

The Grass Carp Aquaculture Manual



*Ana Silvia Pedrazzani
Nathieli Cozer &
Antonio Ostrensky*

FAI Farms Limited

The Barn, Wytham, Oxford OX2 8QJ, United Kingdom.

www.faifarms.com

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1 ABOUT FAI

FAI is a global organisation committed to promoting sustainable and ethical practices within agriculture and aquaculture. Since 2018, FAI has been at the forefront of driving aquatic welfare world-wide, conducting research, developing best practice, and providing consulting services to enhance the well-being of farmed fish and shrimp.

Together with other aquatic animals, grass carp are sentient beings with the ability to feel pain, fear and stress. As such, farmers are facing increasing demand to improve the quality of life of the fish in their care. Research and on-farm experiences also prove time and time again improved fish welfare not only leads to happier, healthier fish but to a better product for consumers and greater financial returns for farmers. By promoting fish welfare FAI aims to not only reduce animal suffering but to help produce more food in a sustainable way.

One of FAI's primary goals is to uncover welfare science, synthesise this knowledge and put it in the hands of farmers. FAI believes farmers have the greatest impact on the welfare of animals in their care and its fish welfare project – with partners in Brazil, Central America, China, Egypt, Thailand and Vietnam - is educating and empowering farmers to improve welfare and achieve better production outcomes in tilapia, shrimp and carp aquaculture.

To address the challenge of identifying the welfare status of fish, FAI's team of aquaculture experts has developed welfare assessment protocols based around nutrition, physical environment, health and behaviour. These protocols have been translated into user-friendly mobile apps for use on farms. FAI also runs a free e-learning platform to help equip farmers with the knowledge and skills needed to continually improve welfare and production outcomes.

The Grass Carp Aquaculture Manual is intended as a tool to disseminate knowledge and best-practice guidelines among farmers; ensuring carp are reared using humane and sustainable practices. Not only does this help protect important ecosystems and improve food quality and safety, but also reduces costs and increases profitability for carp farmers.

Our thanks to the manual's authors for sharing their expertise and experience in this area and to FAI's Aquaculture Welfare Project team who collated and edited this publication; Sara Barrento (FAI E-learning and Aquaculture Programme Manager), Murilo Quintiliano (FAI COO) and Amy Wilson (FAI Sustainability Programme Assistant).

2 AUTHOR INFORMATION

Antonio Ostrensky Neto

Ostrensky obtained his degree in Oceanology from the Federal University of Rio Grande in 1988, followed by a master's degree in 1991 and a doctorate in Zoology in 1997 from the Federal University of Paraná, Brazil. He worked as a consultant for the Ministry of Agriculture, Livestock and Supply and with the National Programme for the Development of Aquaculture for the United Nations Development Programme (UNDP), and developed technical advisory work for the Food and Agriculture Organization of the United Nations (FAO). He has been a professor in the Department of Animal Science since 1997 and a permanent professor in the Postgraduate Programme in Zoology and Animal Science, both at the Federal University of Paraná. He has also worked in molecular biology and is currently coordinating the incubation of a start-up (ATGC Genética Ambiental Ltda). He is one of the founders and current coordinator of the Integrated Group for Aquaculture and Environmental Studies (GIA) and co-founder of Wai Ora Aquaculture and Environmental Technology LTD.



Ana Silvia Pedrazzani

Pedrazzani has a degree in veterinary medicine from the Pontifical Catholic University of Paraná (2005), Brazil. She holds a master's (2007) and PhD (2014) in Veterinary Sciences from the Federal University of Paraná, emphasising fish farming and welfare. She completed a sandwich doctorate at the Vero Beach Marine Laboratory of the Florida Institute of Technology, USA, working on breeding marine ornamental fish. Subsequently, she conducted post-doctoral research at the Marine Ornamental Fish Laboratory of the Federal University of Santa Catarina, Brazil and the Animal Welfare Laboratory (LABEA) at the Federal University of Paraná, where, in partnership with FAI Farms, she developed the first Nile tilapia welfare protocol, a finalist in the Aquaculture Awards (2020). The FAO recommended her job as a reference in the area. She is co-founder and director of Wai Ora Aquaculture and Environmental Technology LTD, which researches aquatic animal welfare and sustainable technological alternatives for aquaculture.



Nathieli Cozer

Cozer obtained her degree in Fisheries engineering from the State University of Western Paraná in 2007, followed by a master's degree in 2012 in Fisheries Resources from the same institution and a doctorate in animal science in 2019 from the Federal University of Paraná, Brazil. She has experience in aquaculture, emphasising shrimp farming and tilapia farming. Cozer works in monitoring invasive species in hydroelectric plants, mainly the mollusc *Limnoperna fortunei*, and in the area of bio-monitoring using environmental DNA sequencing. She is a post-doctoral researcher in the Postgraduate Programme in Animal Science (UFPR). She contributes to managing research projects at the Integrated Group of Aquaculture and Environmental Studies – GIA, at the Federal University of Paraná – and is a researcher at Wai Ora Aquaculture and Environmental Technology LTD. She also holds the position of Administrative Manager at the company ATGC Environmental Genetics in the management and elaboration of R&D projects.



3 PREFACE

This manual provides valuable information on grass carp production. It covers everything from grass carp's natural distribution and reproductive behaviours to its biology and anatomy. This manual also explores diseases that can affect grass carp and emphasises the crucial connection between animal welfare and stress in fish.

With a detailed discussion on cultivation systems, this manual includes pond structures and fish farming techniques in both polyculture and monoculture. It provides practical insights into important aspects such as water quality monitoring, feeding, nutrition, health checks and behaviour assessments at every stage of grass carp farming – from broodstock management to pre-slaughter and slaughter processes. The emphasis is on responsible harvesting techniques, transportation considerations and humane slaughter methods.

With its extensive range of topics, from the intricacies of grass carp biology to the practical aspects of pond management and sustainable farming practices, this manual offers a comprehensive guide for both novice and experienced fish farmers, and enthusiasts interested in cultivating grass carp sustainably and ensuring optimal animal welfare.

We would like to acknowledge the authors and all partners who contributed to the creation of this guideline on fish farming and animal welfare. The valuable information presented here is a result of their expertise and dedicated efforts.

FAI Farms Aquaculture Welfare Project Team

4 INTRODUCTION

We present our Grass Carp Cultivation Manual, a meticulously crafted and developed work intended to serve as a reliable and comprehensive source of information.

The manual caters for a diverse audience. It includes fish farmers, both seasoned and new to the industry, and aquaculture investors. Educators and rural extension workers active in this field will also find it a valuable resource.

Equally beneficial for certifiers of sustainable aquaculture practices, it provides clear guidelines and well-defined criteria. Animal welfare professionals will discover recommendations ensuring that fish are produced and farmed in conditions that respect their biological and emotional needs. Aquaculture consultants can expand their advice and guidance with our up-to-date information.

Equally significant, representatives from organisations focused on the regulation, conservation and promotion of sustainable aquaculture, as well as enthusiasts and students of biological sciences and aquaculture, will find the manual instructive and practical to use.

All these audiences will find practical tips, precise guidance and valuable information in this work to enhance their knowledge and skills in cultivating and managing grass carp. With a practical focus and accessible language, our manual aims to contribute to the spread of more efficient, sustainable and ethical aquaculture methods.

5 FISH FARMING AND ANIMAL WELFARE

Animal welfare can be defined as the general physical and mental state of an animal in relation to the conditions in which it lives, is raised and dies. This concept reflects how well the animal can manage and respond to its environment. Animal welfare as a concept includes aspects related to quality of life, comfort, meeting basic needs, and the ability to express the natural behaviours of its species (Figure 1).

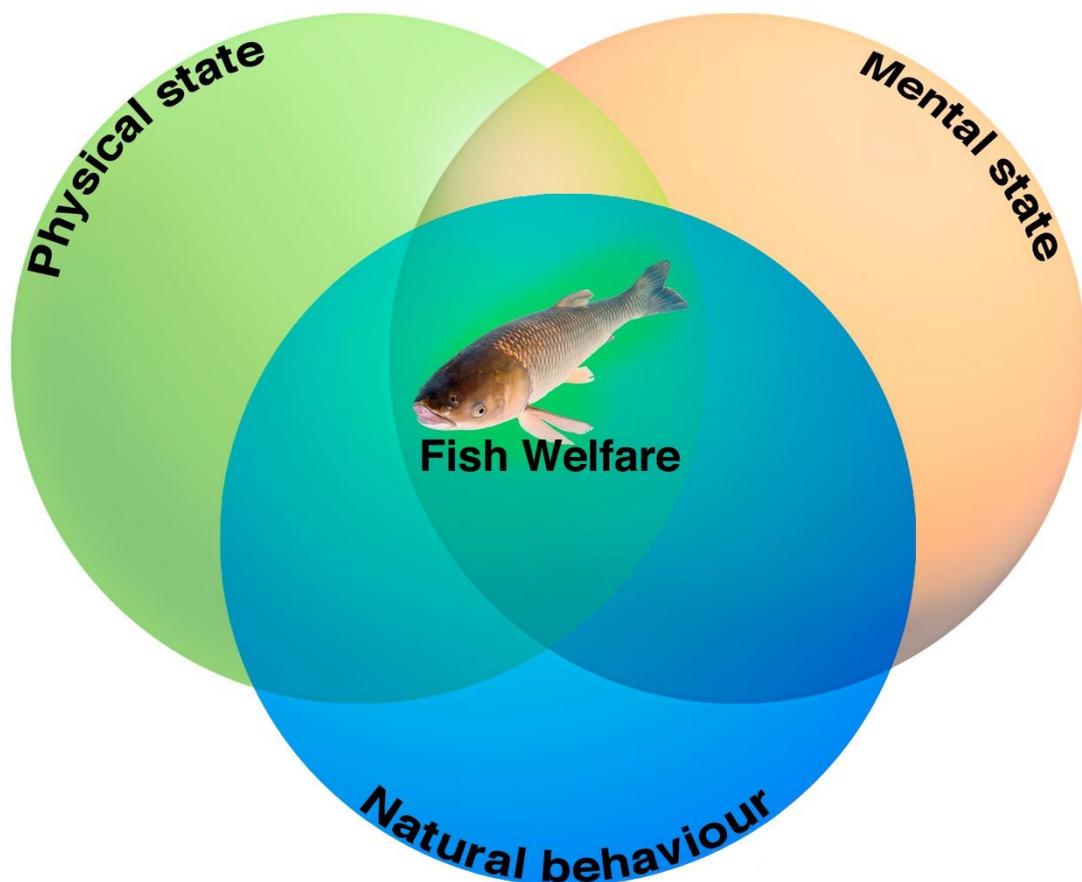


Figure 1. Factors affecting the degree of animal welfare, in this case fish.

The welfare of fish has become an increasing ethical, social and scientific concern, reflecting a growing understanding that fish are capable of feeling and being aware of their feelings and emotions and can perceive pain, comfort and discomfort. It is essential to understand that despite behavioural differences from terrestrial vertebrates, fish can also differentiate between good or bad, pleasurable or unpleasant. This ability, known as sentience, has been scientifically proven in many fish species. The fact that an animal is sentient is a prerequisite for considering and caring about its welfare, its needs and its wants.

The fundamental principles of animal welfare are called ‘The Five Freedoms of Animals’, as defined by the Farm Animal Welfare Committee (FAWC). They are:

1. Freedom from hunger and thirst (where applicable)

Nutritional freedom, access to a quality diet

Feeding should meet the nutritional needs of the species, considering the growth stage they are in. A balanced and nutritious diet is fundamental for grass carp's welfare and healthy growth. In addition to the quality of the feed, it is essential to adopt proper nutritional management practices, such as controlling the quantity and frequency of feeding, avoiding waste, and ensuring that all fish receive enough food.

2. Freedom from discomfort

Environmental freedom

Appropriate environment. It is crucial to provide an environment that meets the physical and psychological needs of the animal, including adequate space, shelter, temperature and lighting. Providing sufficient space in tanks or ponds is also crucial to avoid overcrowding, which can lead to stress and competition for food. Adequate space allows grass carp to swim freely, promoting their healthy development.

3. Freedom from pain, injury, and disease

The absence of pain, diseases and injuries implies maintaining good health conditions for the fish and ensuring continuous monitoring. Detecting signs of illness or stress and adopting preventive and treatment measures when necessary are crucial practices for the welfare of grass carp. Conducting calm and careful management, especially during reproduction, biometrics, harvesting or transfer, minimises stress and trauma in fish, ensuring their welfare throughout the production process.

4. Freedom to express normal behaviour

Behavioural freedom

Expression of the species' natural behaviour. We should allow animals to express typical and natural behaviours of their species, allowing for freedom of movement, social interaction and recreational activities.

5. Freedom from fear and distress

Psychological freedom

Absence of suffering and pain. To ensure welfare in a production environment, we must avoid causing pain, excessive stress or unnecessary suffering to animals through painful procedures, inadequate living conditions or cruel practices. The fifth freedom states that the mental health of an animal is just as important as its physical health. Preventing overcrowding and providing a safe space for all animals are ways to ensure they feel protected.

Thus, it is crucial to balance the needs of animals with the demands of aquaculture, research, commerce and other human activities, always aiming to promote a more compassionate and responsible coexistence with animals. That is, we must apply the principles of animal welfare and ensure proper care for fish, as they deserve consideration and care in the same way as other species used for animal production.

These principles provide a valuable and practical approach to assessing the degree of welfare directly on farms and during the transport and slaughter of fish. Through them, it is possible to develop a

protocol for evaluating the degree of welfare, considering the Five Freedoms of Animals. In addition to being species-specific, the indicators that make up a welfare assessment protocol should also reflect the critical points and physiological and environmental demands of each cultivation phase. For example, Figure 2 illustrates some of the main critical points during the fattening phase of grass carp in a semi-intensive production system. Throughout the manual, we address the indicators shown in the figure and their ideal and tolerance levels for each production phase.

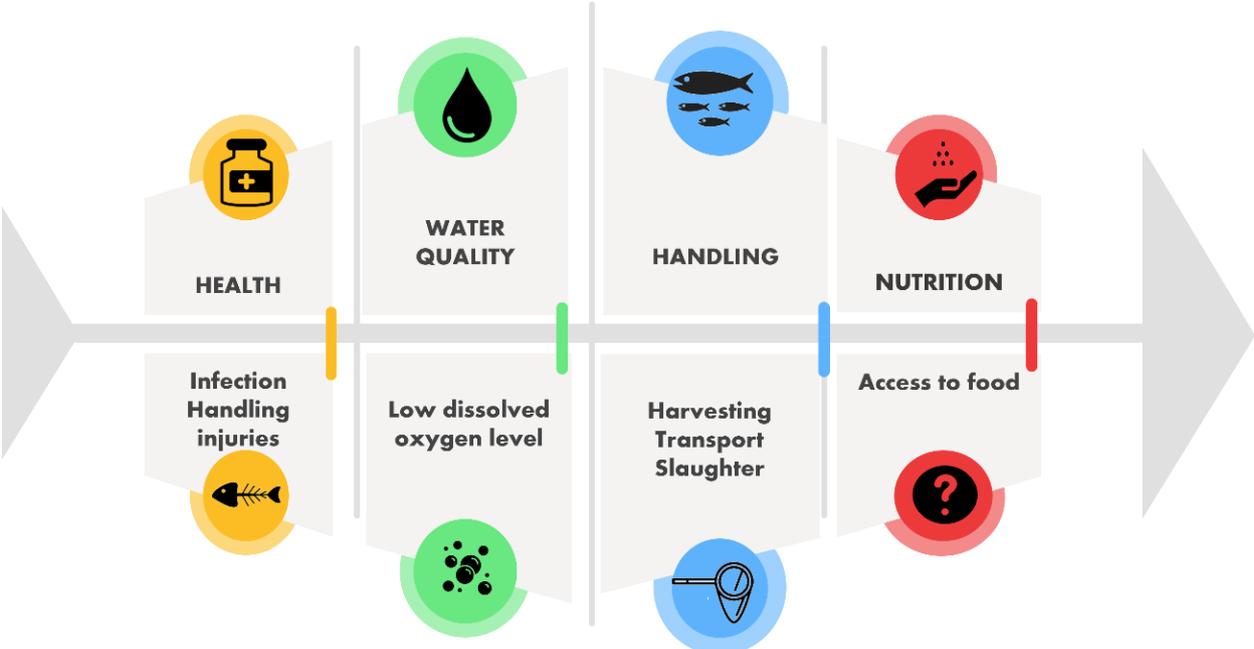


Figure 2. Main critical points of grass carp welfare during the fattening phase in semi-intensive aquaculture production.

6 THE GRASS CARP



Figure 3. The grass carp, *Ctenopharyngodon idella*.

Known in Chinese as Tsao Yu, Hwuan Yu, or Bai Hwuan, the grass carp (as it is called in the West) is the main species of fish cultivated worldwide (Figure 3). 'Yu' in Chinese means fish. The name variation reflects its preferred food ('tsao' in Chinese is grass), its medicinal function ('hwuan' in Chinese is soothing), or its body colour ('bai' is white or shiny grey).

The grass carp has become a star in global aquaculture, not only for its gastronomic value but also for its remarkable biological characteristics and ease of cultivation. Known for having large, easily removable bones (Figure 4) and its tasty and delicate flesh (Figure 5), it is an excellent source of unsaturated fatty acids. These fatty acids are nutritionally rich food that significantly promotes cardiovascular and cerebrovascular health. The flesh of the grass carp is also rich in selenium, an essential mineral that contributes to the prevention of ageing and the strengthening of the immune system.



Figure 4. Skeleton of the grass carp.



Figure 5. The grass carp has delicate and highly flavourful flesh.

It is also noteworthy for its rapid growth rate. Picture a fish that, at the beginning of spring, measures about 20 cm in length but by autumn reaches 45 cm or more. Adults of this species can typically reach impressive sizes, growing up to 1.2 m and weighing more than 18 kg. The largest recorded specimens were around 1.8 m long and weighed 45 kg.

In terms of longevity the grass carp is also impressive. Typically, their life expectancy ranges from 5 to 9 years, but there are records of individuals living for over two decades, especially when the animals are in environments conducive to their development.

6.1 Natural distribution

Originating from the Far East of the Pacific, the grass carp (*Ctenopharyngodon idella*) belongs to the *Cyprinidae* family (the same as other carp) and is the sole species of the genus *Ctenopharyngodon*. Its native distribution extends from northern Vietnam to the Amur River, on the border between China and Russia (Figure 6). The species is mainly found in large rivers such as the Yangtze, Pearl River and Heilongjiang, as well as associated lakes and marshes.

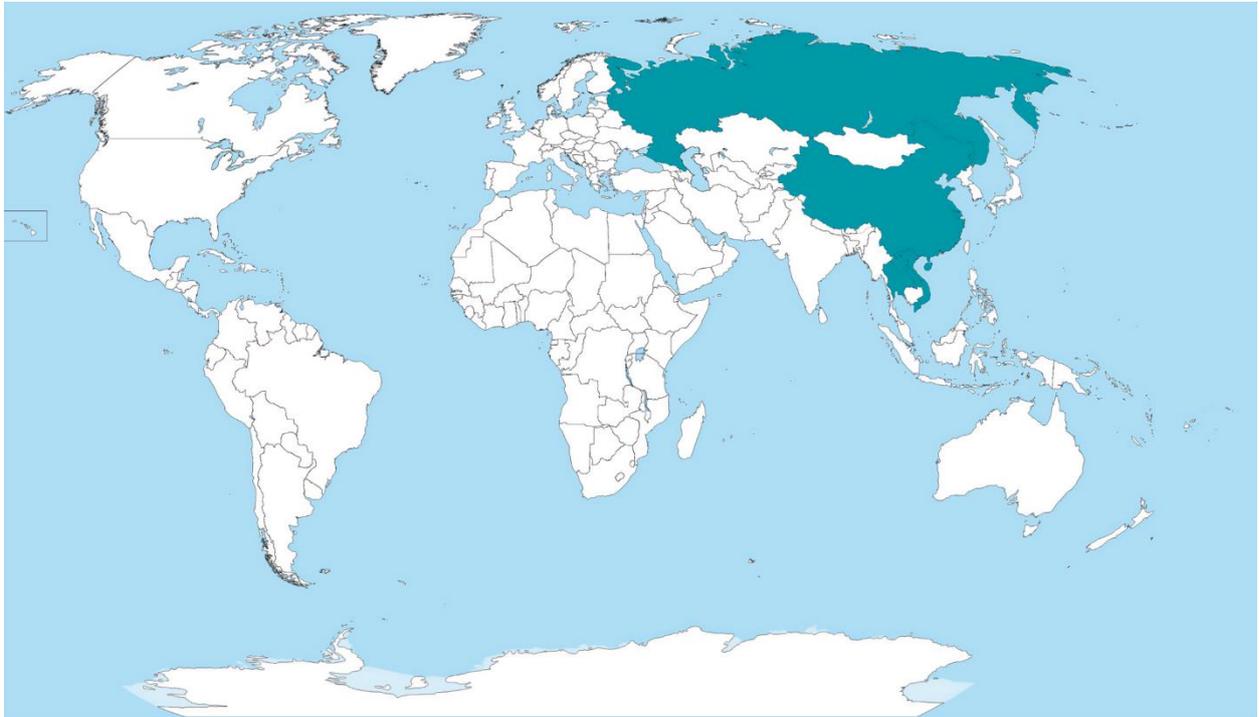


Figure 6. Natural distribution zone of the grass carp, *Ctenopharyngodon idella*.

6.2 Reproduction in natural conditions

The grass carp is a migratory species that demonstrates a wide temperature tolerance range and spawns in conditions varying from 20 to 30°C, although the adults can withstand temperatures between 0 and 38°C.

Grass carp typically reach sexual maturity at around 2–8 years, when they weigh between 4 and 8 kg. This broad time and weight range is due to specific environmental conditions and other factors, such as food availability, genetic factors and interaction with other species.

Sexually mature fish leave their growing areas and migrate upstream to spawn in flowing water environments.

The reproduction of grass carp generally occurs in late spring or early summer, when the water temperature rises and the days become longer. The photoperiod is an essential factor in the reproduction of migratory fish, and this is no different in the case of grass carp.

During the breeding season the females' ovaries become filled with developing ova, evident by their swollen and taut abdomens. This indicates that the fish is ready for spawning.

Besides physical changes, the ventral area's colour may also change, becoming paler than other life stages when it usually exhibits more intense colouration.

The belly may also show some swelling in males, though not as pronounced as in females due to the lesser amount of reproductive material. They also develop rough tubercles on their opercula and scales, making their pectoral fins thicker. These sexual characteristics in both males and females disappear after the mating season.

Natural spawning is impossible in stagnant waters. Increased water flow and rising temperature trigger reproductive migration and spawning, a collective process. Mating usually occurs near the water's surface in groups ranging from 4 to 12 fish. Males chase females, nudging their bellies and even jumping out of the water. This mating and spawning behaviour can last from less than an hour to two days.

The entire reproductive process is hormonally controlled. The carp's nervous system responds to environmental cues by releasing hormones that act on the gonads to initiate spawning. In aquaculture settings hormones induce this process, especially when ideal environmental conditions are absent.

Males appear to have a slightly different sexual maturation cycle than females, being less sensitive to environmental conditions. Nevertheless, the process of preparing the testicles for reproduction takes about 3 months.

A female grass carp can release about half a million eggs, which and this number tends to increase throughout her life. At the peak of their reproductive life a single grass carp female can produce up to about 1.5 million eggs at the peak of her reproductive life. In cultivated carp, fecundity is even higher and can exceed 100 eggs per gram of body weight.

In nature, most of these eggs will not produce individuals reaching adulthood due to predation or unsuitable conditions, such as facing temperatures below 18°C in the early stages of life.

Once the eggs are fertilised they float downstream, suspended by turbulence. The survival of the eggs and young fish depends on these long, turbulent rivers, as the eggs are likely to die if they sink. In an ideal environment, such as the Yangtze River, the eggs need to float in the water for about 2 days to hatch, travelling a distance of 100 to 150 km. Once hatched, the larvae alternate between swimming and sinking. In a short time they fill their swim bladder and become post-larvae, and begin swimming towards calmer areas such as lakes, marshes and floodplains, which act as natural nurseries.

The species has a rapid growth rate. The post-larvae feed on zooplankton, and when they reach approximately 5 to 7 cm in length the juveniles shift to a predominantly herbivorous diet.

The breeders and juveniles migrate to the middle and lower layers of water bodies such as ponds, streams, lakes and reservoirs, seeking areas rich in vegetation, which serves as food and ensures ideal conditions for their growth. The fish will spend most of their lives in still or slow-flowing water environments with abundant vegetation (Figure 7) and high turbidity.



Figure 7. Representation of the grass carp in its natural habitat.

6.3 The use of grass carp in controlling aquatic vegetation

Due to their large appetite for aquatic plants, grass carp (*Ctenopharyngodon idella*) have been introduced in at least 100 countries across North America, South America, Africa, Asia, Europe and Oceania. This introduction was for meat production but primarily for controlling aquatic weeds.

The diet of grass carp is predominantly herbivorous, focusing mainly on aquatic plants and even submerged terrestrial vegetation. However, this species can also diversify its diet, consuming detritus, insects and other small invertebrates when necessary.

Remarkably, grass carp can ingest an amount of vegetation equivalent to about 40% of their body weight daily. In environments with abundant plants, the carp can consume more than double their weight in a single day, leading to rapid population growth.

Such voracity makes the grass carp a potentially invasive species, especially in ecosystems where it is introduced without proper protective and control measures.

Their ability to consume large amounts of aquatic vegetation can destroy habitat and disrupt the local flora and entire food chain, negatively impacting other species, including many native fish and aquatic birds that rely on these plants for survival. Therefore, any initiative to introduce this species into new environments must be undertaken with extreme caution and only if certain criteria are fulfilled.

An innovative technique has been employed to mitigate these risks: triploid grass carp. Triploidy is a state where organisms have an extra set of chromosomes, rendering them sterile and therefore

unable to reproduce. This characteristic is beneficial when trying to prevent the uncontrolled proliferation of the species in new habitats.

The fact that these triploid carp are sterile eliminates the risk of a carp superpopulation that could unbalance the aquatic ecosystem. Thus, by not reproducing, these carp do not become a threat to other native species and do not contribute to the ecological imbalance of the environment where they are introduced.

6.4 Grass carp in aquaculture

Grass carp occupies a prominent place in the industry and even in the history of global aquaculture. Today, it is the world's most widely cultivated fish species and is probably the first species cultivated in global fish farming. Archaeological discoveries in China believed to be remnants of ancient grass carp cultivation ponds provide evidence that dates back at least 6,000 years. This finding highlights the region's deep historical roots of grass carp aquaculture. Grass carp are also mentioned in ancient texts dating back over 2,000 years. The extensive longevity in the production of grass carp demonstrates not just its cultural and historical significance but also its adaptability and economic importance.

In fact, in China, this fish is an essential part of the local aquaculture culture, along with three other species: the silver carp *Hypophthalmichthys molitrix*, the bighead carp *H. nobilis*, and the black carp *Mylopharyngodon piceus*, which are collectively referred to as Asian carps in Western literature.

Artificial reproduction of these carps was successfully achieved for the first time in China in the early 1940s, and large-scale aquaculture began in 1958.

Grass carp can be cultivated in various farming systems, such as ponds, enclosures and cages. However, the captive production cycle involves many more stages, such as reproduction, larviculture and fingerling production (Figure 8). All these stages are addressed in this manual.

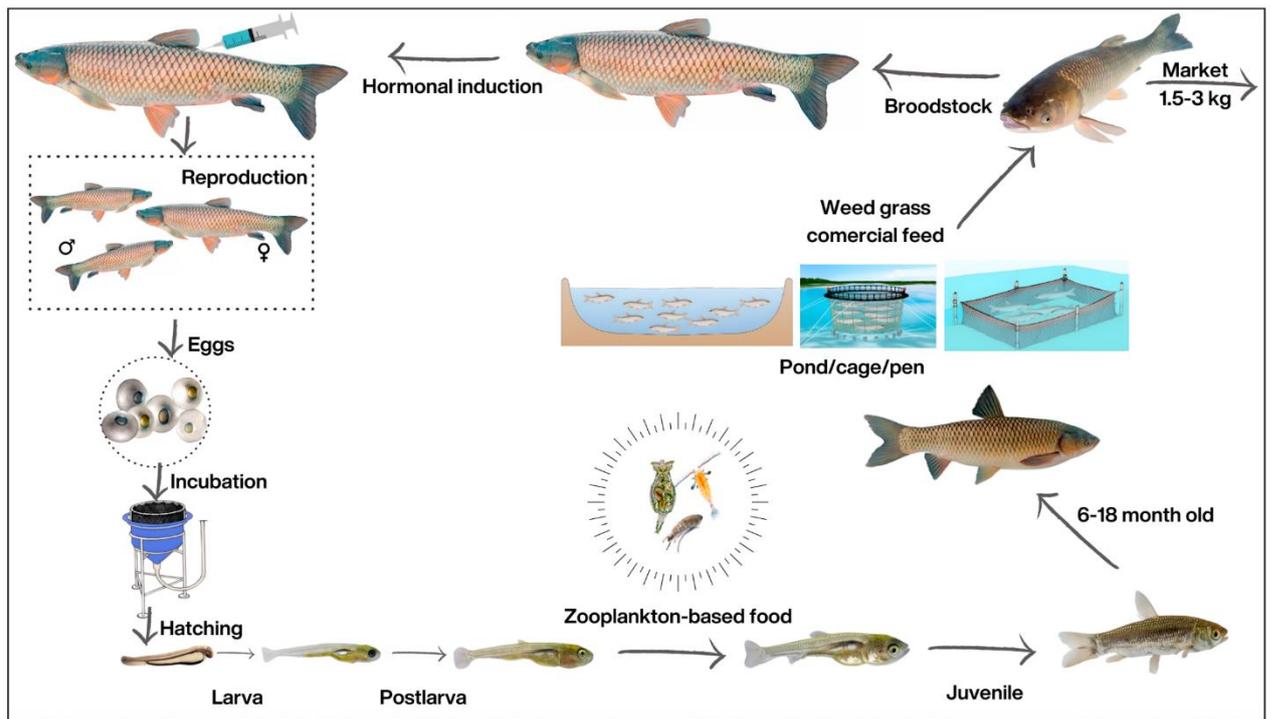


Figure 8. Grass carp production cycle.

In 2020, the global production of cultivated grass carp reached an impressive 5.79 million tonnes. This underscores its colossal role in the aquaculture sector, ranking just behind – and nearly equal to – the marine shrimp *Litopenaeus vannamei*, which amounted to 5.80 million tonnes in the same year.

These massive numbers highlight the fundamental role that grass carp plays as a pillar of food security in many regions, especially in Asia, where its production is primarily a crucial source of dietary protein for local communities, given that exports of this species are relatively insignificant.

7 GRASS CARP BIOLOGY

7.1 Life stages

7.1.1 Eggs

Oocytes – eggs before fertilisation – have a 1.2–1.3 mm diameter and contain a yolk surrounded by a two-layer membrane. The outer layer is adhesive until fertilisation.

The fertilised eggs are 3.8–4.0 mm in diameter, and the yolk is separated from the membrane by absorbed water (Figure 9).

The spawning containing eggs can vary in colour from greyish blue to bright orange.

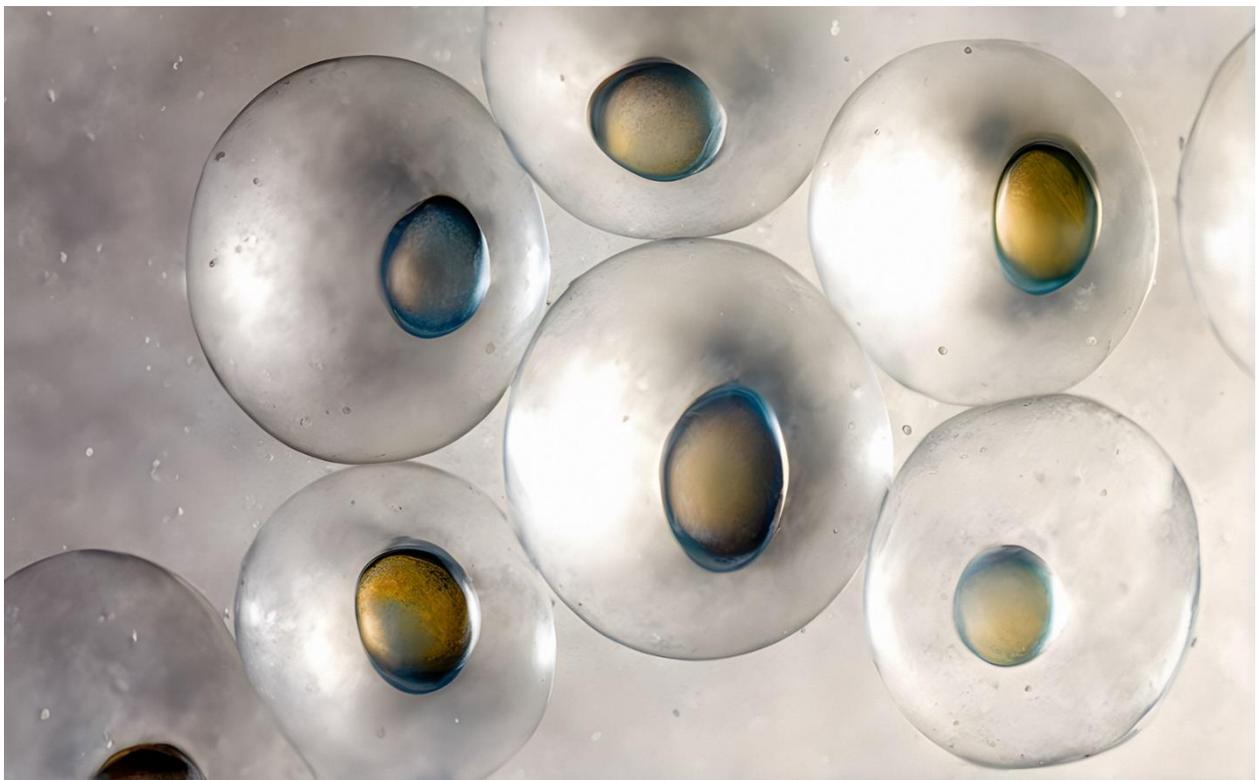


Figure 9. Fertilised grass carp eggs.

7.1.2 Larvae (1–3 days)

The larvae hatch from the eggs at a length of 5.0–5.5 mm.

They are transparent, completely depigmented at this stage, and have a visible yolk sac (Figure 10). The larval development is significantly temperature-dependent: in water at a temperature of 24°C the larvae reach 10.5 mm in 5 days. At 25°C, they can grow to 12.4 mm in the same period.

Their gills are functional once the fish reaches about 7.5 mm, and their eyes become pigmented with golden irises. During this period, the proto larvae also begins to swim. Although they still primarily feed on the yolk sac, from the second day the larvae can also start to ingest tiny plankton organisms.



Figure 10. A grass carp larva (a proto larva) shortly after hatching.

7.1.3 Transition to post-larva

At about 4 days old and measuring approximately 8 mm, the larvae from Figure 11A are still relatively transparent but become larger and more pigmented each day. At this stage they already have a caudal fin. The yolk sac is still present; the intestine is not yet fully formed; the swim bladder is quite evident.

As the yolk sac rapidly depletes (from 9 mm onwards), the mesolarvae become dependent on exogenous food, consuming algae and zooplankton, and by around the 5th day they feed almost exclusively on zooplankton (Figure 11B).

By the 20th day of life the fins are formed and the post-larvae are about 1.5 cm long (Figure 11C).

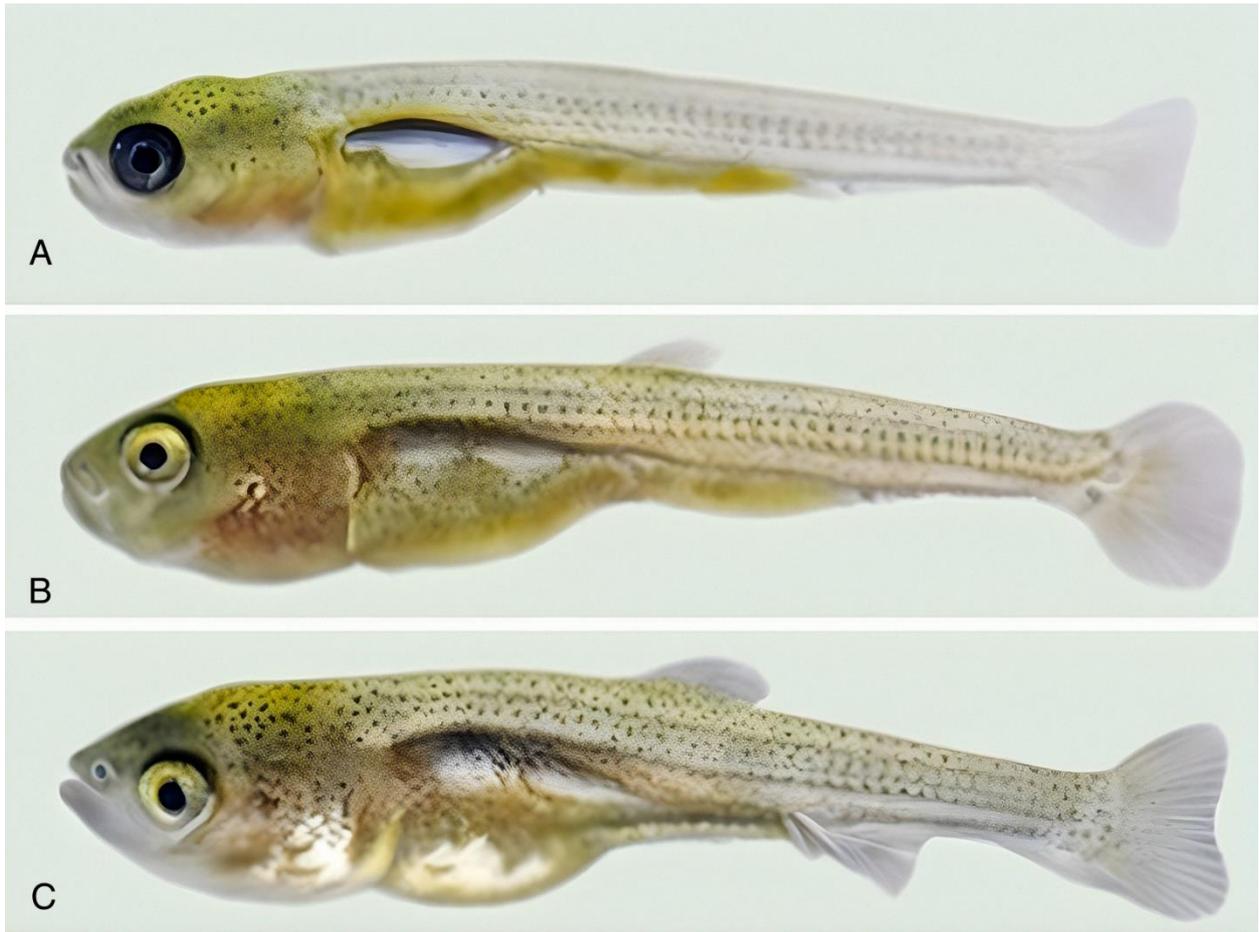


Figure 11. Evolution from larva (mesolarva) to post-larva. Source: Ma et al. (2023), modified. A – four-day-old grass carp larva measuring approximately 8 mm, caudal fin is starting to develop, the yolk sac is still present; the intestine is not yet fully formed; the swim bladder is quite evident. B – five-day-old grass carp mesolarva, feeds exclusively on zooplankton as the yolk sac depletes. C – 20-day-old post-larva, the fins are formed, and the post-larva is about 1.5 cm long.

7.1.4 Fry (20-30 days)

Between the 20th and 30th days of life the fry are about 1.5 to 2.3 cm long. Their fins and scales are developed (Figure 12). The pharyngeal teeth are formed, and the jaw is set. The swim bladder and intestine resemble those of an adult. Fry feed on zooplankton and aquatic insect larvae. At 2 cm in length, grass carp fry begins to feed on aquatic plants.



Figure 12. Grass carp fry. Between the 20th and 30th days of life the fry is about 1.5 to 2.3 cm long.

7.1.5 Juveniles (45–60 days)

Juveniles measure 3.7 to 6.7 cm in length and resemble miniature adults (Figure 13). Around the 50th day the scales are complete, and approximately on the 55th day, at about 6.7 cm in length, the juveniles become identical to an adult, except they are still immature individuals, i.e., their ovaries and testicles are not yet developed, and thus they are not yet capable of reproducing. Juveniles can feed on animals (e.g., insects and zooplankton), but from 5.5 cm in length, they primarily feed on plants.



Figure 13. Grass carp juvenile.

7.1.6 Adult

Studies conducted in China's Heilongjiang, Yangtze and Zhujiang rivers show that grass carp populations can exhibit significant morphological differences depending on their origin. That is, animals of the same species but from different rivers show notable differences in shape and size. To understand this, imagine having three cousins: one is tall and thin, another is shorter and more robust, and the third is average height. They are from the same family, but each has distinct

characteristics. The same happens with grass carp. Different lineages exhibit different characteristics and may grow more or less than those of a different lineage.

But, as a general rule, the head of the adult grass carp is slightly flattened (Figure 14). The moderately small eyes are centred on the side of the head. Large overlapping scales cover the body, and the colour ranges from blackish to olive-brown, with greyish or silvery-white hues on the sides and belly. The scales on the dorsal region and sides are outlined by pigment, giving a hatched effect and aiding in camouflage.

The body is elongated and narrow with a terminal mouth, meaning its mouth is located in the middle of the head and points forward. Grass carp have large circular scales with a black spot at the base.



Figure 14. Adult grass carp.

7.2 Morphology

Understanding the morphological structures of the fish being cultivated is essential for anyone interested in fish farming. This is not only because it can lead to increased production efficiency, but also to ensure the welfare of the fish by providing an environment that respects their anatomical and behavioural needs.

Grass carp have several distinct morphological characteristics (Figure 15) that make them well adapted to their environment and lifestyle. They have elongated, chubby, torpedo-shaped and slightly laterally compressed body forms, contributing to their agile swimming ability among aquatic vegetation. The complete lateral line contains 40 to 42 scales. The anal fin is positioned closer to the tail than in most other carp species. The body is dark olive-green, with shades of brownish yellow on the sides and a white belly.

7.2.1 Head

The head is triangular in shape and slightly flattened dorsally and is thus optimised for hydrodynamic efficiency, allowing efficient swimming and foraging.

- **Mouth:** The terminal mouth is slightly oblique, with firm, non-fleshy lips and without barbels. It is protrusible, devoid of teeth, and the upper lip is thick, specially adapted for scraping aquatic vegetation.
- **Pharyngeal teeth:** Located in the pharynx, these teeth are designed for chopping and grinding vegetation, facilitating digestion.
- **Eyes:** Positioned laterally, providing a wide field of view.

7.2.2 Fins

The grass carp's moderately large pectoral and pelvic fins enhance its ability to execute swift manoeuvres and maintain balance in the water. Its dorsal fin is notably long and unbroken, in contrast to the shorter anal fin.

7.2.3 Scales

Large and oval in size, they protect effectively against parasites and physical injuries.

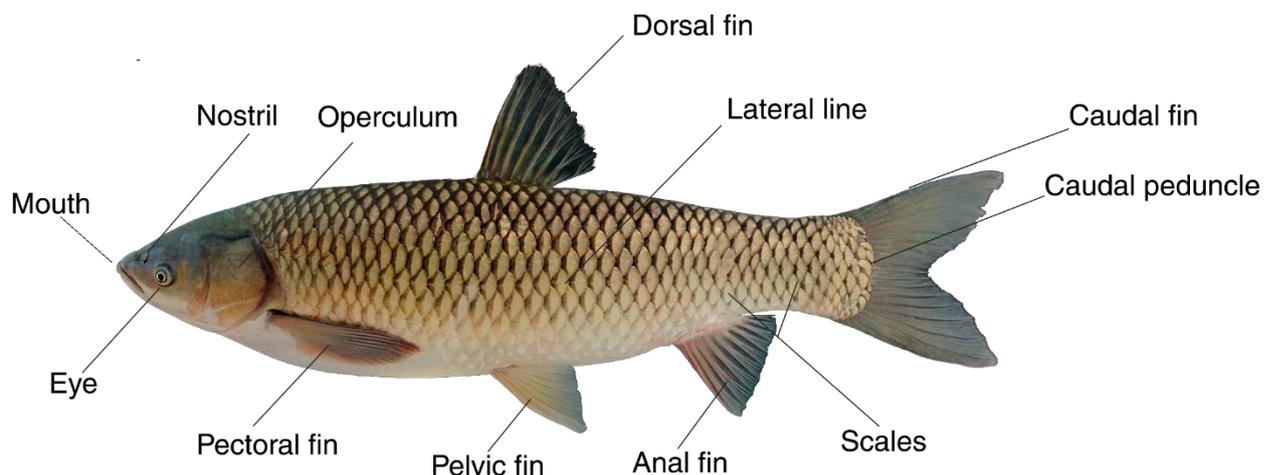


Figure 15. External morphology of grass carp.

7.3 Anatomy

The organs of grass carp are similar to those of other fish but they have some specific adaptations for their herbivorous lifestyle. Notable in the anatomy of a grass carp (Figure 16) is the length of its intestine, which can be two to even 10 times longer than the fish's body length, depending on age, nutritional factors and genetics. Long intestines are characteristic of predominantly herbivorous fish.

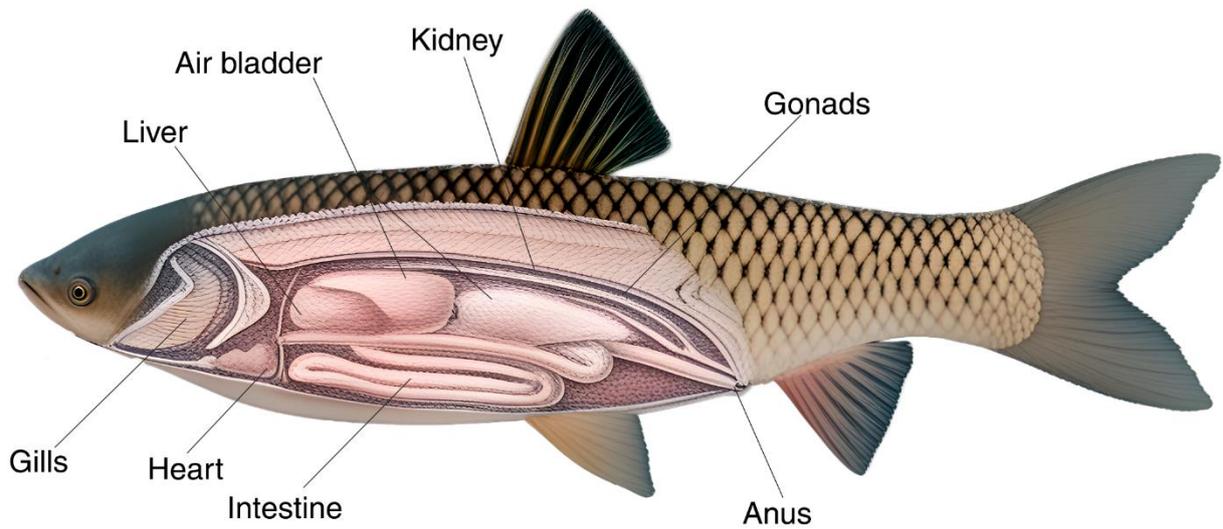


Figure 16. Schematic representation of grass carp anatomy.

