g/m^3) concentrations in the water body;
- Further, knowing the area of the water body (S) and the depth of the productive layer (h), it is possible to calculate the average seasonal amount of phytoplankton and zooplankton in the entire water body;
- Using P/B-factors (which is the ratio of the amount of production per time interval to the average biomass for this period), it is possible to calculate the total output of phytoplankton and zooplankton in the entire water body per season. It has been revealed that for the phytoplankton in the South of Ukraine and Russia, Central Asia and the Caucasus, the P/B (Ph) coefficient amounts to 300 (ranging from 150 to 400), while for zooplankton P/B (Z) is 20 (ranging from 10 to 25).
- Assumption in the calculation are that:
    - fish can consume 50 percent of produced phytoplankton or zooplankton (L), while the feed conversion rate (FCR) for phytoplankton and zooplankton amounts to 50 and 7 respectively;
    - the average weight (W1) of caught fish at the age of 5+ (a five-year plan) is 4 kg for silver carp and 4.5 kg for bighead carp; and
    - commercial return (R) after 3 years from stocked two-year-old fish of 150 g wet weight (W0) is at the 25 percent level.

Let us consider an example of the calculation of fish productivity by phytoplankton (FPh) and zooplankton (FZ) for a 1 000 ha water reservoir with 2 m depth of the productive layer, containing an average phytoplankton and zooplankton concentration of $3g/m^3$ and $1\ g/m^3$, respectively. Then

- $FPh = Ph \times S \times h \times P/B(Ph) \times (L / FCR)$
    - Which equals $0.003\ kg/m3 \times 10\ 000\ 000\ m^2 \times 2m \times 300 \times (0.5 / 50) = 180\ 000$ kg
- $FZ= Z \times S \times h \times P/B(Z) \times (L/FCR)$
    - Which equals $0.001\ kg/m3 \times 10\ 000\ 000\ m^2 \times 2m \times 20 \times (0.5 / 7) = 28\ 571$ kg

Assuming that silver carp feeds on phytoplankton and 50 percent of its productivity is provided with detritus, then annual potential fish productivity in a 1 000 ha water reservoir can be 180 + 180 / 2 = 270 tonnes.

The required annual number of fish stocking material (N, specimens) can be calculated knowing the fish productivity:

- N = FPh / (W1-W0) × R
    - Which equals 270 000 kg / (4 kg – 0.15 kg) × 0.25 = 280 519 individuals

At this, it has been proceeded from the fact that the natural mortality of silver and bighead carp is mainly observed at the first year of its life in the water body, and the technology supposes annual stocking of an equal number of fish. Thus, the full utilization of the food reserve of the water body will occur from the third year of its fishery exploitation.

Similar calculations can be made on bighead carp on the basis of fish productivity on zooplankton (also assuming that detritus amounts to 50 percent of its potential fish productivity) then annual potential fish productivity in a 1 000 ha water reservoir can be 28 571 + (2 8571 / 2) = 42 856.5 kg.

The required annual number of fish stocking material for bighead carp (N, specimens) can be calculated knowing the fish productivity:

- N = FZ / (W1-W0) × R
    - Which equals 42 856.5 kg / (4.5 kg - 0.15 kg) × 0.25 = 39 408 individuals.

Thus, based on natural feed availability and stocking annually about 280 000 and 40 000 two-year-old specimens of silver and bighead carp in spring, respectively, with a total weight of 48 tonnes, in 3.5 years in autumn it is possible to harvest approximately 325 tonnes of valuable commercial fish with an average weight of 4–4.5 kg from a water body of 1 000 ha area.

Another consideration is that grass carp compete with the natural ichthyofauna for food, amounting to minus 10–100 percent of productivity on this species. In addition, supplementary stocking of the water body with grass carp can also elevate its fish productivity by 30–100 kg/ha, depending on the abundance macrophytes. It is important to understand that in southern regions with high development of phytoplankton biomass and high P/BPh ratios, the main role in the formation of fish products belongs to silver carp. In regions with colder climates, it is advisable to stock water bodies with hybrid of silver or bighead carp that is more productive in these conditions and possesses a wider range of nutrition.

An important point in the sustainable use of established commercial stocks of silver and bighead carp is their effective harvesting from water bodies, which is ensured by specialized harvest in the autumn-winter period. If it seems impossible or difficult to catch all the fish that have reached marketable (table) size, the subsequent stocking should be carried out considering the number of the non-caught part of the population. The rate of growth should be also monitored, if it decreases or the weight of fish does not reach the target values, it is necessary to reduce the annual amount of stocked material or intensify the harvesting.

In general, it is urgent to note the high economic efficiency of silver and bighead carp use in the course of the improvement of fish productivity of inland water bodies, especially well-warmed shallow water bodies of the lowland type. Their fishery related use allows to significantly raise the gross yield of fish from these freshwater bodies, thereby ensuring their sustainable rational operation without causing significant damage to the aquatic ecosystems.

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