7.3.1 Pharyngeal teeth

A notable feature of the grass carp is the presence of two rows of comb-like teeth on the pharyngeal arches in the throat (pharynx, to be more precise) (Figure 17). These strong teeth resemble the teeth of a comb. Grass carp use these pharyngeal teeth to grind and consume aquatic vegetation.

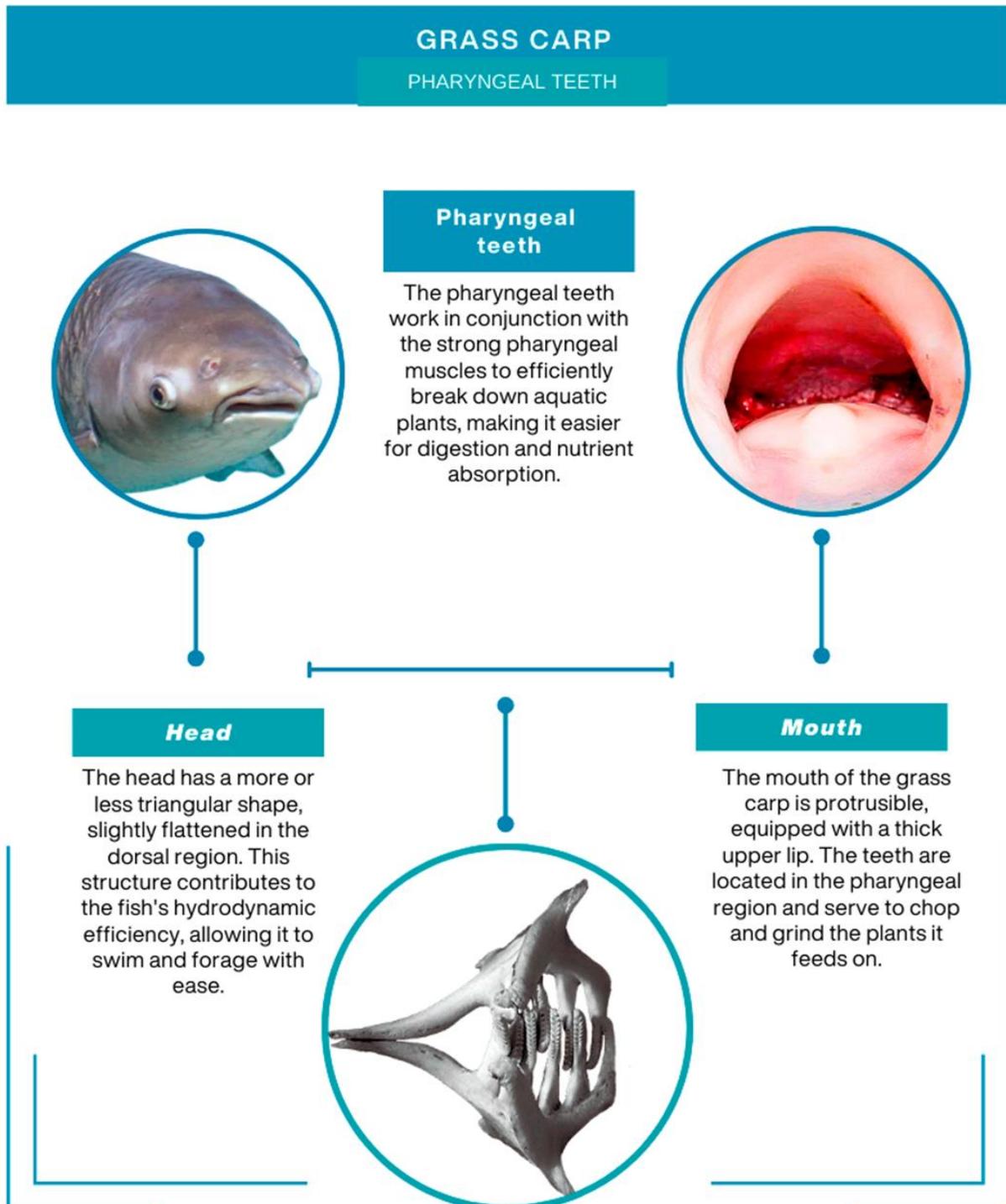


Figure 17. The scientific name of grass carp, 'Ctenopharyngodon', is a combination of three Greek words : 'cteno', meaning 'comb', 'pharyngo', meaning 'throat' or 'pharynx', and 'odon', meaning 'tooth'.

The jaw of the grass carp functions as an intricate system of levers and cords, composed of 11 muscles working in harmony. Some of these muscles are attached to the fish's skull, and others to its pectoral structure. There is no direct bony connection of the jaw to the skull, only ligamentous connections. Two muscles stand out: the 'levator', which raises the jaw to aid in chewing, and the 'retractor', which pulls the jaw back (Figure 18). These muscles move the pharyngeal arch where the pharyngeal teeth are located, functioning as a grinder for the leaves and stems ingested by the grass carp.

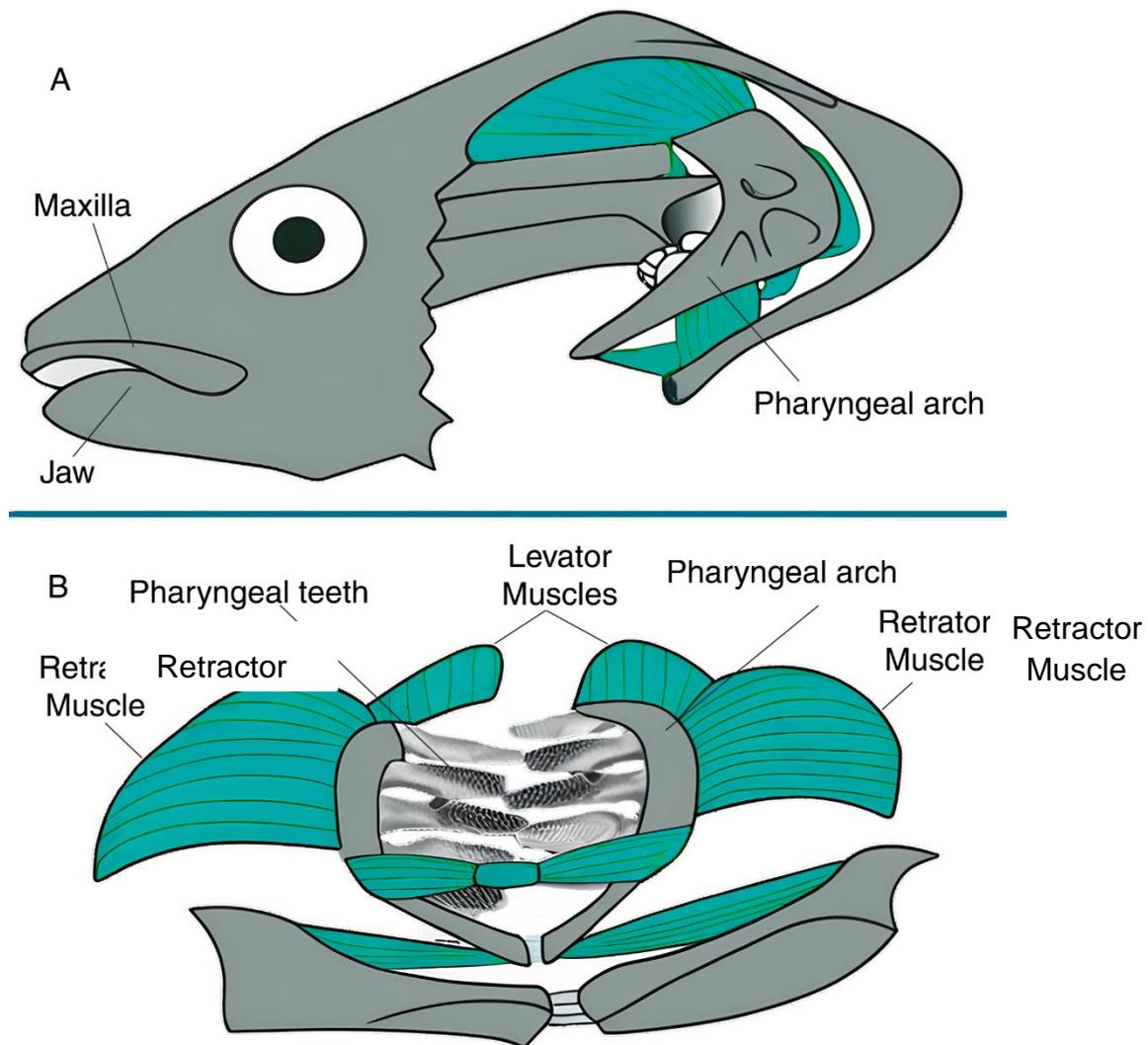


Figure 18. Anatomy of the grass carp. A) Side view. B) Muscle and bone structure. In green, the muscles responsible for transforming the pharyngeal teeth into real grass crushers. Adapted from Gidmark et al. (2015).

7.3.2 Intestine

The intestine is a simple coiled tube with several convolutions. It is divided into three parts: anterior intestine, mid-intestine and rectum. The intestine is the primary organ for food digestion and nutrient absorption. It occupies a ventral position in the body cavity and is entirely separate from the swim bladder. The intestine of a grass carp is also lined with a layer of mucus that helps protect the fish from bacteria and other harmful organisms. The mucus also helps lubricate the intestine, facilitating the passage of food.

7.3.3 Liver

The liver is located at the top of the gastrointestinal tract and primarily functions in the metabolism and storage of nutrients.

7.3.4 Swim bladder

The swim bladder is large and located dorsally to the intestine, and is responsible for the fish's buoyancy. It has two chambers and is of the physoclistous type, comprising a closed system without direct connection to the digestive system. In fish with physoclistous swim bladders, gas is secreted into the bladder via a specialised set of capillaries called the '*rete mirabile*'. This mechanism enables the fish to regulate its buoyancy by secreting or absorbing gases in the swim bladder, a critical function for maintaining their vertical position in the water column.

7.3.5 Gills

Grass carp breathe through their gills, located on the sides of the head, under the operculum. The gills consist of gill filaments covered by a thin membrane. Water enters through the mouth of the grass carp and passes over the gills, where oxygen is absorbed and carbon dioxide is released.

The circulatory system of the grass carp is closed, meaning blood flows through vessels. Its heart is a simple but efficient muscular organ with two chambers: an atrium and a ventricle.

This typical arrangement in bony fish works as follows: The atrium is an upper chamber that receives venous blood passing through the various body tissues via systemic veins. Blood is pushed into the next chamber, the ventricle, when the atrium contracts. When the ventricle contracts, blood is forced into the arteries and proceeds to the gills, which will be oxygenated.

7.3.6 Kidney

The kidney of the grass carp is an elongated organ extending along the body cavity, located below the spinal column. It performs a range of vital functions, similar to the kidneys of other bony fish, such as blood filtration, regulation of water and ion balance, and production of blood cells and hormones.

7.3.7 Gonads

Grass carp are oviparous fish, meaning they lay eggs.

Females have ovaries, which produce oocytes and can occupy the entire extent of the fish's abdominal cavity during breeding.

Male grass carp have testicles, which produce sperm.

8 DISEASES AFFECTING GRASS CARP

8.1 The relationship between welfare and stress in fish

The relationship between animal welfare and stress is crucial to understanding how the environment and living conditions impact their emotional, behavioural and physiological state. An environment that promotes welfare enables animals to develop strategies for adapting to stress.

Such an environment includes providing access to refuges, engaging in enriching activities (such as using grass for dietary supplementation), fostering positive social interactions and practising gentle handling. These elements not only help reduce stress but also significantly enhance the overall welfare of the carp throughout their lifespan.

Inadequate living conditions, overcrowding, lack of environmental enrichment, social isolation, abrupt changes, excessive noise, lack of access to essential resources (like food and overexposure to light) and improper handling practices can trigger animal stress.

Fish reaction to stressful situations occurs in three distinct stages: primary, secondary and tertiary. This process begins in the hormonal system and expands to affect the organism globally (Figure 19).

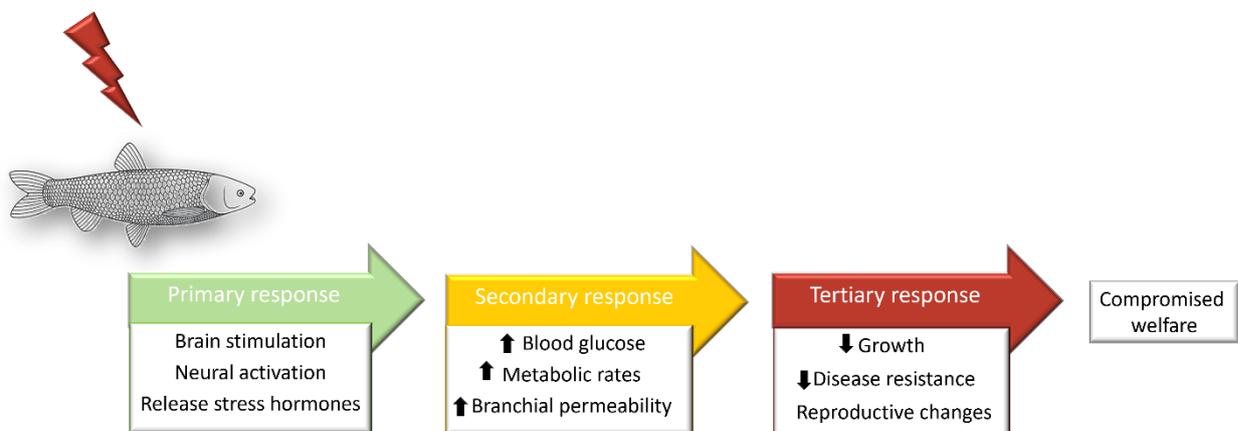


Figure 19. Primary, secondary and tertiary responses to stress triggered by external factors in grass carp and the changes occurring in the fish's body at each stage.

The primary stage involves the activation of brain areas, resulting in the release of hormonal substances like catecholamines and corticosteroids.

The secondary stage is characterised by the immediate impact of these hormones on blood tissues, increased heart and respiratory rates, mobilisation of energy resources, and even disruption of the fish's osmotic balance.

Lastly, the tertiary stage manifests in population terms, reflecting inhibited development, reproduction and immune response in a large portion of the fish in a group, increasing susceptibility to diseases. The reduced ability of the fish to handle subsequent or additional stressors is also considered a tertiary response to stress.

Animals with opportunities to develop resilience to stress are more adept at dealing with specific stressful situations, such as a sudden drop in temperature. An appropriate and enriched environment allows animals to develop skills and competencies that strengthen their resilience and, consequently, their welfare. Conversely, if the organism does not overcome the challenge, stress becomes chronic, indicating low animal welfare. Chronic stress due to inadequate housing conditions, improper management or negative social interactions can suppress the animals' immune system, making them more susceptible to infections and diseases.

Besides diseases, fish exhibit various behavioural responses to different stressors. Stress in fish can manifest through abnormal behaviours (stereotypes) or as aggression, inactivity or hyperactivity, changes in food consumption, increased respiratory frequency, alterations in body colouration or swimming patterns, escape, seeking shelter, and submissive posture, among others. These behaviours indicate discomfort, i.e., welfare is negatively impacted, compromising the fish's quality of life.

Stress indicators, such as hormonal levels and heart rate, are frequently used to assess animal welfare in laboratory studies. However, in practical farm routines, physical assessment of the fish is more feasible, where a thorough inspection of the fish's external organs like eyes, fins, and skin should be performed. Significant changes in these indicators may suggest an impact on the animal's welfare.

8.2 Health vs. diseases and the impact on animal welfare

'Disease' can be defined as a mere absence or imbalance in health. It might also be regarded as an abnormal physical condition stemming from environmental stress and the fish's immune response to pathogenic agents.

Therefore, 'health' and 'disease' are subjective concepts influenced by various simultaneous factors. The onset of disease does not always precisely coincide with its clinical signs, and it might be detected accidentally or only after the death of some fish in the pond.

As discussed, a high degree of animal welfare is essential to ensure that animals live healthily, free from excessive stress and with a satisfactory quality of life.

Promoting the welfare of grass carp in aquaculture benefits the fish in terms of health and growth and contributes to the sustainability of production and the quality of the products offered to the market. Attention to these aspects is crucial for the success of grass carp farming.

Proper nutrition, rational and humane management, and good environmental conditions will make animals more resistant to diseases. Good health means both high levels of welfare and high profitability rates in production. Thus, improving animal welfare involves identifying and mitigating

sources of stress, providing a suitable environment and adopting management practices that promote natural and healthy animal behaviour.

8.3 Diseases affecting grass carp

Other measures should be adopted to prevent diseases, such as:

- **Source:** Acquiring animals from well-sourced, disease-free stocks from companies that follow good production and hygiene practices.
- **Visual inspection:** Conduct a detailed visual inspection to identify lesions characteristic of disease on receipt of the animals and check the mortality rate during transport.
- **Water quality:** Maintaining the animals in a system that ensures water quality during acclimation and transfers, avoiding potential shocks from parameters like temperature and pH.
- **Best management practices:** Implementing appropriate hygiene and management practices at all stages, from reception to harvest and slaughter of the animals.
- **Personal qualification:** Training staff for early identification of signs of diseases and abnormalities in the behaviour of the fish.

In addition to these actions, the main approaches to preventing and controlling diseases in aquaculture involve using vaccines and medications. However, with increasing attention to the quality of aquatic products and environmental pollution, there are questions about using chemicals in water for disease prevention. Sometimes medication access is restricted, or local legislation does not permit its use. Therefore, it is clear here that prevention is better than cure. [Table 1](#) addresses the primary infectious diseases caused by viruses, bacteria and parasites that affect grass carp.

Table 1. Primary infectious diseases affecting grass carp.

Disease	Agent	Clinical signs	Prevention and control
Haemorrhagic disease of grass carp (GCRV)	Aquareovirus	Red muscle, mouth, fins and operculum caused by haemorrhage, exophthalmia, body darkening, haemorrhagic or pale gills. Internal haemorrhage may occur throughout the musculature, liver, spleen, kidney and intestines; Fry and one-year fingerlings are most susceptible, resulting in mortality of 30–50% of infected animals	Vaccination through injection; disinfecting ponds, reducing density, disinfecting fish seed and culture environment with chlorine compounds, quicklime and potassium permanganate; Chinese rhubarb (<i>Rheum officinale</i>); sweet gum leaves (<i>Liquidambar taiwaniana</i>); cork tree bark (<i>Phellodendron</i> sp) and skullcap root (<i>Scutellaria baicalensis</i>)
Spring viremia of carp (SVCV)	RNA Reovirus Mononegavirales, Rhabdoviridae, Vesiculovirus	Exophthalmia; abdominal distension; petechial haemorrhage or darkening of the skin, gills, eyes and internal organs; degeneration of the gill lamellae (discolouration); abdominal swelling (abdominal dropsy); hepatic necrosis; raised scales, slow and difficult balance and swimming. Typically, infected fish are close to an oxygenation zone in the pond (at the water entrance)	There is no treatment. Maintaining adequate diets and quality of water helps prevent the disease. Some disinfectants can be efficient in inactivating the virus on utensils and in the environment without the presence of fish, such as 3% formalin for 5 min, 2% sodium hydroxide for 10 min, 540 mg of chlorine in a litre of water for 20 minutes
Septicemia	<i>Aeromonas sobria</i> ; <i>Aeromonas hydrophila</i> ; <i>Yersinia ruckeri</i> ; Vibrio sp.	Hyperaemia, congestion and ulceration at different positions of the body such as jaws, mouth cavity, operculum, fin-base and musculature; exophthalmia; swollen anus; expanded belly; erected scales; gill rotten; reduced feeding; disordered swimming, high mortality of fish	Disinfect the fish and culture environment with quicklime and potassium permanganate; 5 mg/kg enrofloxacin orally for three consecutive days
Enteritis	<i>Aeromonas intestinalis</i> <i>punctata</i> f.	Expanded and red spot on the abdomen; enteritis; red and swollen anus with yellow mucus when compressed; losing appetite; slow swimming; congested and decayed fins	Prevention is achieved by disinfecting the culture environment using bleaching powder and quicklime. Treatment with sulphamethoxazole blended with food into floatable medicated pellet: 1g/10 kg of fish on the first day and 1g/20 kg for the next 5 days; or garlic (1–2 kg garlic/100 kg fish daily for 6 consecutive days), alternatively use Chinese dry or fresh herbs: <i>Euphorbia</i>

Disease	Agent	Clinical signs	Prevention and control
			<i>humifusa, Aclypha australis, Polygonum hydropiper</i> for 3 straight days.
Gill-rot disease	<i>Myxococcus piscicola</i>	The body of diseased fish is characterised by darkening, head in particular. It is characterised by necrotic gill tissue, usually with mud attached, pale coloration of the gill filaments, then sloughing and accumulation of excess slime. The gill covers of seriously infected fish are inflamed and are eroded by the bacteria forming small transparent patches. Signs of congestion and inflammation of the inner membrane of the operculum	For prevention purposes, fish can be vaccinated. Bleaching powder baskets should be hung around the feeding platforms, or bleaching powder should be spread into the pond water to a concentration of 1 ppm. Fish can be bathed in 2–2.5% saline water. For treatment, disinfect the pond water and the pond sides weekly with dissolved bleaching powder at a rate of 0.25 kg/mu or quicklime once (20 ppm) or spray the pond surface with erythromycin (0.3 ppm) and mix in fish feed for 6 days. (4 g/ 100 kg of fish on the 1st day; 2 g/ 100 kg for the next 5 days). Alternatively pulverised Chinese herbs can be used (<i>Galla chinensis, Sapium sebiferum</i> and <i>Rheum officinale</i>)
Erythroderma (red-skin disease)	(red- <i>Pseudomonas fluorescens</i>	External haemorrhage and inflammation; skin bleeding; losing scales, on the sides of the abdomen; congested fins and rotten fin rays; necrosis of the terminal of fins; red blotches around the upper and lower jaws	Prevention through careful handling during transportation and stocking; disinfection of pond with bleaching powder; bathing fingerlings in a 5–10 ppm bleaching powder solution for 30 min. The fingerlings may be vaccinated. Treatment with sulphathiazole mixed in feed (5g/100 kg fish) for 5 consecutive days; Chinese gall (<i>Galla chinensis</i>) applied to pond surface (2–4 ppm)
Bothriocephalosis	Tapeworm <i>Bothriocephalus</i> sp.	Worms accumulate in the anterior intestine, which may become obstructed or perforated; weakness; reduced feeding; opening mouth; eggs or body parts of the tapeworms in faeces or the gut of the fish; very high mortalities	Disinfection of pond with quicklime; drying the ponds annually or treating drained wet ponds with calcium chloride (about 70 kg/ha) or calcium hydroxide (about 2 t/acre) to kill the copepod intermediate host. According to the manufacturer's instructions, fish can be treated with anthelmintics such as praziquantel

Disease	Agent	Clinical signs	Prevention and control
Dactylogyriasis	Helminth <i>Dactylogyrus</i> sp.	Weak physically; dark body colour; pale gill; open operculum; slow swimming; anaemia; reduced feeding and difficulty breathing	Prevention bath before stocking fingerlings in potassium permanganate (20 ppm) for 15–30 min. Control with a single dose of formalin (250 ppm for 35–40 min) or two repeated applications of potassium permanganate (5 ppm) in static water with a 2-day interval
Ichthyophthiriasis	Protozoan <i>Ichthyophthirius multifiliis</i>	Many white pustules attached to skin and gill filaments; white membrane covering skin in severe infection; excess of mucus, necrosis, congestion of gills; rotting and splitting of fins; slow swimming near to surface; itch; cornea inflammation and blindness; loss of appetite; dyspnoeal; high mortality	Prevention involves pond disinfection using quicklime and quarantining fingerlings before stocking. For control, a formalin bath (25 ppm) may be administered twice daily, but this should not be applied to grow-out fish. Alternatively, infected fish can be treated with sea water (salinity exceeding 1%)
Lernaesis	Anchor worm <i>Lernaea ctenopharyngodontis</i>	The parasite can be seen with the naked eye adhered to the skin. In these areas, the tissue is swollen, difficulty in swimming and moving slowly, reduced appetite, and emaciation	Bath of the fish attacked by worms with a 10–20 potassium permanganate solution for 1.5–2 hr before stocking, according to water temperature. (low concentrations being better at higher temperatures)
Sinergasiliasis	Copepod <i>Sinergasilus major</i>	Difficulty in breathing; inflammation, deformation and necrotic gill filament; focal anaemia of gills; Swimming madly in circles in the water surface, often jumping; death of exhaustion	Due to the strict selectivity of the pathogen, rotary farming prevents infection. Before stocking, bathe the fingerlings with a solution of copper sulphate and ferrous sulphate (7 ppm) in a 5:2 proportion for 30 min. Spray the pond with a solution of copper and ferrous sulphate (0.7 ppm) in a proportion of 5:2

Figure 20 illustrates various changes in the bodies of diseased grass carp.

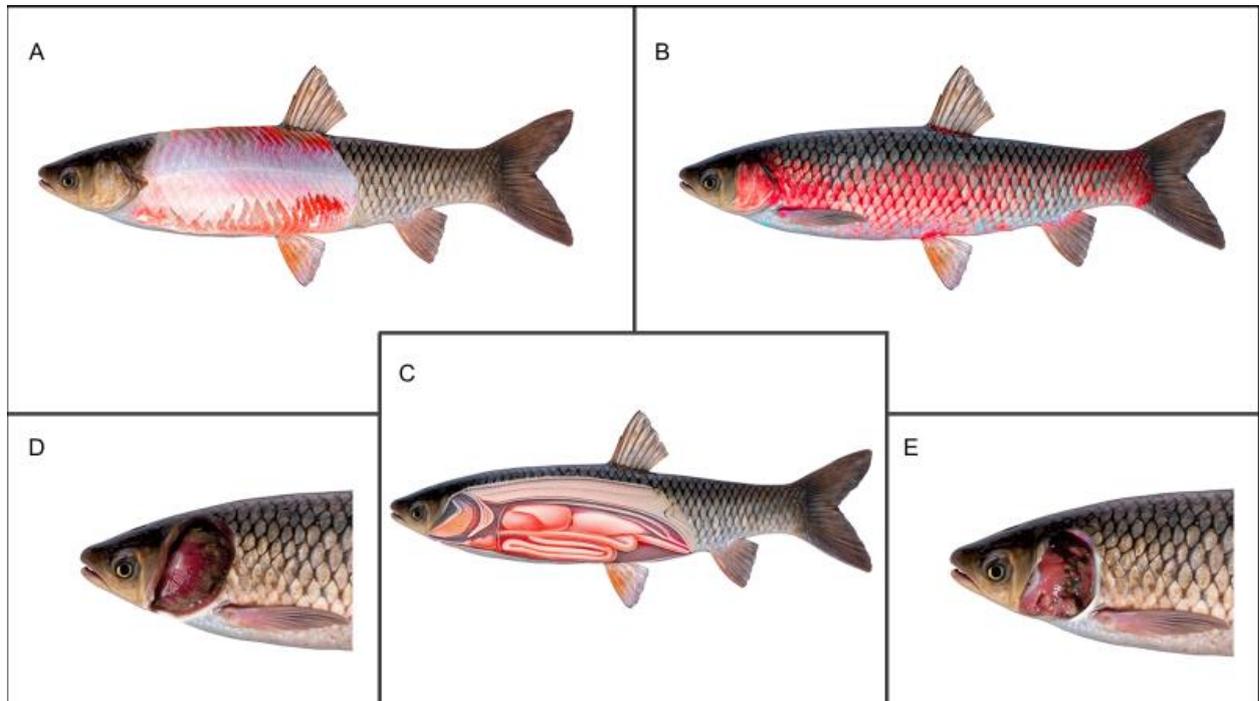


Figure 20. Changes in diseased grass carp bodies. Source: Adapted from FAO, 2023.

In Figure 20 we can observe: (A) Signs of hyperaemia and congestion in the musculature and fins; (B) Haemorrhaging on the skin and at the base of the fins; (C) Anaemia of the gills, hyperaemia and haemorrhage of internal organs; (D) Darkening of the gill filaments, covered in mud and mucus; (E) Gill necrosis, putrid and pale filaments.

9 CULTIVATION SYSTEMS AND REGIMENS

- **Cultivation systems:** This term refers to the infrastructure and physical structures used in aquaculture, determining the environment in which aquatic organisms are farmed. Such systems can range from earth-dug ponds, concrete tanks, fibreglass, or various materials of waterproof tarpaulins, to cages in natural water bodies and even advanced recirculation systems in fully controlled settings, known as RAS – Recirculation Aquaculture Systems.
- **Farming regimes:** The farming regime is closely related to the amount of energy that will be applied to the production system. However, this energy must be understood in its various forms, such as financial resources, stocking density, aeration, types and frequency of feeding, water quality management, and the use of medicines and fertilisers, among other factors that directly influence the productivity and health of the farmed organisms. In other words, the so-called ‘intensity’ of cultivation refers to the set of practices, methods and strategies adopted within a specific fish farming system.

9.1 Cultivation systems

The grass carp is an incredibly versatile fish that is adaptable to different cultivation systems, such as earth ponds, enclosures, cages, and even recirculation systems. However, the traditionally lower price of this fish's meat may make the latter unviable.

Each system has its advantages and disadvantages, of course. However, the critical point is that this flexibility makes grass carp a viable option for various scenarios and investment levels.

Choosing the right cultivation system will depend on the objectives of each stakeholder and the resources available. This manual focuses on pond cultivation, the primary system used worldwide. Nevertheless, we also provide a brief introduction to some other systems.

Beyond its tasty and nutritious meat, grass carp are also used for biological control of invasive aquatic plants, as they primarily feed on underwater vegetation. Moreover, grass carp are an excellent choice for sport fishing, making them a species of great interest in various sectors.

To cultivate grass carp efficiently and profitably, regardless of the chosen cultivation system, it is essential to consider available space (i.e., proper stocking density); adopt a balanced diet meeting the fish's nutritional needs; ensure suitable environmental conditions; and implement management practices to maintain sanitary conditions. Combining these factors will ensure grass carp's healthy and rapid growth, benefiting fish farmers and consumers.

9.1.1 Ponds

The fish cultivation system where animals are kept in an enclosed space (usually enclosed by earth but also by plastic materials or cement) and – mandatorily – with a natural bottom is known as ‘earth pond’ or simply ‘pond’.

Ponds vary significantly in size and depth and can be constructed with different materials.

The primary difference between a pond and a tank is that the former has a natural bottom, allowing a close relationship between the chemical, physical and biological processes co-occurring in the soil and water. In tanks, however, the bottom is constructed from artificial impermeable material, preventing soil-water interaction.

The natural bottom allows microorganisms to thrive, which can contribute to – or even harm – the overall health of the fish. Therefore, careful management is required to prevent the accumulation of debris and food residues, which could cause water quality to deteriorate.

These systems with a natural substrate also allow for more significant interaction of the fish with the environment, which can be beneficial as a form of environmental enrichment, contributing to their welfare. However, this characteristic also makes good management essential to avoid problems such as diseases and parasites.

The image depicted (Figure 21) exemplifies the importance of fish cultivation in ponds for China, as the country is the world's largest aquaculture producer.



Figure 21. The satellite image of Hubei Province in China shows thousands of ponds for fish cultivation. Source: Google Earth.

In many places, fish farming is fully integrated into the urban landscapes of Chinese cities (Figure 22).



Figure 22. Fish farming is integrated into the urban landscapes of many Chinese cities.

Therefore, one should not think that a pond is about digging a hole, filling it with water, and adding fish. Ponds are living systems where the water and the organisms it contains, be they phytoplankton, viruses, bacteria, protozoa, and, of course, the fish themselves, interact with the soil and the organisms and microorganisms living in it. This means that it is essential to understand various parameters and conscious management practices to ensure the cultivated fish's productivity and welfare.

9.1.1.1 Characteristics

- **Design:** Generally rectangular, with a slight slope for drainage. However, the ponds can have other regular or irregular shapes that better use the available land (Figure 23).
- **Excavation:** Preferably done with earth-moving equipment.
- **Bottom material:** Natural soil, sometimes sealed with clay.
- **Water supply and drainage system:** Channels and gates direct water into and out of the ponds.
- **Aeration system:** Aerators are often needed to maintain water quality control, especially the dissolved oxygen concentrations for the pond organisms.
- **Microorganisms:** Bacteria and other microorganisms help decompose organic matter and create a unique ecosystem.
- **Feeding:** Fish are fed with pellets but often use natural food in the ponds.
- **Diseases:** Frequent risk of exposure to pathogens, necessitating the adoption of preventive practices.
- **Water quality:** Constant monitoring of water quality indicators, such as pH, dissolved oxygen, and ammonia, among other parameters.
- **Nutrients:** Nitrogen, phosphorus and other nutrients can affect water quality and the balance between the organisms and microorganisms inhabiting a fish farming pond.
- **Decomposition:** A series of chemical processes constantly occur in the ponds, associated with the decomposition of organic matter, feeding, excretion, and respiration of the fish.



Figure 23. Ponds of different sizes and shapes are used for grass carp cultivation.

9.1.1.2 Pond advantages and disadvantages

Table 2. Advantages and disadvantages of pond fish farming.

Pond factors	Advantages	Disadvantages
Land Investment	Many suitable areas are available for setting up	Need to invest in land acquisition for pond construction
Installation	Permanent and durable structures	Initial installation is time-consuming and expensive
Production Cost	Generally lower than most other available systems. Operational costs are usually predictable	Construction and maintenance costs can be high
Construction	Long lifespan if well constructed	Higher complexity and construction cost. Initial investment can be increased, especially with earth-moving and operational support structures
Yield	Stable yield with proper management	Lower yield per area compared to cages
Zootechnical Aspects	Ease of monitoring and controlling water parameters. Ease of adaptation and rapid growth of grass carp	Requires knowledge of management and monitoring efforts
Environmental Impacts	Well-controlled and minimised risks if well managed	Possible eutrophication and fish escape into the environment if poorly managed
Management	Simple and effective techniques. Sanitary and feeding management facilitated	Requires skilled labour for optimised results
Legislation	Norms generally well established	Need for various licences and environmental compliance

9.1.2 Cages

9.1.2.1 Characteristics

- Cages can be purchased from companies or constructed by fish farmers themselves.
- One of the significant advantages of grass carp cultivation in cages is the immense range of possibilities for setting up cultivation structures, involving everything from simple designs to models involving more appropriate and advanced technologies. What they have in common is that all of them are positioned in water bodies without earth-moving, as with pond cultivation (Figure 24).
- Cages can have various sizes and mesh openings according to the size of the fish.
- For intensive grass carp cultivation in cages, this species is commonly the main one in systems using cages of about 60 m², depths ranging from 2 to 2.5 m, and mesh openings of 25 mm.
- For growing grass carp breeders using floating cages, it is ideal to use cages of 12 or 24 m² and 30 or 60 m³ volumes. These cages should have nets with 20 or 30 mm mesh sizes.
- Cages can be supported by floaters made of foam blocks or plastic drums and are anchored to a dock or fixed to the bottom by a cable.
- The installation site for the cages should offer good water circulation, with a minimum depth of 3 m between the bottom of the cages and the bottom of the water body. The current speed at this location should be about 0.2 m/s. As hydrological conditions change, periodic reorganisation of the cages is advisable to optimise the cultivation environment.
- The density to be used in the cages should be defined based on the size of the fingerlings/juveniles at the time of stocking, the environmental conditions, and the resources available for the management practices to be adopted.
- For example, young fish (fingerlings from about 10–20 g) can be stocked at densities between 50–80 fish m³ in waters preferably above 20°C. If larger fish (80–125 g) are stored, a lower initial density (30–50 fish/m³) can be used. In this case, later redistribution of fish to other cages may be recommended to adjust the stocking density and prevent reduced growth rates and welfare due to high densities. Stocking can also be done with juveniles weighing 250–500 g. In this case, 10–20 fish/m³ density can be used.



Figure 24. Different structures are used for grass carp cultivation in cages in rivers, lakes and reservoirs.

- **Feed efficiency:** In cage cultivation, feed efficiency may not always be as high as in ponds, where fish often have access to more natural food. However, the provision of grass and the removal of waste are facilitated in cages. The required labour is also less than in pond cultivation.
- **Feeding options:** Fish can be fed with pellets or a combination of aquatic vegetation, terrestrial grasses and commercial feeds.
- **Feeding monitoring:** Feeding conditions are closely monitored to ensure the fish can consume the feed within 30 minutes. Subsequent adjustments are based on leftover feed. In practice, adjustments can be made weekly.
- **Water movement:** Water movement through the cages may be restricted by the accumulation of algae and other organisms. Periodic washing of the cages with high-pressure water jets, although laborious and time-consuming, is recommended to reduce the problem of biofouling. Special anti-fouling agents are also often applied to the surfaces of the cages before submersion in water.

- **Cage rotation:** Rotating the cages every two weeks and sun-drying the dirty cages are other practical measures.
- **Use of scraper fish:** Fish with scraping habits can also be used to consume the biofilm formed on the nets or meshes of the cages.
- **Polyculture:** Grass carp are often produced with other species, as with the Wuchang (bluntnose black bream, *Megalobrama amblycephala*) (Figure 25). Silver carp and bighead carp can also be used (at a ratio of 1% of the total fish in the cage) to act as 'cage cleaners'. In this case, the proportion would be 60% grass carp, 39% Wuchang and 1% silver and bighead carp.



Figure 25. The Wuchang (*Megalobrama amblycephala*) is commonly cultivated in cages with grass carp in China.

- **Vietnam:** Other species are used in polyculture in cages along with grass carp, such as mrigal (*Cirrhinus cirrhosus*) and rohu (*Labeo rohita*) (Figure 26).

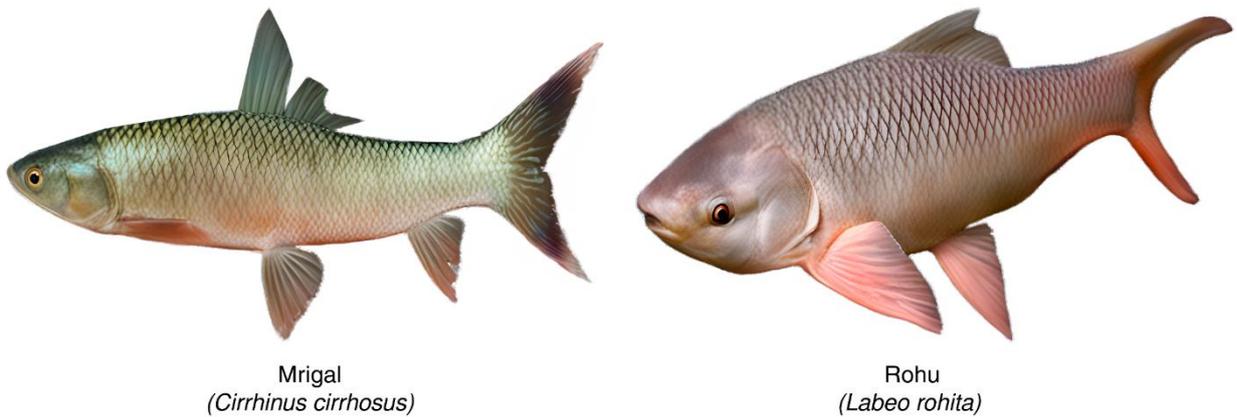


Figure 26. Indian carp species cultivated in cages in polyculture with grass carp in Vietnam.

- **Growth in cages:** In cages, grass carp can reach 1–1.5 kg in one year and 4.0–5.5 kg after two years of cultivation.
- **Harvesting:** Harvest occurs when the fish reach commercial size, which varies from country to country but typically happens when the fish weigh about 2–5 kg. The cages are then brought to the river or lake shore, where they are partially removed from the water. A scoop net is used to capture the fish. The harvest can be partial or complete.

9.1.2.2 Advantages and disadvantages

Table 3. Advantages and disadvantages of cultivating grass carp in cages.

Cage factors	Advantages	Disadvantages
Installation Areas	There is no need for land acquisition investments, as they are installed in natural areas and public spaces. Enables efficient use of various environments and aquatic spaces	Requires public access to reservoirs or lakes where they are to be installed, which is not always available. Limited to areas protected from winds and tides
Installation	A system that can be quickly and efficiently installed, less laboriously than other systems	Higher initial costs (for the acquisition of cages, nets and support structures)
Production Cost	Generally higher than other systems	This can be inflated by the greater possibility of thefts and losses due to disease
Yield	High production rates per area and volume of water	Greater dependence on inputs such as feed and variations in environmental conditions
Construction	Simple infrastructure and use of low-cost materials	Material susceptible to wear and damage
Zootechnical Aspects	Facilitates controlled feeding and monitoring	No control over water quality. Higher risk of diseases due to high density
Environmental Impacts	-	There is a significant risk of fish escaping into the environment. Possible environmental impact due to the accumulation of waste on the bottom. Fish are exposed to environmental effects in the water body itself and surrounding areas
Management	Ease of monitoring and management. Greater control over feeding. Easier harvesting	Higher risk of diseases due to high density
Legislation	Many countries already have defined regulations	Regulations and environmental and water use licensing tend to be quite stringent

9.1.3 Net pens

Fish cultivation in net pens involves the management of fish stocked in a part of a floodplain enclosed on one or more sides with a bamboo structure, other fences, or nets. A distinctive feature of this cultivation type is that the base of the net pen is fixed in the mud at the bottom of the water body, and the water inside the net pen is well connected with the water outside, allowing flow between the net pen and the floodplain (Figure 27 and Figure 28).

The practice of fish cultivation in net pens is fairly recent. Its origin dates to the 1920s in Japan and it was later adopted in China and other Asian countries. Recently, countries like the Philippines, Indonesia, Thailand and Malaysia have used this technology for commercial fish production.

Commercial fish cultivation in net pens also has significant potential in countries like Bangladesh. For instance, in the Philippines, production was found to be four times higher in net pens than in ponds. However, this cultivation system has not gained much popularity and has been falling into disuse over time, because installation site requirements are so specific.

While cages can be installed in various types of aquatic environments, provided they are protected from adverse weather conditions like strong winds and water levels of rivers or lakes, net pens have the same protection requirements but also need a location shallow enough for the screens to be installed.



Figure 27. Net pens for grass carp cultivation are set up in a lake.

9.1.3.1 Characteristics

- **Design:** The design and construction of net pens are based on a basic concept: a fixed structure formed by nets supported by a rigid frame. The nets are anchored to the bottom through wooden stakes (usually bamboo), so that the lake's bottom is also the net pen's bottom (Figure 28).
- **Size:** Theoretically, net pens have no size or area limit, given the ease of construction and low cost.
- **Shape:** Most are rectangular or square, but some can be circular or cylindrical.
- **Location:** To decide on the cultivation site, several factors need to be considered, such as predominant wind direction, flood risk, typhoons, stratification, soil type, siltation, depth variation, occurrences of toxic algal blooms, presence of predators, pests and competitors, agricultural or industrial pollutants, navigation routes, and property rights, to name the most important. The difficulty of balancing all these factors explains why net pens have lost importance among the main cultivation systems employed in grass carp production.
- **Density:** The density of fingerlings/juveniles in the net pens usually ranges from 20,000–50,000 per ha, and the animals are cultivated to commercial size.
- **Management:** Environmental management in net pens is limited by the intrinsic characteristics of the system itself. For example, applying fertilisers, doing liming or using pesticides is impossible, as it is an open system, with water being continuously exchanged between the internal compartment and the external environment.
- **Feeding:** Regular provision of grass and feed is necessary for feeding the fish.

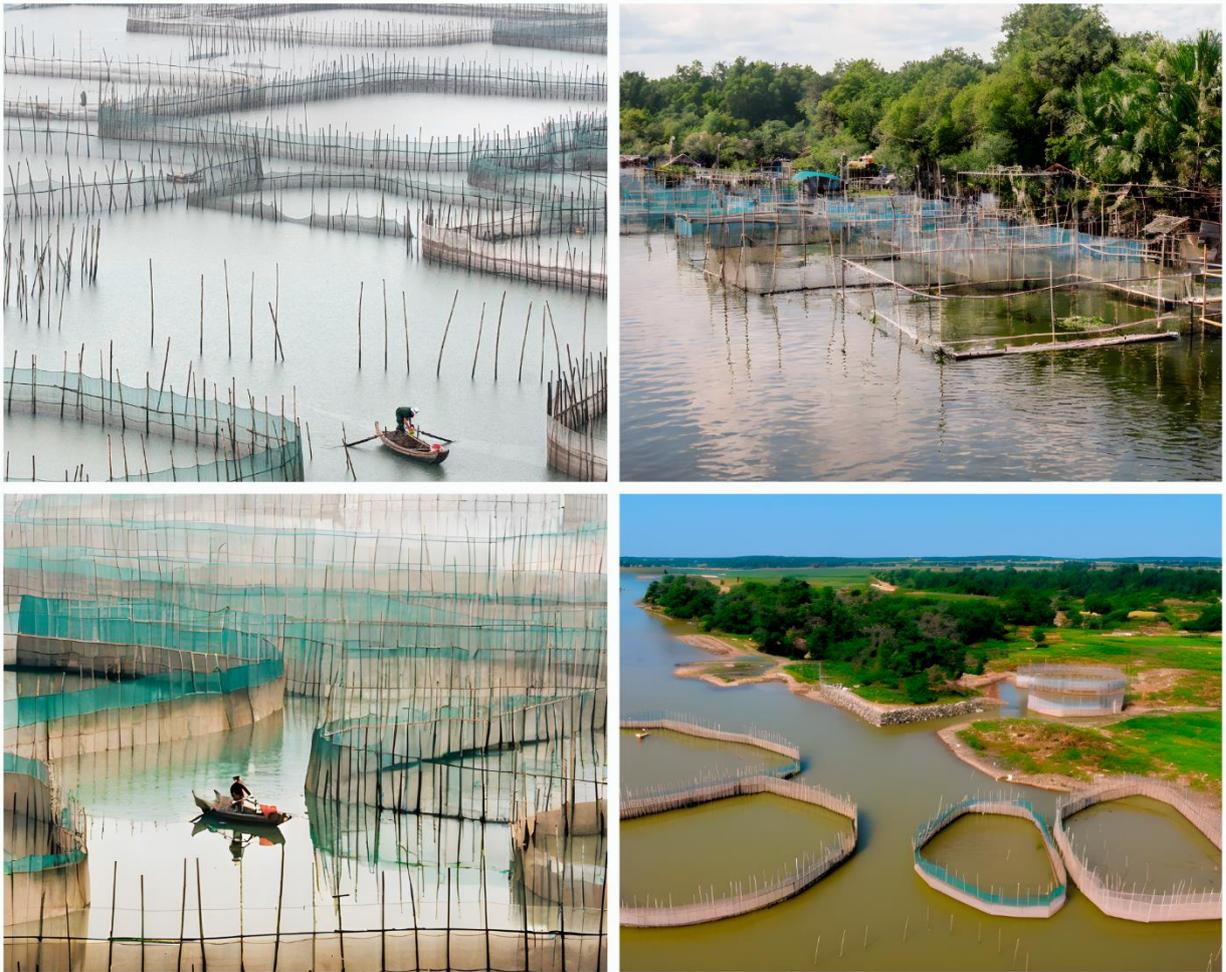


Figure 28. Net pens used in fish farming.

9.1.3.2 Advantages and disadvantages

Table 4. Advantages and disadvantages of cultivating grass carp in net pens.

Net pens factors	Advantages	Disadvantages
Installation Areas	There is no need for land acquisition investments, as they are installed in natural areas and public spaces.	The number of areas is always limited by the need for shallow and protected zones, so there is low versatility of application.
Production Cost	Generally, very low, accessible to low-income rural communities.	Productivity is also usually very low.
Yield	As production costs are low, profits are proportionally high.	Yield greatly depends on natural productivity and water body conditions.
Construction	Simple infrastructure and use of low-cost materials.	Offers little protection to fish and management conditions.
Zootechnical Aspects	Easy adaptation and rapid growth of grass carp. Possibility of polyculture with other species.	Offers few options for controlling variables of zootechnical interest.
Environmental Impacts	Generally low environmental impact.	Significant risk of fish escaping into the environment.
Management	Simple and effective techniques.	Offers few options for stock management.
Legislation	In most places where it is practised, it is adopted by traditional communities, who face few legal restrictions.	Net pen cultivation has been banned in some countries and some regions of China.

9.2 Fish farming regimes

The definition of the cultivation regime and the classification of one's enterprise usually make no difference to fish farmers. However, applying the concept of production regime is essential for management and to maximise the results achieved in aquaculture ventures.

Table 5 highlights the variation between the application of natural foods and balanced feeds across different fish farming regimes. The extensive regime has a total dependence (100%) on raw foods, with no obligation to use supplementary or balanced foods. This reliance on natural foods decreases as we move towards more controlled and intensive regimes. In the semi-intensive regime, for example, there is a balance between the use of natural and supplementary foods, representing approximately 50% of the diet. (It is essential to understand that these percentages are merely illustrative and not fixed values that define one regime over another.) In contrast in the intensive regime, fish are entirely dependent (100%) on supplementary or balanced foods, with little or no contribution from natural foods.

Table 5. A comparative scenario of feed application under four types of culture regimes. The percentages are illustrative only.

Fish culture regime system	Natural food (%)	Supplementary/ balanced food (%)
Extensive system	100	0
Improved extensive system	70	30
Semi-intensive system	50	50
Intensive system	0	100

Table 6 shows the implications of intensifying management practices as one progresses from extensive to more intensive regimes.

In the extensive regime, interventions are minimal, with natural pond maintenance, little to no strict control of stocking densities, no fertilisers or external feeding, and irregular harvesting.

In the improved extensive regime there are some interventions, such as removing unwanted fish and aquatic plants and sporadic application of fertilisers and feed, but no oxygen is supplied.

The semi-intensive regime increases control with regular feeding and fertiliser application and more structured management of harvest and restocking.

In the intensive regime there is a higher degree of intervention, with total control of stocking density, balanced feeding, rigorous maintenance of minimum concentrations of dissolved oxygen in the water, and execution of planned and controlled harvesting.

Table 6. Other differences between the practices adopted in different cultivation regimes.

Culture regime	Pond preparation	Stocking density	Fertiliser application	Feed application	Water/oxygen supply	Fish Harvesting
Extensive	Weed fish and aquatic weeds are not removed	Uncontrolled	No fertiliser is applied	No fish feed from external resources is used	No oxygen is supplied	Irregular harvest
Improved extensive	Weed fish and aquatic weeds are removed	Semi controlled	Fertilisers are applied irregularly	Fish feed is used irregularly	No oxygen is supplied	Fish harvested several times a year
Semi-intensive	Weed fish and aquatic weeds are removed	Controlled	Fertilisers are applied regularly	Fish feed is used regularly	Oxygen is supplied if necessary	Fish are partially harvested with irregular restocking
Intensive	Complete removal of weed fish and aquatic weeds	Controlled with high stocking density	No fertiliser is applied	Balanced diet/feed is the only source of food	Full-time arrangement for oxygen supply	Fish are partially harvested with regular restocking several times a year

The intensification of fish farming involves more than just stocking density and feeding practices, as commonly thought. It encompasses a broader set of integrated practices focused on optimizing production, ensuring fish welfare, and promoting environmental sustainability. This means farmers must also prioritize managing water quality and animal welfare alongside stocking density and feeding. Neglecting these aspects can lead to greater problems, so farmers need to consider all factors to achieve optimized and sustainable production. Simply increasing stocking density and feeding won't suffice. In addition to the factors already mentioned, several additional factors could be cited:

- **Water quality monitoring:** Water quality plays a central role in fish farming. Temperature, pH, alkalinity, ammonia, nitrites, and nitrates must be monitored and maintained within ideal ranges. In more intensive regimes, the need for treatments and adjustments is more frequent.
- **Disease control:** With the intensification of production regimes, the risk of disease outbreaks increases due to the closer proximity and interaction among fish. Therefore, biosecurity practices, vaccination, and judicious use of medications become essential.

- **Aeration and circulation:** In more intensive regimes, adequate water aeration and circulation are fundamental to maintaining optimal oxygen levels and preventing waste accumulation at the pond's bottom
- **Advanced infrastructure:** Intensifying production regimes also involves investing in more technologically advanced production systems, filtration systems, UV, ozonation, and other technological equipment that helps maintain clean water free from pathogens.
- **Waste management:** In more intensive production regimes, effective management of waste, such as fish excreta and leftover food is crucial to prevent eutrophication and deterioration of water quality.
- **Training and skills development:** Qualified labour is essential in more intensive regimes. Personnel need to be regularly trained on best practices and updated technologies.
- **Biological control:** In some systems, auxiliary organisms like shrimps or other fish are introduced to help control pests or clean the bottom of ponds. This strategy is more common in less intensive regimes but can also be practised in more intensive fish farming regimes.
- **Economic aspects:** Financial management and cost-benefit analysis become more complex as the regime intensifies, given the higher input of investments in equipment, inputs and labour.

In this manual, our central focus is on the semi-intensive production regimes of grass carp in ponds, although the principles presented here also apply to other regimes and cultivation systems.

Examples of grass carp farming regimes Grass carp cultivation is a fish farming activity with great economic potential but requires careful technical management for farmers to optimise productivity in an economically profitable way. In aquaculture systems, different production regimes can be adopted, from the simplest, based solely on the natural productivity of the ponds, to the most intensive, which uses a range of techniques to maximise yield.

Each approach has its set of practices, costs and results, with productivity varying from 150 kg/ha/year to an impressive 20,000 kg/ha/year, depending on the level of investment in inputs and technology:

- **Natural productivity:** In grass carp cultivation ponds, the production is based only on available natural foods, which usually results in low production indices ranging from 150 to 300 kg/ha/year, depending on water quality.

- **Liming:** The simple use of liming and mineral fertilisation with 7.5 to 11 kg of phosphorus (P) and 15 kg of nitrogen (N) per hectare, applied six times during the growth season, can increase this productivity to 500-800 kg/ha/year.
- **Liming + fertilisation + mineral + a supplemental diet with cereals:** These practices can raise productivity to about 1,500 kg/ha/year.
- **Managing feed and water quality:** With the use of the previous practices, plus balanced feed for grass carp, supplemental aeration so that there is no lack of oxygen in the pond and periodic water renewal, productivity can exceed 20,000 kg/ha/year.

9.3 Biological control of vegetation

One method – which not precisely a cultivation system and can be classified as an extensive regime, and is quite common – is related to using grass carp to control aquatic plants (native or invasive) in lakes and reservoirs. In this case, therefore, the main objective is not to produce the fish but to promote the removal of vegetation from the environment (as illustrated in Figure 29). It is important to note, however, that grass carp is more suitable for controlling floating aquatic plants than submerged types.

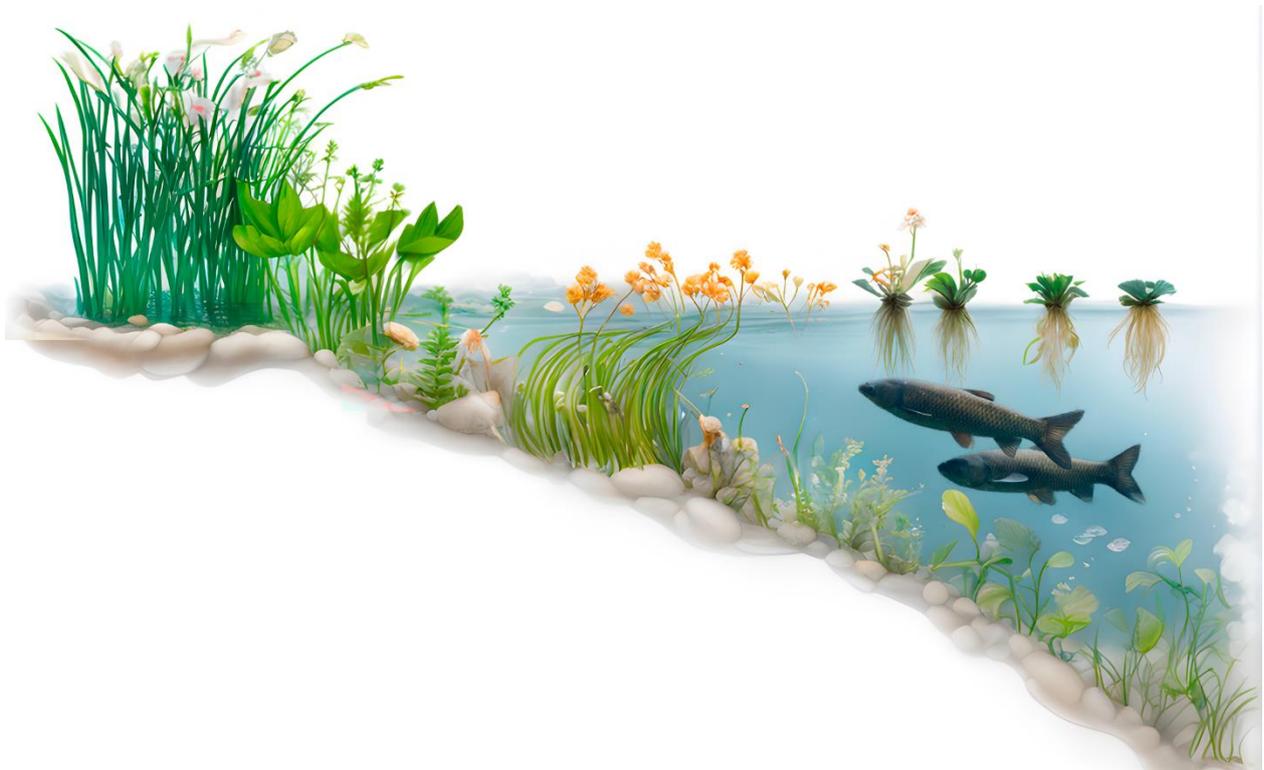


Figure 29. Grass carp are used to control vegetation in aquatic environments.

Juvenile grass carp (20 to 30 cm) are commonly stocked at densities ranging between 2 and 250 fish/ha. The exact numbers vary depending on the species of vegetation present, the density of the plants, and the time available to eliminate them.

Most plants can be controlled with approximately 50 grass carp per hectare. Softer aquatic vegetation is quickly eradicated at about 150 grass carp per hectare densities. In lakes or ponds infested with *Chara* spp., it may be necessary to use 247 grass carps/ha.

Grass carp are ineffective in controlling cattails, lilies and other emergent vegetation. However, as the preferred vegetation is consumed, the diet of the herbivorous carp shifts to less palatable foods, which include coarser woody stem plants like smartweed and very young cattails.

As the degree of infestation by aquatic plants increases, there is a need to populate the pond with more carp to control the vegetation effectively. In these cases, it is advisable to stock juvenile fish in the density ranges indicated in Table 7.

Table 7. Standard stocking rates of 5 to 15 cm grass carp for lake vegetation control.

Weed Infestation Level	Small Carps (5 to 15 cm)
Light	2–20 per hectare
Moderate	30–70 per hectare
Severe	100–250 per hectare

Table 8 provides a comprehensive guide on the effectiveness of grass carp in managing different species of aquatic plants. It segments aquatic plant species based on the efficacy of control through grass carp, and details stocking recommendations to optimise control efficiency.

Table 8. Species of plants controlled by grass carp in lakes and the stocking recommendations. Source: Wright and Reeves (2004).

Grass Carp Control Type	Plant Species	Stocking Recommendation
Easily controlled	Hydrilla, Elodea, Najias, bladderwort, Egeria, Potomogeton	Stock according to standard rates. Control occurs in one season, except in severe cases.
Difficult to control	Watermilfoil (parrot feather, Eurasian, etc.), slender spikerush, duckweed, red ludwigia	Stock at 1 to 2 times higher than the standard rate. Control may take more than one season

Grass Carp Control Type	Plant Species	Stocking Recommendation
Controlled using extreme densities	Filamentous algae (Lyngbya, Pitophora, etc.)	Stock from 60 to 250 animals per hectare
Generally not controlled	Cattails, alligator weed, watermeal, water shield, American lotus, water lilies, willow, water primrose, water lettuce, water hyacinth	Use other control measures and stock grass carp at standard rates. Grass carp will help prevent other weeds from replacing them and sometimes suppress the growth of these resistant forms by eating the young shoots

In the case of controlling hydrilla, for instance, to ensure that consumption by fish is greater than the plant's growth rate in severe infestation levels, it is necessary to use about 20 carp per hectare of lake area. This density allows the preservation of some less preferred plant species by carp, as shown in Figure 30. However, it is important to note that stocking densities higher than 25–30 grass carp per hectare of vegetation generally have the effect of completely removing all aquatic vegetation, which may not be ideal.

Although grass carp can live for many years, their effectiveness in vegetation control decreases significantly after 5 to 7 years. Lakes must usually be restocked with grass carp every 5 to 7 years. In new lakes, 3–5 grass carp per hectare can be stocked to prevent submerged aquatic vegetation from establishing, thereby controlling aquatic weeds for 5 to 7 years.

Once the objectives with grass carp are achieved, it is necessary to reduce the number of fish in the lake so that the animals do not suffer from lack of food or cause environmental impacts through indiscriminate consumption of plant species present in the environment.



Figure 30. Grass carp efficiently control aquatic macrophytes in lakes and reservoirs at appropriate densities.

The use of grass carp as a biological control in lakes, however, can bring a significant side effect: the increase in the amount of nutrients (a phenomenon known as eutrophication) in the lake water, associated with increased water transparency – a consequence of the removal of macrophytes – can cause a cascade effect on the local environment. Under these conditions, there can be growth in phytoplankton populations, leading to a further reduction in water transparency and changes in the dissolved oxygen cycle, affecting the lake's biodiversity and the fish's welfare (Table 9). Often, it becomes necessary to use aerators in these environments to avoid compromising water quality.

National and subnational legislation tends to allow only the use of sterile fish when biological control is carried out in large lakes to prevent the fish from reproducing and spreading beyond the intended limits. If non-sterile animals escape into natural environments, they can cause large-scale alterations in local ecosystems and risk spreading diseases that can affect the native fish fauna.

Grass carp become sterile when the eggs are subjected to stress. This process makes the genes triploid instead of diploid. Triploid means the eggs contain three sets of chromosomes instead of the usual two. Triploid fish cannot produce viable offspring, so their use is environmentally safe.

Table 9. Positive and negative environmental impacts associated with using grass carp for biological control of aquatic vegetation.

Type	Positive Impact	Negative Impact
Ecological Interactions		<ul style="list-style-type: none"> • Competition for food and habitat between herbivorous carps and other fishes • Massive ingestion of some species and possible eutrophication
Population Explosion	Increase in the population of natural predators of grass carp	<ul style="list-style-type: none"> • Deterioration of water quality • Reduction in populations of other species
Water Quality		<ul style="list-style-type: none"> • Increase in water turbidity, nitrogen and phosphorus levels • Increase in phytoplankton biomass
Ecological Interactions		<ul style="list-style-type: none"> • Competition for food and habitat between herbivorous carps and other fishes • Massive ingestion of some species and possible eutrophication

10 MONOCULTURE VS. POLY CULTURE

In aquaculture, fish can be produced in either monoculture or polyculture systems. In monoculture ponds, only one species of fish is grown. In polyculture, two or more species are stocked together in the same pond.

Generally, monoculture is a simpler cultivation model with fewer variables involved, and polyculture is a more complex model, which tends to value natural feeding and the biological cycles that occur in cultivation ponds.

Another possibility to consider is monoculture or polyculture of mixed ages. In this method, different fish species and groups of fish of different ages within a given species can be cultivated together. The purpose here is to optimise pond resource use and enable continuous fish production for consumption throughout the season. However, a disadvantage is having to separate fish of various sizes and species in the same pond simultaneously, which can become a complex and laborious process. In addition, smaller fish may have difficulties at feeding time and suffer from cannibalism.

10.1 Monoculture VS Polyculture in grass carp

Grass carp have historically been cultivated in polyculture systems in different regions of the world. In these polyculture systems the cultivation cycles lasted up to three years. However, it is also possible to produce this fish in monoculture systems. The choice of model will depend on a series of factors, but mainly on the goals of the producer/entrepreneur and the available resources.

More recent innovations propose cultivation methods that mix grass carp with other species in models that employ higher densities and more significant resource inputs (such as feed, aeration, and water renewal) to significantly increase production and even the quality of grass carp meat. However, more studies are needed to understand the real impact of this type of system on fish welfare.

10.1.1 Criteria and factors to be considered

- **Producer/entrepreneur objectives:**
Clear objectives must be predefined regarding species to be produced, productivity to be achieved, product quality, and financial profitability, among other factors.
- **Available resources:**
Polyculture requires more resources, such as space, water, and labour.
- **Management of biological resources:**
Polyculture demands more knowledge about the integrated processes related to the climatic and environmental conditions of the region and the cultivation ponds.
- **Understanding the consumer:**
The consumer is the one who will pay for all the work and investments made by the fish farmer. Therefore, they need to be seen as protagonists of the business. The producer must know which species are most appreciated, how they prefer the fish (fresh, frozen, in cuts, etc.), and the price range they are willing to pay.
- **Suppliers:**
The fish farmer will need various inputs for their venture, such as feed, fry and equipment. Suppliers must be reliable and offer quality products. Additionally, suppliers close to the property can facilitate logistics.
- **Competitors:**
The price achieved by the product will be defined by the market. Therefore, it is relevant that the producer knows their competitors and assesses how they can differentiate themselves in this market. In some situations, forming partnerships with other producers for joint purchasing of inputs or joint sales can be beneficial.
- **Species in demand:**
Ultimately, the consumer defines the species to be cultivated. Each country/region has its preferences, and the fish farmers must know their customers' preferences.
- **Sale weight and size:**
The fish can be sold in sizes ranging from 1 to 3 kg, depending on the species and market. Knowing the preferences of local and regional markets is, once again, crucial.

10.1.2 Advantages and disadvantages

10.1.2.1 Monoculture

- **Advantage – Simplicity of management**
Managing a single fish species is less complex than managing several species simultaneously.
- **Advantage – Specialisation**
Producers can specialise in cultivating a single species, leading to more outstanding technical and economic efficiency. They can also focus on the most significant commercial interest species or economic viability to the detriment of lesser interest/value species.
- **Advantage – Control**
Monoculture allows more specific control of pond conditions, feeding and sanitary management, as all interventions are geared towards a single species.
- **Advantage – Simplicity**
With only one species in the pond, it becomes simpler to monitor the growth, health and behaviour of the fish, ensuring better welfare conditions.
- **Disadvantage – Increased risks**
Monoculture is more susceptible to losses due to diseases or pests.
- **Disadvantage – Inefficient use of resources**
Grass carp are omnivorous, meaning they feed on various foods. Monoculture can lead to food inefficiency, as there are losses and leftovers of nutrients (such as zooplankton or benthic organisms, for example) that could be used to produce other species simultaneously.

10.1.2.2 Polyculture

- **Advantage – Diversification**
Polyculture diversifies production, which can reduce the economic risks of the venture.
- **Advantage – Efficient use of resources**
The different species of fish can feed on other foods, leading to greater efficiency in using available food resources in the pond.
- **Advantage – Risk reduction**
The diversity of species can reduce susceptibility to specific diseases and help stabilise the pond's ecosystem.

- **Advantage – Improvement of water quality**
Polyculture helps improve water quality, as the different fish species can perform various functions in the pond's ecosystem.
- **Advantage – Diversification of production**
Polyculture allows the simultaneous production of different species, which can diversify the producer's income source and cater to other markets and demands.
- **Disadvantage – Complex management**
Polyculture requires in-depth knowledge of the needs and characteristics of each cultivated species, which can make management more complex.
- **Disadvantage – Reduced productivity**
Polyculture can reduce the total productivity of the system, as the different species of fish end up competing for space and food. If not properly managed, this can result in reduced growth rates, decreased immunity, and increased susceptibility to diseases and predation for some species.

10.1.3 Monoculture

Among the different species of carp, the common carp is the one that best adapts to intensive monoculture cultivation, while the Chinese and Indian carp are usually cultivated in a polyculture model. Monocultures of grass carp on a commercial scale are rare, and little information is available.

This situation does not mean that technically this fish cannot be produced in monoculture, being fed totally or partially with protein-rich pelleted feeds, mechanical aeration, and frequent water renewal in the same way as other species cultivated individually in a particular system.

As grass carp tend to be a cheap fish, it is not always worth investing more resources in a monoculture, which explains the preference for using the species in polyculture, where production costs are diluted.

Therefore, before opting for a monoculture system, it is highly recommended that the fish farmer carry out a technical-financial viability analysis of the venture, considering the production costs and the price achieved by the final product in their region.

If opting to produce grass carp in a monoculture model, the fish farmer needs to be aware that the zootechnical indices to be achieved will fundamentally depend on the system and production regime employed. In other words, the energy and resources inputted into the system are inputs and care in management.

Monoculture ponds have no standard stocking rate, and they vary from 1,600 to 20,000 grass carps/ha (Table 10). Densities even higher than these can be used, provided there is strict control of feeding and water quality. Generally, the most profitable stocking densities for monoculture tend to be around 15,000 fish/ha, with the stocking of juveniles between 100 and 600 g.

Table 10. Technical specifications relating to the monoculture of grass carp.

Particulars	Nursery pond	Rearing pond	Stocking pond
Area (ha)	0.02 to 0.05	0.04 to 0.10	0.50 to 2.00
Depth (m)	1.0	1.0 to 1.5	1.2 to 2.0
Stocking stage of fish	Post-larva (6 mm)	Fry (20–25 mm)	Fingerling (80–300 mm)
Stocking density per sector	5 to 10 million post-larvae	200,000 to 300,000 fry	1,600 to 20,000 fingerling
Stocking period	Up to 1 month	3 to 4 months	24 to 42 months
Production	1.0 to 1.5 million fry	120,000 to 180,000 fingerlings	3,000 to 30,000 kg fish per hectare

10.1.4 Polyculture

10.1.4.1 A complex relationship

A fish farming pond is a highly complex ecosystem featuring an intricate web of biological, physical and chemical relationships among its organisms and a series of naturally occurring events. This cycle encompasses the production, consumption and decomposition of organic and inorganic materials (Figure 31).

Primary production occurs when photosynthetic organisms, such as bacteria, plants and microalgae, convert inorganic materials (dissolved nutrients) into organic materials (their tissues).

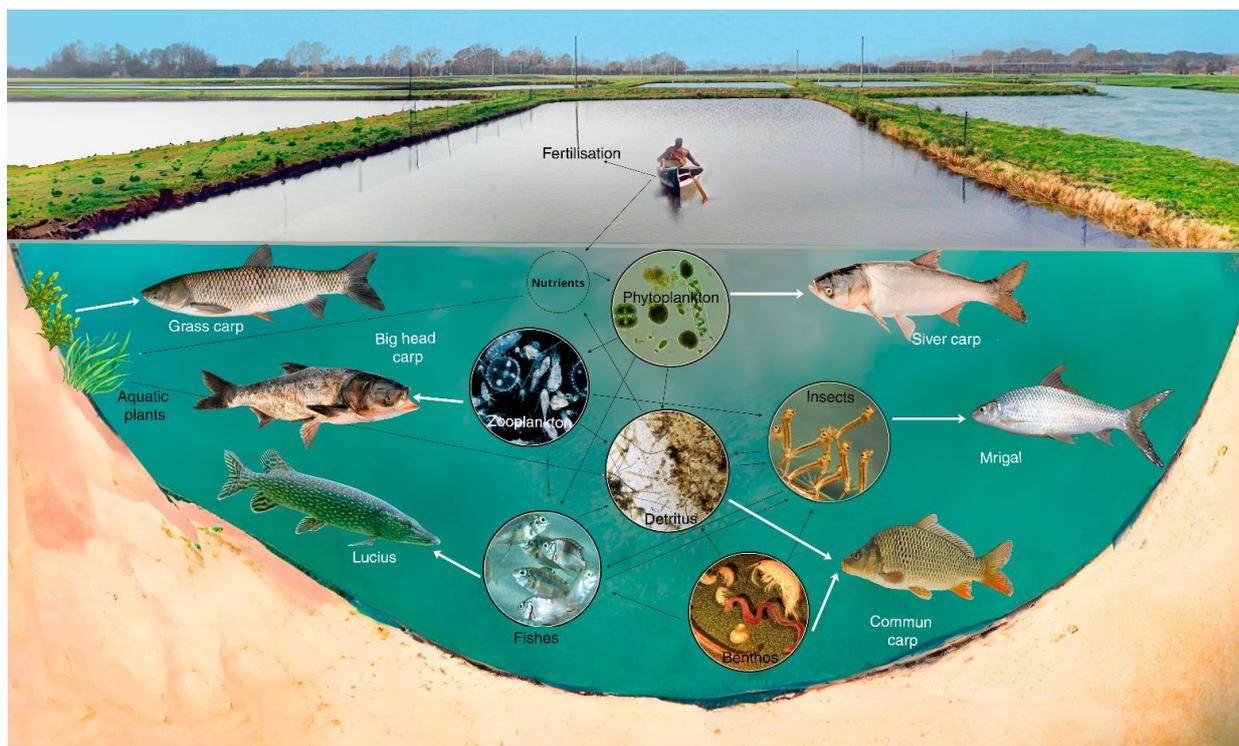


Figure 31. Representation of a polyculture involving six different fish species, each with their specific feeding habits and niches.

In contrast, consumption is carried out both by organisms that perform photosynthesis and those that do not. Plants, for example, also break down organic compounds they produce to release energy. Heterotrophic organisms (those that do not perform photosynthesis) are mostly animals, fungi and bacteria that depend on organic material for their survival and development.

There are two types of consumers in our pond: macroconsumers and microconsumers.

Macroconsumers are primarily animals, such as fish and insects, with diverse diets – herbivorous, carnivorous, detritivores or omnivorous.

Microconsumers, like bacteria and fungi, act in the decomposition of organic compounds.

In the ecosystem of a fish farming pond, bacteria and fungi play a central role in the decomposition of organic matter, such as food leftovers and fish excrement. The action of these microorganisms breaks down this matter into simpler components, called detritus, while releasing nutrients like nitrogen and phosphorus into the water. These nutrients, in turn, can be used by phytoplankton for their growth, serving as the basis for the aquatic food chain.

The success of fish farming in ponds depends significantly on how the fish farmer manages this biological cycle. Understanding the cycle allows for a more efficient approach to the fish's food chain, essentially the series of organisms that consume each other.

In this scenario, managing this cycle by cultivating more than one species simultaneously (polyculture) becomes more complex than growing just one species (monoculture). At the same time,

polyculture allows for better and more efficient use of resources and the chain of biological relationships that occur in the pond. However, the compatibility between species must be carefully analysed, avoiding carnivorous animals and, consequently, the predation of herbivorous species.

10.1.4.2 Nutritional management

- **Natural foods**

The key to success in extensive and semi-intensive management of grass carp polyculture is to ensure abundant natural foods in the pond's different layers (top, middle and bottom). Species like catla, silver carp and bighead carp inhabit the top layer, rohu prefers the middle layer, and mrigal, common carp/mirror carp, pangas and shrimps (yes, they can also be used in grass carp polyculture) generally occupy the bottom layer. Sarpunti and grass carp, on the other hand, tend to circulate through all layers.

- **Use of natural food**

If polyculture involves fish and shrimps that only inhabit the bottom of the pond and are stocked in excess, there will be competition for food and habitat, causing other layers to be underused. Therefore, choosing a diverse mix of species is crucial to maximising food and habitat use in all water layers and optimising space in the pond.

- **Nutrients**

Some species' excreted products help produce natural food for others, and bottom-dwelling fish contribute to water fertility by releasing nutrients in the sediments.

- **Lack of natural foods**

Regarding supplementary feeding, besides commercial feeds, species like rohu and mrigal readily accept rice bran, while catla and silver carp prefer phytoplankton and zooplankton. Grass carp, for their part, can easily switch their habit to consume feeds formulated for other carp species. The problem is that such feeds often have low nutritional value or low digestibility for grass carp, so that this transition is often unacceptable. Feeding grass carp with unusual foods can also cause various metabolic disorders in the fish and, consequently, generate low zootechnical levels.

10.1.4.3 Deciding what species to use

The producer should use practical criteria to determine which fish species to use in polyculture. These criteria include:

- **Economic importance**
The commercial value of species may vary by region.
- **Feeding habits**
Some fish prefer to feed at the surface, others in the middle of the water column or even at the bottom of the pond. Mixing species with different habits helps optimise the process.
- **Function in polyculture**
Species can be classified as primary or secondary, depending on their role in the ecological balance of the system. Thus, species of lesser economic interest may play an essential role in the functioning of a polyculture system.
- **Nutritional status**
It is vital to combine species that will receive supplementary feeding and others that will feed exclusively on the natural resources present in the pond.
- **Size and growth**
Each species will have a specific commercial size and distinct growth rate. Therefore, it is crucial to plan the species to be used properly.
- **Environmental tolerance**
Each species is tolerant to a specific temperature and overall water quality.
- **Legal restrictions**
Exotic species may have legal restrictions for use in certain regions. Knowing and respecting such limitations is essential to avoid subsequent problems.

These practical criteria not only help in selecting species but also provide helpful information for pond management. Remember, the key to successful polyculture is balance, achieved through careful species selection, considering both the biological aspects and economic impacts of a species.

10.1.4.4 Most used species

In some countries of Central and Eastern Europe (CEE), Central Asia and the Caucasus (CCA), traditional carp culture comprises almost a monoculture, making up 90–95% of production. The remaining 5–10% is traditionally divided between fish species defined as ‘peaceful’ and as ‘predators’.

In Western countries, there was a significant shift from this trend starting in the 1960s–1970s, when monoculture migrated to polyculture following the introduction of the leading Chinese carp. Since then, polyculture has involved common carp, the primary Chinese carp (grass carp, silver carp and bighead carp), and other peaceful and predatory fish indicated in Figure 32 to Figure 35.

Fish species such as common carp, breams and the Chinese carp are frequently cultivated in polycultures, while other species are merely secondary members of this environment, being inserted occasionally. There are also smaller fish, such as rasbora, bleak minnows and tetra, found in natural waters, and they can end up entering the ponds with the water and accidentally becoming part of the polyculture. They are called ‘trash fish’, as they often compete for food with larger fish but can also serve as food for the predatory species. Those that survive and remain in the ponds can be fished out and sold as bait for sport fishing. Thus, even the ‘trash fish’ can generate income.

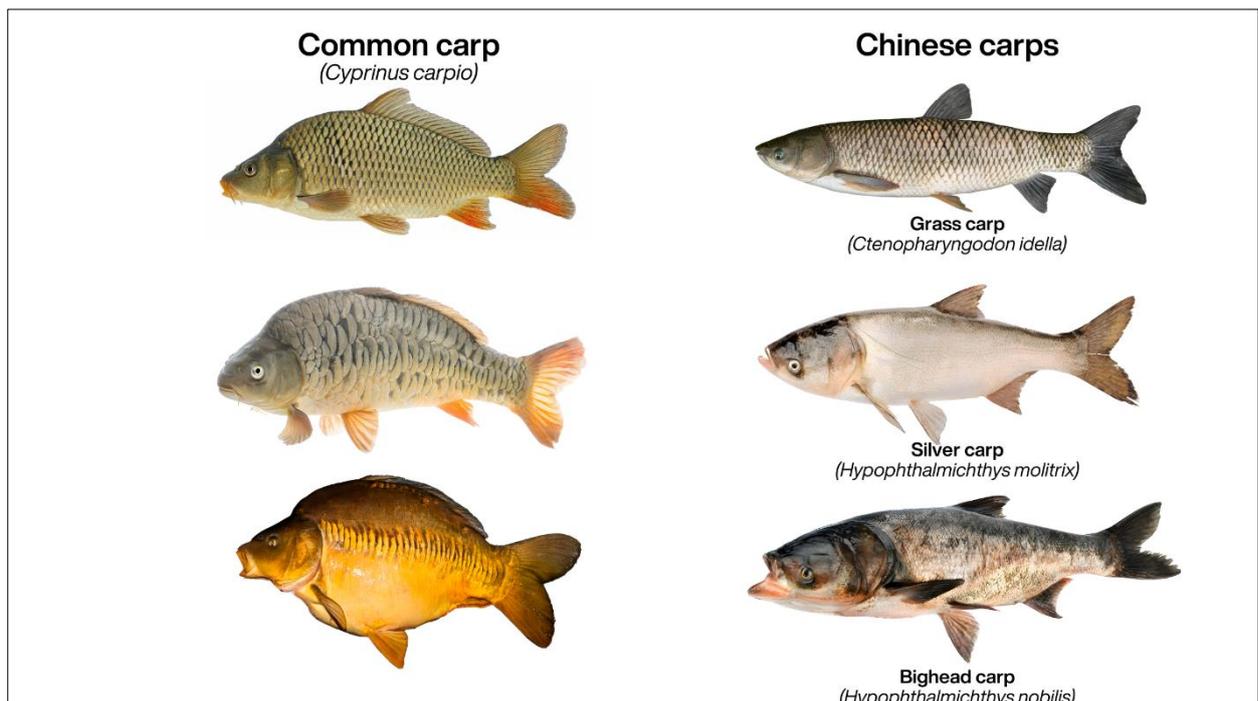


Figure 32. The main Chinese carp species conventionally used in polycultures; the three pictures on the left show different shapes of the common carp.

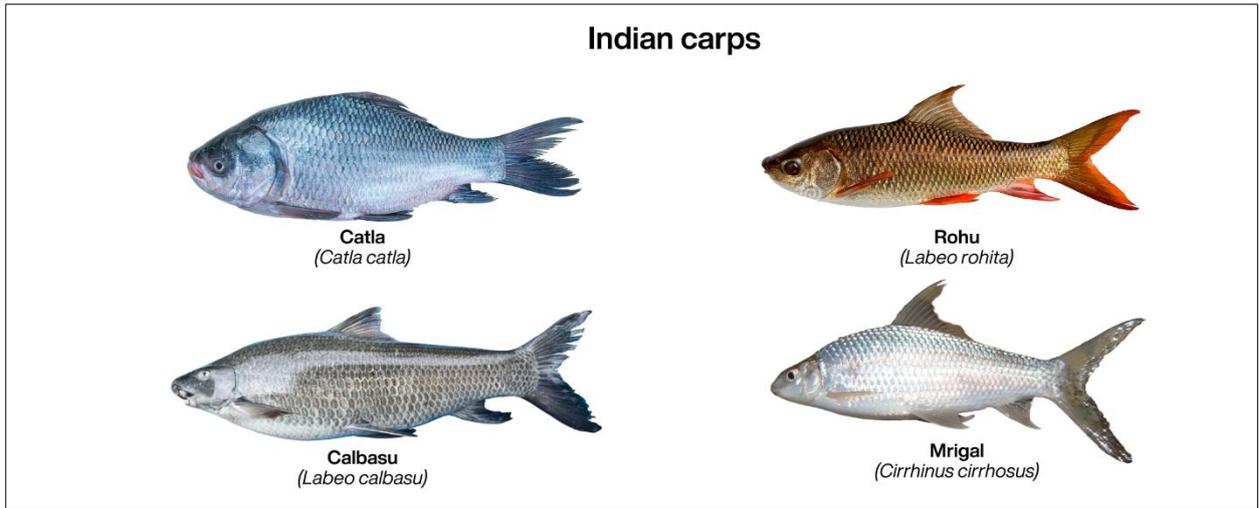


Figure 33. The main Indian carp species used in polyculture with grass carp.

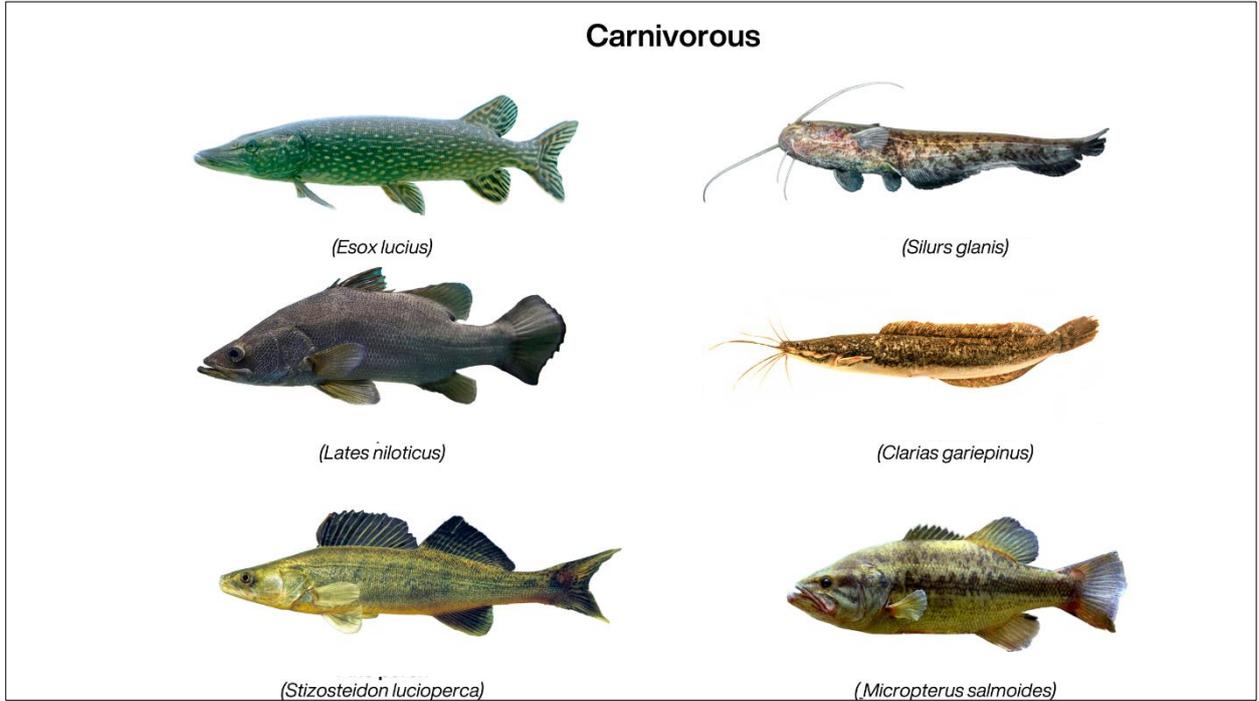


Figure 34. Examples of carnivorous species commonly cultivated with grass carp.

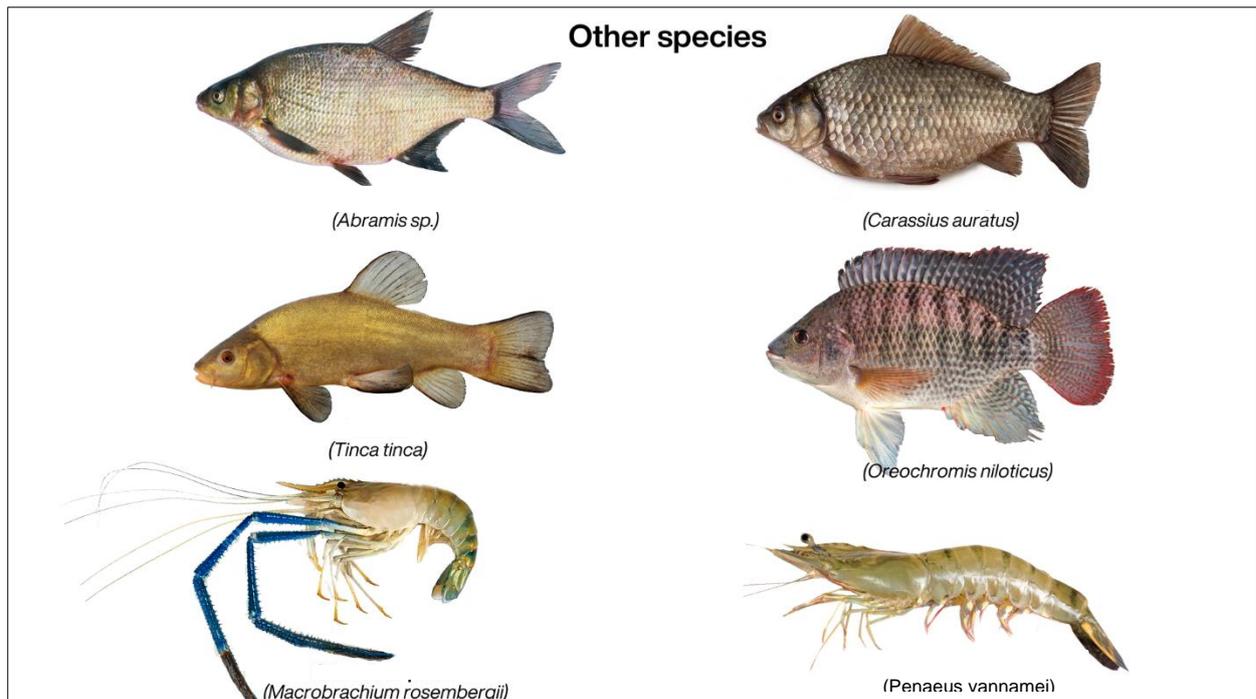


Figure 35. Other species commonly cultivated in polyculture with grass carp.

10.1.4.5 Proportions, densities and cultivation time

Understanding the fish production cycle until it reaches the so-called ‘table point’ is crucial for aquaculture projects.

Various factors, including physical, human and economic conditions, influence fish production in polyculture systems. The specific combination of species to be used in each case will vary, depending on the region and the goals of the fish farmers.

Furthermore, it is also important to highlight that the proportion between grass carp and other fish species in a polyculture system and even the initial density of each species in the system vary according to the intensity of the production regime. That is, they vary depending on the number of resources allocated to the cultivation system in the form of energy, natural foods, fertilisers, soil and water correction products, feeds, aeration, and care in management, among other factors.

All these aspects should be considered when defining a production strategy in a scenario with numerous possible productive combinations.

We will use the zootechnical indices proposed by Woynarovich et al. (2010) to guide the readers more practically and objectively. These indices should not be followed as a rigid rule but as a guide or reference, especially for those first entering the activity.

The tables aim to present numbers, trends and stocking ranges of the different age groups of fish. They also aim to show some observable survival trends, number and expected weight of fish produced under extensive, semi-intensive and intensive conditions.

The following factors must be considered to understand the tables:

- **Primary or secondary species**

Grass carp can be used in polyculture as a primary or secondary species. It can account for up to 80% of the organisms stocked in a pond as a primary species. As a secondary species, the proportion of grass carp should meet the project's needs; for example, when grass carp are stocked in a minimal number to keep the ponds clean of macrophytes.

- **Cultivation time**

This fundamentally depends on climatic conditions. In warmer zones, the production cycle can be completed in two years. The entire cultivation cycle extends for at least three years in countries with more defined seasonal climates and colder weather.

- **Summer fish**

When authors refer to 'summer fish', they assume that the stocking of the ponds took place in spring. Therefore, by the end of the following summer the fish will be 1.5 years old; by the end of the second summer they will be 2.5 years old, and by the end of the third summer 3.5 years old.

- **Harvesting**

The timing of harvesting varies according to the production cycle and the desired size of the fish but usually occurs at the end of summer or the beginning of autumn when the fish reach their peak growth rates for that annual cycle.

- **Larger fish:** When the goal is to cultivate fish that exceed 2.5 kg, producers commonly opt for an additional year of cultivation.

10.2 Advanced fry production patterns for polyculture systems

Table 11¹. Pike: Production period: 4–6 weeks.

Intensity of stocking	Stocking per hectare				Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Extensive	Feeding post-larvae		100,000	-	10	40	Advanced fry	1.50	25,000	40	40
Intensive			500,000	-				0.25	125,000	30	30

Table 12. Pikeperch: Production period: 4–6 weeks.

Intensity of stocking	Stocking per hectare				Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Extensive	Eggs on nests	-	500,000	-	5	20	Advanced fry	1.00	65,000	65	65
Intensive			2,000,000	-				0.25	250,000	65	65
Extensive	Feeding larvae	-	250,000	-	10	40	Advanced fry	1.00	65,000	65	65
Intensive			1,000,000	-				0.25	250,000	65	65

Table 13. European catfish. Production period: 46 weeks.

Intensity of stocking	Stocking per hectare				Expected survival rate (%)		Harvesting per hectare				
	Age Group	Avg. size (g/fish)	Number	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Extensive	Feeding larvae	-	50,000	-	20	40	Advanced fry	2.00	15,000	30	30
Intensive			250,000	-				0.50	75,000	40	40

Table 14. Carps (common, silver, and grass carp). Production period is 36 weeks.

Intensity of stocking	Stocking per hectare				Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Extensive	Feeding larvae	-	500,000	-	30	40	Advanced fry	2.00	175,000	350	350
Intensive			2,000,000	-				0.50	700,000	350	350

¹ All the tables shown here were developed by Woynarovich, A., Moth-Poulsen, T., Péteri, A., 2010. *Carp polyculture in Central and Eastern Europe, the Caucasus and Central Asia: a manual*. Food and Agriculture Organization of the United Nations.

Table 15. Breams. Production period: 46 weeks.

Intensity of stocking	Stocking per hectare				Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Extensive	Feeding larvae	-	500,000	-	30	40	Advanced fry	1.00	175,000	175	175
Intensive			1,000,000	-				0.50	350,000	175	175

Table 16. Advanced fry production patterns of tench. Production period: 46 weeks.

Intensity of stocking	Stocking per hectare				Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Extensive	Feeding larvae	-	500,000	-	30	40	Advanced fry	0.50	175,000	90	90
Intensive			1,000,000	-				0.25	350,000	90	90

10.3 One-summer-old fish production patterns

Table 17. Extensive production of medium-size one-summer-old fish: 10–12 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. Size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	Fry	1.0	4,500	30	5	50	70	One-summer-old fish	50	2,700	135	130
Silver carp		1.0	4,500	30	5	50	70		50	2,700	135	130
Bighead carp		1.0	4,500	30	5	50	70		50	2,700	135	130
Grass carp		1.0	4,500	30	5	50	70		50	2,700	135	130
Predators		0.5	1,500	10	-	50	70		50	900	45	45
Total	-	-	15,000	100	15	-	-	-	-	9,000	450	435

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 18. Semi-intensive production of small one-summer-old fish: 10–12 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	Fry	1.0	50,000	50	50	50	70	One-summer-old fish	25	30,000	750	700
Silver carp		1.0	35,000	35	35	50	70		25	21,000	530	495
Bighead carp		1.0	10,000	10	10	50	70		25	6,000	150	140
Grass carp		0.5	5,000	5	-	50	70		25	3,000	80	80
Predators												
Total	-	-	100,000	100	95	-	-	-	-	60,000	1,510	1,415

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 19. Semi-intensive production of large one-summer-old fish: 10–12 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	Fry	1.0	12,500	50	10	50	70	One-summer-old fish	100	7,500	750	740
Silver carp		1.0	9,000	36	10	50	70		100	5,400	540	530
Bighead carp		1.0	2,500	10	-	50	70		100	1,500	150	150
Grass carp		0.5	1,000	4	-	50	70		100	600	60	60
Predators												
Total	-	-	25,000	100	20	-	-	-	-	15,000	1,500	1,480

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 20. Extensive production of two-summer-old fish: 22–24 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	One-summer-old fish	25	1,000	29	25	60	80	Two-summer-old fish	250	700	175	150
Silver carp		25	1,000	29	25	60	80		250	700	175	150
Bighead carp		25	1,000	29	25	60	80		250	700	175	150
Grass carp		25	1,000	29	25	60	80		250	700	175	150
Predators		20	500	14	10	60	80		250	350	90	80
Total	-	-	3,500	100	85	-	-	-	-	2,450	615	530

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 21. Semi-intensive production of two-summer-old fish: 22–24 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net Weight (kg)
Common carp	One-summer-old fish	25	5,000	50	125	60	80	Two-summer-old fish	250	3,500	875	750
Silver carp		25	3,500	35	90	60	80		250	2,450	610	520
Bighead carp		25	1,000	10	25	60	80		250	700	175	150
Grass carp		25	1,000	10	25	60	80		250	700	175	150
Predators		20	500	5	10	60	80		250	350	90	80
Total	-	-	10,000	100	250	-	-	-	-	7,000	1,750	1,500

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 22. Semi-intensive production of large two-summer-old fish: 22–24 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	One-summer-old fish	100	1,500	50	150	80	90	Two-summer-old fish	750	1,280	960	810
Silver carp		100	1,000	33	100	80	90		750	850	640	540
Bighead carp		100	350	12	35	80	90		750	300	225	190
Grass carp		75	150	5	10	80	90		750	130	100	90
Predators												
Total	-	-	3,000	100	295	-	-	-	-	2,560	1,925	1,630

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

10.4 Table fish production patterns

Table 23. Semi-intensive production from large one-summer-old fish: 22–24 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	One-summer-old fish	100	750	50	75	80	90	Table fish	1,250	640	800	725
Silver carp		100	450	30	45	80	90		1,250	380	475	430
Bighead carp		100	200	13	20	80	90		1,250	170	215	195
Grass carp		75	100	7	10	80	90		1,250	90	110	100
Predators												
Total	-	-	1,500	100	150	-	-	-	-	1,280	1,600	1,450

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 24. Extensive production from two-summer-old fish: 22–24 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	Two-summer-old fish	250	210	30	50	80	90	Table fish	1,250	180	225	175
Silver carp Bighead carp		250	210	30	50	80	90		1,250	180	225	175
Grass carp		250	210	30	50	80	90		1,250	180	225	175
Predators		200	70	10	10	80	90		1,250	60	75	65
Total	-	-	700	100	160	-	-	-	-	600	750	590

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 25. Semi-intensive production from two-summer-old fish: 22–24 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net weight (kg)
Common carp	Two-summer-old fish	250	1,000	50	250	80	90	Table fish	1,250	850	1,065	815
Silver carp Bighead carp		250	700	35	175	80	90		1,250	600	750	575
Grass carp		250	200	10	50	80	90		1,250	170	215	165
Predators		200	100	5	20	80	90		1,250	80	100	80
Total	-	-	2,000	100	495	-	-	-	-	1,700	2,130	1,635

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

Table 26. Two-summer-old fish: 12–18 weeks.

Species	Stocking per hectare					Expected survival rate (%)		Harvesting per hectare				
	Age group	Avg. size (g/fish)	Number	%	Total weight (kg)	Low	High	Age group	Avg. size (g/fish)	Number	Total weight (kg)	Net Weight (kg)
Common carp	Two-summer-old fish	750	2,000	50	1,500	80	90	Table fish	1,250	1,700	2,125	625
Silver carp Bighead carp		750	1,250	31	935	80	90		1,250	1,060	1,325	390
Grass carp		750	500	13	375	80	90		1,250	430	540	165
Predators		600	250	6	150	80	90		1,250	210	260	110
Total	-	-	4,000	100	2,960	-	-	-	-	3,400	4,250	1,290

Observation: The ratio between silver and bighead carp should be 80–90% and 10–20%.

10.5 Other combinations with grass carp as the main species

There are numerous options other than those presented above for combinations and proportions among species to be cultivated in a polyculture model. Three more options are given below.

- **Three species**

Grass carp: 150 g/fish (66%), bighead carp: 100 g/fish (32%), and common carp: 100 g/fish (2%). The total recommended density in this case is 3–15 fish per m², depending on available resources.

- **Four species**

Grass carp: 150 g/fish (55%); silver carp: 100 g/fish (30%); bighead carp: 100 g/fish (7%); common carp: 100 g/fish (8%). The total recommended density in this case is 3–15 fish per m², depending on available resources.

- **Five species**

Grass carp: 150 g/fish (75%); silver carp: 100g/fish (8%), bighead carp: 100 g/fish (6%); carnivorous fish: 150 g/fish (6%), crucian carp: 60 g/fish (5%). The total recommended density in this case is 3 fish per m².

- **Six species**

Grass carp: 100 g/fish (70–80%); silver carp: 70 g/fish (5–8%), common carp: 70 g/fish (5–8%), bighead carp: 80 g/fish (3–5%), bream: 60 g/fish (3–5%), African catfish: 50 g/fish (1–3%). The total recommended density, in this case, is 3–10 fish per m², depending on available resources.

However, it is essential to note here that, in terms of welfare, the use of carnivorous species is not recommended, as constantly remaining in the same habitat as a predator with minimal resources to defend itself affects the psychological freedom of the animal, potentially causing chronic stress.

10.6 Other combinations with grass carp as a secondary species

- **Combination 1**
Silver carp: 21% to 19%
Catla: 10.5% to 13%
Rohu: 21% to 19%
Mrigal: 10.5% to 13%
Common carp: 5.3% to 6.5%
Grass carp: 5.3% to 4.8%
Sarpunti: 26.4% to 24.2%
Total: 3,800 to 6,200 fish per hectare.
- **Combination 2**
Silver carp: 21% to 19%
Bighead carp: 10.5% to 13%
Rohu: 21% to 19%
Mrigal: 10.5% to 13%
Common carp: 5.3% to 6.5%
Grass carp: 5.3% to 4.8%
Sarpunti: 26.4% to 24.2%
Total: 3,800 to 6,200 fish per hectare.
- **Combination 3:**
Silver carp: 27.8% to 27.3%
Catla: 11.1% to 14.5%
Rohu: 27.8% to 27.3%
Mrigal: 16.7% to 14.5%
Common carp: 11.1% to 10.9%
Grass carp: 5.6% to 5.5%
Total: 3,600 to 5,500 fish per hectare.
- **Combination 4:**
Silver carp: 9.4%
Catla: 3.1%
Grass carp: 3.1%
Rohu: 6.3%
Shing: 78.1%
Total: 12,800 fish per hectare.

10.6.1 Deciding what species to use

The following aspects should be considered when selecting species to be used in polycultures:

- **Natural food**
Even if the chosen species is fed, they should be able to make adequate use of the natural food available in the ponds, as this is one of the principles that justify polyculture.
- **Supplementary foods**
Preferably, the chosen species should be capable of feeding satisfactorily on cheap and readily available supplemental foods in the region.
- **Growth rate**
Species with a short food chain and rapid growth should be chosen.
- **Market value**
Preference should be given to species with higher local demand and market value.
- **Availability of fry**
Preference should be given to species whose fry/juveniles are easily found in the local or regional market.
- **Disease resistance**
Species highly resistant to different diseases should be prioritised.
- **Competition**
In a polyculture, preference should be given to species that do not compete for the same type of food.
- **Experience of fish farmers**
The choice of the number of fish and of species to be cultivated also needs to consider the prior experience of the producers.
- **Financial capacity**
The definition of the species and the production regime to be employed will also depend on the financial capacity of the fish farmers.

11 WATER QUALITY

11.1 The ecosystem of a grass carp polyculture pond

A cultivation pond is very similar to a lake, but it is a lake from which many organisms that could have been living there have been intentionally removed. For instance, we do not want the presence of birds, mammals and reptiles that may prey on or compete with cultivated fish for space or resources, such as oxygen and feed.

Yet, this 'lake', now transformed into a carp cultivation pond, is far from containing only the fish of the cultivated species. Many organisms coexist and compete for the same resources, affecting the welfare and zootechnical performance of the farmed fish, even if we cannot see most of them with the naked eye.

Phytoplankton also known as microalgae (Figure 36) consists of unicellular organisms that obtain their food through photosynthesis, using sunlight and nutrients present in the water.

Zooplankton (Figure 37) is mainly made up of microscopic organisms that feed on phytoplankton or other zooplankton animals. Both phyto- and zooplankton serve as natural food for the filter-feeding fish used in polyculture. There is also an endless number of bacteria living in the water and soil of the pond.

11.1.1 Phytoplankton

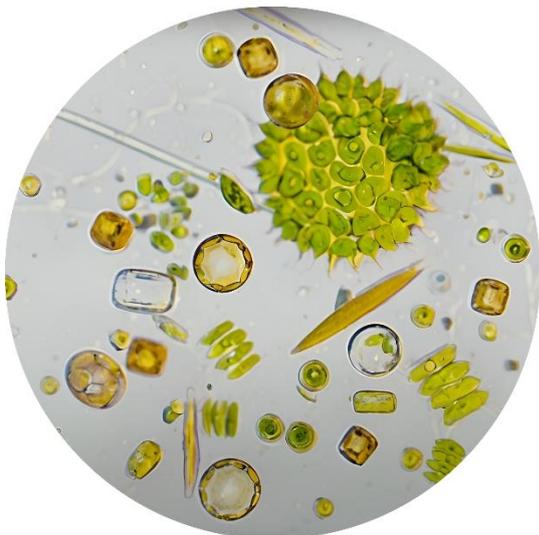


Figure 36. Phytoplankton.

- **Daily cycle**

During photosynthesis, phytoplankton extracts nutrients (mainly nitrogen, phosphorus and carbon dioxide (CO₂)) from the water and, using the energy of the sunlight that reaches the pond, produces organic substances for its nutrition (such as glucose). The by-product of this biochemical process is oxygen, which phytoplankton releases into the water. It is used for the respiration of all organisms present in the ponds, whether by bacteria, zooplankton, carp, or even the phytoplankton itself. Thus, the oxygen released during the day tends to be more significant as the number of phytoplankton and direct solar energy on the pond's surface increases.

However, if phytoplankton 'produces' oxygen during the day, at night, photosynthesis stops due to the lack of sunlight, and the oxygen factory shuts down until the sun rises again. This process results in dissolved oxygen concentrations in a pond's water increasing during the day and falling (sometimes dangerously) at night. Meanwhile, CO₂, which is toxic to fish and can alter the water's pH, follows the opposite pattern, falling during the day and rising at night. When unbalanced by excess organic matter or phytoplankton in the pond, this cycle can even threaten the survival of the cultivated fish.

- **Challenge**

One of the daily challenges of a fish farmer is to try to keep this system in balance, as the more fish there are in the pond, the more food will be needed. The problem is that part of this food or its components return to the cultivation environment through uneaten leftovers, faeces, and fish excretion products. These compounds contain many of the nutrients that phytoplankton needs for photosynthesis, and in excess, can also cause significant environmental imbalance.

- **Water colour**

Thanks to all these processes happening and intensifying throughout the production cycle, the tendency is for the water in the pond to gradually change colour, acquiring different shades of green, grey, blue, brownish-yellow or brownish-green due to the increase in populations of bacteria, zooplankton, and mainly phytoplankton.

- **Die-off**

When phytoplankton concentrations are very high there is also an increased possibility of extreme events, known as 'die-off'. This phenomenon is characterised by the sudden, unidentifiable death of large phytoplankton. Bacteria feast on the dead phytoplankton and radically increase their biomass in the ponds. They will also consume large amounts of dissolved oxygen in the water.

11.1.2 Zooplankton



Figure 37. Zooplankton.

- **Composition**

Zooplankton is a diverse group of aquatic microscopic or nearly microscopic organisms that inhabit the water column in marine and freshwater environments, including fish cultivation ponds. These organisms are primarily heterotrophic (i.e., they feed on organic matter) and include small crustaceans, such as cladocerans (water fleas) and copepods, as well as larvae of various aquatic and terrestrial animals (such as insects), rotifers and protozoans.

- **Importance 1**

Zooplankton plays a fundamental role in aquatic ecosystems, acting as a crucial link in the food chain, transferring energy from primary producers (such as phytoplankton) to larger consumers, including many fish species. Zooplankton also contributes to nutrient cycling processes and regulating algae populations in water bodies.

- **Importance 2**

Zooplankton is fundamental in feeding fish cultivated in polyculture with grass carp, as in the early growth stages.

11.1.3 Benthic organisms



Figure 38. Benthic organisms.

Many invertebrates (Figure 38) such as molluscs, crustaceans, aquatic insects and vertebrates like small fish colonise the bottom of a grass carp polyculture pond.

- **Invertebrates**
This category includes various organisms, such as annelids, insect larvae, molluscs (like snails and mussels), and crustaceans (such as crabs and shrimps).
- **Microorganisms**
Bacteria and fungi that live in the pond substrate play a vital role in the decomposition of organic matter.

11.1.3.1 Ecological functions

The presence of these organisms and their activities have several important implications for the pond ecosystem:

- **Decomposition**
They aid in decomposing organic matter, such as plant remains, uneaten food and fish faeces, transforming it into nutrients available for other aquatic organisms.
- **Nutrient recycling**
These animals contribute to nutrient cycling in the pond ecosystem by feeding on organic matter and excreting waste.
- **Sediment aeration**
Some of these organisms, such as earthworms, help aerate the pond soil, which is beneficial for the roots of aquatic plants and the overall health of the system.

11.1.3.2 Contribution to grass carp polyculture

- **Natural feeding**
Many benthic animals serve as a natural food source for grass carp, contributing to the diversity and quality of their diet.
- **Water quality maintenance**
By decomposing organic matter and recycling nutrients, these organisms help maintain water quality, which is essential for the health of cultivated fish.
- **Indicators of pond health**
The presence and diversity of these organisms indicate pond health. A balanced population suggests a healthy and well-managed environment.

11.1.4 Bacteria

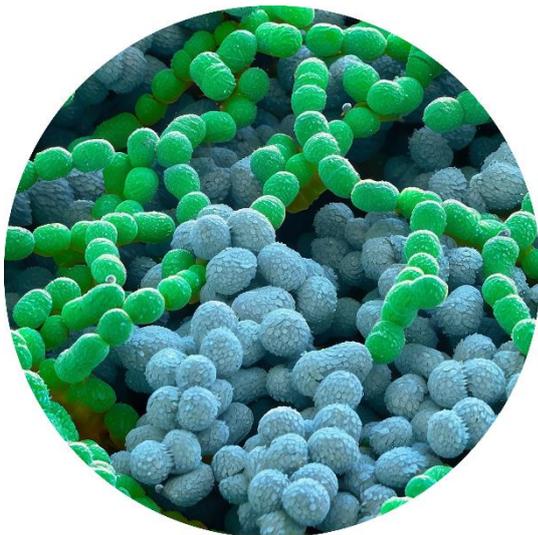


Figure 39. Bacteria.

Phyto, zooplankton and some benthic species have very short life cycles and are constantly born and dying in large numbers. These dead organisms transform into decomposing organic matter and join uneaten feed and fish faeces, serving as a food source for decomposer bacteria, which multiply in vast numbers whenever the organic matter in this cultivation system increases.

Besides decomposer bacteria (Figure 39), there are others of direct interest to fish farming living and multiplying in the pond, such as iron-reducing bacteria (which give a rust colouration to the soil), nitrifying and denitrifying bacteria, playing an essential role in the nitrogen cycle, and anaerobic bacteria (which multiply in the soil without oxygen and produce toxic gases like sulphides and methane).

11.2 The cultivation pond is a 'living organism'

It is through this intense and constant 'tug of war' involving the different organisms present in an excavated pond that this cultivation system behaves like a giant 'living organism', breathing intensely and responding to changes in water quality with changes in the dynamics of the populations of the beings that live there, including the carp.

Water quality in a pond constantly changes due to changes in cultivated fish, phyto- and zooplankton populations, and bacteria. On the other hand, every time water quality changes, the entire biota (set of living beings) of the pond can also be affected, even though grass carp are a species known for their low demand in terms of water quality.

But to make things even more complex, in a pond, unlike a tank where the bottom is made of concrete or lined with plastic, fibreglass, or other inert material, the bottom here is composed of soil very rich in macro and microorganisms, which will also significantly affect water quality.

In addition to microbiological interaction, there is a chemical interaction between soil and water in a pond. The water also tends to become acidic in places where the pond or dam soil is acidic. Similarly, in locations where the soil is rich in limestone, the waters are naturally enriched by this mineral and become alkaline.

11.3 Temperature



Figure 40. Thermometer for measuring water temperature.

11.3.1 What is it?

Temperature is a physical measure representing the average kinetic energy of particles in a material. In simpler terms, it indicates how hot or cold an object or environment is. Temperature is directly related to particle movement: the faster the particles move in a material, the higher its temperature. In aquaculture, temperature is measured in degrees Celsius (°C) or degrees Fahrenheit (°F) (Figure 40).

The temperature of aquatic animals adjusts to the temperature of their environment. They exhibit low tolerance or even intolerance to rapid temperature changes and fluctuations. This means that water is an ideal habitat for them, given that water is a poor conductor of heat and can absorb substantial amounts of thermal energy without significant changes in its temperature.

11.3.2 Fish tolerance to temperature

Fish are ectothermic animals. Their body temperature is directly related to the temperature of the aquatic environment in which they live. They cannot actively regulate their internal temperature. This means that if the temperature of the surrounding water changes, their body temperature also adjusts to this change.

Fish reactions to temperature variations are divided into four categories: ideal temperature, preferred temperature, tolerance range, and resistance.

- **Ideal temperature:** the one at which the fish's bodily functions operate at peak efficiency. The ideal temperature is the one that leads to a maximum growth rate, efficient food conversion, and better fish conditions. An appropriate temperature regime enhances disease resistance and tolerance to toxins (metabolites and pollutants).
- **Preferred temperature:** is the one in which fish will choose to stay longer when given the option. This is specific to each species and usually coincides with the temperature range that promotes optimal growth.
- **Tolerance range** refers to the spectrum of temperatures where half of the fish population can survive. This range varies according to the acclimation process the fish have undergone.
- **Thermal resistance:** defines the critical temperature limit that a fish can endure. This limit is identified by changing the water temperature by 1°C per minute until the fish lose balance, indicating the point of maximum resistance. Survival outside this range is almost impossible.

Other definitions are fundamental when it comes to water temperature in aquaculture:

- **Unfavourable temperatures**
Fish response to adverse temperatures depends on the intensity and duration of the temperature change outside normal limits. This can manifest in decreased productivity, ranging from high stress levels to complete metabolic dysfunction and mortality. To a limited degree, fish can increase their thermal tolerance range through acclimation.

- **Temperature variations**

Temperature fluctuations can cause high mortalities in fish embryos and larvae. Short-term elevations in temperature also increase the incidence of deformities in fish larvae. Typical long-term responses to unfavourable temperatures include slow growth, low food conversion efficiency, reduced production, poor condition, and increased susceptibility to diseases, particularly bacterial and fungal infections. Fish can suffer from thermal shock when there is a sudden change from a high to a low temperature. This shock can destroy red blood cells, causing the release of haemoglobin into the plasma – a phenomenon known as hemolysis. This can lead to a decreased capacity of the blood to carry oxygen to the body's tissues, potentially causing conditions such as anaemia. Hemolysis can also overwhelm the kidneys, as they need to filter out the excess haemoglobin released, which, in severe cases, can lead to renal failure and death of the fish.

- **Extreme temperatures**

The acute response of fish to exposure to critical high or low temperatures includes loss of balance and metabolic dysfunction. An increased breathing rate is also observed when fish are exposed to critical upper thermal limits.

- **Sublethal temperatures**

Short-term responses to sublethal temperatures are behavioural changes, including decreased activity and loss of appetite. A decrease in oxygen saturation, coupled with an increase in water temperature, results in an increased heart rate, with fish often seeking air at the water's surface.

11.3.3 The impact of temperature in biological systems and the environment

Temperature regulates the biochemical reactions of fish and other aquatic organisms, directly influencing their survival and growth rates.

- **Fish metabolic rate**

As we have seen, fish are ectothermic animals, meaning their metabolic rate is strongly influenced by water temperature. Higher temperatures generally increase metabolism, accelerating growth and increasing oxygen and waste production demand.

- **Oxygen solubility**

The solubility of oxygen in water decreases with increasing temperature. This happens because, as the temperature increases, water molecules gain more kinetic energy, which reduces the water's ability to maintain oxygen molecules dissolved. In other words, water 'holds' fewer oxygen molecules when it is warmer, as the water molecules move more quickly and cannot hold as many oxygen molecules in solution. Therefore, fish may have less oxygen available at higher temperatures, which is crucial for respiration.

- **Fish development and health**

Different fish species have ideal growth, reproduction and immunity temperature ranges. Temperatures outside these ranges can cause stress, reduce reproductive efficiency and increase disease susceptibility.

- **Biological processes in the ecosystem**

Temperature affects processes such as nitrification (the conversion of ammonia (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-)) and the activity of beneficial bacteria in biological filtration systems. Inadequate temperatures can impair these processes, affecting water quality.

- **Chemical interactions**

Temperature influences the solubility and reactivity of chemicals in water, including toxins like ammonia and nitrite.

- **Other organisms**

The above principle also applies to other organisms in the aquatic environment, where temperature affects nutrient dynamics, essential for the nutrition and growth of fish. Therefore, productivity in aquaculture is linked to maintaining an appropriate temperature.

- **Chemical interactions**

Temperature influences the form and toxicity of ammonia: with an increase in temperature, ammonia can become more toxic. The same applies to the toxicity of metals, which can increase with temperature.

Temperature variations All water bodies are subject to daily and seasonal temperature variations. Natural temperature variation depends both directly and indirectly on prevailing climatic conditions. Direct changes in water temperature result from alterations in ambient air temperature, while indirect changes may occur due to the influx of water at different temperatures. For example, increased rainwater flow, water released from snowmelt, or after a hailstorm can alter the temperature regime.

The rate at which a body of water resists thermal change depends on its volume and the surface-to-volume ratio. These factors also influence the homogeneity of temperature within the water body. High resistance to external disturbances, such as wind and wind reach, can result in thermal stratification (water layers separating into different strata at distinct temperatures) within the water body.

11.3.4 Measurement

- **Standard unit:**

The standard unit for measuring water temperature in degrees Celsius (°C), typically gauged using mercury, alcohol or electronic thermometers (Figure 41). For aquaculture purposes, water temperature records should incorporate spatial and temporal records, covering seasonal maximum and minimum values and thermal homogeneity, which implies measuring the temperature at various points and depths of the pond.



Figure 41. Digital thermometer for measuring water temperature.

11.3.5 Control

- **Costs**

Controlling temperature in pond cultivation conditions is prohibitively expensive and unfeasible. When stocking natural waters, it is essential to ensure that the species to be cultivated can perform optimally within the water body's thermal range. When transferring fish to new water, proper acclimatisation to any change in water temperature is vital.

11.3.6 Grass carp and temperature

- **Adaptation capacity**

Grass carp are notable for their remarkable adaptability to different temperatures, thriving in conditions ranging from 0 to 38°C. However, water temperature is a determining factor for their appetite and nutrient absorption. The 20–30°C temperature range is ideal for reproduction, 22–29°C for larval rearing, and 20–33°C for fingerling and fattening stages. Conversely, temperatures below 5°C significantly reduce food intake, ceasing entirely below this threshold.

- **High temperatures**

During the warmer months it is essential to monitor and control excessive water heating, as it can lead to high mortality rates. Overheating can be managed by controlling water levels or installing shading structures, such as solar protection nets, to create a stable aquatic environment conducive to healthy grass carp development.

11.4 Dissolved oxygen



Figure 42. Gaseous oxygen present in the water.

11.4.1 What is it?

- **Definition**

Dissolved oxygen is the amount of gaseous oxygen present (Figure 42) in the water and available for use in the respiration of cultivated fish and all other organisms and microorganisms in the pond.

11.4.2 Cycle

- **Production and consumption**

Oxygen production by phytoplankton through photosynthesis is the primary source (about 80%) that supplies a fish cultivation pond. On the other hand, phytoplankton is also responsible for about 60% of the oxygen consumed in that pond. Bacteria and other microorganisms consume about 25 to 30%, and the cultivated fish account for about 5 to 10% of the total. Thus, for a pond to be self-sufficient in dissolved oxygen, the photosynthesis conducted throughout the day should be high enough to meet the demand of all organisms during the night when there is no photosynthesis. The problem is that the farmer cannot guarantee that this will happen.

11.4.3 Units

- **Absolute concentrations**

This is a direct measurement of DO in the water and refers to the amount of dissolved oxygen in the water, expressed in milligrams per litre (mg/L) or parts per million (ppm).

- **Percentage of saturation**

This relative measure expresses the amount of dissolved oxygen as a percentage of the maximum amount of oxygen that water can naturally retain under the current conditions of temperature, atmospheric pressure and water salinity. DO saturation varies primarily according to temperature: colder waters can keep more oxygen than warmer waters. Therefore, a saturation percentage of 100% indicates that the water contains the maximum amount of oxygen it can dissolve at that specific temperature and pressure.

11.4.4 Factors influencing oxygen saturant percentage

- **Temperature**

The solubility of DO is severely affected by temperature; at 760 mmHg at 10°C, fresh water can contain 11.29 mg/L, while at 30°C this value decreases to 7.56 mg/L.

- **Altitude/Atmospheric pressure**

At sea level (0 metres altitude), standard atmospheric pressure is approximately 760 mmHg. At this level, water at 20°C can contain about 9 mg/L of dissolved oxygen under saturation conditions. At a higher altitude (2,500 m, for example), atmospheric pressure is

significantly lower, around 560 mmHg. At this altitude, under the same temperature conditions (20°C), the water can contain only about 7 mg/L of dissolved oxygen, or even less depending on the exact conditions.

- **Salinity**

The solubility of oxygen decreases with increasing salinity; for each increase of 9 g/L (or parts per thousand) of salinity, the solubility of DO decreases by 5%.

11.4.5 **Supersaturation**

Values above 100% DO saturation in pond waters can occur and indicate a state of supersaturation. That is, the water, at that moment, contains more oxygen than it is capable of retaining under those conditions of temperature, altitude and salinity. For example, the saturation of DO in water at sea level (atmospheric pressure of 760 mm Hg) is 14.16 mg/L at 0°C, 12.8 mg/L at 5°C, 10.1 mg/L at 15°C, and 9.09 mg/L at 20°C. Oxygen concentrations that are higher in such conditions would indicate supersaturation, an unstable situation that can have the following causes:

- **Increased photosynthetic activity**

On sunny days or in nutrient-rich waters, phytoplankton or aquatic plants may produce excess oxygen through photosynthesis.

- **Excessive aeration**

Though less common, specific aeration or oxygenation systems can infuse water with more oxygen than it can naturally hold under equilibrium conditions.

- **Water discharges**

Waters released from dams or other structures can become supersaturated due to intense mixing and turbulence.

11.4.6 **Subsaturation**

The subsaturation of DO in cultivation ponds is a condition where the oxygen concentration in the water is below the saturation level for those conditions of temperature, altitude and salinity. It is widespread in cultivation ponds and can occur due to the following factors:

- **Biological oxygen demand (BOD)**

The decomposition of organic matter, such as food residues and fish excrements, consumes oxygen. Ponds with high organic load or overfeeding can have a high BOD, leading to OD subsaturation.

- **High fish density**

A high fish density increases the oxygen demand and can reduce OD concentrations. In this case, O₂ is biochemically reduced by carbon compounds, forming carbon dioxide (CO₂).

- **Water temperature**
Warmer waters have a lower capacity to retain oxygen. Additionally, fish metabolism is faster at higher temperatures, increasing the oxygen demand.
- **Reduced water agitation**
Lack of movement on the water surface facilitates the incorporation of oxygen from the air into the water.
- **Natural processes**
In some instances, natural phenomena such as water stratification and thermal inversions can contribute to the subsaturation of dissolved oxygen (DO) in cultivation ponds. Stratification can restrict the circulation of oxygen and nutrients, negatively affecting water quality and fish health. Thermal inversions refer to situations where warmer, less dense water layers overlap colder, denser layers, limiting the mixing between them and therefore the distribution of oxygen and other essential nutrients.

11.4.7 Measurement

Various techniques and equipment can be used to measure dissolved oxygen (DO) in cultivation ponds. Each method has advantages and limitations, and the choice of the most suitable method depends on factors such as available resources, required accuracy, ease of use, and the nature of the aquaculture system.

- **Automatic sensors and continuous monitoring systems**
In more advanced aquaculture systems, automatic sensors can continuously monitor DO concentrations. These sensors are often integrated into control systems that can automatically adjust aeration or other pond conditions to maintain ideal DO levels.
- **Dissolved oxygen meter (or DO probe)**
One of the most common and accurate methods for measuring DO in ponds. A dissolved oxygen meter is an electronic instrument that directly measures the amount of dissolved oxygen in the water (Figure 43). The device has a probe inserted into the water, providing instant and accurate readings (if well calibrated) of DO levels, usually in milligrams per litre (mg/L) and as a percentage of saturation.
- **Winkler method**
This traditional chemical method involves collecting a water sample and adding substances that react with the dissolved oxygen. The result is then determined by titrating the sample with a chemical solution until a colour change occurs. This method is more time-consuming and requires technical knowledge but is entirely accurate and valuable, especially when electronic equipment is not available or is impractical.
- **Titrimetric or colorimetric kits**
These kits are more straightforward and less precise than the previous methods but offer a

quick and low-cost way to estimate DO levels. They generally use the Winkler method but through portable and easy-to-use titrimetric kits.



Figure 43. Electronic dissolved oxygen meter.

11.4.8 The daily cycle of DO in ponds

The daily cycle of dissolved oxygen (DO) in aquaculture ponds is a natural phenomenon reflecting the complex interactions between aquatic organisms, environmental conditions and biological processes. This cycle is primarily influenced by the photosynthesis of aquatic plants and phytoplankton and the respiration of all organisms in the pond ecosystem.

- **In the morning**, DO concentrations are usually at their lowest. This is because photosynthesis, which produces oxygen, ceases at night due to a lack of light, while respiration, which consumes oxygen, continues. Consequently, it can drop to levels that may be stressful and even lethal for fish.
- **During the day**, as daylight emerges, aquatic plants and phytoplankton begin to photosynthesise, producing oxygen. This causes DO concentrations to gradually increase throughout the day, peaking in the early afternoon when photosynthetic activity is most intense.

- **In the late afternoon and night**, as the sun sets, photosynthesis decreases until it stops completely. However, the respiration of plants, fish, microorganisms and other aquatic organisms continues to consume oxygen. This causes DO concentrations to fall again, reaching their lowest levels late at night or early in the morning.

This daily DO cycle can be affected by several factors, including the density of plants and phytoplankton in the pond, water temperature and quality, fish density, and the intensity of sunlight.

11.4.9 Effects

- **Stress**
Low DO concentrations cause stress in fish, reducing appetite, growth and productivity and increasing susceptibility to infectious diseases.

11.4.10 Symptoms

- **Fecundity**
Fish kept in low DO concentrations show reduced fecundity and viability of eggs and sperm. Gametogenesis is also impaired.
- **Larval hatching**
The success of hatching and larval survival are severely affected by low DO concentrations.
- **Larval deformities**
Deformities such as micro-, mono-, or anophthalmia and hydration of the embryonic yolk sac and pericardium are observed under low DO conditions.
- **Fish at the surface**
When DO concentrations in water drop significantly, it is typical for juveniles and adults to rise to the surface to seek air directly from the atmosphere.
- **Colouration**
Fish colouration can change (stress colouration), and swimming activity may increase or decrease.
- **Growth**
Carp growth is impaired if DO drops to 25% of the saturation level.
- **Survival**
Low DO concentrations can result in mass mortalities in aquaculture conditions.
- **Supersaturation**
Excessively high DO concentrations (> 20 mg/L) are toxic to fish, causing physiological

dysfunctions, including gas bubble disease and abnormalities in the development of fertilised eggs and larvae.

11.4.11 Control

- **Aeration and water agitation**

Mechanical aeration should be used in ponds and reservoirs to ensure conditions necessary for fish health. Aerators should be used efficiently, especially on hot, windless days. They can be turned on between 3 pm and 5 pm to increase DO concentrations or in the early morning to prevent them from falling to critical levels. On cloudy days, aeration can be advanced to maintain adequate concentrations.

- **Power of aerators**

The installed power of aerators should be proportional to the biomass of fish intended to be produced. For example, if the planned biomass is 8,000 kg/ha, the aerators should be sized so that their installed power is at least 5 hp. If the scheduled productivity is 10,000 kg/ha, they should have a power of at least 6 hp/ha.

- **Water renewal**

More frequent and smaller volume exchanges are more efficient for controlling water quality. When renewing pond water, the farmer should remove the less oxygenated water and replace the volume with new (more oxygen-rich) water.

- **Aerators installation**

Aerators should be installed and ready for use in ponds that do not have significant water exchange (below 5%/day) and receive high feeding rates (above 6 kg of feed per 1,000 m²).

- **Monitoring food consumption**

Observe the relationship between grass carp food intake and DO concentrations. When DO is below 3 mg/L, fish may stop feeding. Between 3 and 4 mg/L, food intake may be reduced by half.

- **Ponds design**

An intelligent way to promote more efficient natural aeration in cultivation ponds is in the ponds' design. They should be designed in such a way as to make the most of the winds. For this, it should be ensured that the most extended length of the ponds is parallel to the direction of the prevailing winds in the location.

11.5 pH

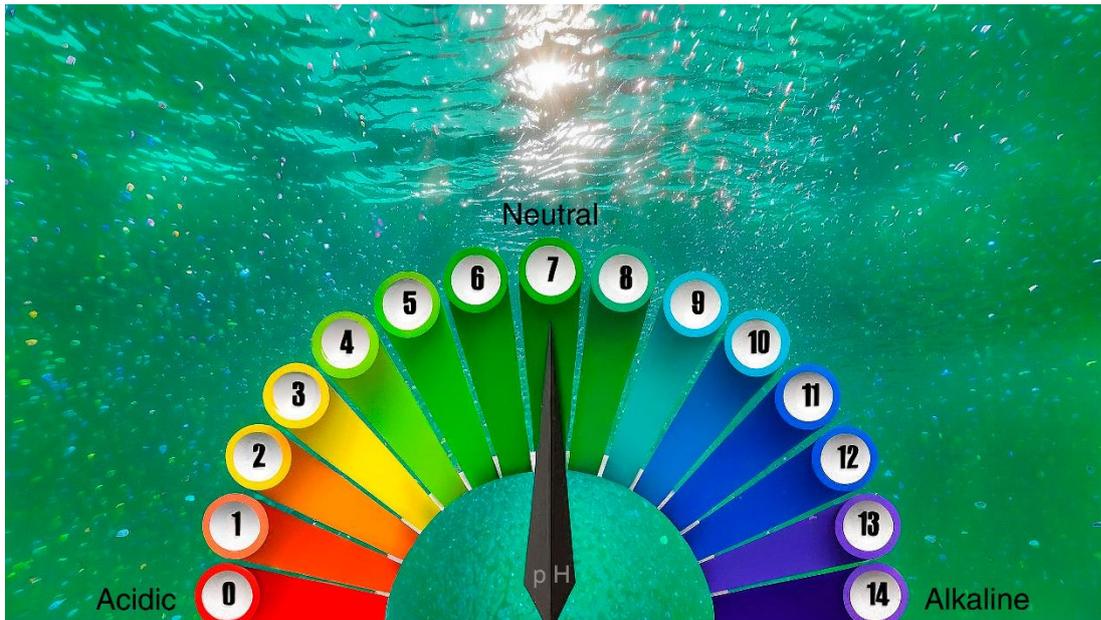


Figure 44. pH scale

11.5.1 What is it?

pH, or hydrogen potential, measures the concentration of H^+ ions in water. It seems more complicated than it is: pure water contains only oxygen and hydrogen in its formula (H_2O). It comprises positive ions (H^+) and negative ions (OH^-).



Positive ions (H^+) behave like acids, i.e., they make the water acidic. Negative ions have the opposite effect, i.e., they make the water alkaline (Figure 44).

11.5.2 Interactions

- **Alkalinity**

The most critical interaction of pH is with alkalinity, as these two factors largely determine the ionic character of water. Waters with low buffering capacity, i.e., low carbonate and bicarbonate alkalinity, are more susceptible to pH variations and, therefore, to changes in water quality.

- **Ammonia**

The influence of pH on ionisation is most notable in ammonia, where a 2-unit increase in pH

can result in a tenfold increase in the dissociation of the harmless ammonium ion (NH_4^+) to the highly toxic ammonia gas (NH_3).

- **CO₂ and H₂S**

The degree of ionisation of carbon dioxide and hydrogen sulphide is similarly affected by pH.

- **Metals**

The ionisation solubility, and chemical species of many aquatic toxins are controlled by pH. For example, the toxicity of heavy metals such as zinc, copper and aluminium is more common in acidic waters, as these metals are more soluble and prone to speciation.

11.5.3 Effects

- **Standard**

Most freshwater fish tolerate pH ranges between 6 and 9, provided other conditions, particularly ammonia concentration, are optimal. The toxic effects of pH above or below this range generally result from disturbances in the fish's ion balance.

- **Physiology**

Extreme pH fluctuations in the environment alter blood pH, affecting the physiological ability of fish to control ion loss through diffusion and reducing the capacity for ion influx across the gill surface. The direct effect, both at high and low pH, is a persistent decrease in plasma concentrations of essential sodium and chloride ions for the active transport of excretion products, whose accumulation is a component of the toxic syndrome.

- **Reproductive effects**

Acid stress can interfere with physiology, resulting in reproductive failures. Depression of pH below 6.5 disrupts calcium metabolism, affecting egg maturation and protein deposition in developing oocytes.

- **Asphyxiation**

Gill membrane damage caused by pH below 5 impairs gas exchange, ion regulation, and excretion, leading to hypoxia and mortality due to changes in blood chemistry and structural damage to the gills. Therefore, carp show signs of asphyxiation, with increased respiratory frequency when exposed to excessively acidic pH.

- **Excretion**

The toxic effects of high pH are related to the reduced ability to eliminate ammonia.

- **Stress**

Prolonged exposure to sublethal elevations or depressions in pH results in elevated stress levels, manifesting as low growth and reduced disease resistance. Recovery from pH stress

depends on restoring internal ionic balance, facilitated by adjusting the environmental pH to optimal values.

- **Mucus**
With pH variation at levels unsuitable for the species, fish skin and gills show excess mucus.
- **Other**
pH is a genuinely relevant parameter for aquaculture and for the welfare of farmed fish, as it regulates the speed of a series of chemical and biological reactions and processes that occur in ponds and the fish themselves, including the functioning of enzymes involved in numerous vital processes of carps; the concentration of toxic gaseous ammonia and the chemical forms of sulphur present in the water; the speed of decomposition of organic matter in the water or soil, among other processes.
- **Death**
When fish die, they remain with their mouths open and their eyes bulging, similar signs to those shown when death occurs due to lack of oxygen. This happens because the fish's gills are very fragile and sensitive, and acidic pH destroys the gill tissue and prevents gas exchange. In other words, the fish die of asphyxiation in these cases even though the water is rich in dissolved oxygen.

11.5.4 Variation

- **Acid, neutral, alkaline**
The pH of water can range from 0 to 14. Pure water, without any other element, has a neutral pH (7). This is because it has a perfect balance of positive and negative ions. However, with the presence of other compounds in the water (carbon dioxide, carbonates, nutrients, etc.), this balance is disrupted. The balance can shift towards the acidic side (0–6.9) or the alkaline side (7.1–14) depending on the elements present.
- **pH unit**
A decrease of one unit in pH represents a tenfold increase in the concentration of hydrogen ions, while an increase of one unit in pH indicates a tenfold increase in the activity of hydroxyl ions.

11.5.5 Water source

Measuring pH is essential when assessing a potential water source for fish or investigating fish health problems, as the effect of pH is often observed through the likelihood or severity of other water quality issues.

- **Surface waters**
The pH of surface waters depends on the physical and chemical properties of the geological formations they come in contact with, such as soils and rocks. Low pH values are caused by

mineral acids, typical of streams and dams in igneous regions, and are also found in waters rich in organic acids produced by plant decomposition. Low pH values can also originate from anthropogenic sources, such as industrial effluents and acid rain. Usually, precipitation is acidic due to carbon dioxide, but in areas polluted by industrial emissions of sulphur dioxide or nitrogen oxides, the pH of rain can decrease even further. The pH of surface waters rich in aquatic vegetation can vary daily due to photosynthetic activity.

- **Groundwater**

Most groundwater contains dissolved carbon dioxide, bicarbonate and carbonate, with pH values between 5 and 8. However, groundwater in contact with silicate minerals has a lower buffering capacity, carbon dioxide concentration, and pH lower than water from carbonate rock deposits. Groundwater with a high pH (> 8.5) often has a high sodium carbonate content.

11.5.6 Cycle

- **Ponds**

Daily pH fluctuations are common in pond cultivation conditions, with the intensity depending on the density of phytoplankton. The ideal to ensure high welfare for grass carp is a water pH of 7.0 to 8.5.

- **pH and CO₂**

As the intensity of sunlight begins to decrease, the photosynthesis performed by phytoplankton also reduces, and less CO₂ is removed from the water. At night, as fish, bacteria, and mainly plankton respiration add more CO₂ to the water, the pH begins to fall. Therefore, pH peaks in cultivation ponds are usually reached in the late afternoon, while the minimum values occur in the early morning.

11.5.7 Interactions

- **Enzymes**

Enzymes are proteins that all living beings possess, and their function is to accelerate the speed of reactions occurring in the organism, such as those associated with obtaining and using the energy present in feeds and natural foods. All fish enzymes operate within quite specific pH ranges. Therefore, if the pH of the fish's blood varies, the enzymes may stop working, and the metabolism of these animals can collapse, leading to death.

- **Osmotic balance**

Changes in the pH of the cultivation water can also affect the fish's ability to maintain the internal salt balance. Therefore, extreme values or rapid pH variations can cause shock and massive mortalities, especially in early life stages.

- **High pH**

In ponds with an excess of phytoplankton (very green waters) and low total alkalinity (< 20 mg as CaCO₃/L), the pH can rise to values above 11 in the late afternoon, particularly following an exceptionally sunny day. This increase can hinder food consumption and impact fish growth. While direct mortality due to this elevation in pH is typically not observed in ponds, as fish tend to seek refuge in more deep, more acidic waters, the rise in pH heightens the toxicity of ammonia. Consequently, this makes fish more vulnerable to acute poisoning, diseases, handling and transport challenges.

11.5.8 Measurement

- **Methods**

pH measurement can be done using colorimetric or potentiometric methods, using either portable analysis kits or an electronic device called a pH meter (Figure 45).

- **Colorimetry**

Colorimetric measurement uses weak organic acids and bases, whose colour changes with pH. Commonly used indicators show a distinct colour change in a 1-unit pH range. Many portable test kits are based on colorimetric pH measurements. However, the low resolution of this method limits its application to more general purposes.



Figure 45. Portable pH meter.

11.5.9 Technique

- Measure the pH by collecting water about 20 cm from the surface.
- If possible, measure the pH twice daily, in the early morning (around 6:00–7:00 am) and the late afternoon (around 5:00 pm).
- The water pH can be lowered using aluminium sulphate. However, the amount used will depend on the water's alkalinity. The higher the alkalinity, the greater the amount of aluminium sulphate needed. As this is an expensive product, its use can also increase production costs. On the other hand, increasing the pH of pond water can be done using products like hydrated lime and even limestone.

11.5.10 Control

- **Alkalinity increase**
Controlling pH variations depends on the water's buffering capacity. Increasing alkalinity by adding lime or calcium carbonate in neutral or acidic waters increases the buffering capacity and the pH.

11.6 Alkalinity

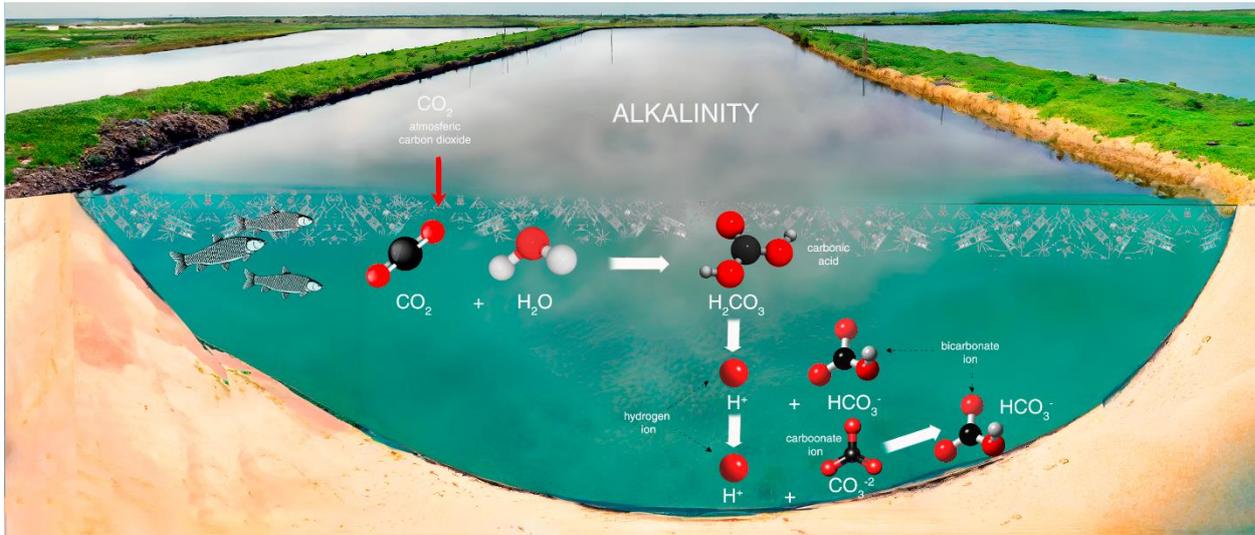


Figure 46. Alkalinity in the pond.

11.6.1 What is it?

- **Definition**

Alkalinity is a measure of the water's capacity to neutralise strong acids and is a summarised measure of the water's anionic character. For ease of understanding, alkalinity can also be seen as the water's concentration of carbonates and bicarbonates (Figure 46).

- **Carbonates and bicarbonates**

Alkalinity is mainly attributed to the presence of bicarbonate (HCO₃⁻), carbonate (CO₃²⁻), and, at high pH, hydroxide ions (OH⁻). As water's alkalinity is governed by the concentration of these conjugate base ions, which are most often bicarbonate, bicarbonate alkalinity is the most common form among the three. At pH values below 8.3, bicarbonate ion concentration predominates, while at pH values above 8.3 and 9.6, carbonate and hydroxide ion concentrations are significant, respectively. Bicarbonate alkalinity is almost zero at pH 4. Other ions contributing to water alkalinity include borates, silicates, phosphates and organic bases.

- **Alkalinity vs. pH**

Many confuse alkalinity with alkaline pH, but the two differ. Technically speaking, alkalinity is defined as the 'measure of the concentrations of titratable bases (mainly, carbonates and bicarbonates) present in the water'. On the other hand, alkaline pH, as previously explained, is higher than 7.

11.6.2 Units

- **Most common units**

Alkalinity is expressed in parts per million (ppm) or milligrams per litre (mg/L) as calcium carbonate (CaCO₃).

- **mEq/L**

Alkalinity can also be expressed in milliequivalents per litre (mEq/L).

11.6.3 Reference values

- **Low alkalinity waters**

Waters with an alkalinity below 20 mg CaCO₃/L are considered less suitable for aquaculture due to unstable water chemistry.

- **High alkalinity waters**

The upper limit of alkalinity is defined mainly by the individual needs of the species and the magnitude of the concomitant increase in the pH value. Freshwater fish in waters with 100–150 mg CaCO₃/L expend less energy on osmoregulation, resulting in better growth.

However, the upper limit of alkalinity can also be related to its effect on osmoregulation at high ionic concentrations. The recommended range for grass carp is 25 to 100 mg/L CaCO₃.

11.6.4 Interactions

- **Water composition and temperature**

The alkalinity of water depends on the activity and ratio of acid and base ions, influenced by the water's complete chemical composition and physical parameters, such as temperature.

- **Metals**

Alkalinity indirectly affects metal speciation and the formation of metal complexes, influencing metal bioavailability.

- **Ammonia and nitrite**

The nitrification of ammonia into nitrite and then into nitrate produces acid that, unless the system is adequately buffered, can cause the pH to drop below 6.

11.6.5 Importance

- **Fish physiological functions**

Although alkalinity has no direct effect, it is an essential criterion for determining the impact and concentration of other constituents and water quality criteria and, therefore, the overall suitability of a water source for fish cultivation.

- **Buffering power**

Although other essential reasons exist, alkalinity actively buffers the pH of water. In other words, alkalinity prevents the water's pH from varying too much or too quickly. The higher the water's alkalinity, the greater its 'buffering power', i.e., its ability to prevent pH variation (Figure 47).

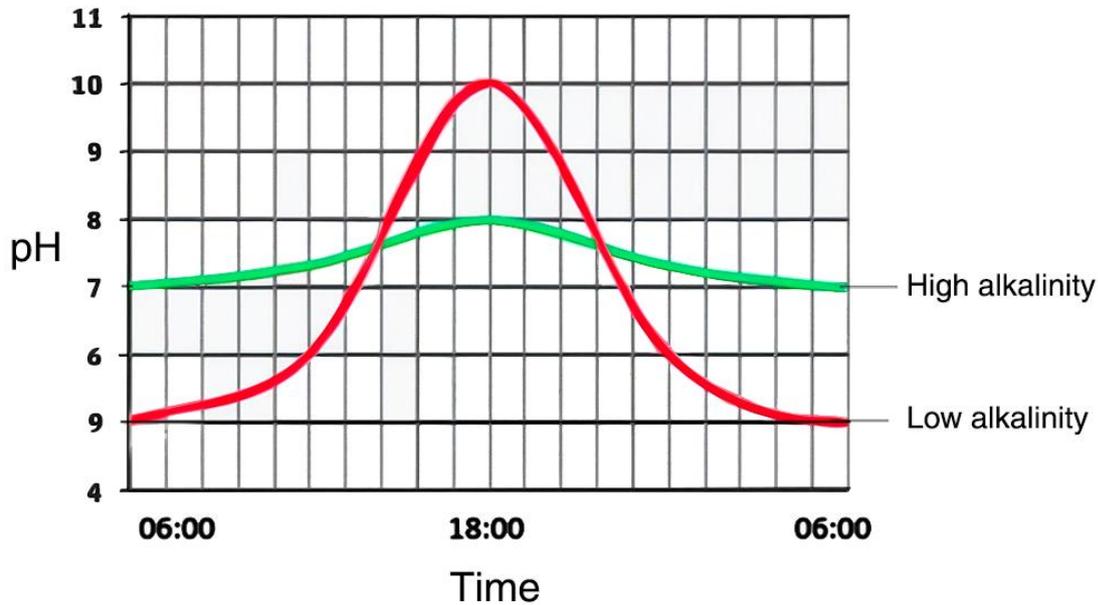


Figure 47. pH Variation in pond water concerning alkaline reserve (alkalinity).

- **Respiration**

As explained, fish, plankton and bacteria absorb O₂ when they breathe and release CO₂, which forms carbonic acid in water and dissociates to form carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻) ions, releasing H⁺ ions, according to the chemical equation below. On the other hand, the higher the alkalinity, the less the water's pH tends to vary.



- **CO₂**

Carbon dioxide is an essential gas for phytoplankton to perform photosynthesis and thus sustain the natural food chain in carp cultivation ponds. The ideal alkalinity for carp cultivations varies from 20 to 100 mg/L CaCO₃. This range is sufficient to maintain water pH within the carp's comfort limits and successfully produce natural foods. Values below 25 mg/L CaCO₃ can compromise fish welfare, as it becomes more difficult to prevent water pH variation at levels that will affect the health of the animals.

11.6.6 Measurement

Titrimetric kits

These are the most common for measuring alkalinity. They work by adding a known acid concentration to the water sample and measuring the amount of acid required to reach a certain pH turning point, indicated by a colour change. This method is considered accurate and is widely used in aquaculture.

Colorimetric kits

Although less standard for alkalinity than other water parameters, some can be used to measure alkalinity. They function by changing colour based on the concentration of specific ions in the water but are generally less accurate than titrimetric methods for alkalinity.

Acid titration

This method involves adding a known acid concentration to the water sample until a colour turning point indicates a pH change. The volume of acid used to reach this point is proportional to the water's alkalinity. This method can be performed manually or with an automatic titrator.

Digital meters

Some digital meters can directly measure the water's alkalinity. These devices are more expensive than other methods but provide rapid and precise readings.

Alkalinity test strips

Similar to pH test strips, these strips change colour when immersed in water. The resulting colour is then compared to a scale to determine the alkalinity (Figure 48).



Figure 48. Titration kit for determining water alkalinity.

11.6.7 Control

- **Monitoring**

It is recommended that alkalinity analysis be conducted: 1) immediately after filling a pond; 2) two weeks after applying lime; 3) at least once a month in all ponds, for monitoring purposes; and 4) whenever there are significant pH variations (above 3 units) or when the pH is above 9.

- **Low alkalinity**

If the alkalinity is very low, one can attempt to correct the issue by adding products containing carbonates (limestone is the most common and inexpensive) or bicarbonates to the water. The amount should be scaled according to the water volume of the pond. Limestone, a common source of CaCO_3 , is not very soluble in water. Its dissolution rate increases with smaller particle sizes, although the total alkalinity typically does not exceed 50–60 mg CaCO_3/L through artificial liming.

- **Ca(OH)_2**

Hydrated lime can also be applied (in portioned amounts not exceeding 150 kg/ha/day) for the same purpose. Hydrated lime increases the water's pH and transforms CO_2 into bicarbonate ions.

- **Advantages**

Controlling alkalinity allows the producer to make fewer water changes to maintain water quality and, in addition to saving energy, promotes a higher degree of fish welfare.

11.7 Colour, turbidity and transparency



Figure 49. Colour, turbidity and transparency of water are all properties related to light.

In aquaculture there is often confusion between water colour, turbidity and transparency, though each describes different aspects of water quality all directly related to light (Figure 49).

Water colour is influenced by dissolved substances, such as organic compounds, while turbidity is related to the number of suspended particles that scatter light. On the other hand, transparency refers to the depth at which objects can be seen in the water. Although all these factors are essential for monitoring pond water quality, transparency is often considered the most practical indicator for day-to-day fish farming. It provides a quick assessment of water quality and can indicate changes in the ecosystem dynamics of the pond, such as algae growth or the presence of sediments, directly affecting the health and behaviour of the fish.

11.7.1 What is it?

- **Colour**

Water colour can be defined as how light, reflected or absorbed by water, stimulates our eyes, and is interpreted by our brain. Water colour is a qualitative characteristic indicating the presence of dissolved or suspended substances that affect the water's visual appearance. It can range from colourless to shades including yellow, brown and green, depending on the chemical and biological composition of the water. These colours are generally the result of the presence of natural organic compounds, such as humic and fulvic acids, minerals, phytoplankton, and other microorganisms. Water colour is an important indicator of water quality, influencing processes such as aquatic photosynthesis, and may indicate alterations in aquatic ecosystems, pollution or changes in nutrient dynamics. In aquaculture environments, water colour can be monitored to ensure optimal conditions for the health and growth of aquatic organisms.

- **Turbidity**

Turbidity refers to the measure of water clarity or clearness, influenced by the number of suspended particles that scatter and absorb light. Therefore, turbidity is a measure of light scattering caused by particles suspended in the water. The greater the suspended particles, the higher the turbidity. It is measured using a turbidimeter (Figure 50) and expressed in Nephelometric Turbidity Units (NTU). The particles that cause water turbidity can be of organic origin, such as algae and plant detritus, or inorganic, like sediments and mineral particles. Turbidity can be an important indicator of water quality, as elevated levels may reduce the penetration of sunlight, affecting processes like photosynthesis and the habitat of aquatic organisms. Furthermore, high turbidity can signal the presence of pollutants and the deterioration of environmental conditions. Effective turbidity management is essential for maintaining the health and welfare of fish and other aquatic organisms in fish cultivation ponds.



Figure 50. Turbidity meter.

- **Transparency**

It is a measure, usually in centimetres, of the light penetration capacity in water. The more transparent the water, the deeper the light can penetrate. Transparency can be affected by several factors, including the presence of suspended particles, plankton, sediments or dissolved substances. In aquaculture water transparency is a vital indicator, as it affects the penetration of sunlight necessary for the photosynthesis of aquatic plants and algae and can be a sign of the health of the aquatic ecosystem, directly influencing the life of the cultivated organisms.

11.7.2 Light in fish farming ponds

The transparency of most interest in fish farming is directly related to the presence or absence of plankton in the pond water. Plankton is also a type of suspended particulate material that alters the pond's water colour, increasing turbidity and decreasing transparency. This type of turbidity, as long as it is not excessive, is desirable, as it means more natural food and less expenditure on feed.

As the concentration of microalgae increases, the water in the pond undergoes successive changes in its colouration. Initially, the colour of the pond water is similar to that of the river, or the supply channel used for its capture. As microalgae populations increase this colouration becomes more intense, essentially defined by the colour of the predominant microalgae, generally tending towards green or brown.

11.7.3 Colour

- **What causes it?**

The colour of water is a physical characteristic that depends on sediments, suspended particulate material, and dissolved salts and organic compounds.

- **Is water colourless?**

Pure water, viewed in a glass in a well-lit environment, appears completely colourless. However, when a large quantity of the same water is placed in a larger environment, such as a pool, it seems to acquire a bluish tint. This phenomenon is caused by the absorption and scattering of sunlight that reaches the water's surface. Moreover, rainwater or river water is not pure, as it contains many dissolved and suspended substances, which can also affect its colour and transparency.

11.7.4 Turbidity

- **Types**

There are two basic types of turbidity in ponds: one effectively resulting from microalgae bloom (phytoplankton) and the other caused by suspended sediment particles (mud).

- **Sediments**

Turbidity due to a high load of fine suspended sediments generally harms fish cultivation. This turbidity prevents light penetration into the water and the development of phytoplankton. The suspended mud particles can also contain large amounts of associated organic matter, leading to the development of bacteria that consume large quantities of oxygen to decompose this organic matter.

- **Reducing turbidity**

In some cases, it may be necessary to reduce the load of suspended particulate material entering the ponds beforehand. One way to do this is by constructing the supply channel in a zigzag pattern to reduce the speed of the incoming water and force the settling of heavier particles. Another alternative is filtering the water through screens or sand and gravel filters placed in the supply channel or at the entrance of the ponds.

11.7.5 Transparency

- **Light is energy**

Light is essential for all living beings, especially for chlorophyll organisms and, in particular, for phytoplankton. That is why transparency is an enormous factor in fish farming. In ponds, the primary source of light is sunlight.

- **Absorption of light 1**

The greater the amount of material dissolved or suspended in the water, the greater the

amount of light that will be absorbed. The more significant the fraction of light absorbed, the lower the transparency of that water.

- **Absorption of light 2**

Water transparency in a pond decreases with depth and turbidity. The deeper the pond and the muddier the water, the less light can reach the bottom, as it is absorbed or refracted (diverted from its original direction) before that.

11.7.5.1 Secchi disk

The Secchi disk, a device used to measure water transparency, is a disk, usually made of plastic, 25 cm in diameter, painted black and white in opposite quarters. The disk can be attached to a graduated rope or a tape measure for measurement and a weight to make it sink (Figure 51). The disk can also be attached to a rigid graduated rod, ideal for shallow depths.

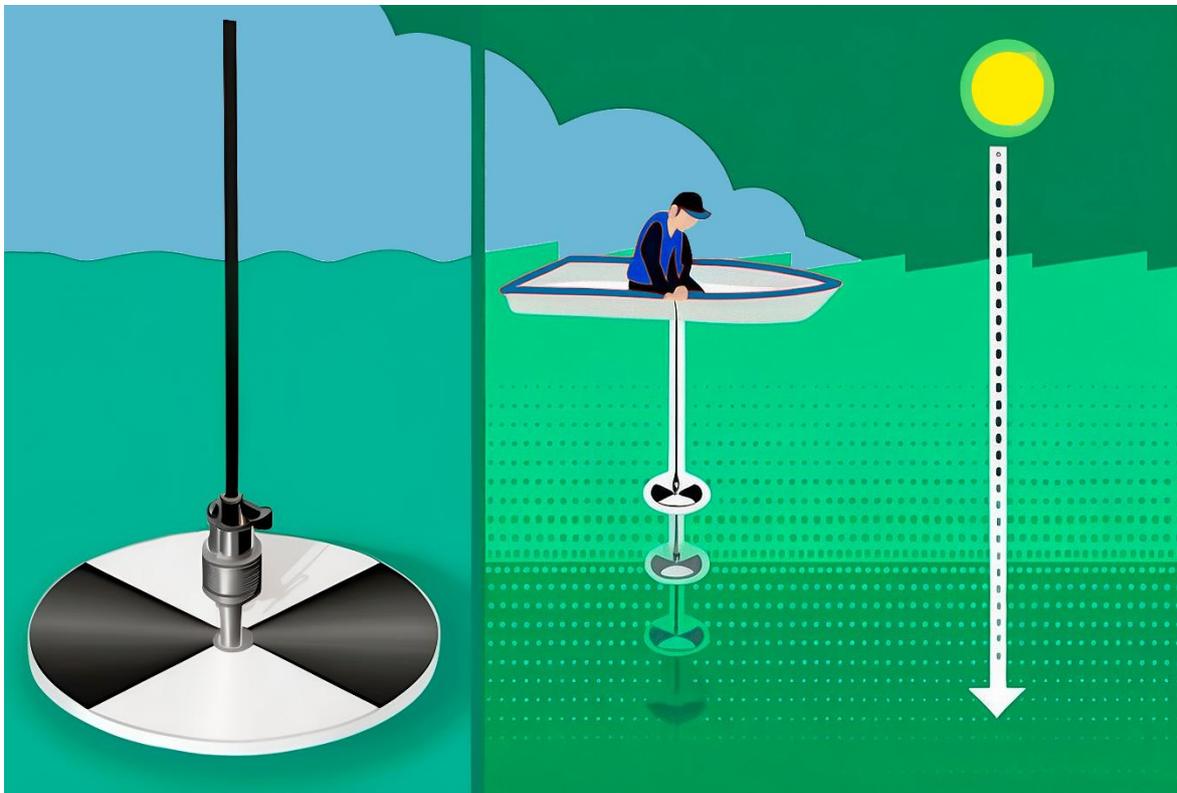


Figure 51. The Secchi disk is an easily constructed device for monitoring water transparency in ponds.

- **Interpretation**

As long as the turbidity is caused by the presence of microalgae and not mud, the ideal measure of water transparency in grass carp cultivations is between 30 and 60 cm. Very reduced transparencies (less than 24 cm) may indicate very high concentrations of phytoplankton, increasing the risk of sharp drops in oxygen concentrations at night. Very high transparencies (above 65 cm) indicate waters that are very poor in natural foods. Moreover, the carp's welfare can be compromised by the excess of ultraviolet light, which can

cause severe burns on the skin, and by the greater exposure of the fish to predators in these cases.

11.7.5.2 Measurement

- Water transparency should be measured daily in each pond, preferably between 10:00 am and 2:00 pm and at sunny moments.
- The technician should stand with their back to the sun and lower the disk into the water, observing it from above and ensuring their own shadow does not interfere with the measurement. Then, they record on a spreadsheet the depth at which they can no longer see the disk. At that moment, they can also note information about the water colour (green, muddy, greyish, tea-coloured or colourless, for example).
- If the transparency is excessively high, sunlight can reach the bottom and stimulate the growth of unwanted plants (macrophytes) or filamentous algae, which can take over the entire pond.
- On sunny days and in ponds with high densities of phytoplankton (ponds with transparency below 30 cm), the water tends to become supersaturated with DO. The problem is that oxygen concentrations can drop to critical levels at night due to increased consumption by phytoplankton and other organisms in the pond. Pond management needs to be done in a way to avoid extreme situations.

11.7.5.3 Control

- If the transparency is below ideal and the water is very greenish, the farmer should reduce the food provided until its condition improves and promote partial water exchange.
- In case of erosion of the slopes, control should be implemented by planting grass or other appropriate vegetation on the sides.
- In case of runoff reaching the pond, contour ploughing or even diversions can be made to prevent the entry of water-containing sediments.
- The area should be fenced off if there are animals (e.g. cattle, horses) on the property to prevent them from accessing the ponds.
- Agricultural gypsum can be used, in a concentration of 750–1,250 kg/ha, every two weeks to reduce the amount of suspended sediments (mud). This procedure may be necessary to increase the amount of natural food in the pond.
- Other products for this purpose include manure (500–1,000 kg/ha), limestone (2,000–5,000 kg/ha), and aluminium sulphate ($Al_2(SO_4)_3$ – 200–500 kg/ha).

11.8 Nitrogen cycle

In aquaculture, nitrogen almost invariably originates from the proteins in the feed provided to the farmed animals. Nitrogen, in turn, is essential for phytoplankton and zooplankton, bacteria and farmed fish. A portion of the nitrogen returns to the environment as excretion products from the carps, such as urine and faeces, and also through the protein present in the uneaten feed residues. As organisms die and decompose, the nitrogen in their tissues also returns to the water. Thus, nitrogen concentrations in the water and soil tend to increase continuously during fish cultivation.

Nitrogen is present in water in the form of protein, ammonia ($\text{NH}_3 + \text{NH}_4^+$), nitrite (NO_2^-), nitrate (NO_3^-) and gaseous nitrogen (N_2). A specific group of bacteria (*Nitrosomonas*) degrades proteins and releases the nitrogen present in them, in the form of ammonia. Ammonia, in turn, is a highly toxic compound for fish.

Once released, ammonia begins to undergo a biochemical oxidation process, carried out by another specific group of bacteria (*Nitrobacter*), which are transformed into nitrite (which is also quite toxic for fish) and subsequently into nitrate (which is much less harmful), in a process that consumes large amounts of DO (dissolved oxygen) (Figure 52). Finally, denitrifying bacteria transform nitrate into gaseous nitrogen, which tends to diffuse from the water into the atmosphere. This last process, however, is of little importance in aquaculture ponds, with the elimination of nitrogenous compounds more commonly achieved through water renewal in the cultivation system.

In the absence of oxygen, however, anaerobic bacteria reverse the process, transforming nitrate into nitrite and then back into ammonia. In a situation like this, with low DO concentrations and high ammonia concentrations, the carp's growth, survival, and welfare can be seriously compromised.

There are also opposite situations where the number of nitrogenous compounds in the pond water is below what is necessary to maintain the natural food production chain. In this case, adding nitrogenous compounds (in the form of fertilisers) directly into the water may be required to correct this problem and stimulate plankton growth.

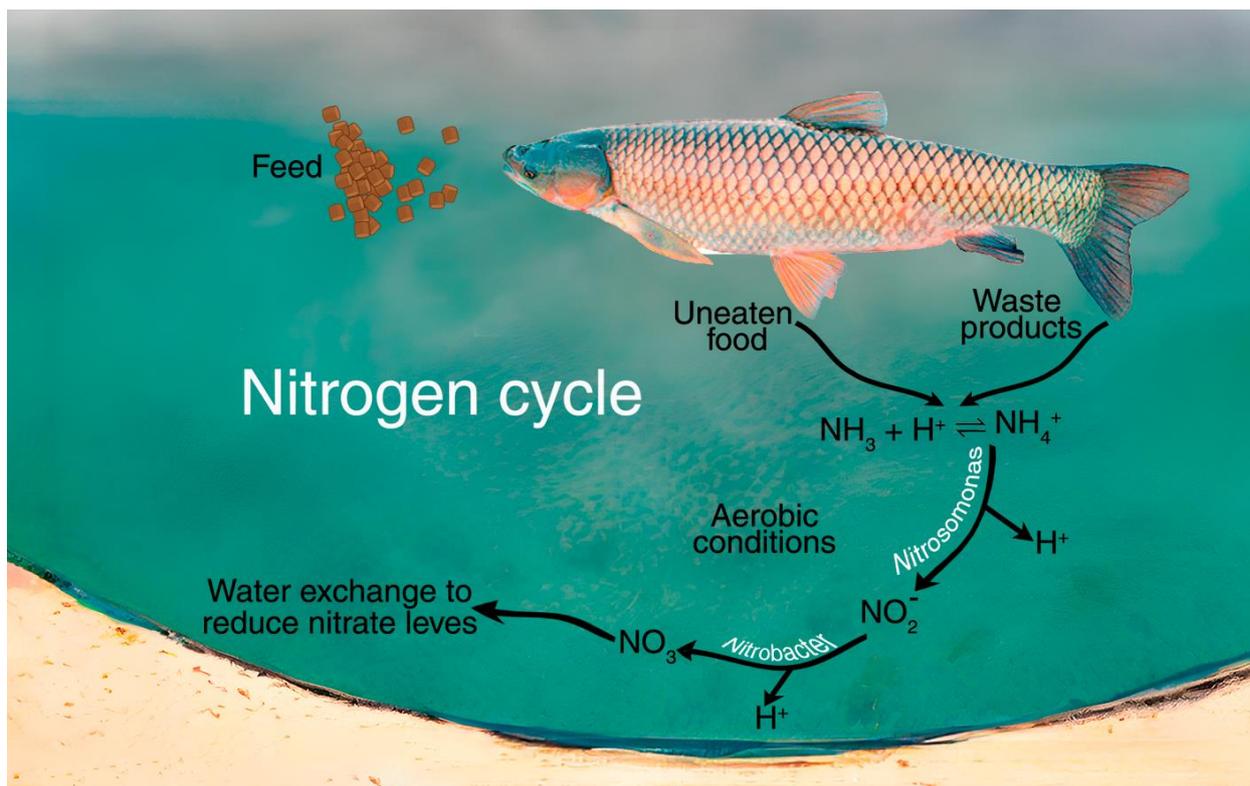


Figure 52. Schematic representation of the nitrogen cycle in grass carp cultivation ponds. Feed is the fish's primary protein source, and the uneaten remnants and the fish's excretion products (faeces and urine) are released in the form of ammonia, which is highly toxic to the fish. In oxygen-rich environments, the bacteria *Nitrosomonas* and *Nitrobacter* convert this ammonia into nitrite and nitrate, which is less harmful.

11.8.1 Ammonia

11.8.1.1 What is it?

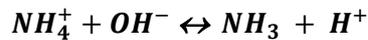
Ammonia is a by-product of the metabolic process by which fish break down proteins obtained through feeding. It is produced mainly in the muscles and liver of fish and by bacteria in the intestine. Most of this ammonia (about 90%) is eliminated by the gills of freshwater fish.

11.8.1.2 Ammonium vs ammonia

Ammonia is a compound considered to be one of the primary pollutants of aquatic systems. It is highly soluble in water and reacts with it to form ammonium ions.

Besides the processes described earlier, ammonia can also reach a cultivation system directly through the water used to supply tanks and ponds if this water is contaminated by industrial or agricultural effluents rich in nitrogen.

Ammonia is found in two chemical forms in water: ionised (ammonium ion, NH_4^+) and un-ionised (or gaseous ammonia, NH_3). The sum of these two forms is called total ammonia ($\text{TA} = \text{NH}_3 + \text{NH}_4^+$). Both the ammonium ion and gaseous ammonia are toxic to fish. However, the gaseous form (NH_3) is more toxic precisely because it can pass directly from the water to the fish's blood during respiration, which does not happen with the ammonium ion. The chemical dissociation reaction of ammonia is represented by:



When the pH is alkaline, the equation shifts to the right, and the proportions of NH_3 increase. The more alkaline the pH, the higher the percentage of gaseous ammonia in the water and the greater its toxicity to fish.

11.8.1.3 Interactions

- **pH**
The main factor controlling the toxicity of ammonia is pH, which, along with temperature, determines the proportion of un-ionised ammonia. Un-ionised ammonia (NH_3) increases with the rise in pH and temperature, which can be significant in pond cultivation conditions where the photosynthetic activity of phytoplankton causes an increase in pH.
- **Dissolved oxygen**
Low concentrations of DO (dissolved oxygen) increase the toxicity of NH_3 (ammonia).
- **CO_2**
High concentrations of free carbon dioxide in the water result in a reduction of pH and, therefore, a decrease in ammonia's toxicity.
- **Salinity**
Higher salt concentrations in the water reduce the fraction of NH_3 .

11.8.1.4 Excretion

- **Gills**
Ammonia is a toxic compound and thus needs to be eliminated from the fish's body; most of it is excreted through diffusion across the gills.
- **Accumulation**
As ammonia concentrations in the water increase, fish experience a reduced ability to excrete ammonia, so that it accumulates in their bloodstream. This has a toxic effect on the central nervous system, which can be detrimental to the stability of enzymes and membranes, the health of the gills, and the organism's balance.

11.8.1.5 Effects

- **Susceptibility**
Generally, juveniles are more sensitive to high concentrations of NH_3 than adults or even eggs.
- **Acute toxicity**
Concentrations of NH_3 above 1.0 mg/L for short periods affect feeding behaviour, swimming activity and balance, increase respiratory rate, and can cause fish death.
- **Chronic toxicity**
Prolonged exposure to concentrations greater than 0.06 mg/L can cause a reduction in egg-hatching success. Such concentrations can also interfere with growth, disease resistance and fecundity. Concentrations of NH_3 above the species' tolerance limits can endanger fish survival.
- **Other observed effects**
Other effects can include osmotic imbalance (damage to osmoregulation), altering nerve impulse transmission and modifying blood parameters. These effects can lead to excretion difficulties, ammonia accumulation in the muscles and blood, and respiratory problems. Ammonia can also induce changes in cellular pH, impacting enzymatic activity and potentially leading to death. Increased stress indicators in the blood, damage to the gills, inflammation, and damage to tissues and blood vessels, along with increased susceptibility to infections, are also observed.

11.8.1.6 Measurement

- **Colorimetric methods**
These are the most common and accessible methods, using chemical reagents that react with ammonia in the water, resulting in a colour change. The intensity of the colour is proportional to the ammonia concentration. Colorimetric test kits are practical for on-site use and are widely used in aquaculture.
- **Indophenol method**
A spectrophotometer is used to measure the colour intensity produced by the chemical reaction of ammonia with a specific reagent. This method is more accurate and is used mainly in laboratories. In the most widely used method, ammonia reacts with chlorophenol and hypochlorite in the presence of sodium nitroprusside to form indophenol blue, whose colour intensity is measured spectrophotometrically. This method is considered a reference standard for ammonia measurement.
- **Ion-selective electrodes (ISE)**
These are specialised probes that measure the concentration of specific ions (in this case, ammonia) in the water (Figure 53). Although more expensive, they offer rapid and precise measurements.

- **Reactive strip tests**

Paper strips, treated with chemical reagents, change colour when immersed in pond water, indicating specific water quality parameters. This method is quick and easy but less accurate than other methods.



Figure 53. Ammonia meter. The device already provides results in mg/L of NH₃.

11.8.1.7 Technique

- The methods used for ammonia analysis typically measure the total ammonia concentration (NH₄⁺ + NH₃). To determine the proportion of un-ionised ammonia, NH₃ (the most toxic form of ammonia), it is also necessary to measure the water's pH and temperature.
- The gaseous ammonia concentration can be calculated using the formula $NH_3 = (\text{total ammonia} \times \text{tabulated value}) / 100$.
- Compare the values found in [Table 27](#). In this table, find the pH closest to the value measured in the analysed sample. Then, find the temperature most proximate to the value measured in the sample. Multiply the total ammonia concentration indicated by the analysis kit by the value obtained in the table and divide this value by 100. The result will be the NH₃ concentration present in the analysed sample.

- For example, the analysis kit indicated the presence of 2 mg/L of total ammonia in the sample; the pH was 8, the water temperature was 26°C, and the NH₃ concentration = 2 (mg/l) x 5.7 (tabulated value) / 100 = 0.11. In other words, the concentration of toxic ammonia would be dangerously elevated from the point of view of the farmed fish's welfare.

Table 27. Percentage of un-ionised ammonia (NH₃) present in total ammonia (NH₄⁺ + NH₃).

		Temperature (°C)												
		20	21	22	23	24	25	26	27	28	29	30	31	32
pH	6.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	6.5	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3
	7.0	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.6	0.7	0.7	0.8	0.8	0.9
	7.5	1.2	1.3	1.4	1.5	1.6	1.7	1.9	2.0	2.1	2.3	2.5	2.6	2.8
	8.0	3.7	4.0	4.3	4.5	5.0	5.3	5.7	6.1	6.5	6.9	7.4	7.8	8.3
	8.5	11.0	11.8	12.5	13.3	14.2	15.1	16.0	17.0	18.0	19.0	20.1	21.2	22.4
	9.0	28.1	29.6	31.2	32.7	34.3	35.9	37.6	39.2	40.9	42.6	44.3	46.0	47.7
	9.5	55.3	57.1	58.9	60.6	62.3	64.0	65.6	67.1	68.7	70.1	71.5	72.9	74.2
	10.0	79.7	80.8	81.9	83.0	83.9	84.9	85.8	86.6	87.4	88.1	88.8	89.5	90.1

11.8.1.8 Control

- **Monitoring**

It is recommended to monitor ammonia and nitrite concentrations in the water at least once a week or whenever the amount of feed offered is greater than 60 kg per hectare/day.

- **Elimination**

It is tough to eliminate ammonia in huge ponds, as even water renewal does not usually yield immediate results, especially in areas distant from the water entry point in the pond. In this case the best approach is prevention, i.e., promoting pond management to prevent the problem from occurring in the first place.

- **Management**

However, if ammonia concentrations are elevated, the following can be done: 1) promote

water renewal; 2) increase aeration; 3) reduce pH; 4) suspend pond fertilisation; 5) temporarily suspend feed supply.

11.8.2 Nitrite

11.8.2.1 What is it?

Nitrite (NO_2^-) is an intermediate substance in transforming ammonia into nitrate in water, especially in fishponds. This process involves two main stages, called nitrification and denitrification, which are carried out by different types of bacteria. Thus, nitrite indicates that these processes are occurring in the pond.

In nitrification, certain bacteria that require a lot of oxygen, such as *Nitrosomonas* spp. and *Nitrobacter* spp., convert ammonia first into nitrite and then into nitrate. In denitrification, a reverse process, other bacteria that can live without oxygen use nitrate, transforming it into different substances while decomposing organic materials in oxygen-free conditions.

Nitrite is more prone to be detected in fish cultivation systems than nitrate and is usually a result of inefficient nitrification in systems with high nitrogen loading rates in the form of protein present in feeds and fresh foods or high stocking densities.

11.8.2.2 Interactions

- **DO**

Nitrification, an oxidation reaction, depends on the presence of dissolved oxygen. In conditions of low dissolved oxygen, which are not favourable for nitrification, nitrite tends to accumulate. Nitrite can also be produced from the reduction of nitrate.

- **Bacteria**

The nitrite accumulation can also be attributed to the differential colonisation of bacterial species. For example, the ammonia-oxidising bacteria *Nitrosomonas* group tends to colonise ponds more quickly than the *Nitrobacter* group, which oxidises nitrite. This is particularly evident when filling ponds or recolonising after treating fish with antibiotics. The *Nitrobacter* spp. bacteria are sensitive to environmental disturbances, especially temperature changes. A rapid decrease in water temperature can result in sudden nitrite accumulation. This effect can produce seasonal variations in nitrite concentrations in ponds subjected to low water temperatures in winter. Therefore, it is essential to monitor pond conditions to prevent imbalances that may lead to nitrite accumulation.

11.8.2.3 Effects

- **Toxicity**

Nitrite is also toxic to carp, but generally to a lesser extent than NH_3 . The safe concentrations of nitrite to ensure the welfare of carps range from 0.0 to 0.9 mg/L. Although carps can tolerate concentrations of 1 mg/L NO_2^- , their welfare may already be compromised in this concentration range. Above 1 mg/L of NO_2^- , there is a significant compromise in the degree of welfare and risk of fish death.

- **Methaemoglobinemia**

Haemoglobin is the pigment in the blood of fish which gives it a red colour. It is responsible for carrying O_2 that enters the bloodstream through the gills to the tissues and for removing toxic CO_2 , which is also eliminated from the fish's body through the gills. Nitrite in solution enters the fish's circulatory system through the gills. The amount of permeable nitrite depends on the fish species and the environmental pH. Nitrite in the blood oxidises the ferrous ion (Fe^{2+}) in haemoglobin to ferric ion (Fe^{3+}), resulting in methaemoglobin, which is incapable of reversibly combining with oxygen, thus impairing oxygen transport. This can cause asphyxiation and death to fish, even if DO concentrations in the water are high. Methaemoglobin has a characteristic brown colour, noticeable when its concentration in the blood exceeds 20 to 30% of the total haemoglobin, which has given nitrite poisoning the common name of 'brown blood disease' or methaemoglobinemia.

- **Clinical signs**

Signs of fish poisoning by nitrite include blood and gills turning a very dark red, almost brown colour; fish swimming sideways or staying still near the surface; seeking air at the waterline even when oxygen concentrations in the water are high; increased mortality rates.

- **Other effects**

At sublethal concentrations, nitrite poisoning presents as a stress response, leading to reduced productivity, activity and growth, as well as poor health. Higher concentrations result in acute anoxia, loss of balance and mortality.

11.8.2.4 Measurement

- **Colorimetric test kits** are the most common and accessible methods, beneficial for smaller aquaculture operations or quick testing. These kits typically involve the addition of chemical reagents to a water sample, which react with nitrite and change colour. The colour intensity indicates the concentration of nitrite.

- **Spectrophotometry**

This is a more precise method used in laboratories. It involves nitrite reacting with specific reagents to form a coloured compound, whose intensity is measured using a spectrophotometer.

- **Enzymatic methods**
Less common but highly precise, these methods involve specific enzymatic reactions that produce a colour change or a quantifiable signal when nitrite is present.
- **Ion-selective electrodes (ISE)**
These are more sophisticated and expensive devices that directly measure the concentration of nitrite ions in water (Figure 54).
- **Reactive strips**
Simple and fast, these strips change colour when immersed in water, indicating the presence and concentration of nitrite.
- **Interpretation of results**
The interpretation of nitrite concentrations should also be done in conjunction with measurements of alkalinity, pH, dissolved oxygen and temperature.



Figure 54. Nitrite meter.

11.8.2.5 Control

- **Salt**

In high nitrite concentrations, dissolving sea salt in the pond water is an alternative. Salt (NaCl) reduces the uptake of nitrite by fish. The amount of salt added should be sufficient to maintain a ratio of 6 to 10 chloride units (Cl⁻) for every unit of NO₂⁻. For example, for a concentration of 5 mg/L of NO₂⁻ in water, it would be advisable to add 83 g/m³ of sea salt to the pond water.

- **Feeding**

The feed should be sufficient to meet the nutritional needs of the fish but not excessive. Feeding should also be temporarily suspended when nitrite concentrations exceed 0.5 mg/L.

- **Renewal**

Increasing the daily water renewal rates of the pond is recommended. An effective way to combat nitrite accumulation is to increase the daily water exchange to about 10% of the pond volume.

- **Aeration**

High nitrite concentrations can be reduced by aerating the water with the other suggested measures.

12 REPRODUCTION IN CAPTIVITY

12.1 Introduction

Before the development of induced breeding techniques, rivers served as the sole source of grass carp larvae for aquaculture, as these fish do not naturally reproduce in captivity. In such cases, however, the collection of larvae or fry from rivers and lakes often resulted in an assortment of desirable and undesirable fish species, besides the fact that their availability was entirely subject to a series of uncertainties and wholly uncontrollable factors.

Today, there are various strategies for assisted reproduction of grass carp in captivity, aiming to ensure controlled and practical outcomes, and this is achieved through hormonal induction of spawning.

Through this method, females and males receive a hormone injection (using crude fish pituitary extract or synthetic hormones like HCG (human chorionic gonadotropin)) to stimulate ovulation and sperm development. Following this induction, breeders are placed in tanks for natural spawning, manually manipulated for egg collection, and then taken for incubation.

12.2 Broodstock management

When creating a genetic material bank, strict standards must be adhered to. Among the main recommendations for establishing a quality broodstock, we highlight:

- **Ponds:** The broodstock of grass carp can be grown in ponds with an average area of 2–3 hectares, where the filling and draining processes should ideally not exceed 3–4 days.
- **Age 1**
It is not advisable to keep fish of different ages in the same pond, as this can lead to growth retardation in older fish, which are more sensitive to feeding conditions.
- **Weight gain**
Selection for the broodstock should favour those fish that have shown the highest growth rate up to that point. Three-year-old fish are expected to exceed an average weight of 2 kg, and four-year-old animals should weigh over 3 kg. This is another reason not to mix many breeders of different ages. However, an alternative here is to tag the breeders to track their individual development.
- **Age 2**
A broodstock can, at least in theory, be used up to the 15th year of the fish's life, although the peak of fertility occurs around the 11th year. The challenge here is that large and heavy

fish are more difficult to handle and manipulate safely without compromising their welfare and even the dynamics of a reproduction laboratory, which can hinder the use of older fish.

- **Survival**

The survival rate of a broodstock for three-year-old carp should exceed 90%.

- **Feeding**

Inadequate feeding can lead to functional disorders that delay growth and maturation and, in extreme cases, even cause mortality. The diet should be based on high-quality commercial feeds, but it is also essential to provide green vegetation, whether aquatic or terrestrial in origin.

- **Density**

The density to be maintained in the maintenance ponds should be low (0.2 to 2.9 kg of fish/m²). This will allow the fish to develop their reproductive potential fully.

- **Broodstock size**

During the spawning period significant losses can occur as a result of excessive handling, potentially reaching up to 20% of the broodstock. Therefore, good management practices should be prioritised, avoiding stress and injuries while handling the animals. It is also prudent to have a reserve stock of females in the broodstock (since the handling of females is always significantly more extensive than that of males), which should be planned at a minimum of 50% and preferably 100% above what is necessary.

12.3 Selection of fish for breeding

Male and female breeders can be cultivated in the same tanks or ponds. However, before breeding, they must be collected (Figure 55), weighed and individually identified.



Figure 55. Collection of breeders from a pond, using a seine net, before induced reproduction in the laboratory.

Sex identification in carp can be challenging, as secondary sexual characteristics are not distinctly discernible, even during the breeding season. Some morphological features are listed in [Table 28](#), but they are unreliable.

Table 28. External distinguishing features of sexes of grass carp

Female characteristics	Male characteristics
Pectoral fins are relatively small and weak. The outermost ray is not very thick	Pectoral fins are relatively long and prominent, with well-developed, thick outermost rays
The inner surface of the pectoral fin, facing the body, feels smooth to the touch	The inner surface of the pectoral fin, facing the body, feels rough to the touch
The abdomen has a bulge extending past the pelvis to the genital aperture and is soft to the touch. There is no median ridge in front of the vent. Note: The bulge may also be due to fat deposits around the gut and is not a reliable indicator of mature gonad	The abdomen generally lacks a conspicuous bulge and is not very soft to the touch. A median ridge appears in front of the vent
The genital aperture is protruding, swollen and shows pinkish margins. Note: This method is challenging to apply to grass carp	The vent is not protruding and appears pit-like.
Ova are visible inside the genital aperture when pressure is applied to the abdomen. The vent may also be swollen and reddish (Figure 56)	Milky white milt can be exuded through the genital aperture by applying gentle pressure to the abdomen
The body appears stouter compared to males of the same age	The body seems thinner and more linear compared to females of the same age



Figure 56. Grass carp female ready for hormonal induction. Notice the swollen belly and the swollen and reddened genital opening.

A method used to determine sex and maturity level for breeding is the microscopic examination of an egg sample taken directly from the ovaries of females. This procedure uses a 2 mm diameter glass or plastic catheter, carefully inserted into the genital opening and guided to the end portion of the ovary to collect some oocytes (Figure 57). A sample is then aspirated into the glass tube and analysed under a magnifying glass or microscope. This invasive procedure must be conducted in full hygienic conditions, and the fish should have been previously anaesthetised, as with the fish in the figure.



Figure 57. Obtaining a sample for assessing the developmental stage of oocytes from a female.

From examining this sample, it is possible to observe that the yolk and the centre of each oocyte become visible within about 5 minutes. If the centre of the oocyte is more to the side, it is a good sign that the fish is ready to release the eggs. However, if the centre of the oocyte is right in the middle, it is less likely that the fish is prepared for reproduction, even with hormonal stimulation.

Important facts about sexual maturity include:

- **Maturity 1:** Males generally reach sexual maturity in a shorter lifespan than females.
- **Maturity 2:** Gonadal maturation occurs in still waters. Therefore, it is feasible to maintain a broodstock in ponds or even cages. However, without hormonal induction, the maturation process will not be completed, and the fish will not spawn.
- **Body weight:** The body weight at first sexual maturation varies between 3 and 8 kg.

12.4 Stages of gonadal maturation

The gonads of the grass carp go through seven stages of maturation, as detailed in Table 29. Ovarian development accelerates significantly from the third stage onwards as day length and water temperature increase. By Stage V, the weight of the ovary can represent 20 to 30% of the female's total body weight. In males, this rate varies between 5 and 10% at the same stage. In other words, females require much more energy than males throughout the reproductive process.

Table 29. Stages of gonadal maturation of grass carp.

Stage of maturation	Condition of gonad	
	Male	Female
I Immature	Transparent, long, narrow strip	Transparent, long, narrow strip
II Immature	Thickened strip, translucent	Thickened strip, translucent
III Maturing	Opaque, granular	Opaque, granular, somewhat greyish, occupying about one-third of the body cavity
IV Maturing	Dull greyish	Dull greyish in colour, testes discernible as granules, occupying about half of the body cavity (Figure 58)
V Mature	Dull grey to greenish	Dull grey to greenish, occupying almost the entire body cavity, oocytes distinctly round
VI Spawning	Testes soft and milky white, milt flowing out upon applying slight pressure on the belly	The ovarian wall has loose eggs that ooze through the genital aperture upon applying pressure on the belly
VII Spent	Testicle blood-shot, pinkish brown	Ovary blood-shot, pinkish brown mass but considerably shrunk



Figure 58. Oocytes of grass carp at stage IV of maturation.

12.5 Transport and handling of breeding stock

Transporting and handling broodstock, especially females, is a delicate task that requires attention and care. Below we discuss some techniques and fundamentals that help to ensure that fish are transported with as little stress as possible.

Sensitivity of broodstock

Females, in particular, are susceptible animals when they are 'pregnant' (pregnant is the term used in aquaculture to describe females with eggs). It is, therefore, crucial never to transport them roughly, to use fishing nets for this purpose or use bags with several animals 'piled up' inside.

Transport stretcher

To transport the broodstock, we recommend using a net of wood or iron pipes covered with tarpaulin or other waterproof material. The tarpaulin should be 85 cm long, 30 cm deep and 15–17 cm wide. The feet of the structure are 45 cm long, and the carrying handles are 60 cm long. The broodstock should be placed carefully in the transport net with water, avoiding sudden movements that could cause stress or injury.

Weighing

When weighing breeding stock, the process should be quick and careful to minimise stress.

12.5.1 Recommendations for transporting breeding stock²

Table 30. Indicators for the transport of adults from carp grass.

Category	Indicators	Level	Values of reference
Environmental	Temperature (°C)	Optimum	20–25
		Acceptable	15–19 or 26–33
	pH	Optimum	7.0–8.5
		Acceptable	6.0–6.9 or 8.6–9.0
	Oxygen saturation (%)	Optimum	72–96
		Acceptable	48–71

² The tables in the 'Recommendations' section show welfare indicators for each stage of the grass carp production process. The aim of including these tables is to provide scientifically based guidelines (Pedrazzani et al., 2022) for optimising the welfare of farmed fish. The tables present two levels of recommendation for each indicator: 'Optimum' and 'Acceptable'. The 'Optimum' level aims to guarantee the maximum welfare of the fish in relation to the specific indicator, while the 'Acceptable' level, although it may compromise their degree of welfare, would not cause their death.

Category	Indicators	Level	Values of reference
	Stocking density (kg/m ³)	Optimum	≤ 300
		Acceptable	301–400
	Non-ionised ammonia (NH ₃)	Optimum	0.000–0.025
		Acceptable	0.026–0.059
Sanitary	Skin	Optimum	Apparently, normal, healthy and bright
		Acceptable	Pallor, darkening, loss of scales or localised injury
	Fins	Optimum	normal appearance, healthy
		Acceptable	Light lesions or splitting
	Mortality (%)	Optimum	0–10
		Acceptable	11–20
Nutritional	Fasting time (hours) – diet feed	Optimum	12–24
		Acceptable	8–11
	Fasting time (hours) – vegetable diet	Optimum	24–30 12–23 or 31–36 ≤ 11 or ≥ 37
Behavioural	Sedation for transport	Acceptable	Sedation (loss of reaction to touch and visual perception)
		Optimum	Light anaesthesia (loss of balance and normal swimming movement interspersed with irregular lateral swimming)
	Swimming behaviour after transport	Acceptable	Constant swimming
		Optimum	Erratic swimming, panting

12.6 Use of crude pituitary extract

The most common method for inducing carp reproduction is hypophysation. This process involves injecting mature male and female grass carp with extracts from the pituitary gland taken from other equally mature fish.

The pituitary gland releases gonadotropic hormones that regulate gonadal development and stimulate the ovaries and testes to release gametes (sperm and eggs).

The application of pituitary extracts in fish reproduction has been a standard practice in aquaculture for nearly a century, used to induce ovulation and spawning. This process involves using extracts prepared from the pituitary glands of fish, which may be from the same or different species, and administering them through injections.

12.6.1 Obtaining the pituitary glands

12.6.1.1 The pituitary gland

The pituitary gland is an endocrine gland (which means that the gland specialises in producing and secreting hormones directly into the bloodstream) located in the lower part of the fish's brain (Figure 59).

Pituitary glands can be obtained from freshly slaughtered donor fish or from fish well preserved on ice or in cold chambers. They can also be obtained from commercial suppliers, who sell them dried, which makes them easier to handle and store.

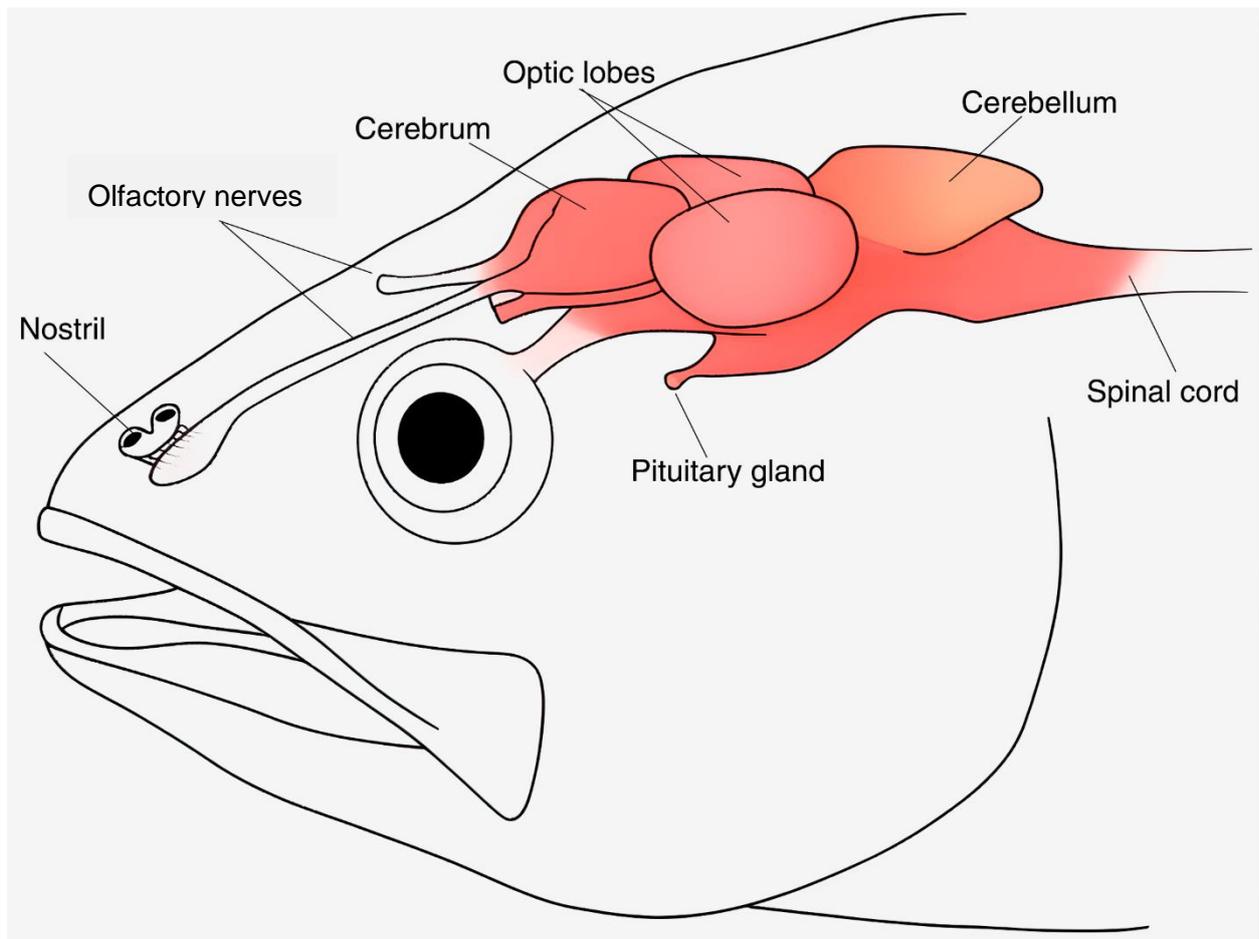


Figure 59. The location of the pituitary gland in fish.

12.6.1.2 Collection of the pituitary gland

The most efficient method for accessing the entire gland in a donor fish is to see or cut off the top of the skull of a previously humanely slaughtered animal (methods discussed in section 15.3 of this manual), thus exposing the brain. The brain should be carefully removed with tweezers, revealing the pituitary gland. The gland should then be removed with tweezers (Figure 60). It is crucial to be careful during this step to avoid damaging the gland, which could result in the loss of water-soluble gonadotrophins.

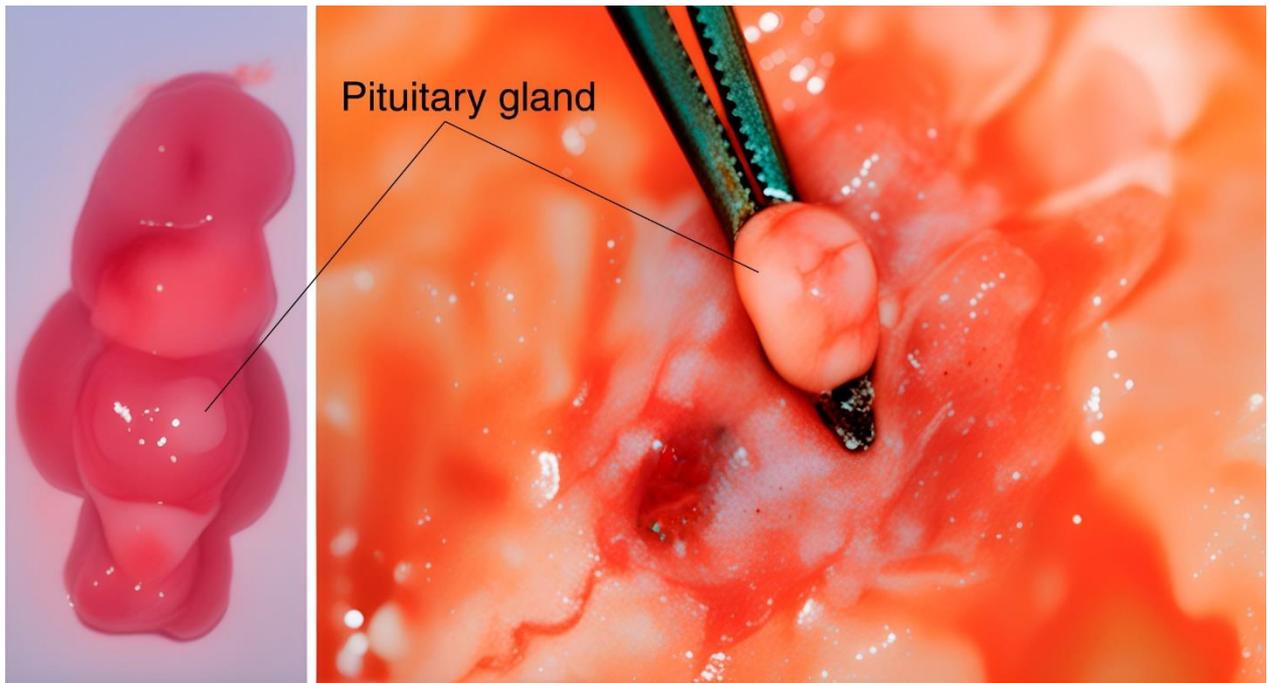


Figure 60. Removal of the pituitary gland from a donor fish. On the left is the ventral part of the fish's brain. On the right, the pituitary gland being removed.

If the gland is not to be used immediately it can be preserved, maintaining its hormonal characteristics, according to the following protocol:

- **Tissue removal**
Any adjacent tissue should be removed, especially fatty tissue.
- **Acetone 1**
Transfer the cleaned glands to a bottle containing acetone.
- **Acetone 2**
Keep the glands in this bottle for 8–10 hours. After that, replace the acetone in the bottle with fresh acetone.
- **Acetone 3**
Keep the glands in acetone for a further 8–10 hours.
- **Acetone 4**
Repeat the procedure for the last time, keeping the glands in clean acetone for 4–6 hours.
- **Packaging**
Drain the acetone and then spread the glands on a flat dish (porcelain or glass). The acetone evaporates quickly. Store the now-dry glands (without an acetone smell) in an airtight container, either glass or plastic, with clean, dry cotton at the bottom. Next, the container should be sealed (Figure 61).



Figure 61. Fish hypophyses are dried and ready for use.

- **Labelling**

A label should be affixed to the bottle with information written on it about origin and date, average gland weight, collector, etc.

- **Storage**

The glands must be protected from vapour and moisture. For this reason, it is recommended that the vials be kept tightly closed in a plastic bag with a sachet of moisture-absorbent silica gel or in a desiccator.

- **Alcohol**

Another way of preserving the glands is to use absolute alcohol. If you choose alcohol, it is essential to change it after 24 and 48 hours to ensure proper preservation. In this case, the pituitary glands should be stored immersed in the alcohol and not dry.

12.6.2 Preparation of pituitary extract

The pituitary gland extract must be prepared to induce spawning and, if not used immediately, preserved so it can be injected into the breeding fish (Figure 62).

- **Preparation**

The glands are removed from the jars in which they are stored, dried on filter paper (if they have been held in alcohol) and then macerated.

- **Materials needed**

To prepare the extract, you will need a pestle and mortar to grind the dried glands into a fine powder. You will also need a syringe: The most suitable type of hypodermic syringe for this practice has a capacity of 2 ml and graduations of 0.1 ml, preferably with a locking system to make it easier to use. The needle size will depend on the size of the fish to be injected. Generally, needle no. 22 is used for carp between 1 and 3 kg, no. 19 for those over 3 kg and no. 24 for those under 1 kg.

- **Counting the glands**

The dried glands must be weighed and measured, and the dosage is calculated based on the average weight of the glands.

- **The weight of the pituitary glands**

The dosage of the gonadotropic hormone extract is expressed in milligrams or as number of dried pituitary glands. The dried pituitary gland of a 1.5–2 kg common carp weighs around 2.5–3 mg. This is the weight considered as a unit when the dosage is expressed in terms of the number of glands.

- **Grinding**

The counted glands are placed in the mortar. Use the pestle to grind the glands into a fine powder.

- **Physiological saline solution**

Physiological saline solution should be added to the powder of the pituitary gland. This solution helps to dissolve the hormone. Saline solution is sold cheaply in pharmacies but can also be prepared by diluting 7–8 g of common salt in 1 litre of boiled and filtered drinking water.

- **Obtaining the pituitary extract**

Around 0.5 mL of physiological solution should be used for each gland, and the maximum amount of solvent should not exceed 5 mL. The saline solution is mixed well to dissolve the crushed hormone, which takes 10 to 30 minutes.

- **Obtaining the suspension**

The suspension of pituitary extract is removed from the mortar using a syringe, taking care to avoid removing 'debris', i.e. gland tissues that have not been properly ground. Alternatively, the residue can be removed by centrifuging or decanting the suspended material. However, simple care when handling the syringe is usually enough.



Figure 62. Preparing the pituitary glands to obtain solutions containing natural hormones to induce the reproduction of grass carp.

12.6.3 Dosages and procedures

- **Females:** The female usually receives two injections of pituitary gland extract. The first dose is equivalent to 10% of the second.
- **Males:** The male usually receives a single injection, which should be administered when the female gets her second dose. The males must not accept the hormone earlier than the females, as this could result in sperm release before the females are ready to ovulate.
- **Additional preparatory doses:** In some instances, when the oocytes are almost ready to spawn, but the ovary still needs to distend towards the lower part of the body cavity, two or more preparatory doses may be necessary before a definitive dose can be applied. If it is necessary to administer more than one preliminary dose, this should be equivalent to 10% the total dose.
- **Dosage, key considerations:**
 - In general terms, for a total dose, you need around 3.5–5.5 mg (1.5–3 glands) of pituitary gland per kg of weight in the case of large breeders weighing more than 5 kg; 1.5 mg (0.5 gland) for medium-sized fish (2–5 kg); and 0.75 mg (0.25 gland) for small fish (up to 2 kg).

- Figure 63 shows the average relationship between dosage of pituitary gland extract and total length of female grass carp.

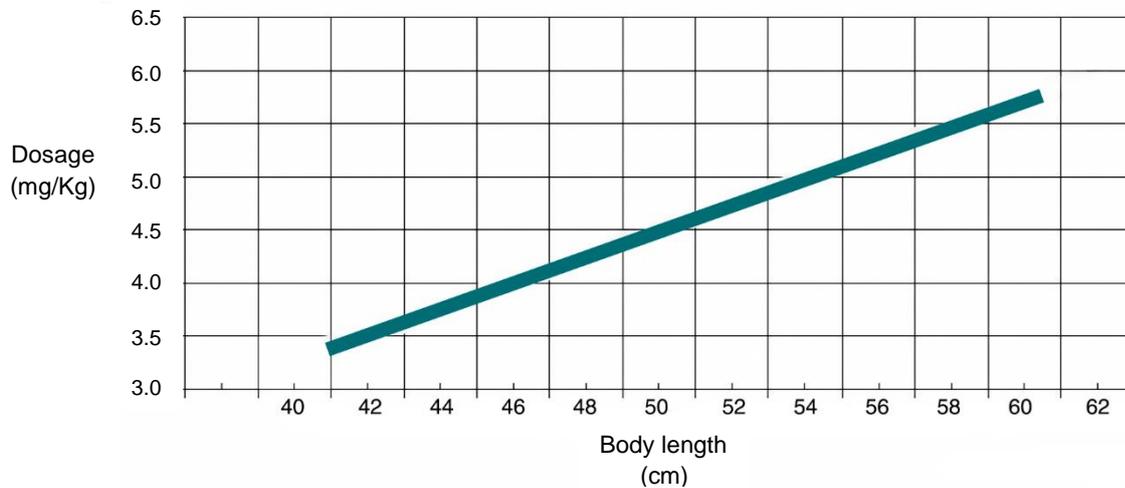


Figure 63. The relationship between average dosages of pituitary and total body length in grass carp.

- **Timing:** In the case of two injections (one preparatory and a second definitive dose) in the female – with an interval of six hours between them – and just a single dose in the males, spawning takes place 3 to 6 hours after the second injection.
- **Care 1:** Each fish should be taken individually to a small field table with a bed made of foam or cloth. Its head should be covered with a damp towel to calm the animal and reduce stress. Ideally, the fish should be anaesthetised beforehand to prevent them from feeling pain and hurting themselves during the procedure.
- **Care 2:** Usually, two people are needed during the injection process: one holds the fish's head against the cushion while the other presses the caudal peduncle with one hand and applies the injection with the other.
- **Cautions:** The operation should be carried out close to the tanks or hapas where the fish will be released after the final injection. Males and females should be kept separately in these tanks. In the case of natural (collective) spawning, the broodstock should be kept together in the same tank.
- **Single dose:** Spawning usually occurs 6 to 9 hours after the injection when a single high dose is administered.

12.6.3.1 Distributed dose method

In this method, which is not widely used for grass carp but also works, the hormones are applied in several doses. The sequence and quantity of doses can vary as follows:

- 50 and 50%, with an interval of 6–8 hours between them;
- 40 and 60%, 6–8 hours apart;
- 10, 30 and 60%, at intervals of 6 hours each;
- 33, 33 and 34%, at intervals of 6 hours each;
- 20, 30 and 50%, at 6 hours each.

12.7 Synthetic hormones

Hormonal induction of ovulation and spawning can also be carried out using human chorionic gonadotropin (HCG) (Figure 64), a hormone that can be bought in pharmacies under different trade names (e.g. Pregnyl, Choragon, Novarel, Ovidrel, Profasi, Gonasi).

Another option is to use Ovatide, a combination of a gonadotrophin-releasing hormone (GnRH) analogue and a dopamine antagonist (such as pimozide).

These hormones make the process much simpler and more practical, and even though they do not guarantee the same results as those obtained from pituitary extract, they can offer an excellent benefit/cost ratio for fry producers.



Figure 64. Injectable HCG, a viable alternative to crude pituitary extract in the hormonal induction of fish reproduction.

12.7.1 Doses

In the case of HCG, the recommended dosage for grass carp is just 800 to 1,000 IU per kg of body weight, administered in two doses eight hours apart. Only 10–15% of the total amount of HCG is administered in the first dose. Males are injected when females receive their second injection.

In the case of Ovotide, the recommended dose to be administered to females is 0.7 ml/kg body weight.

Spawning tends to occur between 8 and 12 hours after administration of the final dose. The eggs hatch between 20 and 24 hours after fertilisation, at temperatures between 22 and 29°C.

12.7.2 Care

Fish that are well prepared and ready for hormone treatment respond better to HCG administration. For example:

- Fish caught during the spawning migration respond better than individuals from captive herds.
- Well-fed and nourished captive fish respond better and more quickly to HCG than malnourished ones.
- Environmental manipulation, such as simulating rain over the broodstock holding tank, can also increase the success of HCG treatment.

12.8 Hormone application techniques

Spawning induction involves weighing the broodstock, deciding on the number of doses to be applied, applying the hormone and deciding on the spawning method.

- **Injection**

Different injection techniques, such as intramuscular, intraperitoneal and intracranial, can be used for grass carp. However, intramuscular injection is the most common and has proven effective and easy to perform. Intramuscular injections are commonly applied in the caudal peduncle region or below the fish's dorsal fin.

- **Scales**

The injection should be made below the scale, taking care not to puncture or tear it during the procedure.

- **Angle**

The injection should be applied at an angle of 45° to the surface of the fish.

- **Position**

Injections designed to stimulate spawning may be administered intramuscularly or intraperitoneally (within the abdominal cavity), each method presenting benefits. For injections of up to 2 mL, the intramuscular approach is efficient and should be performed between the dorsal fin base and the fish's lateral line. Conversely, the intraperitoneal method is preferable for larger volumes, owing to the space available in the abdominal cavity.

- **Anaesthetics**

anaesthetics such as MS 222 can be used to increase survival and avoid compromising the welfare of the fish during the handling process.

- **Transfer**

After the second injection, the fish should be released into spawning or holding ponds for artificial insemination ('stripping').

12.8.1 Weighing

The broodstock is removed from the holding tank where they were, separated by sex and weighed individually, as the doses of hormones must be applied according to the weight of each animal (Figure 65).



Figure 65. For weighing, the breeder places the fish in a plastic bag like the ones used to store feed.

12.9 The concept of degree hours

The concept of degree hours is a crucial metric in induced fish reproduction. It relates to water temperature and is used to assess the time required for specific biological events, such as ovulation.

This metric is a practical guide to enhance cultivation conditions and boost the effectiveness of hormonal treatments and other interventions. The application of degree hours is calculated by multiplying the time, in hours, by the water temperature in degrees Celsius, as explained below:

- **Degree hours and treatment**

In the case of grass carp, the degree hours index ranges between 340 and 360 when only a single, decisive dose is administered. This number drops to 240–260 if a preparatory dose is applied up to 24 hours before the definitive dose. The degree hours value reduces to 200–220 in cases where two decisive injections are administered within a 6–8-hour interval.

- **Degree hours and female size**

Smaller females ovulate before larger ones. This concept is particularly notable when there is a significant size difference between females, for example between 1–2 kg and 7–10 kg.

- **Calculating**

With the ideal degree hours value for grass carp set at 240 and the water temperature in the tank maintained at 25°C, the necessary time for ovulation can be calculated. The calculation is done by dividing the degree hours value (240) by the water temperature (25°C). Therefore, the calculation is 240-degree hours ÷ 25°C, resulting in 9.6 hours. In other words, under these specific temperature conditions, ovulation of grass carp is expected to occur at around 9

hours and 36 minutes. This adjusted calculation provides a more precise understanding of the environmental conditions impacting the fish's development and physiology. Figure 66 shows the relationship between the duration of final maturation of females and water temperature. After the second injection, the time required for ovulation in females strongly depends on water temperature. Typically, at temperatures of 23–25°C, ovulation occurs after 8–11 hours.

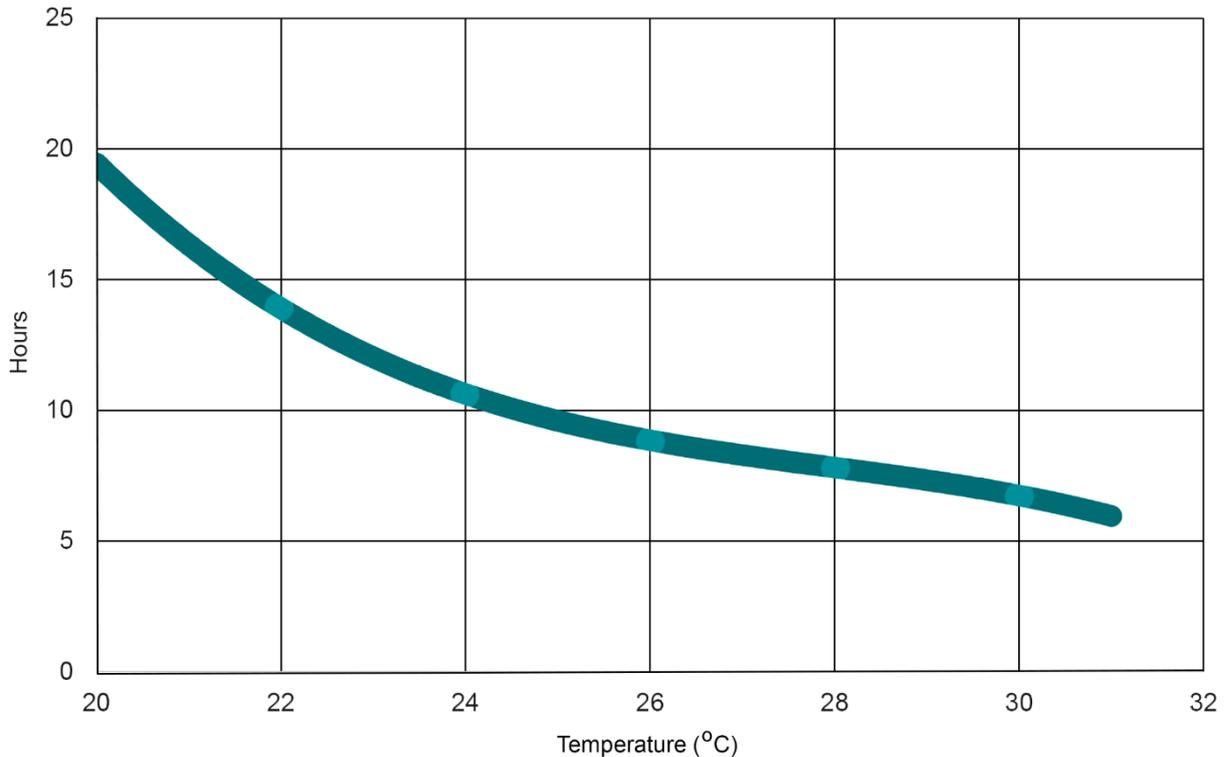


Figure 66. The relationship between the time (in hours) required for the final maturation of the ovaries in female grass carp and the water temperature.

12.10 Oocytes vs ovules vs eggs

The term 'oocyte' refers to the still developing female germ cell. In fish like grass carp, the oocyte undergoes various maturation phases until it becomes a mature ovule. During this period, the oocyte is not ready for fertilisation and is usually involved in a cell division processes known as meiosis.

An 'ovule' is an oocyte that has completed its development and is ready to be fertilised by a sperm. At the time of ovulation, the oocyte completes its second meiotic division and transforms into a mature ovule. The ovule contains all necessary female genetic information and is prepared to merge with the sperm, including the male part of the genetic information to be passed on to the offspring.

An 'egg' comes into existence post-fertilisation of the ovule and the formation of the zygote, which will develop into a new fish. Thus:

- **Oocyte:** The developing female germ cell, not yet ready for fertilisation.

- **Ovule:** The fully developed female germ cell, ready for fertilisation.
- **Egg:** The cell that results from the fertilisation process between a fully developed ovule and a spermatozoon initiates embryonic development and contains all the genetic material necessary for the development of a new being.
- **Fertilisation:** As soon as the ovule contacts water, it swells, closing the micropyle (a pore where the sperm enters to fertilise the ovule, generating the egg, as seen in Figure 67). In grass carp, this occurs in 45 to 60 seconds. Therefore, swift action is crucial.

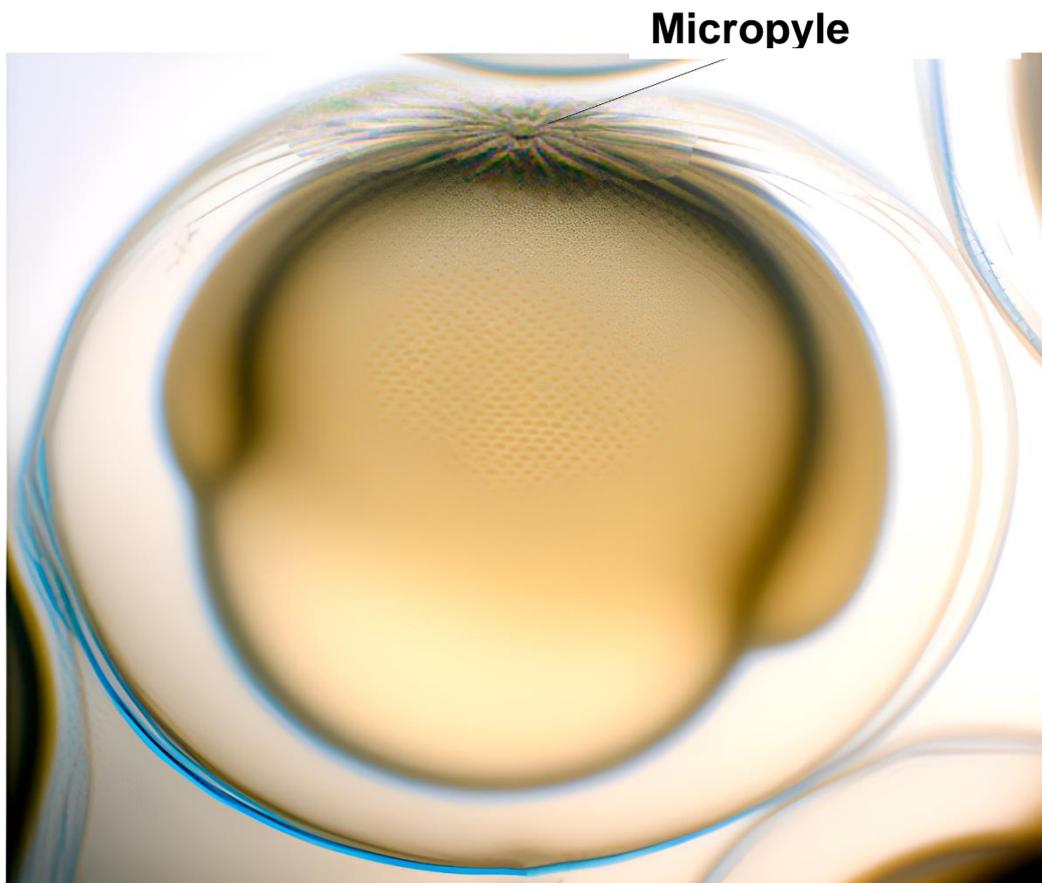


Figure 67. The micropyle is a small opening on the surface of the fish ovule that allows sperm entry during fertilisation. After a few seconds, the ovules hydrate in water, and the micropyle closes. If the sperm has not passed through the micropyle by then, the ovule will no longer be fertilised. The figure shows an egg with the embryo already developing.

12.11 Spawning methods

Following mandatory hormonal induction, there are two possibilities for obtaining spawns: through collective natural spawning, with minimal interventions from the breeders, or through gamete extrusion.

12.11.1 Natural spawning

It is recommended to use combinations such as one female, weighing 2–3 kg, with two or up to three males to enhance success in induced spawning; two females for three males, or at most, three females for four males. If the breeders weigh less than 2 kg, placing three to five females with four to six males in the same tank is advisable.

In the case of natural spawning, it is common for both the female and male to receive single doses of pituitary extract or synthetic hormones. Subsequently, they are relocated together in the breeding tanks. For grass carp, unlike some other carp species, no substrate is used as spawning occurs naturally (Figure 68). The other minimum conditions to be maintained in the breeding tanks include:

- Dissolved oxygen concentrations above $\geq 48.0\%$;
- pH levels between 7.0 and 8.5;
- Water temperatures range from 20–30°C;
- Simulating rain over the surface of the breeding tank;
- High turbidity (2,000–2,500 ppm).

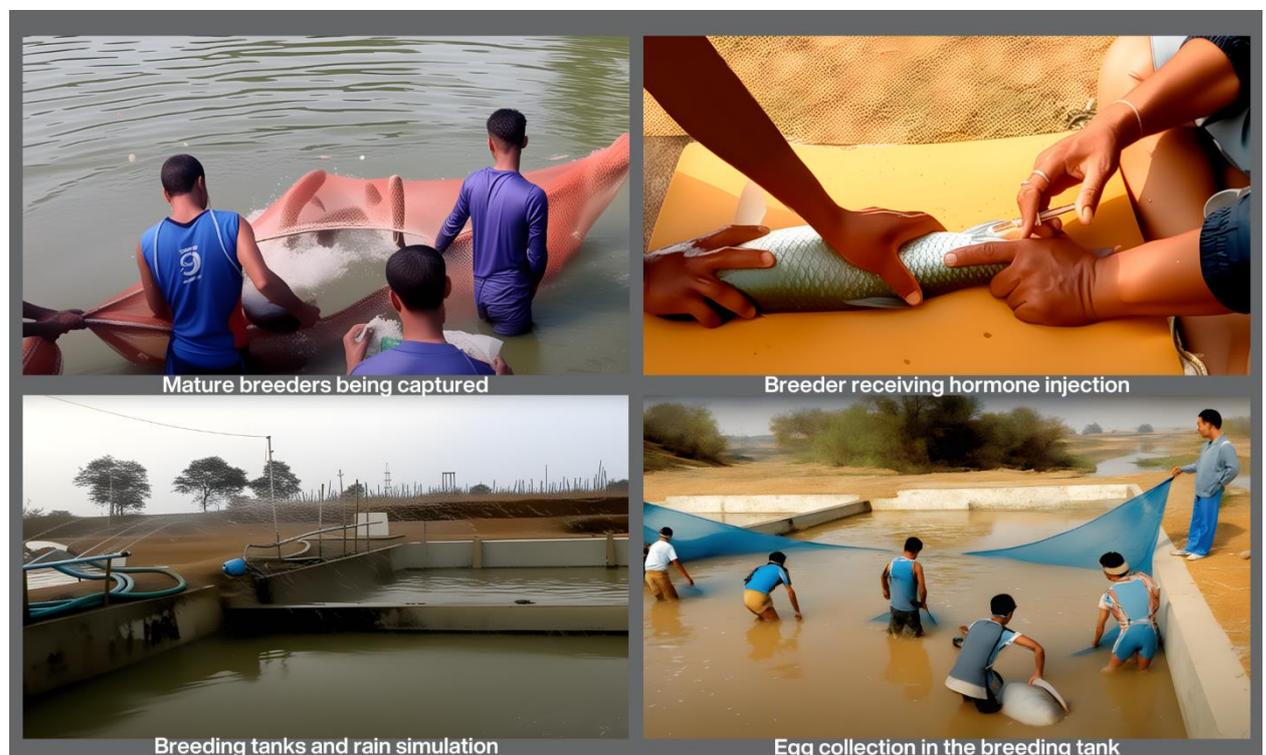


Figure 68. Process of massive natural reproduction of grass carp, from the selection of breeders to the collection of eggs.

Approximately 6 to 8 hours after administering the decisive hormone dose – a period that can vary depending on the water temperature – signs of reproductive behaviour can be observed in the pair. At this point, the female and male are expected to initiate the mating process, culminating in spawning and fertilisation of the egg.

As the female releases the eggs the male expels his sperm, and thus the eggs are fertilised. Following fertilisation, the eggs swell significantly within 10–15 minutes due to water absorption through the vitelline membrane.

A fine mesh is then used to concentrate the floating eggs in a net and transfer them to a jar and circular tanks with continuously circulating water to simulate a river environment. Within 48 hours, larvae/post-larvae are ready to be collected and packaged for transport (Figure 69) or cultured in the hatching tanks until they reach the fry stage.



Figure 69. Process of massive natural reproduction of grass carp, from egg collection to packaging of larvae/post-larvae.

12.11.2 Artificial spawning ('stripping')

Artificial spawning, also known as 'stripping', is an individual method of obtaining oocytes and sperm. The reproductive cells are extruded manually and removed from the breeders (Figure 70). This procedure applies pressure to their abdomens when they are ready to reproduce. The fertilisation of the eggs can then be done using either dry or wet methods (Figure 71).

It is crucial to emphasise the importance of carefully handling the manipulated fish. Improper handling and prolonged exposure to air can lead to stress and physical injuries to the animals, compromising their welfare, health and longevity.

12.11.2.1 Dry fertilisation

This method is widely used as it is very effective.

- The females are examined 3–4 hours after the second hormonal injection to check if they are ready for spawning.
- When ready, the abdomen becomes very soft, and the oocytes are quickly released with light pressure. Once a female is identified as ready, the egg extraction procedure should be done quickly to prevent reabsorption or accidental ovulation.
- First, the oocytes are collected in a plastic, aluminium or enamelled basin. Then, the semen is collected by pressing the males' abdomens (Figure 70).
- The semen and eggs are then carefully mixed using a plastic spoon or a feather.
- A few minutes later, the fertilised eggs are washed to remove excess semen and blood clots.
- They are then transferred to incubators.
- **Caution:** Attention must be paid to the time after ovulation, as the ovules deteriorate rapidly. Approximately 50% of the grass carp oocytes become unsuitable for fertilisation after 30–40 minutes.
- **Hydration:** Once fertilised, the eggs naturally absorb water, causing the internal membrane to expand relative to the outer capsule. This process takes 2 to 3 hours, resulting in eggs with a diameter of about 5.5 mm.



Figure 70. Gamete extrusion and artificial fertilisation of grass carp.

12.11.2.2 Wet fertilisation

After they have been released in water, the viability of ovules and spermatozoa is short, approximately 60 to 90 seconds, making it crucial that the release of gametes is almost simultaneous. An alternative to this process is wet fertilisation, a method that uses a 0.3% saline solution to extend the viability of the spermatozoa for up to 2–3 minutes.

- **Extrusion:** In this case, the semen is first collected in a plastic or enamelled container containing the saline solution. Then the oocytes are collected in the same tray.
- **Quantity of water or saline solution:** Care must be taken with the amount of water or solution added to the semen and egg mixture. If it is too much, the spermatozoa can disperse

and fail to enter the ovules. If too little, the micropyles (small openings at the apex of the ovule, where fertilisation occurs) can be blocked by other ovules or ovarian mucus.



Figure 71. Process of induced artificial spawning of grass carp in controlled environmental conditions.

12.12 Fecundity

- **Average fecundity**

Absolute and relative fecundity varies with the age and weight of the fish, but the average fecundity rate of grass carp is typically around 70,000–200,000 eggs per kilogram of body weight.

- **Unfertilised eggs**

Some eggs always end up unfertilised or may undergo initial divisions (cleavages).

Following this initial development stage, the process halts completely and the eggs become opaque. This change signifies that the embryo either failed to develop or has died. Common causes for such outcomes include the unsynchronised release of gametes by both males and females, insufficient or poor-quality sperm, females not being in the ideal stages of maturity, or infestations of fungi or bacteria.

- **Number of eggs**

The females can be weighed before and after spawning to calculate the weight difference and thus estimate the quantity of eggs produced. The average weight of the eggs must be determined through a small sample, which should be weighed and counted individually. The weight difference of the female is then multiplied by the average weight of each egg to estimate the total number of eggs spawned.

- **Dependence**

The fecundity of carp depends on the fish's size, age and readiness to spawn.

12.13 Additional tips

12.13.1 Pituitaries

- Pituitaries collected from carcasses of immature fish generally do not yield satisfactory results.
- Hormone concentration in the pituitary decreases after spawning. Therefore, glands should not be collected from fish that have just spawned.
- Pituitaries should not be collected from fish in advanced stages of decomposition.
- Care must be taken not to damage the pituitary at the time of collection, as a damaged gland loses its potency.
- A significant problem with pituitaries is that they are generally collected from fish at different stages of maturity, and therefore the potency of the extracts can vary considerably between different batches.

- There is no qualitative difference between the pituitary glands of male and female donor fish.
- If kept in acetone and stored in freezers at -20 °C, the pituitaries can maintain their potency for up to 15 years.

12.13.2 Selection and handling of breeders

- Regarding the selection of breeders, it is essential always to choose healthy fish.
- Breeders can be collected from ponds or cultivation tanks and stocked in maintenance tanks, separated by sex, to increase the effectiveness of the reproductive process.
- Once again, we emphasise that careful handling of the breeders is crucial to avoid injuries and stress, which can negatively affect the spawning process. This care not only ensures the welfare of the fish but also optimises the chances of successful spawning, making the process more efficient and economical.
- Injuries or neglect can result in high mortality rates, ranging from 10% to 100% in extreme cases.
- The leading causes of mortality are injuries during collection, contamination, and inflammatory processes, especially in high water temperatures (> 30°C).
- Unlike females, males can be used 2–3 times during the spawning season.
- After spawning, the tanks with the breeders should be partially emptied. The breeders are then captured, and the fish farmer needs to count and note the number of females that have completed the spawning process.

12.13.3 Storage

- Semen can be collected up to an hour before egg extraction, which is crucial to avoid contamination and contact with water.
- Temperature is essential for sperm viability; at temperatures between 0 and 2°C, sperm maintains its motility for several days.
- The same cannot be done with oocytes, which will lose efficiency.

12.13.4 Hormonal dosages

- A slight hormone excess in the decisive dose does not harm the fish. Therefore, an overdose of 10–15% is generally administered as a safety measure. For the total or 100% dose, 1 to 1.5 pituitary glands (i.e., 3.0–4.5 mg) per kilogram of female weight are usually administered. If the calculated dose requires more than five glands, one more is usually added for safety.

12.13.5 Hormone application

- Hormonal induction injections can be done at any time of day or night, as spawning can be scheduled to take place at any hour.
- Avoiding the lateral line area is crucial when applying pituitary extract or synthetic hormones.
- If more than one injection is necessary, it is ideal for the second to be applied on the opposite side to the first.

12.13.6 Anaesthesia

- An efficient and quick technique for this is the use of synthetic anaesthetics like MS 222. Prepare a swab with the anaesthetic by folding a cotton swab soaked in MS 222 anaesthetic solution (use 0.2 to 0.3 g of MS 222 in 50 to 80 mL of water) and wrap it in a mosquito net-type mesh. Insert the moistened swab into the female's mouth. The fish will fall asleep shortly.
- Essential oils diluted in alcohol and added to water can also be used for this purpose (such as clove oil – eugenol, a compound that acts on the central nervous system of fish, causing sedation and loss of consciousness – or peppermint oil). The ideal concentration of clove oil is 7.5 mL per 100 litres of water.

12.14 Issues related to grass carp reproduction

As grass carp aquaculture progresses, more fish bred in captivity are being artificially reproduced. However, the handling and management of these fish often present critical challenges. The methods employed can potentially induce stress, pain and injuries in the animals, thereby compromising their welfare and, in some cases, may even result in death.

Table 31 shows some methods that may cause pain, stress, suffering and injuries, as well as their advantages, disadvantages, and alternatives that promote a higher degree of welfare during the reproduction of grass carp.

Table 31. Advantages and disadvantages for animal welfare of methods used for fish reproduction.

Methods	Principles	Is anaesthesia generally performed?	Advantages	Disadvantages	How to minimise stress
Determining sex and maturity for reproduction	Microscopic examination of an egg sample taken directly from the ovaries of females using a glass catheter, exposure of animals to air	No	Enables developmental stage of oocytes/eggs in a female to be determined	It can cause pain, injuries during collection, and death of animals due to secondary infection	The use of natural reproduction and non-invasive methods is recommended. If not possible, use anaesthetics during procedures and adhere to strict hygiene standards
Hormonal induction	Application of injections, exposure of animals to air	No	Scheduled spawning	Can cause pain and death of animals	
Artificial spawning	Obtaining oocytes and sperm through extrusion, i.e., manually removed from breeders by applying pressure to the abdomens, exposure to air	No	Year-round reproduction of animals	This can lead to stress or injuries, compromising their welfare, health and longevity	

12.15 Recommendations for captive breeding

12.15.1 Environmental Indicators

Table 32. Environmental indicators for grass carp breeders.

Indicators	Level	Reference values
Temperature (°C) during spawning and maintenance	Optimum	20–30
	Acceptable	15–19 or 31–34
pH	Optimum	7.0–8.5
	Acceptable	6.0–6.9 or 8.6–9.0
Transparency (cm)	Optimum	30–60
	Acceptable	20–29 or 61–70
Dissolved oxygen (% saturation)	Optimum	≥ 48.0
	Acceptable	35.0–47.9
Salinity (ups)	Optimum	0.0–0.7
	Acceptable	0.8–8.0
Stocking density (kg/m ²)	Optimum	0.2–2.9
	Acceptable	3.0–5.9
Un-ionised ammonia (mg/L of NH ₃)	Optimum	0.000–0.025
	Acceptable	0.026–0.050
Nitrite (mg/L of NO ₂ ⁻)	Optimum	0.0–0.9
	Acceptable	1.0–1.5
Alkalinity (mg/L of CaCO ₃)	Optimum	25–100
	Acceptable	≥ 101
Photoperiod (Light: Dark)	Optimum	12L:12D – 14L:10D
	Acceptable	15L:9D – 16L:8D
Terrestrial predators	Optimum	Absence
	Acceptable	Controlled presence
Predators and other aquatic interspecific inhabitants	Optimum	Absence
	Acceptable	Controlled presence

12.15.2 Health indicators

Table 33. Health indicators for grass carp breeders.

Indicators	Level	Description or reference values
Eyes	Optimum	Normal and healthy appearance
	Acceptable	Hemorrhage, exophthalmos or unilateral traumatic injury
Jaw/lips	Optimum	Normal and healthy appearance
	Acceptable	Bleeding, redness, mild injury, or mild deformity (without affecting diet)
Operculum	Optimum	Normal and healthy appearance
	Acceptable	Absence of tissue (<25%)
Skin	Optimum	Normal, healthy appearance, scar tissue
	Acceptable	Punctual loss of scales, ulcers, or superficial lesions < 1 cm ²
Fins	Optimum	Normal and healthy appearance
	Acceptable	Scar tissue, mild necrosis or splitting
Gills	Optimum	Normal and healthy appearance
	Acceptable	Light injury, mild necrosis, splitting or thickening
Abdomen	Optimum	Normal and healthy appearance
	Acceptable	Discreet hollow distension, redness
Anus	Optimum	Normal and healthy appearance
	Acceptable	Faecal residue, swelling and redness
Invasive procedures	Optimum	No invasive procedure
	Acceptable	Hormonal injection, natural spawn
Sexual maturation	Optimum	Mature animals. Male: First ray of pectoral fin thickening, nuptial tubercles in pectoral, dorsal fin, or head. Semen is released quickly by pressing the abdomen. Body length ≥ 50 cm. Female: soft pectoral fin, swollen and tender abdomen, pink orifices; decidual tubercles may occur. Body length ≥ 54 cm
Mortality (%)	Optimum	≤ 10

Indicators	Level	Description or reference values
	Acceptable	11-24

12.15.3 Nutritional indicators

Table 34. Nutritional indicators for grass carp breeders.

Indicators	Level	Reference values
Crude protein (%)	Optimum	30-40
	Acceptable	25-29 or 41-45
Amount of feed during maintenance (% biomass)	Optimum	2.5-5.9
	Acceptable	1.0-2.4
Amount of feed during mating (% biomass)	Optimum	1.0-2.0
	Acceptable	0.5-0.9
Amount of natural food - grass (% biomass)	Optimum	100-200
	Acceptable	50-99
Feeding frequency (times/day)	Optimum	Once, twice a day or more
	Acceptable	Every other day
Food distribution in the pond/tank area (% of surface area reach)	Optimum	> 75 of surface area
	Acceptable	50-75 of surface area
Feed seizure (minutes)	Optimum	3-5
	Acceptable	2-3 or 5-7
Forage seizure (minutes)	Optimum	120-180
	Acceptable	60-119 or 181-240

12.15.4 Behaviour indicators

Table 35. Behaviour indicators for grass carp breeders.

Handling	Behaviour		
	Indicators	Scores	Criteria
Environmental acclimation Formation of breeding groups	Respiratory frequency (Opercular beating rate)	Optimum	40-70
		Acceptable	20-39 or 71-90 ≤ 20 or ≥ 91
Hormonal injection Gamete extrusion	Anaesthesia - surgical stage (reduction of opercular beating rate; lack of balance and swimming)	Optimum	Induction in 1-3 min; recovery in ≤ 5 min
		Acceptable	Induction and or recovery in > 5 min

13 LARVICULTURE, NURSERY, AND FRY REARING

13.1 Facilities

The laboratory designated for breeding and egg incubation must have adequate space and the necessary facilities to conduct related activities. One of the most critical installations is a reservoir capable of providing a backup water flow for at least 12 hours. The reservoir water must also be pre-treated, undergoing sedimentation or filtration to remove unwanted organisms, debris and excess sediment.

Ensuring a water level difference of at least 1.5 m between the reservoir and the laboratory is crucial. This measure guarantees adequate water pressure in the equipment, thus preventing the accumulation of eggs at the bottom of the tanks during incubation.

If the laboratory is located in an area with significant temperature fluctuations, installing an automatic water heating system is also advisable.

Internally, the laboratory space should be planned to accommodate various plastic or concrete tanks to manage breeding fish during the breeding process (Figure 72).

Before the spawning season, it is crucial to clean the systems and disinfect all laboratory equipment thoroughly.



Figure 72. A breeding and larviculture laboratory must have space for tanks and incubators.

13.2 Incubators

Incubators are essential for creating a controlled environment that fosters the healthy and safe development of grass carp eggs and larvae. They make it possible to adjust critical factors such as temperature, dissolved oxygen levels and water flow, thereby creating stable and ideal conditions for hatching and the initial growth of the larvae. Depending on the specific needs of your grass carp breeding and larviculture project, various incubator options are available and can be integrated into the laboratory environment.

13.2.1 Funnel-type incubator

Funnel-type incubators (conical jars) are typically made from laminated fibreglass and feature a tube with a screen and a tripod with tubular support for stability. Water enters the incubator at the bottom via a threaded connection and exits at the top.

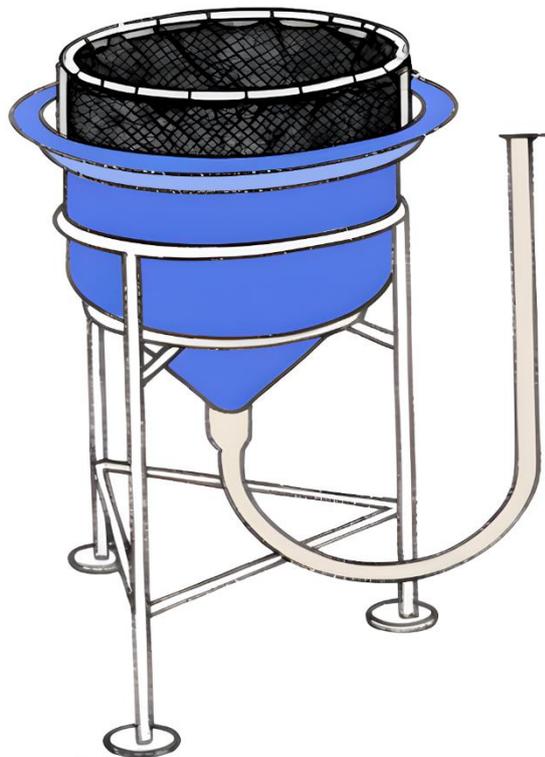


Figure 73. Funnel incubator (also called a conical jar).

This incubator model offers versatility in its construction, as it can be made from different materials such as fibreglass, galvanised steel sheets, or a combination of both. For example, the conical base can be made of galvanised steel, while the main body may comprise plastic drums, all fixed together with rivets. The capacity of these incubators can vary significantly, from 40 to 80 litres, and in specific cases they can hold 200 litres or more.

The control of the water flow entering the incubator is meticulously adjusted to minimise any possibility of damage to the eggs. The current keeps the mass of eggs in suspension, allowing them to move freely through the water column. An overflow system is positioned at the top of the incubator to prevent the eggs (Figure 74) and the larvae from being carried away by the current. This system has a mesh screen, which can be made of fabric or stainless steel, with mesh openings varying from 80 to 120 mm. Practice shows that a 200-litre incubator can safely accommodate up to about 500,000 eggs.



Figure 74. The eggs will significantly increase in volume three hours after fertilisation due to hydration.

13.2.2 Zug-Weiss vertical incubator

This practical and versatile incubation system offers high efficiency with relatively low operational costs.

The equipment is vertical and resembles an inverted bottle, optimising the available space for incubation. It operates with an upward water flow mechanism. Water enters the bottom of the incubator, flowing upwards to keep the eggs suspended (Figure 75) which is essential to ensure adequate oxygenation, vital for the healthy development of the larvae. This type of incubator, being transparent, allows for the visualisation of the eggs and proper control of the water flow, maintaining the buoyancy of the eggs during embryonic development. As the eggs hydrate and the embryos develop, the eggs become heavier and more prominent (Figure 76 and Figure 77).

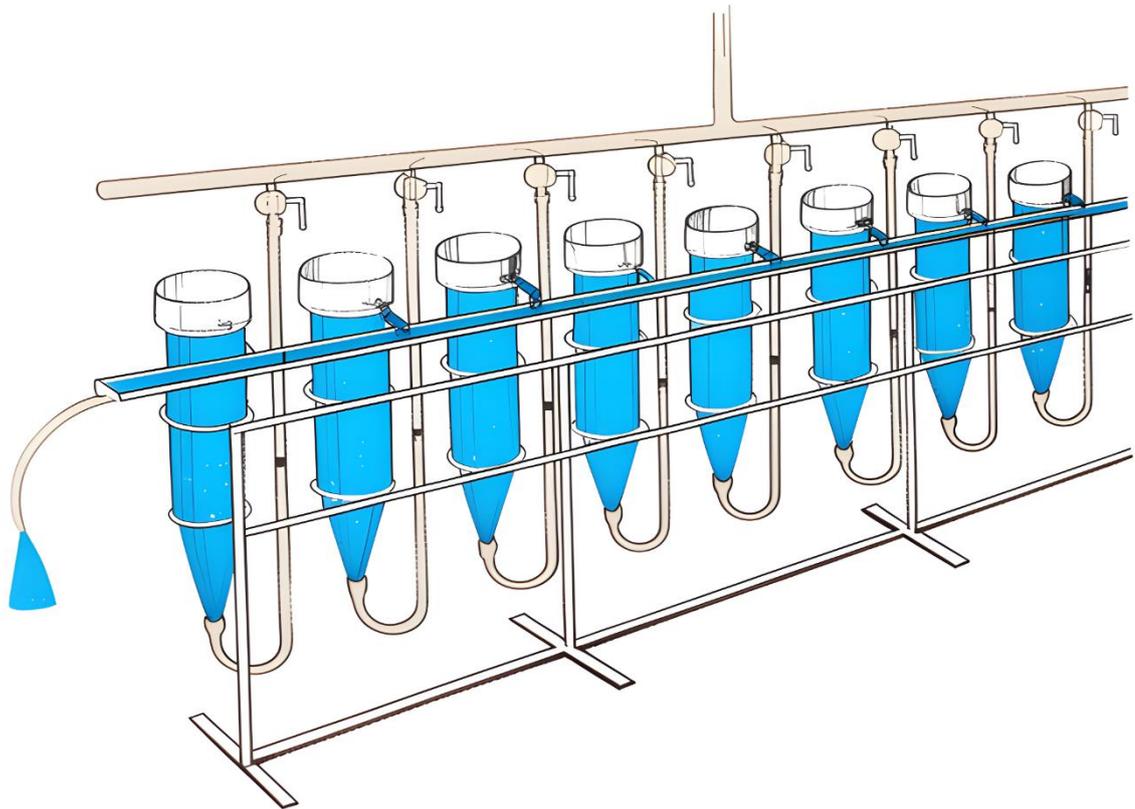


Figure 75. Zug-Weiss vertical incubator system.

- **Hatching and removal of larvae**

The water outlet of the jar usually has a fine mesh screen. This mesh prevents the hatched larvae from being carried out of the jar, so they can be safely collected manually for the next growth phase. At the same time, a structure can be attached to the water outlet enabling the removal of larvae using only the water flow.

- **Flow rate adjustment**

An essential feature of this incubator is the ability to adjust the water flow rate, accommodating different types and sizes of eggs. The flow is calibrated to keep the eggs suspended, thus ensuring effective gas exchange (Figure 76).

- **Scale of operation**

The simple yet robust design makes this incubator ideal for small to medium-scale aquaculture operations. It can be configured to house multiple jars, making it adaptable to the production size.

- **Versatility**

One of the most significant advantages of this system is its versatility. It can be assembled with a variable number of jars in a single configuration, allowing the producer to adapt the system to the size of their production.

- **Efficiency**

This system allows for mass incubation efficiently and with minimal handling, which benefits fish welfare and operational efficiency.

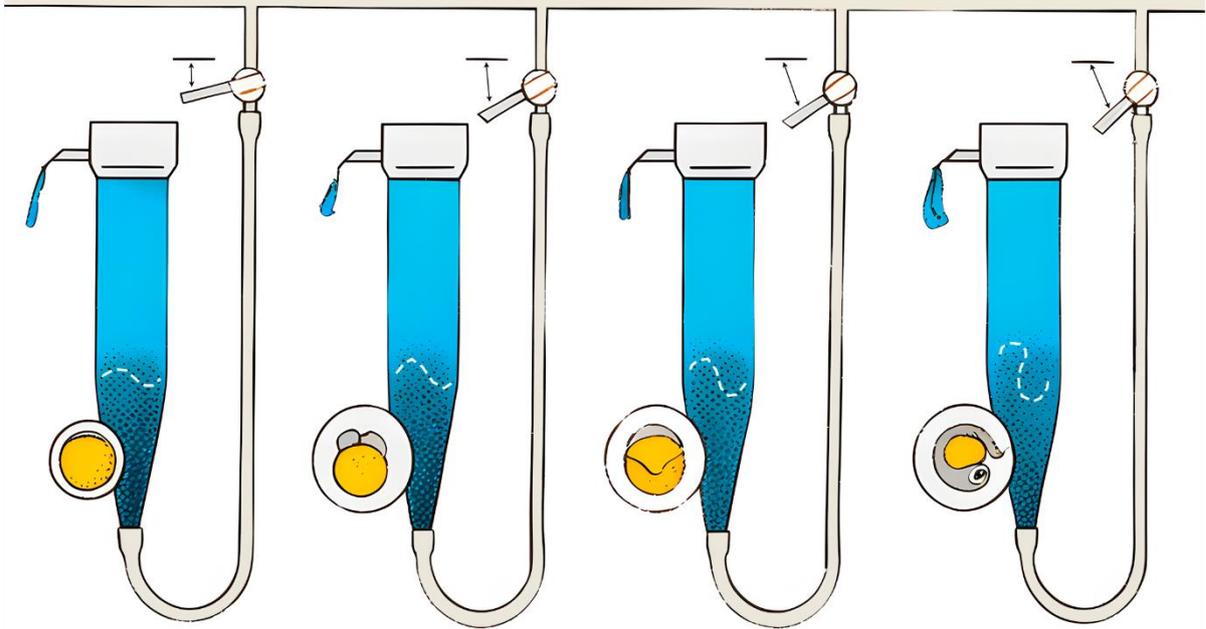


Figure 76. In the Zug-Weiss-type incubator, as the embryo develops the eggs become heavier.

The upward water flow in the incubator can be increased to maintain their suspension.

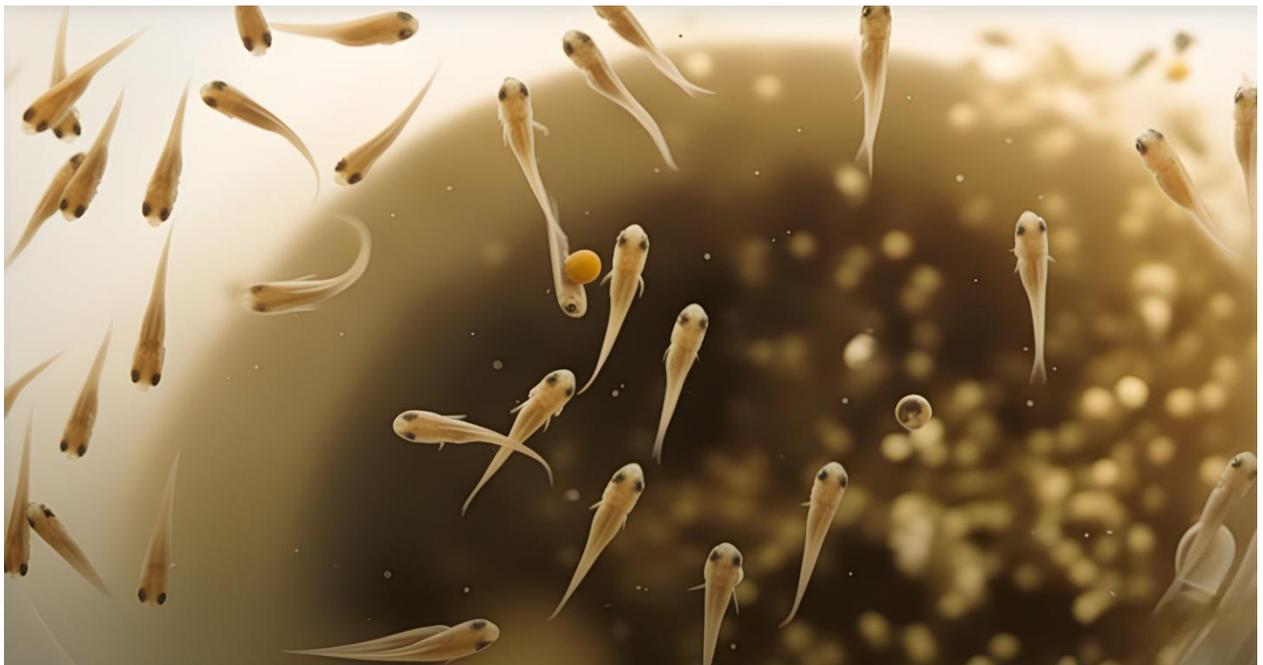


Figure 77. Grass carp larvae in an incubator.

13.2.3 Carp breeding-cum-incubation pool

Initially developed in China and proven efficient, this system optimises large-scale breeding processes. It is based on two main components: the spawning tank and the incubation tank. There are other auxiliary structures, schematically shown in Figure 78, including an essential water reservoir for the system's operation. The total capacity is generally about 30,000 litres but can be adapted to larger or smaller volumes.

The innovative aspect of this system is its water circulation method. Water flows from the spawning tank directly to the incubation tank and then to the post-larval reception tank just using gravity. This natural and continuous flow is an effective method for transferring different stages of fish development.

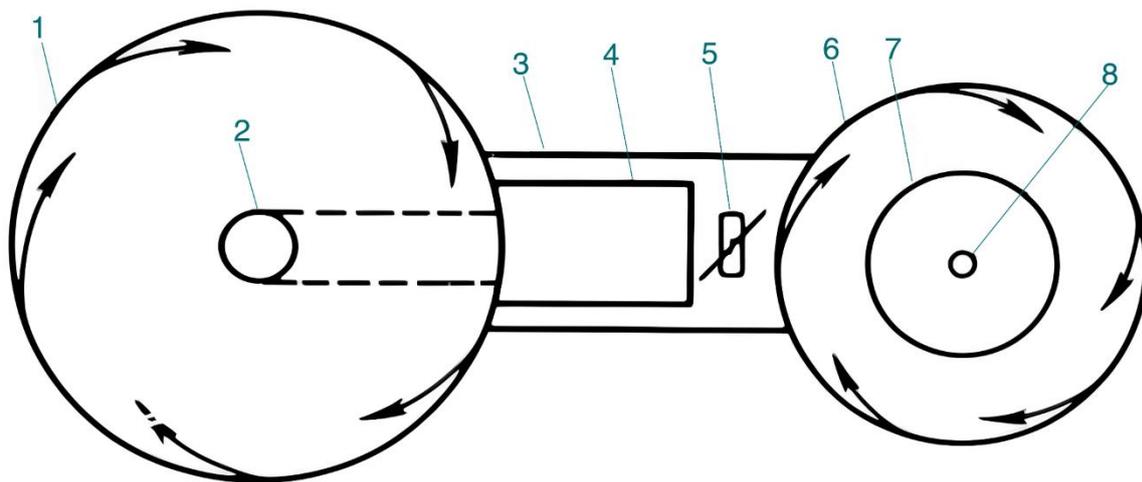


Figure 78. Schematic outline of circular spawning-incubation complex: comprising 1 - spawning tank; 2 - bottom drain (pipe); 3 - concrete tank for installation of egg collector; 4 - egg collector; 5 - discharge pipe with a valve; 6 - incubation tank; 7 - filter; 8 - discharge pipe. Source: Jeney and Bekh (2020).

The spawning tank is 8 m in diameter, constructed with brick masonry, and has a depth of 1.2 m. The incubation tank is also circular, with an internal diameter between 3 and 4 m. It comprises two chambers: the outer chamber is made of masonry or concrete, while the inner chamber features a fixed mesh screen (Figure 79).

In this tank, water circulates in centrifugal or circular motions, somewhat simulating the natural conditions found in rivers. This continuous water flow system minimises the need for manual intervention.

The system offers several advantages, such as minimal handling of fish and eggs, making the process more natural and reducing animal stress. Furthermore, the continuous water flow and the capacity for mass incubation significantly improve the system's overall efficiency. These features are crucial for large-scale production and ensuring the welfare of the cultivated fish. Additionally:

- The percentage of females producing eggs increases to 80–85%, compared to 50% according to average data over several years with conventional technology.
- Effective fecundity increases by 10–15% due to a more complete yield of eggs.
- Egg quality improves, resulting in a 10–15% increase in the yield of larvae aged 3 to 4 days.
- Mortality of breeding fish is eliminated, provided operations are conducted carefully.
- The technology requires less labour to be applied.
- The technology offers the most remarkable advantages during periods of unstable weather.



Figure 79. The carp breeding-cum-incubation pool.

13.3 Care during egg incubation

- After loading the eggs, water flow must be controlled to ensure constant movement of the eggs while preventing them from being dragged or clustering in stagnant zones of the tank.
- The quality of the eggs should be periodically assessed, and the percentage of successful fertilisation should be calculated.
- The incubation period varies with water temperature. At temperatures between 21 and 25°C incubation takes 23 to 33 hours. After hatching, the free embryos become active and make 'sailing' movements, rising to the upper water layers.
- The survival rate from egg fertilisation to 3–4-day-old larvae should be at least 50%.
- Larvae or post-larvae should be transported to fry systems in transport boxes or polyethylene bags filled with water and oxygen.
- The mortality rate during transport should not exceed 10%.

13.4 Nursery

In the initial life stage of grass carp, lasting between 2 and 4 days at 24–32°C temperatures, the larvae sustain themselves on their yolk sac. This period extends from hatching until the larvae reach the water's surface, fill their swim bladders with air, and begin to swim horizontally. At this point they transform into post-larvae, and their digestive tracts are ready to digest external food.

At this stage, the producer has two options: transfer the post-larvae to nursery ponds or hapas (see section 12.4.3 of this manual for more about hapas) or keep them where they were incubated. The advantage of moving them is that, in a well-prepared external environment, the abundance of zooplankton can meet the initial feeding needs of the post-larvae, even at high stocking densities.

The stocking rate in a pond varies according to management practices. Continuous zooplankton supply through fertilisation and supplemental feeding can achieve a density of up to 250,000 post-larvae per hectare. However, it is crucial to have aerators to compensate for any potential oxygen deficiency.

13.4.1 Tanks

Tanks designed for rearing grass carp post-larvae and fry can be made of various materials and come in different shapes. They must have systems to maintain and drain the water level, ensuring a constant flow. To prevent the larvae from being drained out of the tanks, filters made of kapron mesh (or nylon 6.6) with mesh sizes between 18 and 25 should be installed directly at the spillway.

The pipes supplying water to the tanks must also be equipped with filters, preventing the entry of unwanted organisms and debris. The recommended water flow is about 12 cubic metres per hour for rearing up to one million larvae.

Maintaining tank hygiene is fundamental. The bottom should be cleaned daily using a siphon to remove food residues and debris. This will not harm the larvae as they are active enough to avoid the siphon during this cleaning. Moreover, more modern tank designs feature pumping systems that facilitate the removal of sediments and accumulated residues at the tank's conical bottom.

13.4.2 Nursery ponds

Nursery ponds typically range from 0.2–1.0 hectares in size, with a depth between 1.2 and 1.8 m. They must have an adequate supply of high-quality water and a filtration system to prevent the entry of undesirable organisms. Ideally, nurseries should be protected with screens on the top and sides (Figure 80). Unscreened nurseries (Figure 81) expose post-larvae and fry to predation by birds and aquatic mammals.



Figure 80. Tanks and nursery ponds are used for rearing post-larvae and fry, covered with screens to protect the fish from avian predation.



Figure 81. In uncovered nurseries, fish are highly vulnerable to predation.

13.4.2.1 Soil preparation in nursery ponds

Soil preparation in nursery ponds is essential for ensuring a healthy and productive environment for fry production. This process goes beyond simple cleaning, involving specific actions to optimise the soil and water conditions.

During winter, it is advisable to drain the ponds for cleaning and remove plants and other debris that may compromise water quality and fish welfare. This period provides the ideal opportunity to inspect the overall condition of the pond, identifying any areas that may require maintenance.

After this initial cleaning, it is recommended to disinfect the pond bottom and the submerged parts of the dikes or slopes. A common technique is the application of lime, with rates varying from 200 to 400 kg per hectare with the pond still empty. If there are puddles at the bottom even after the pond drainage, the lime dosage in these areas can be increased.

Lime not only disinfects the area but also positively influences the soil structure and the chemical composition of the water that will fill the pond.

Soil preparation is an additional, often neglected step involving soil aeration. This process can significantly improve plankton production once the pond is filled, providing an abundant natural food source for the fry.

13.4.2.2 Fertilisation

The pond should be fertilised to promote the growth of algae and zooplankton, serving as natural food for the carp. This environment preparation typically takes place 5 to 10 days before introducing the fish, depending on water temperature conditions. To minimise the risk of cultivating undesirable organisms, the pond should not be fertilised too far in advance.

Before fertiliser is applied, the pond should be filled to half of its capacity.

Fertilisers can be of animal origin, such as manure, with a usual dose of 3,000–5,000 kg per hectare; of plant origin, known as ‘green manure’, with a dosage of about 4,500 kg per hectare; or chemical, based on nitrogen and phosphorus.

Organic fertilisers contain their own bacteria and organic debris that can serve as direct nutrients for zooplankton organisms, with rotifers being the most important for grass carp in their early feeding stages. Ideally, manure should be used in liquid form mixed with water. If this is not possible, it can be moistened and spread directly over the pond from the edges.

Chemical fertilisers containing nitrogen, such as urea or ammonium nitrate, should be diluted in the pond water and applied at 150 to 200 kg per hectare (Table 36 and Table 37). Half of this amount should be added during the pond filling, and the rest should be divided into two applications, one after the first week and another after the second week of larval care.

The solubility of phosphorus-based fertilisers is more complex, so these should be dissolved beforehand, preferably in hot water, and dispersed over as large an area of the pond as possible. Phosphorus should be added at 100 kg per hectare and applied during the pond filling (Table 36 and Table 37).

Table 36. Recommended quantities of manure and fertilisers. Source: Jeney and Bekh (2020).

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 37. Frequently used fertilisers. Source: Jeney and Bekh (2020).

Type of fertiliser	Description
Nitrogenous fertilisers	<p>Liquid ammonia (NH₄OH) or fertilisers with a nitrogen content of 12–16%</p> <p>This is a water solution of ammonia, an essential product of small-scale nitrogenous fertiliser factories with simple synthesising procedures and low costs. Ammonia is in an unsteady state when it is in water and is easy to volatilise, so it could almost lose its effect through the volatilisation if exposed to the air for an extended period</p>
Nitrogenous fertilisers	<p>Ammonium sulphate ((NH₄)₂SO₄) with a nitrogen content of 20–21%</p> <p>Produced from liquid ammonia directly neutralised with diluted sulphuric acid. When pure, it is a white crystal apt to dissolve in water. 100 kg of water can dissolve 75 kg of ammonium sulphate. With a small amount of moisture absorption, it is convenient to preserve and apply</p>
Nitrogenous fertilisers	<p>Urea (CO(NH₂)₂) with a nitrogen content of 44–46%</p> <p>Ammonia and carbon dioxide are combined and synthesised into urea under high heat and pressure conditions. The resulting substance takes the form of white crystals with a solid moisture-absorbing capability. Once dissolved in water urea does not ionise, so it cannot be absorbed by plants in that state. It becomes usable by plants only after being broken down by the enzyme urease, which is secreted by urea-decomposing bacteria and then transformed into ammonium carbonate. The rate at which urea converts is temperature dependent. At 20°C, urea can be fully converted into ammonium carbonate within 4 to 5 days; at 30°C, this process takes only 2 days</p>
Phosphoric fertilisers	<p>Calcium superphosphate (Ca(H₂PO₄)₂·2H₂O) with 12–18% available phosphorus (P₂O₅).</p> <p>This type of fertiliser also has a secondary component of CaSO₄·2H₂O, making up about 50% of its composition. Typically appearing as a white powder, this compound is corrosive and tends to absorb moisture. It emits an acidic odour due to some free acid within the product</p>

13.4.3 Hapas

Hapas are structures of fine mesh, usually nylon or another synthetic material, which can be placed in a fishpond or cultivation lagoon (Figure 82). These structures can act as a sort of nursery for fish, isolating them from the external environment while still allowing water, and consequently oxygen and natural food sources like zooplankton, to pass through. During the fry stage of grass carp, the use of hapas offers several advantages:

- **Controlled environment:** Since a hapa is a controlled environment, managing water conditions to optimise fry development becomes more straightforward.

- **Protection against predators:** The hapa acts as a physical barrier, protecting the fry from potential predators in the pond, such as larger fish and other aquatic animals.
- **Ease of monitoring:** With fry confined to a defined space, activities such as stock counting, growth observation and health assessment of the fish become easier and more efficient.
- **Controlled feeding:** Artificial feed can be better distributed and monitored within the hapa than in the pond, ensuring all fry have equal access to food.
- **Simplified management:** Transferring fry to other ponds or directly to the market becomes more practical as they are gathered in a smaller, controlled space.
- **Optimal use of space:** Using hapas allows for better space optimisation within the pond, as different stages of the fish life cycle can be managed in the same pond but in separate hapas.



Figure 82. Hapas for post-larvae cultivation.

13.5 Feeding the post-larvae

Artificial feeds in the nursery phase are necessary, but ideally fish should be fed a combination of natural foods (zooplankton) and feed. With artificial feed, it is assumed that 50% of weight gain will be provided by the feed, with the remaining 50% coming from live zooplankton.

However, post-larvae overgrow, and their food consumption increases as they develop. After this initial period, zooplankton alone will not be sufficient to meet the daily food needs of the fry. If there is an insufficient supply of food, the initially swift growth of the fish may come to a halt, leading to weakening and increased vulnerability to diseases. This situation can be averted by providing adequate supplementary feeding.

Every 5 days, controlled catches should be performed to collect at least 50 post-larvae or fry and determine their average weight.

13.5.1 In incubators

13.5.1.1 Boiled egg yolk

An old but effective method involves using boiled egg yolks to start feeding post-larvae in incubators:

- **Boiling the egg:** First, cook the eggs in water for 6 to 8 minutes.
- **Preparation:** After boiling, remove the yolk and place it in a fine mesh fabric sieve, similar to those used in plankton nets with a mesh size of 100 to 150 micrometres. Press the yolk through the sieve into water, creating an egg yolk suspension.
- **Feeding the fish:** The next step is to pour this suspension into the container where the post-larvae are being raised. However, this should only be done when the post-larvae have filled their swim bladders with air and are ready to feed.

13.5.1.2 Fish meal

Another option is to use finely ground fish meal when the fish are stocked. Fish meal has its pros and cons compared to live foods:

- **Rich in nutrients:** It is a rich source of proteins, essential amino acids, lipids and essential fatty acids but poor in micronutrients.
- **Palatability:** Post-larvae usually accept high palatability, facilitating the transition from natural to artificial feeding.
- **Storage and nutrition:** Easy to store with a more constant nutritional composition than live foods. However, it can rapidly alter water quality, requiring intense water exchange to avoid harming the fish.

13.5.2 In outdoor areas rich in natural foods

The feeding programme for post-larvae stocked in hapas, tanks or nursery ponds should follow these main guidelines:

- **Natural food:** For economic reasons, living organisms present a highly efficient solution for feeding post-larvae. The primary food in this early life stage, in outdoor environments, are tiny zooplankton organisms such as rotifers, protozoans, invertebrate eggs and small copepods (Figure 83) in concentrations of at least 200–300 individuals per litre.



Figure 83. Zooplankton organisms serve as food for grass carp post-larvae and fry.

- **Commercial feeds, key considerations:**
 - In most countries where grass carp is commercially cultivated, initial feeds on the market meet the nutritional needs of the species post-larvae.
 - Artificial feed should begin on the day of stocking the post-larvae in nursery ponds. Initially, in small amounts, the post-larvae get accustomed to the taste or palate of the artificial feed (Figure 84).
 - The feed should provide the necessary energy and protein nutrients for growth, containing, in the case of post-larvae, 41 to 50% protein. The composition of the feed is similar to that used during the fry stage, although the protein content is usually higher in the second half of the growth period, which will last three to four weeks.

- **Particle size:** The size of the feed particles is crucial for successful feeding of post-larvae and fry. Initially, it is recommended to use crumbled feed with smaller particles of 120 to 180 μm . From the second week, fish can consume 250–600 μm foods. For post-larvae older than 15 days, the ideal food size ranges from 700 to 1,000 μm . Choosing the wrong size can lead to problems such as low feed efficiency, waste, poor nutrition, difficulty in ingestion, and impaired growth. As the fish develop, it is appropriate to transition to a pelleted feed.



Figure 84. Crumbled feed is used in feeding grass carp post-larvae.

- **Feed quantity, key considerations:**
 - The amount of artificial feed to be applied depends on the abundance of zooplankton, the survival rate, and the age of the post-larvae. As a reference, about 3–9% of the biomass per day should be offered, depending on the size and density of the post-larvae and the quality and temperature of the water. The colder the water, the lower the appetite of the fish.
 - The amount of feed also depends on the appetite of the post-larvae, and the quantity can be gradually doubled in the last days of cultivation. However, overfeeding should be avoided.
- **Feeding frequency:** It is recommended to feed the larvae 4–5 times a day during daylight hours.
- **Feed residue:** The nutrients in the feed, as they disintegrate and become soluble, will also serve as nutrients for the zooplankton. Therefore, even if the fish do not entirely consume the feed, it will indirectly feed them, in this case, through natural foods.
- **Feeding programme:** The feeding programme with feeds must be well planned, as it is critical to maximising the growth and health of the larvae, thus ensuring more efficient and sustainable grass carp cultivation (Figure 85).



Figure 85. Manual feeding with crumbled feed of grass carp post-larvae in hapas.

13.6 Feeding the fry

- **Stocking strategy:** Direct stocking of fry in grow-out and finishing systems is not recommended. Ideally, fish should first be kept in rearing systems until they reach 11–15 cm in length or more.
- **Fry stage feeding:** In the fry stage, when the fish reach about 3–7 cm in length, grass carp are primarily fed *Wolffia arrhiza*, a widespread aquatic plant known as duckweed. The initial daily feeding rate is 3–5% of the biomass (feed) and 4–7% of the biomass for natural food, gradually increasing according to the demand of the fish.
- **Diet transition:** Feeding is usually changed to another duckweed species (*Lemna minor*) when the fish reach 70–100 mm long. After this, the carp are fed tender aquatic plants and terrestrial grasses (Figure 86).
- **Growth duration:** The fry stage lasts 4 to 6 months in mild temperatures but can be considerably shortened in higher temperatures or if low population densities are used.
- **Survival rate:** The expected survival rate throughout the entire fry cultivation period should be over 95%.



Figure 86. Two species of duckweed are generally used to feed grass carp fry.

13.7 Monitoring the health of post-larvae

Due to high stocking densities, intense metabolic processes at this life stage of fish, and the quite unstable biological balance in nursery ponds, there is an increased risk of disease outbreaks, including infectious diseases.

Therefore, breeders must frequently assess the behaviour of their stock and use this assessment to indicate the fish's health status. The water quality of the nursery pond, especially oxygen concentration, should also be systematically monitored.

Among other indicators, unusual swimming behaviours, larvae resting on the water's surface or congregating at water inlets or outlets may suggest that the fish are under intense stress or in poor health.

For a more detailed investigation, samples of fish exhibiting this abnormal behaviour should be chosen for analysis.

Fish breeders must be trained to recognise some of the more typical diseases of grass carp. However, having an experienced veterinarian for more complex cases is always helpful.

During feeding, parasitic diseases such as trichomoniasis, costiasis, chilodonellosis and ichthyophthiriasis can cause problems. The appearance of these parasites is a sign for the fish breeder that something is wrong with the larvae. They may be associated with inadequate feeding, poor water quality and excessively high stocking density, among other factors. Lack of appetite, poor feed conversion and low growth rates can also be characteristics of parasitic infestation.

13.8 Predators of young forms

In outdoor environments, post-larvae and fry are subject to various types of predators and pests, including other fish and invertebrates, such as species of cyclopoids and predatory insects. As they often swim in schools and near the surface they also become easy prey for birds. Therefore, the survival and initial growth of post-larvae, fry and juveniles is a technically complicated process that requires labour, but reducing stocked fish losses is essential.

- **Carnivorous cyclops:** These are small zooplanktonic crustaceans. In some species, the adults can attack the larvae and early-stage fry of carp. They cause wounds to the delicate fish skin with their spiny appendages, sometimes even leading to their death (Figure 87).



Figure 87. Cyclops are zooplankton that can prey on fish post-larvae.

- **Fish:** Various species of fish feed on post-larvae and fry. One such fish is the gambusia. Also known as 'mosquito fish', it consumes many larvae and post-larvae of other fish because they have a similar shape to mosquito larvae. Very young fish cannot escape this danger (Figure 88).



Figure 88. Gambusia, the mosquito fish.

- **Predatory insects:** Larvae of diving beetles (aquatic beetles), larvae and nymphs of dragonflies, and water bugs (water scorpions) (Figure 89).



Figure 89. Dragonfly larva preying on a fish.

- **Birds:** Several species of birds (such as herons, cormorants, kingfishers, eagles, hawks, darters and night herons, among others) can prey on post-larvae, fry and even adult fish (Figure 90). In this case, predation can be high as grass carp post-larvae and fry swim in schools and near the surface (Figure 91). One way to avoid this problem is to use tanks or ponds covered with protective netting.



Figure 90. The kingfisher is a great predator of fish.



Figure 91. Grass carp post-larvae and fry become an easy target for piscivorous birds as they swim in schools and near the surface.

13.9 Grow to fry or juveniles?

After about 2–4 weeks of cultivation, depending on water temperature, employed density and correct feeding, among other factors, the fish will be approximately 2–3 cm in total length and weigh about 0.1–0.3 g. Once they reach this size they have reached the fry stage and begin to have the minimum conditions to be transferred to the fattening and finishing systems. However, there is the option to perform a new cultivation phase called rearing. In this case, the fry (2–3 cm) is cultivated until the juvenile stage (about 13 cm) before being sold or transferred to the fattening and finishing systems.

Although in some cases these two stages can be combined in a single pond or tank, it is generally more advantageous to separate them. This recommendation is because the dietary needs, stocking rates and environmental demands vary between post-larvae and fry.

Usually, the larger the fish sent to the fattening and finishing phase (Figure 92), the better its yield and the lower the mortality rates.



Figure 92. The larger the size of grass carp fry and juveniles when transferred to the fattening and finishing cultivation systems , the greater the chance of survival and the shorter the cultivation time.

13.10 Harvesting and transfer to rearing tanks

The harvesting and transfer of the fry should be executed with the utmost care. A day before removal, feeding should be stopped to prepare the fish for transport. In the case of hapas, the great advantage is that harvesting can be carried out using just a net or even by removing the net that makes up the hapa. Feeding should be gradually reduced and then stopped for larger ponds, which require emptying over more than one day. There are two possibilities for harvesting fry kept in nursery ponds: complete emptying of the pond using a fixed net or partial emptying using a seine net.

13.10.1 Complete emptying of the nursery pond

In this case, the first step in collecting fish cultivated in nursery ponds is the total drainage of the pond through the main outlet, over which a net with an appropriate mesh opening should be placed to capture the fry.

The water flow must be controlled so as not to press the fry against the net. Fish captured should not be left in contact with the net for too long. The net should be frequently emptied, and the fish transferred to transport boxes or plastic bags, avoiding crowding of the animals. In both cases, an oxygen supply should be provided to ensure the health and welfare of the fry.

13.10.2 Partial emptying of the nursery pond

Nursery ponds of a few hundred square metres can be harvested with a cloth net dragged through the pond after partially reducing the water level. Most fry can be collected this way, but some may escape and remain in the pond. Therefore, in these cases it may be necessary to empty the pond at the end of the operation and collect the remaining fish.

In the case of larger ponds, it is advisable to wait until the fry accumulates in front of the drainage structure. Seine nets can be used several times for capture.

13.10.3 Transfer

- The transfer should be made using transport boxes with constant aeration or oxygenation.
- Preferably, the cooler periods of the day should be chosen to avoid excessive stress on the fish during their handling.

13.11 Rearing

The rearing phase of grass carp is a transition period between fry and fattening. In this phase, the fish are carefully managed to reach the suitable size and weight for the next phase. The most important aspects related to the rearing phase of grass carp are:

- **Feeding:** Grass carp fry are herbivores and feed on aquatic plants, such as algae, macrophytes and grasses. However, to ensure optimal growth, it is recommended to provide a balanced feed that meets the nutritional needs of the fish. The feed can be provided more than 3 times a day in quantities that vary according to the age and size of the fish.
- **Management:** Proper management of the ponds is essential to ensure the welfare and health of the fish. It is important to control water quality, stocking density and potential predators.

- **Monitoring:** It is crucial to ensure the pond is in ideal conditions. This concept includes water quality measurements, such as pH, dissolved oxygen, and temperature, which should be regularly monitored. Maintaining the pond's filtration and protection structures is critical to prevent predator access or fry escape.
- **Health:** The fish should be visually monitored regularly to detect possible diseases. In case of suspected disease, it is essential to consult a veterinarian.

The duration of the rearing phase varies according to cultivation density, feed quality, and management. Generally, grass carp fry reaches a suitable size and weight for the fattening phase in about 6 to 12 months.

13.11.1 Pre-sale stocking

It is common for fry or juveniles to be stored for a few hours or even days in 5 to 10 m³ tanks (Figure 93) lined with rectangular nets of fine material, waiting for orders from fish consumers.



Figure 93. Tanks for storing fry before sale.

13.11.2 Packaging

There are two methods for transporting fingerlings and juveniles: transport boxes, with or without artificial aeration, oxygenation, or water circulation, and sealed, airtight polyethylene bags filled with oxygen (Figure 94).



Figure 94. Transport boxes and plastic bags containing 1/3 water and 2/3 oxygen are used for transporting fingerlings and juveniles.

Transport boxes are designed in a 'splash less' system. The lid is autoclave-type, and the built-in aeration system provides compressed air. This system can be powered by a compressor, or a belt connected to the engine of the transport vehicle. There is also the option to use oxygen cylinders attached to the transport boxes.

Polyethylene bags or other types of thick plastic are filled with 1/3 water and 2/3 oxygen and are usually used for short-distance fish transport.

Before being transported, whether for short or long distances, young fish need to undergo a preparation period. This preparation is crucial to help them endure the confined space they will be in during transport. This conditioning aims to ensure the fish eliminate any food still being digested in their digestive systems. They should be fasted (12–24 hours) before being packaged to achieve this.

Fish in transport systems should be placed in a shaded area to avoid sudden temperature changes.

During conditioning, no natural or artificial feed should be given to the fish. When fed, the startled fingerlings excrete and even regurgitate food, emptying their digestive tracts, deteriorating water quality and compromising survival.

13.11.3 Transport

The fish should be transported in trucks or small vehicles (Figure 95) and this should be done in the shortest time possible, preferably in less than 6 hours, although occasionally, this time may need to be longer.



Figure 95. Transport of fingerlings in transport boxes (above) or plastic bags (below).

13.11.3.1 Recommendation for the transport of larvae and fingerlings

Table 38. Indicators for the transport of grass carp post-larvae and fingerlings.

Category	Indicators	Level	Values of reference	
Environmental	Temperature (°C)	Optimum	22–25	
		Acceptable	15–21 or 26–32	
	pH	Optimum	7.0–8.5	
		Acceptable	6.0–6.9 or 8.6–9.0	
	Oxygen saturation (%)	Optimum	97–133	
		Acceptable	48–96	
	Un-ionised ammonia (NH ₃)	Optimum	0.000–0.025	
		Acceptable	0.026–0.059	
	Sanitary	Skin	Optimum	Normal, healthy, and bright
			Acceptable	Pallor, darkening, loss of scales or localised injury
Fins		Optimum	Normal, healthy	
		Acceptable	Light lesions or splitting	
Mortality (%)		Optimum	0–10	
		Acceptable	11–24	
Nutritional	Fasting time (hours) - diet feed	Optimum	12–24	
		Acceptable	8–11	
	Fasting time (hours) - vegetable diet	Optimum	24–30	
		Acceptable	12–23 or 31–36	
Behavioural	Sedation for transport	Optimum	Sedation (loss of reaction to touch and visual perception)	

Category	Indicators	Level	Values of reference
		Acceptable	Light anaesthesia (loss of balance and normal swimming movement interspersed with irregular lateral swimming)
		Optimum	Constant swimming
	Swimming behaviour after transport	Acceptable	Erratic swimming, panting

13.12 Recommendations for larviculture, nursery, and fingerling rearing

13.12.1 Environmental indicators

Table 39. Environmental indicators for eggs, larvae and post-larvae of grass carp.

Indicators	Level	Reference values
Temperature (°C) for hatching eggs	Optimum	22–29
	Acceptable	16–21 or 30–35
Temperature (°C) for growing larvae and post-larvae	Optimum	24–32
	Acceptable	13–23 or 33–34
pH	Optimum	7.0–8.5
	Acceptable	6.0–6.9 or 8.6–9.0
Dissolved oxygen (% saturation)	Optimum	≥ 63.9
	Acceptable	38.0–63.8
Salinity (ups)	Optimum	0–0.7
	Acceptable	0.8–8.0
Stocking density (post-larvae/m³)	Optimum	120–150
	Acceptable	151–200
Un-ionised ammonia (mg/L of NH₃)	Optimum	0.000–0.025
	Acceptable	0.026–0.059

Indicators	Level	Reference values
Nitrite (mg/L of NO ₂ ⁻)	Optimum	0–0.9
	Acceptable	1.0–1.5
Alkalinity (mg/L of CaCO ₃)	Optimum	25–100
	Acceptable	≥ 101
Photoperiod (Light: Dark) for hatching eggs	Optimum	8L:16D–10L:14D
	Acceptable	11L:13D–12L:12D
Photoperiod (Light: Dark) for growing larvae and post-larvae	Optimum	12L:12D–14L:10D
	Acceptable	15L:9D–16L:8D
Predators and other aquatic interspecific inhabitants*	Optimum	Absence
	Acceptable	Controlled presence

Table 40. Environmental indicators for grass carp fingerlings.

Indicators	Level	Reference values
Temperature (°C) during spawning and maintenance	Optimum	20–33
	Acceptable	16–9 or 34–37
pH	Optimum	7.0–8.5
	Acceptable	6.0–6.9 or 8.6–9.0
Transparency (cm)	Optimum	30–60
	Acceptable	20–29 or 61–70
Dissolved oxygen (% saturation)	Optimum	≥ 49.0
	Acceptable	43.0–48.9
Salinity (ups)	Optimum	0–0.7
	Acceptable	0.8–8.0
Un-ionised ammonia (mg/L of NH ₃)	Optimum	0.000–0.025
	Acceptable	0.026–0.05
Nitrite (mg/L of NO ₂ ⁻)	Optimum	0–0.9
	Acceptable	1.0–1.5

Indicators	Level	Reference values
Alkalinity (mg/L of CaCO₃)	Optimum	25–100
	Acceptable	≥ 101
Terrestrial predators	Optimum	Absence
	Acceptable	Controlled presence
Predators and other aquatic interspecific inhabitants	Optimum	Absence
	Acceptable	Controlled presence

13.12.2 Health indicators

Table 41. Health indicators for eggs, larvae, and post-larvae of grass carp.

Indicators	Level	Reference values
Eggs	Optimum	75 to 100% spherical and translucent
	Acceptable	50 to 75% spherical and translucent
Eyes	Optimum	Fully pigmented eyes with golden irises
	Acceptable	Partially pigmented
Body	Optimum	Fully pigmented (melanophores throughout the dorsal, ventral and mediolateral region of the body)
	Acceptable	Partially pigmented (melanophores for some regions of the body)
Fins	Optimum	Functional and healthy appearance
	Acceptable	Fin malformation
Notochord	Optimum	It is functional as a support axis
	Acceptable	Malformation without movement restriction
Mortality (%)	Optimum	≤ 15
	Acceptable	16–30

Table 42. Health indicators for grass carp fingerlings.

Indicators	Scores	Reference values
Eyes	Optimum	Normal and healthy appearance
	Acceptable	Haemorrhage, unilateral exophthalmos or traumatic injury
Jaws/lips	Optimum	Normal and healthy appearance
	Acceptable	Bleeding, redness or mild injury or deformity (without affecting eating)
Skin	Optimum	Normal and healthy appearance
	Acceptable	Scar tissue, punctual loss of scales, ulcers, or superficial lesions < 1 cm ²
Fins	Optimum	Normal and healthy appearance
	Acceptable	Scar tissue, mild necrosis or splitting
Abdomen	Optimum	Normal and healthy appearance
	Acceptable	Discreet distension, redness
Mortality (%)	Optimum	≤ 10
	Acceptable	11-24

13.12.3 Nutritional indicators

Table 43. Nutritional indicators for eggs, larvae and post-larvae of grass carp up to three weeks after hatching.

Indicators	Level	Reference values
Crude Protein (%)	Optimum	41-50
	Acceptable	36-40
Feed amount* (% body weight)	Optimum	3-9
	Acceptable	1-2
Pond preparation/ fertilisation	Optimum	Performed before each production cycle
	Acceptable	Performed sometimes
Seizure of live food (minutes)	Optimum	30-45
	Acceptable	25-29 or 46-50

* Recommendation: For post-larvae up to 7 days after hatching (body length 5-8 mm and weight up to 30 mg), the ideal feed size is 120 to 180 µm. For post-larvae 8-14 days after hatching (body length 8-12.5 mm and

weight 30–55 mg), the adequate feed size is 250–600 µm. For post-larvae 15–21 days after hatching (body length 11.5–18.6 mm and weight 65–137 mg), the ideal feed size is 700 to 1000 µm.

Table 44. Nutritional indicators for grass carp fingerlings.

Indicators	Level	Reference value
Feed crude protein (%)	Optimum	35–45
	Acceptable	30–34
Formulated feed amount (% biomass)*	Optimum	3–5
	Acceptable	1–2,9
Natural feed amount (% biomass)**	Optimum	4–7
	Acceptable	1–3
Feeding frequency (times/day)	Optimum	≥ 3
	Acceptable	2
Food distribution (% of surface area reach)	Optimum	> 75 of surface area
	Acceptable	50–75 of surface area
Body condition factor (K)	Optimum	≥ 1.20
	Acceptable	– 1.19

13.12.4 Behaviour indicators

Table 45. Behaviour indicators for eggs, larvae and post-larvae of grass carp.

Indicators	Level	Reference values
Eggs floating	Optimum	Eggs are constantly suspended in the water column until they hatch
	Acceptable	Eggs suspended in the water column most of the time
Post-larvae phototaxis (%)	Optimum	95–100 with positive phototaxis
	Acceptable	50–94 with positive phototaxis
Post-larvae behaviour swimming	Optimum	Active post-larvae swimming vertically or horizontally in the water column and with short periods at the tank bottom
	Acceptable	Reduced swimming activity in the water column or some post-larvae present at the tank bottom

Table 46. Behaviour indicators for grass carp fingerlings.

Procedure	Indicators	Level	Reference values
Acclimatisation Massive capture	Respiratory frequency (opercular rate/ min)	Optimum	40–70
		Acceptable	20–39 or 71–90
Massive capture (partial or total)	Swimming behaviour	Optimum	Regular swimming, no or few body parts on the surface
		Acceptable	Restless swimming behaviour, swimming in different directions and or jumping

13.13 Overwintering

In warmer regions, where stable ice formation is not observed even during the harshest winter, the fish farmer has more flexibility in choosing ponds and other cultivation structures. However, in regions with shorter winters, overwintering may be necessary.

Overwintering grass carp is similar to common carp and involves smaller ponds, ranging from 0.2 to 1.0 hectares in area, with a minimum water depth of 1.2 m that do not freeze. If the surface freezes, of course, the water needs to be deeper than this. Another detail is the frequency of water renewal in the pond, which should be every 15 to 20 days. On the other hand, the filling and draining of the pond water should be done within 12 to 24 hours.

The stocking density of grass carp fingerlings in overwintering ponds should not exceed 450,000 to 550,000 per hectare, approximately 10 tonnes per hectare. The survival rate of the fingerlings after winter should be at least 75%. As the animals' ability to feed is reduced during winter, weight loss during this period is expected. However, the weight loss of the fish should not exceed 10 to 12%.

A crucial point to note is that during winter it is essential to monitor the water temperature and oxygen concentration daily. Samples are usually collected at the outlet of the pond. If the fish farmer notices these parameters are deteriorating, measures should be taken to improve the situation. If the pond surface freezes, holes should be opened in the ice. More generally, the fish farmer can increase the water flow or use aerators to improve the conditions of the pond.

Thus, the producer ensures the fish's survival and maintains a healthy environment, favouring their welfare during winter.

14 POND MANAGEMENT

14.1 Types of ponds

Throughout the grass carp's production cycle, three main types of ponds are commonly employed, each with specific characteristics and objectives. Each type involves a unique set of management practices, including different feeding regimes, maintenance of water quality parameters and stocking densities, although the preparation processes for each are fundamentally the same (Figure 96).

- **Nursery pond:** This is the initial stage where post-larvae are cultured until they become fry. This pond is usually smaller, allowing for more stringent control of environmental conditions and ensuring protection and abundant natural food for the fish. The aim is for the post-larvae to survive the most critical life cycle phase.
- **Rearing pond:** The second stage is the rearing pond. Here, fry are kept until they become juveniles. Rearing ponds are generally larger than nursery ponds and smaller than fattening ponds, accommodating an intermediate density of fish compared to the other two. The focus is to grow the carp to a size that allows them to survive and thrive in the fattening ponds. Regarding feeding, although natural food is used, in these ponds the fish are fully adapted to dry feeds.
- **Stocking pond:** Finally, there is the fattening pond, where juvenile fish are cultured until they reach commercial size. These are the broadest ponds designed to support rapid growth and the maximum possible weight gain of the fish. Therefore, the stocking density (number of fish/m²) is lower than that used in the other ponds, but the biomass is much greater.

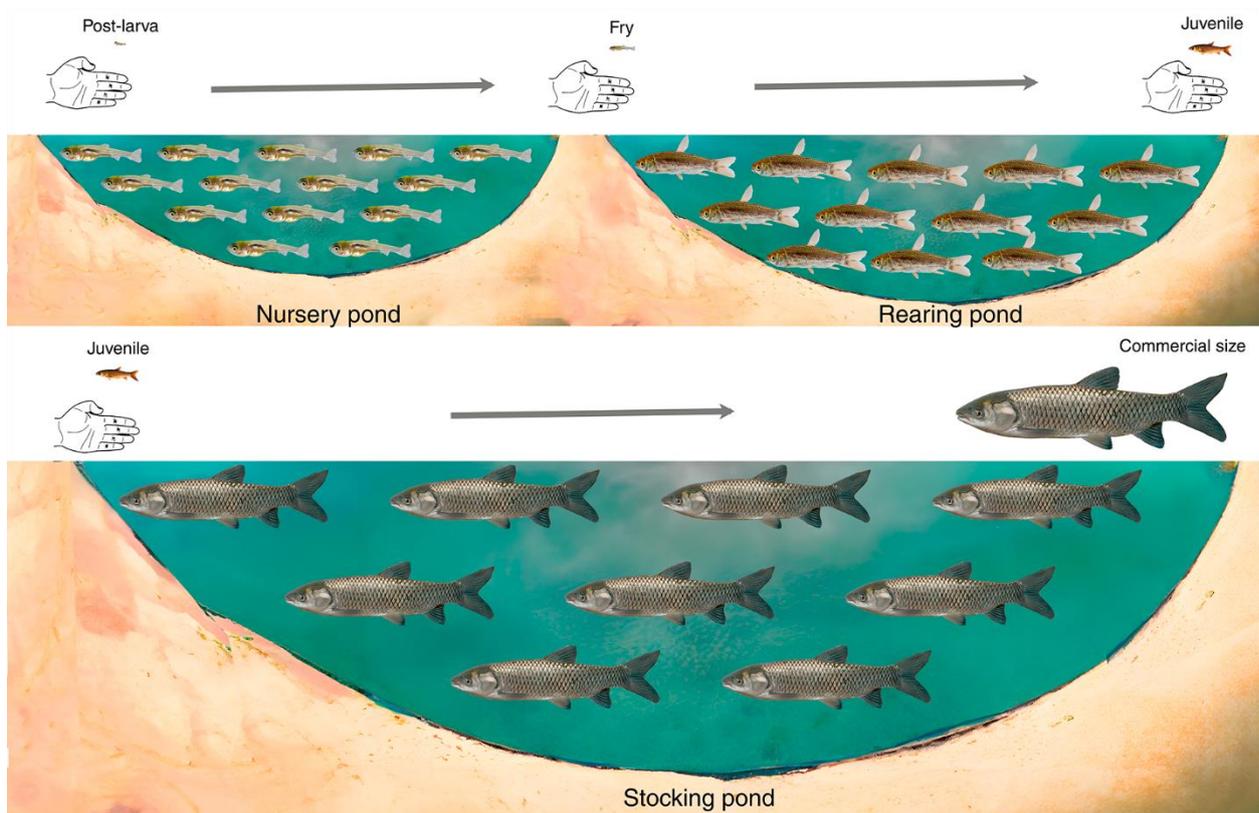


Figure 96. Different types of ponds are used during the grass carp production cycle.

14.2 Choosing the site for pond construction

Selecting the ideal site for setting up grass carp pond aquaculture should ideally consider:

- **Topography:** opt for broad, flat but drainable areas, well ventilated, with abundant sunlight.
- **Water quantity:** The water source should be plentiful, free of upstream pollution, constant throughout the year, and sufficient to fill all the property's ponds in a maximum of 20 days. This recommendation is not a fixed rule but it is a reasonable one. It means that the minimum desirable daily water renewal rate would be 5%/day. Usually, the fish farmer will not need to renew such a volume, but in exceptional situations high water renewals may be necessary, and the venture should be designed to meet these potential critical situations.
- **Soil texture:** Soil texture is an essential factor to consider in fishpond construction. The texture is expressed by the proportion of granulometric components of the soil's mineral phase, sand, silt, and clay. Soils with a mix of textures are considered ideal for aquaculture pond construction. The advised ratio typically ranges from 20–30% clay, 30–50% silt, and 20–30% sand. Such ratios are crucial for preserving the ponds' impermeability and facilitating better management of surplus organic matter at the bottom, and must be adhered to.

14.3 Pond construction

- **Shape:** It is preferable to construct ponds in regular shapes, generally rectangular, with the longest dimension built parallel to the region's prevailing wind direction. However, constructing irregular ponds may be necessary for better land utilisation. Irregular ponds, however, can complicate management, especially for harvesting.
- **Size:** The area of each pond usually varies from 0.5 to 3 hectares. Nursery and fry ponds tend to be smaller. Larger ponds have proportionally lower installation and operating costs. However, they are more challenging to manage and usually achieve lower productivity rates. Small ponds are proportionally more expensive, requiring the same hydraulic structures as large ponds. They also demand a more intensive management routine, involving proportionally higher labour costs than large ponds. On the other hand, small ponds allow for better control of environmental variables and more rigorous production process management. Thus, the size of the ponds should be planned according to the techniques to be applied and the projected financial results.
- **Depth:** The depth should preferably vary from 1.2 m at the shallowest point to a maximum of 2.0 m at the deepest point.
- **Slope:** The ideal slope will depend on the size of the pond. For example, rectangular ponds of about 1 ha have an ideal slope of around 1%. This proportion means a drop of one centimetre per metre towards the drainage gates. Larger ponds will necessarily have lesser slopes; otherwise, the lower portion of these ponds would become too deep. In practice slopes below 0.1% are not recommended, as this creates areas where water pools form, preventing the complete drainage of the ponds.

14.4 Pond preparation

Adequate pond preparation is vital for the ongoing success of fish farming, whether initiating production in new ponds or readying previously used ponds for a subsequent cultivation cycle. Such preparation is crucial in maintaining a healthy environment for the fish, thereby enhancing the productivity and sustainability of the operation. As previously stated, the fundamental principles of pond preparation remain consistent across different pond types, with minor variations between newly constructed ponds and those previously in use.

14.4.1 New ponds

- **Cleaning and debris removal:** Before starting cultivation for the first time, it is essential to clean the pond thoroughly. During construction, removing debris, such as branches, leaves and rubble that may have accumulated by itself, helps ensure the pond's operability and maintain water quality, avoiding contamination.
- **Levelling and imperfection correction:** It is necessary to check if the pond bottom is adequately levelled and correct any imperfections. This contributes to efficient water use, prevents sediment accumulation, and enables complete pond drainage at the end of each production cycle.
- **Soil analysis:** At least one soil analysis should be conducted to check the pH and make necessary corrections. The process of carrying out soil analysis is explained in section 13.4.4.

14.4.2 Emptying the pond and oxidation of organic matter residues

When a production cycle ends, the pond must be completely drained. This management procedure is relevant to eliminating undesirable organisms (fish, insect larvae, pathogenic microorganisms) and allowing oxidation and mineralisation of the excess organic matter at the bottom of the pond.

Excess organic matter at the bottom of ponds during cultivation will be slowly decomposed by microorganisms present in the soil. These microorganisms will consume a large amount of dissolved oxygen and, in extreme cases, can release a series of highly toxic chemical compounds into the water. Therefore, these excesses of organic matter need to be eliminated at the end of each production cycle.

The dark colour, the muddy soil that sinks when one walks in the pond (Figure 97) and the pungent odour of rotten material are indicators of the presence of excess organic matter at the bottom of the pond at the end of cultivation.

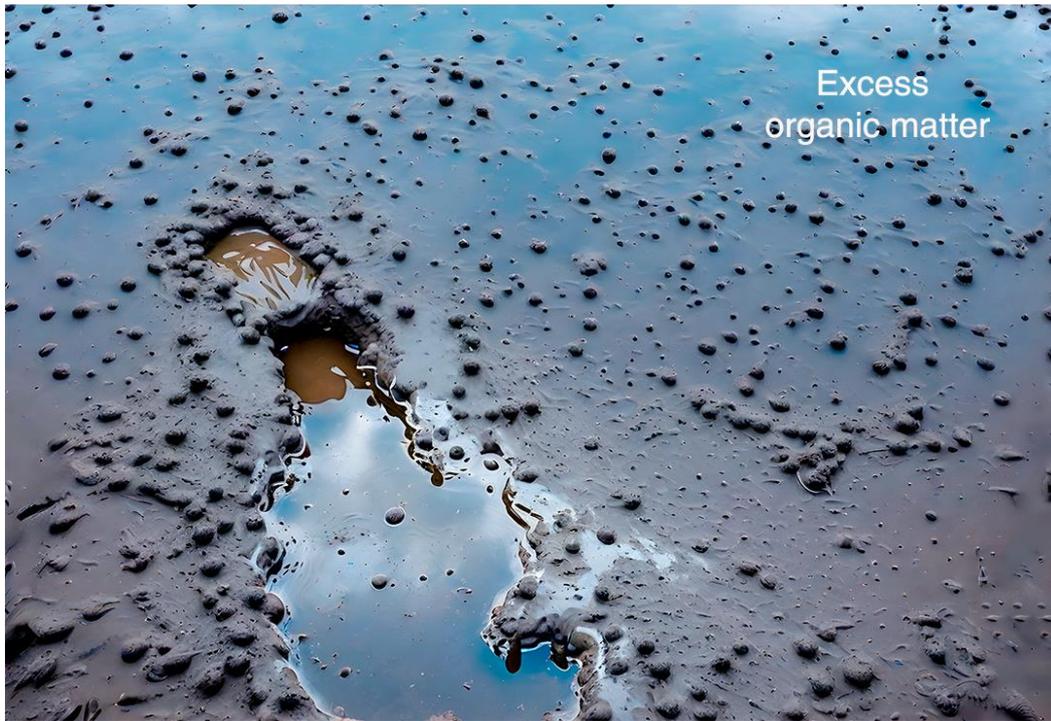


Figure 97. Fishponds after harvesting. Above: with little organic matter. Below: with excess organic matter at the bottom.

- **Sun:** If there is no excess organic matter at the bottom at the end of cultivation, the sun can oxidise the organic matter. Leave the pond dry for a few days of intense sun, and the high incidence of sunlight will destroy the excess organic matter present in the soil.
- **Microorganisms:** If there is an excess of organic matter at the bottom of the pond, the bacteria and microorganisms naturally present in the soil can help decompose this matter from the previous cultivation and release the nutrients. However, this takes time, and the producer generally sees the period without fish in the pond as wasted time. To accelerate decomposition, we can aid these bacteria. For this, the soil must be very moist but not flooded.
- **Technique 1:** To help these microorganisms oxidise the excess organic matter, add nitrogenous fertiliser and lime to the soil. This procedure enhances the bacteria by balancing the C:N (Carbon: Nitrogen) ratio and adjusting the pH to about 7.0, which is ideal for these bacteria and beneficial to fish farming. An effective technique is to apply nitrogenous fertiliser to the moist soil after harvesting, with sodium or calcium nitrate (20–100 kg/ha) being the best option, as they do not release acids and help improve water quality. Along with this, apply lime at 200–300 kg/ha.
- **Carbon and organic matter:** The results of organic matter concentrations sometimes do not appear in soil analyses conducted in laboratories. In these cases, multiply the result for percentage of carbon (C), which will undoubtedly be present in the analysis, by 1.72. The resulting value indicates the percentage of organic matter present in the soil.
- **Organic matter:** Soils ideal for fish farming should contain 1.0–3.0% of organic carbon in their composition (equivalent to 1.7–5.2%), as indicated in Table 47.

Table 47. Classification of organic carbon concentrations in pond soil.

Organic Carbon (%)	Organic Matter (%)	Assessment
>15	> 25.8%	Organic soil
3.1–15	5.3–25.8	Mineral soil with high organic matter content
1.0–3.0	1.7–5.2	Mineral soil with moderate organic matter content, most suitable for fish farming
<1	<1.7	Mineral soil, low in organic matter, which does not support good development of benthic organisms

- **Technique 2:** If the concentration of organic matter in the soil is high (above 4%), apply 11 kg/ha of urea or sodium nitrate (the latter also being a nitrogen source and a more efficient oxidant) to the still-moist soil for each 1% of organic matter exceeding the 3% limit. For instance, if the soil contains 5% organic matter, correcting the excess 2% (5%–3% = 2% to be updated) is necessary. As 11 kg/ha of urea is required for each 1%, and there are 2% to correct, 22 kg/ha of urea should be applied. After one or two days, it is advisable to use 2,000 kg/ha of calcitic or dolomitic lime and plough the pond bed. This procedure reduces organic

matter by up to 18% per cycle. However, if the concentration of organic matter in the soil exceeds 5%, after applying urea, 2,000 kg of calcium hydroxide can be used before stocking the pond.

- **Technique 3:** Conversely, if the concentration of organic matter in the soil is low (common in sandy soils), the technician may apply manure, incorporated through ploughing into the pond bed, at 1,000 kg/ha. In this case, to maintain the levels of organic matter in the soil, apply 32 kg/ha/week of chicken manure, together with 25 kg/ha/week of ammonium nitrate or calcium nitrate, or apply chicken manure supplemented with urea (18.5 kg/ha/week).

14.4.3 Disinfection

While it may not always be necessary to correct the pH of the soil between two production cycles, it is almost certain that pond disinfection will be required after completing a production cycle. Disinfection eliminates undesirable organisms that can cause economic losses, whether by competing with the fish for food, transmitting diseases, or even preying on the cultivated fish.

14.4.3.1 Pond drying and sun exposure

- **Sun:** Once again, the sun is the best and cheapest way to disinfect the pond. Drying the pond eliminates eggs and juvenile forms of fish, insect larvae and other undesirable organisms, which may survive in moist soil but never in dry soil.
- **Technique:** The pond is left to dry for about 5 to 7 days of sun exposure. This procedure will cause cracks of about 5 cm across the pond bed, aiding the oxygenation of the deeper layers of this topsoil horizon (Figure 98). One way to determine if the pond has been exposed to the sun for a sufficient time is to check if it is possible to walk across the entire pond bed without sinking into the mud.



Figure 98. Sun-dried pond soil. Most, if not all, potentially undesirable organisms are eliminated with this simple and efficient practice.

14.4.3.2 Treatment of puddles and water stagnation points

Drying the bottom completely, or even drying the sides of the pond, can be challenging, especially during heavy rain seasons or due to unevenness in the pond. In such cases, chemical disinfection may be necessary. This disinfection is critical as grass carp can be susceptible to diseases.

14.4.3.3 Lime

- **Liming:** For disinfection of the pond bed, calcium oxide (CaO , also called quicklime) or calcium hydroxide (Ca(OH)_2 or hydrated lime) can be used. In contact with water, these products cause a rapid and lethal increase in pH. Quicklime also releases heat on contact with water, further enhancing this disinfecting effect.
- **Technique:** The recommended quantity of lime for eliminating undesirable organisms (Figure 99) during pond preparation is 1–2 t/ha.



Figure 99. It is technically relevant to use quicklime to eliminate undesirable organisms at the bottom, especially if there are undrained puddles of water in the pond.

14.4.3.4 Chlorine

When serious infectious microorganism problems are reported on neighbouring farms, intensive disinfection of the pond bed between cultivations may be recommended, even if no diseases occurred in the previous cycle. Although it is technically impossible to sterilise a fish cultivation pond, the aim is to reduce the concentration of microorganisms on the surface to the greatest extent possible. In such situations, the survival of beneficial bacterial biota for aquaculture in the pond should be a secondary consideration. Chlorine is frequently used in aquaculture to disinfect ponds, mainly when there is suspicion or confirmation of disease in the previous production cycle. However, due to its toxicity, water solubility and volatility, chlorine should be limited to the pond preparation phase, never when fish are present, as this could lead to the death of the entire stock.

- **Chemical forms:** Chlorine can be used in different chemical forms, with sodium hypochlorite (NaOCl) and calcium hypochlorite $\text{Ca}(\text{OCl})_2$ being the most common types used in aquaculture. Calcium hypochlorite (HTH, with 65% active chlorine) is also frequently used.
- **Principles:** Upon reacting with water, both hypochlorites form hypochlorous acid (HOCl), a powerful disinfectant that penetrates the cell walls of bacteria. The disinfecting effect of chlorine depends on the pH, so combining it with substances that reduce the pH makes it even more toxic to microorganisms.
- **Technique:** Calcium hypochlorite can be dissolved in pure or muriatic acid-acidified water to increase its oxidising power. Chlorine should be applied primarily to any remaining water puddles at the bottom. This procedure helps ensure that all pathogens are eliminated. Leave the pond empty for at least 4 days after applying chlorine to allow it to dissipate completely.

Besides chlorine, other chemicals can be used as disinfectants in fish cultivation ponds (Table 48).

Table 48. Most commonly used chemical products for disinfection of fish cultivation ponds and recommended dosages.

DESINFECTANT	DOSAGE (g/m ²)
Sodium hypochlorite (5.%)	100-300
Calcium hypochlorite	10-30
Calcium hydroxide	100-150
Calcium oxide	100-150
Formalin	5-10
Benzalkonium chloride (BKC)	1-1.5
Iodine	1-5
Saponin (7%)	1-25

- **Caution!** When applying chlorine, use personal protective equipment (gloves, face mask, rubber boots, and appropriate clothing such as long-sleeved shirts and long trousers), as chlorine can be quite toxic to the person applying it.

14.4.4 Soil analysis

A distinctive feature of cultivation ponds is that once flooded, the soil significantly influences physical, chemical and biological processes, affecting water quality and directly interfering with cultivation outcomes. Therefore, the producer must conduct periodic soil analyses so that any effects can be corrected. Ideally, in the first years of the farm's operation, soil analyses should be done at least once a year in each pond, as cultivation itself alters soil characteristics, especially in new ponds. Over the years, the pond matures and shows fewer variations in its physical and chemical characteristics. pH checks can be conducted on the property using a soil pH meter, or for more detailed analyses, soil samples can be collected and sent to specialised laboratories.

14.4.4.1 Interpretation of soil analysis results

Table 49 shows various parameters related to soil quality for fish farming. Obviously, not all need to be analysed by the producer, but all can affect the activity to a greater or lesser extent.

Table 49. Main chemical elements and parameters related to soil, along with their classification.
Source: Boyd (1995).

Variable	Very low	Low	Medium	High	Very high
pH	< 4	4-6	6-8	8-9	> 9
Organic matter (%)	< 0.9	0.9-1.7	1.7-4.3	4.3-6.9	> 6.9
Carbon (%)	< 0.5	0.5-1.0	1.0-2.5	2.5-4.0	> 4.0
Nitrogen (%)	< 0.15	0.15-0.25	0.25-0.40	0.40-0.50	> 0.5
Phosphorus (ppm)	< 20	20-40	40-250	250-400	> 400
Sulphur (%)	< 0.05	0.05-0.1	0.1-0.5	0.5-1.5	> 1.5
Calcium (ppm)	< 1,000	1,000-2,000	2,000-4,000	4,000-8,000	> 8,000
Magnesium (ppm)	< 700	700-1,500	1,500-3,000	3,000-4,000	> 4,000
Potassium (ppm)	< 100	100-400	400-1,200	1,200-1,700	> 1,700
Sodium (ppm)	< 2,500	2,500-7,000	7,000-15,000	15,000-25,000	> 25,000

14.4.4.2 pH

Soil pH is determined by its chemical composition. Since water interacts directly with the soil, an inappropriate pH can compromise the growth and survival of fish. The ideal pH range for aquaculture soil is between 6.5 and 7.5. This range is also suitable for microorganisms responsible for the decomposition and mineralisation of organic waste generated in the cultures.

Presently, portable devices automatically measure soil pH, eliminating the need to send samples to laboratories when only a pH analysis is required (Figure 100). However, if a soil pH meter is unavailable one can collect a dry soil sample, mix it with distilled water in a 1:1 ratio, and measure it with a standard water pH meter. The result assists in evaluating the soil condition and planning its amendment through liming.



Figure 100. Portable soil pH meter.

14.4.4.3 Laboratory soil analysis

Conducting soil analysis in specialised laboratories is generally an accessible practice, as the costs are not usually high, and it provides crucial data for proper pond management. Based on the results the producer can make the necessary corrections, ensuring a soil conducive to fish cultivation.

It is crucial to note that a property may have several ponds, and sometimes there are heterogeneous soils within a single pond. Therefore, it is advisable for the technician to collect samples from various pond points, covering all textures (such as sandy, silty, or clayey) and soil colours (be they yellow, white, or red, among others). These individual samples are mixed in a bucket to form a composite sample. The collection process is straightforward, but some guidelines must be followed:

- Remove, with the aid of an auger or cutter, approximately 10 cm of soil depth.
- Collect representative samples from the entire pond in a zigzag pattern (Figure 101).
- Place these samples (known as simple samples) in a clean bucket or plastic bags (never use bags previously used for fertilisers, lime, feed, etc. for this purpose).
- Mix the collected simple samples within the bucket or bag.
- Remove and transfer about 500 g of this composite sample to a plastic bag and label it (with wax paper and pencil identification for the label to be placed inside the bag and with a tag

and pen identification for the label to be stuck on the bag). Labels should include the pond's identification and the sample collection date.

- Send the sample for analysis to a specialised laboratory.

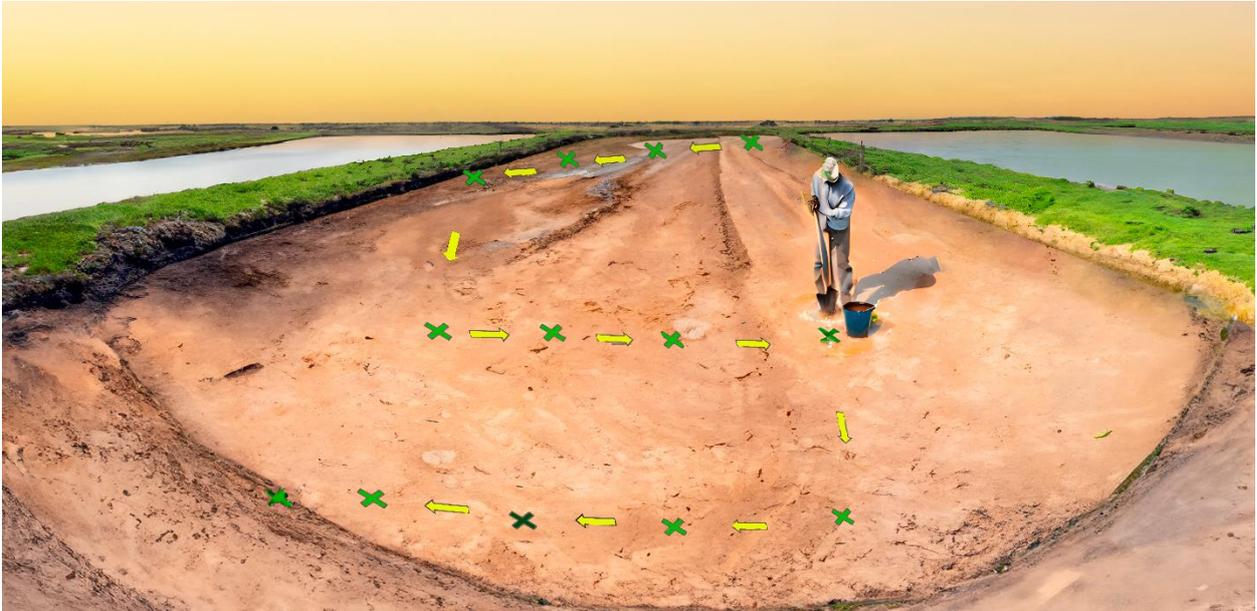


Figure 101. Zigzag soil sampling was used to obtain a composite soil sample from the pond.

14.4.4.4 Organic matter

- **Pond depth:** Very deep ponds typically have lower oxygen levels and a higher concentration of organic matter accumulating at the bottom. This is a crucial reason why ponds are usually constructed with average depths of less than 1.5 m.
- **Soil respiration:** This increases significantly in the first three days after the pond is drained. However in arid soils the decomposition rate decreases; therefore, if the goal is to accelerate the decomposition of organic matter, it is not advisable to leave the soil drying for more than 5 days.

14.4.5 Soil correction

14.4.5.1 pH

- **Objectives:** a) ensure better survival conditions for fish; b) facilitate the success of other management procedures, especially pond fertilisation.
- **Products:** The most accessible and practical method to adjust soil pH is applying a liming material (available in carbonate, oxide or hydroxide), which will neutralise the soil's acidity.

The products commonly used for this purpose are calcitic limestone (CaCO_3), dolomitic limestone ($\text{CaMg}(\text{CO}_3)_2$), quicklime (CaO) or hydrated lime ($\text{Ca}(\text{OH})_2$).

- **Technique:** Soil correction should be based on pH results or more comprehensive laboratory analyses. The limestone should be spread mechanically or manually over the pond surface, including the side slopes (Figure 102).



Figure 102. The effects of limestone will be more evident if it is incorporated into the pond soil, either mechanically (top view) or manually (bottom view).

Limestone has the capability to:

- Increase the soil pH value: In ponds with excessively acidic soil there is a tendency for the water to be acidic, too; it becomes more challenging to promote phytoplankton growth, and fish may experience growth problems.
- Reduce phosphorus retention at the bottom of ponds: A significant portion of the phosphorus used as fertiliser to promote phytoplankton growth may be retained in the soil. Applying limestone increases the pH, causing less phosphorus to be retained in the soil, thus increasing its availability for phytoplankton.
- Increase the amount of carbon dioxide for photosynthesis: limestone or lime added to the soil reacts with water, generating carbon dioxide. Phytoplankton requires this gas for photosynthesis.
- Reduce water turbidity and the amount of suspended material.
- Increase the water's alkalinity.
- Promote the growth and population maintenance of desirable bacteria in the pond.

Other aspects to consider in relation to limestone are:

- **Granularity:** The finer the limestone used the higher its solubility, i.e., the finer, the better.
- **Agricultural gypsum:** Dolomitic limestone (which contains at least 4% magnesium), also known as agricultural gypsum, has advantages over calcitic limestone. In addition to being more soluble in water, it is more effective in maintaining water alkalinity in an environment with elevated pH.
- **Application, key considerations:**
 - Limestone can be applied to the soil during pond preparation and the water during cultivation. When used with the pond full, the limestone increases water alkalinity and the sedimentation of suspended inorganic particles, clarifying the water.
 - Whenever it is necessary to use limestone in already flooded ponds, it is advisable to do so on sunny days and in the morning, as in the afternoon the pH of ponds tends to be naturally higher.

14.5 Fertilisation

Fertilising cultivation ponds is crucial, especially during the life stages when fish depend on zooplankton as a food source. This practice ensures adequate levels of essential nutrients, primarily nitrogen and phosphorus, which can fluctuate throughout the production cycle.

The choice of fertilisers is flexible and can include the same products used in agriculture as long as they promote phytoplankton growth. The amount and frequency of fertilisation should be based on the response of the system, and is often determined through trial and error.

- **Fertiliser applications 1:** In the case of polyculture, periodic fertilisation is essential. Manure/fertiliser can be applied daily, weekly, fortnightly or monthly. Treating pond water with smaller, more frequent doses is considered better and more effective, as smaller doses do not overload the pond ecosystem, keeping all organisms in the active production phase.
- **Fertiliser applications 2:** As manure/fertiliser application frequency decreases, doses should be increased to maintain the same natural fish food production in pond water. However, this general rule cannot be followed if manures/fertilisers are used only fortnightly or monthly. Organisms in the pond cannot process massive doses. Consequently, if fertilisation is carried out only rarely, the productivity of the pond will be lower than its real potential.
- **Results:** Fertilisations carried out during ongoing cultivation generally only start to have effects 2 or 3 days after application, on sunny days. If the days following fertilisation are cloudy, results will only be felt after a more extended period.
- **Solubility, key considerations:**
 - The higher the solubility of the fertiliser employed, the more rapidly it will exert its effects.
 - Liquid fertilisers are up to 4 times more soluble in water than solid fertilisers.
- **Water changes:** When fertilising ponds, water changes should be minimised to prevent the added nutrients from being carried out of the ponds.

14.5.1 Manure

Using organic fertilisers, such as cattle, pig or poultry manure, is another option for pond fertilisation.

Applying fresh manure (organic fertiliser) ensures essential mineral nutrients and carbon, which are often limited in fishponds. Another advantage of manure is that many fish food organisms consume the organic materials directly or indirectly through a very short food chain. Manure and the bacteria that develop on its particles are microscopic foods rich in protein, ideal for zooplankton.

However, it is crucial to use well-rotted manure to minimise the risk of pathogen contamination. Organic fertilisers contain fewer nutrients than chemical ones, and their concentrations vary widely, making it challenging to predict fertilisation results. Moreover, larger quantities of manure are needed to achieve the same effect as chemical fertilisers. For example, 1 kg of urea has the same nitrogen content as 75 kg of cattle manure, and 1 kg of triple superphosphate is equivalent to 167 kg of pig manure in terms of phosphorus (Table 50).

Table 50. Approximate nutrient concentration in fresh manure from various animals. Source: Boyd and Tucker (2012).

Manure origin	Concentration (%)			
	Moisture	N	P ₂ O ₅	K ₂ O
Dairy cattle	85	0.5	0.2	0.5
Beef cattle	85	0.7	0.5	0.5
Horse	78	0.7	0.3	0.6
Pig	87	0.5	0.3	0.4
Sheep	68	1.0	0.5	1.2
Poultry bedding	22	3.5	3.0	2.0

Another problem with the use of manures is that the nutrients are not immediately available, especially if the manure is not fresh. In fresh manure, part is liquid and another part is solid. In the case of cattle manure, for example, 43% of the nitrogen, 4.8% of the phosphorus and 60% of the potassium is in the liquid fraction (Table 51) and will be lost when using dry manure.

Table 51. Nutrient quantity present in one ton of fresh cattle manure. Source: Boyd and Tucker (2012).

Nutrients	Solid Part (690kg)	Liquid part (310kg)	Total (1,000kg)
Nitrogen	2.85	2.15	5.00
Phosphorus	2.38	0.12	2.50
Potassium	2.00	3.00	5.00

- **Origin:** It is vital to check the origin of the manure to ensure the animals that generated it did not receive antibiotics, a common practice in poultry farming for example.
- **Manure curing, key considerationa:**

- Besides the sanitary issue, pre-curing the manure is an excellent alternative to reduce its adverse effects on water quality (specifically, on DO concentrations). Curing promotes the fermentation of organic matter in manures and decreases oxygen consumption by bacteria; makes nutrients more readily available for phytoplankton; reduces or inactivates any pathogenic microorganisms present in this material; and reduces the toxicity of these residues.
- One of the simplest ways to cure cattle manure is to form a pile with the material and moisten it daily. It is essential to turn the material every two days; after 30 days, it will be ready for application. The same can be done for chicken manure or poultry litter. However, the process should be done in a composter. In the case of pig manure, which is more liquid, it can be placed in a settling tank and the liquid part can be used.
- **Logistics:** The use of manure in organic fertilisation can be challenging due to the large quantities required, which can result in high transportation and storage costs. Thus, its use is usually only feasible when manure production occurs locally.
- **Availability, key considerations:**
 - The nutrients in manure are not immediately available to plants, especially if the manure is not fresh. In fresh cattle manure, for example, a large part of the nutrients is in the liquid fraction, which is lost when using dry manure. Therefore, manure is not necessarily more economical or efficient than chemical fertilisers.
 - The nutrients present in the solid fraction of these manures will need to go through the process of bacterial mineralisation before becoming available to phytoplankton. As seen later, mineralisation is a slow process and consumes much oxygen.
- **Advantages:** There are several situations where the characteristics of organic fertilisers are naturally advantageous. For example, sandy soils are generally poor in organic matter and have high water loss through infiltration. In these cases, the use of organic fertilisers is highly recommended. The manure fibres get trapped between the soil particles, helping to reduce losses through infiltration.
- **Technique to apply the manure:** Unlike chemical fertilisers, manure should start to be applied in dry ponds after preparation and soil correction, reaching 1,500–2,500 kg per hectare in post-larvae and fry ponds and 3,000–5,000 kg in juvenile and adult ponds (Figure 103). For post-larvae and juveniles, 100% of the dose should be applied initially. For juveniles and adults, 25% of this value should be used with empty ponds and 75% with flooded ponds.



Figure 103. Dry manure can be applied directly to the soil. In addition to fertilising, it will help reduce water loss through infiltration.

14.5.2 Chemical fertilisers

- **Advantage:** Fertilisers have a standardised content, while the content of manures changes according to the feeding and management of the animals.
- **Disadvantage 1:** Fertilisers do not contain carbon, which could indirectly support photosynthesis.
- **Disadvantage 2:** The active ingredients of fertilisers reach fish food organisms through a longer chain than manures.
- **NPK:** The most common fertilisers usually contain nitrogen (N), phosphorus (P) in the form of phosphorus pentoxide (P_2O_5), and potassium (K) in the form of potassium monoxide (K₂O). Thus, an N:P:K fertiliser called, for example, 20:20:5 contains 20% nitrogen, 20% phosphorus, P_2O_5 , and 5% potassium in the form of K₂O. Different fertilisers have different nutrient concentrations in their formulas, as seen in

- Table 52.

Table 52. Chemical fertilisers used and their respective nutrient concentrations.

Fertiliser	Nutrient Ratio (%)		
	N	P ₂ O ₅	K ₂ O
Urea	45	0	0
Calcium Nitrate	15	0	0
Sodium Nitrate	16	0	0
Ammonium Nitrate	33-35	0	0
Ammonium Sulphate	20-21	0	0
Single Superphosphate	0	18-20	0
Triple Superphosphate	0	44-54	0
Monoammonium Phosphate	11	48	0
Diammonium Phosphate	18	48	0
Calcium Metaphosphate	0	62-64	0
Potassium Nitrate	13	0	44
Potassium Sulphate	0	0	50

- Technique 1 – Initial fertilisation of ponds:** When the water column in the pond reaches a height of 30 cm, fertilise with liquid fertiliser, and wait for 2 days (you may apply 9 kg/ha of urea and 0.9 kg/ha of triple superphosphate, both previously dissolved in water). When the water column reaches 60 cm, apply 14 kg/ha of urea and 1.4 kg/ha of triple superphosphate, also previously dissolved. Repeat the operation when the water column reaches 1.0 m. Wait 3-5 days for increased natural food before transferring the post-larvae. If the pond water does not turn a brown or yellowish colour, 90 kg/ha of lime can be applied to stimulate plankton blooms.
- Technique 2 – Fertiliser mix:** Ponds for fry production are prepared similarly to nursery ponds. Add to the pond cattle manure (3-4 tonnes/ha) and single superphosphate (30-40 kg/ha) ten days before stocking. After stocking, add 500 kg/ha of cattle manure and 10 kg/ha of single superphosphate twice a month. Cattle manure is reduced by about a third or half when using poultry manure.
- Technique 3 – Fertilisation with full ponds:** The gates or water outlet structures must be closed before application. Fertilisers should be applied on alternate days but only on sunny

days; otherwise fertilisation should not be carried out. Fertilisers should preferably be used in the morning. Applying about 1 kg of phosphorus and 20 kg of nitrogen per hectare every three days is usually adequate. However, monitoring the results is crucial. This can be done using a Secchi disk to measure water transparency and, consequently, primary (phytoplankton) and secondary (zooplankton) productivity. Each pond has its peculiarities, so the technician needs to establish a fertilisation programme adapted to the specific conditions of each property.

- **Technique 4 – Punctured bag:** The technique of puncturing the bag containing the fertiliser and placing it at the pond's water inlet offers an efficient and gradual nutrient application method (Figure 104). By employing this method, the fertiliser gradually dissolves when water enters the pond, facilitating a managed and extended release of nutrients. The method ensures a uniform distribution of fertiliser in the pond, which can result in more lasting and practical effects, optimising natural food production.



Figure 104. The bag containing fertilisers is punctured and placed at the pond's water inlet so that the water flow helps dissolve and spread the fertilisers. The same technique can be used for the application of lime.

14.5.3 Tips

- **Initial fertilisation:** If chemical fertilisers are used, the initial fertilisation should be done with some water in the pond (about 40–50 cm depth is sufficient). What this means that fertilisation should be done at the start of the pond-filling process.
- **Dissolve first:** Chemical fertilisers should be permanently dissolved in water before application because this increases their efficiency and reduces losses. The solubility of

fertilisers in water depends on the size of their particle, their chemical composition and the water temperature. Some dissolve quickly, such as urea. Others, such as triple superphosphate, are so insoluble that they must be diluted in hot water. Certain fertilisers are already sold in liquid form, which is an advantage because they are up to 4 times more soluble in water than solid fertilisers.

- **Temperature:** The higher the water temperature, the greater the solubility of the fertiliser. Therefore, less soluble fertilisers, such as triple superphosphate, can be diluted in hot water.

14.6 Pond maintenance

Maintaining the ponds' structures and the dikes' vegetation in good condition is a recommended practice and a necessity for adequately operating a fish farming farm, ensuring the agility and efficiency of day-to-day actions and the health of the cultivated fish.

- **Regular inspection:** Regular inspections are necessary to check for any damage that may compromise the integrity of the pond and its accessory structures (supply channels, water inlet piping, screens, monks, dikes, slopes, drainage channels, settling tanks) (Figure 105).

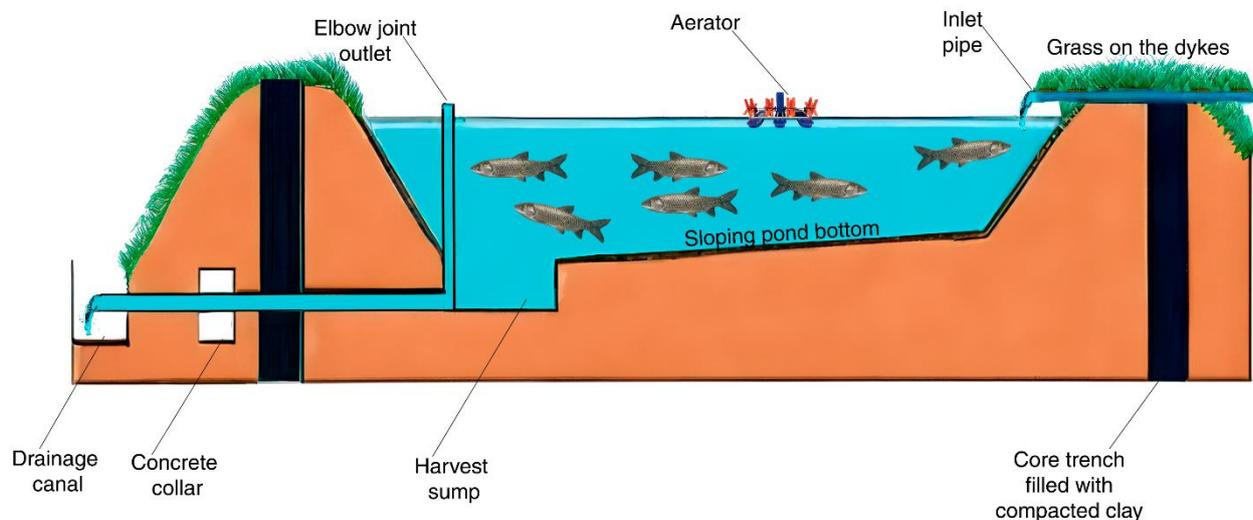


Figure 105. A cross-section of a grass carp pond showing the main structures.

- **Equipment:** It is important for the producer to regularly check the maintenance of equipment, such as aerators, which are fundamental to maintaining the living conditions and health of the cultivated fish.

- **Immediate repairs:** Any signs of wear should be repaired immediately to avoid structural problems (such as leaks and collapses) or operational issues (such as difficulty in water renewal, access of undesirable organisms, and difficulty of access for employees).
- **Drainage systems:** Maintaining the drainage systems is vital to prevent the accumulation of unwanted water, which can lead to issues such as erosion.
- **Control of terrestrial vegetation:** The ground cover vegetation of the slopes helps prevent soil erosion. Therefore, it is crucial to maintain healthy vegetation on the dikes. However, it is also necessary to control the height of this vegetation so that it does not serve as a shelter for predators and does not hinder caretakers' access to the ponds (Figure 106).



Figure 106. It is crucial to keep the dikes of the ponds vegetated to prevent erosion, but it is also necessary to mow to maintain a controlled environment. The cut grass can be used to feed the carp.

14.7 Filling the ponds

With the soil adequately prepared, the ponds will be ready to be filled again. Ideally, the water that supplies the ponds should be previously decanted (in a settling tank or even in the supply channel to avoid excessive entry of particulate material in suspension and the consequent silting of the ponds).

After passing through this decantation, the water should be filtered through screens or nets before entering the ponds. Screens with 2–4 mm mesh openings can be used for this. This procedure is recommended in the case of nursery ponds and fry ponds, as it prevents the entry of undesirable organisms. However, the screens or filters will require constant maintenance to prevent them from clogging (Figure 107).



Figure 107. A net was placed at the pond's water inlet to prevent the entry of unwanted organisms.

14.8 Acquisition of fry/juveniles

- **Fry vs. juveniles:** The larger the fish at the stocking time, the shorter the cultivation times. Moreover, older individuals are less prone to infections. Therefore, priority should be given to acquiring juveniles over fry when starting the fattening and finishing phase in ponds.
- **Supplier:** Always choose suppliers with a good reputation and a history of supplying quality fish.

- **Healthy fish:** When selecting grass carp fry/juveniles or any fish used in polyculture, priority should be given to individuals with agile, healthy movements without injuries or signs of disease. The animals should not show deformities, and the body colour changes as follows:
 - Newly hatched larvae: The larvae are transparent and very small, measuring about 5-7 mm in length. They have relatively large eyes and a yolk sac that provides nutrition in the early days.
 - Developing larvae: As they grow, they build pigmentation along the body. Small dark spots (melanophores) appear scattered throughout the body and tail. The yolk sac is gradually absorbed.
 - Post-larvae and juveniles: After complete absorption of the yolk sac, the post-larvae exhibit a more greyish or silvery colouration. They may have some irregular dark spots along the body. The caudal fin starts to develop and becomes darker at the edges.
 - Juveniles: Grass carp juveniles have a shiny silver colouration, with greenish or yellowish tones on the back. The fins are more developed and may show yellowish or reddish hues. Some irregular dark spots may still be present but tend to fade as the fish grows.
- **Activity:** Healthy fries are restless and swim quickly. The unhealthy ones are calm and less reactive.
- **Appearance:** Healthy fries are shiny; the unhealthy ones are pale.
- **Body:** The body of a healthy fry is smooth. That of the sick is rough.
- **Markings:** Healthy fry usually do not have spots or markings. Red marks can be seen on the body, fins, and gills of unhealthy fry.
- **Response:** If the caudal fins are pressed, healthy fry respond by vigorously shaking their heads. The unhealthy ones shake their heads very slowly.
- **Swimming:** If a current is created in a tank full of water, the healthy fry swim against the current. The unhealthy ones group together in the centre of the tank.

14.9 Transport of fry/juveniles

- **Importance:** Successful transport of fry/juveniles is crucial to validate all the effort invested in reproduction, larviculture and fish production. It is essential to adopt efficient practices that minimise fish stress, thus ensuring their higher survival during and after transport. It is about having good equipment and technically training the team responsible for this critical phase. Understanding the factors that affect the well-being of fish during handling and transport is vital to avoid high mortality rates, which entail financial losses and affect the supplier's reputation.

- **Pre-transport conditions:** The transport of fry/juveniles begins in the ponds and fry tanks, as the management applied to the fish at this stage directly impacts their survival during and after transport. Fish that are poorly fed or exposed to low oxygen concentrations are more likely to have high mortality during this phase. The same applies to fish with parasitic infestations. Inadequate practices during harvesting, such as excessive use of nets and rough handling, also increase the risks of stress and mortality.
- **Preparing the fish:** Fish preparation strategies for transport usually involve the following procedures: fasting before harvesting and transporting, treating fish to eliminate parasites, and maintaining the fish in an adequate environment to complete depuration (emptying of the digestive tract) before transport.
- **Fasting:** Fasting before fish transportation is crucial to reduce oxygen consumption and the excretion of ammonia and carbon dioxide in the water. The fasting period varies with the age and type of fish: longer for adults, shorter for fry and post-larvae. As grass carp consume natural food in ponds, merely suspending feed is insufficient. For this species, purging should be done in suspended net tanks, facilitating subsequent capture.
- **Parasite elimination, key things to consider:**
 - Before transporting fish, it is vital to conduct a microscopic analysis to check for parasites on the gills and body. If parasites are detected, the fish should be treated to minimise post-transport mortality. A typical treatment is a formalin bath, which can be done in the transport boxes or the purging tanks. Monitoring the dissolved oxygen level during treatment is essential, as formalin consumes significant oxygen. Extended salt baths can also be effective against various parasites and bacterial and fungal infections.
 - In situations where transferring fish to purging tanks is not feasible, such as when dealing with large quantities of market fish, treatment should be carried out directly in the transport boxes. After treatment, water should be renewed to avoid prolonged exposure to medications. Besides formalin and salt, other products may be used, but always consult a specialised professional for proper prescriptions and dosages.
- **Water:** Choose clean, contaminant-free water from reliable wells or reservoirs. Use products to condition the water, such as chlorine neutralisers and buffers. Gypsum and calcium chloride can be used to increase water hardness. If the water's pH is too acidic, add sodium bicarbonate and measure the pH before transferring the fish to the transport systems.
- **Salt:** Salinizing the water, in the range of 0–0.7 psu, not only improves the fish's condition but also prevents fungal and bacterial infections. Salinisation also prevents excessive loss of blood salt, which can occur due to the stress of confinement.
- **Fungi:** In cases where there is evidence of fungal presence in fish, copper sulphate baths at a concentration of 8 mg/kg of fish can also be used.

- **Vaccines:** In some countries, authorised commercial vaccines, administered via injection, are available to protect grass carp against bacterial diseases.
- **Transport options:** Fry/juveniles can be transported in specific fish transport tanks or, for smaller ones, in transport bags. In some countries such as India and Bangladesh animals are also transported in jars known as 'hari' or 'Patil' (Figure 108).



Figure 108. Hari/patil jars are typically used for transporting fry in South Asia under certain conditions.

- **Mortality:** Common causes of mortality during fish transport include lack of oxygen, excessive density, injuries or wounds, increased ammonia concentrations, excessively long transport duration, fish stress, and lack of prior acclimatisation.
- **Transport density, key things to consider:**
 - The longer the transport duration, the larger the fish size, or the lower the capacity to maintain dissolved oxygen concentrations in the transport water, the lower the transport density.
 - In tanks with oxygen: 60–80 kg/m³ for fry and 200–250 kg/m³ for juveniles. In plastic bags with oxygen: 120–150 post-larvae/m³ and 80–200 g/L for fry, depending on transport time and temperature factors. Using the Hari/Patil method, the density should be a maximum of 10 fry/L for a transport duration of 3–4 hours.
 - It is important to note that the ideal transport density for fry varies depending on the species and size of the fish, as well as the water conditions. For example, catla and silver carp must be transported at 30% lower densities than those used for other species.

- **Transport tanks:** During loading, keep oxygen above 72% saturation, as consumption is accelerated due to fish stress. As the fish calm down in the first half of the journey, adjust the oxygen flow to avoid excessively high levels. During the second half of the journey perform new measurements and adjustments to maintain oxygen close to saturation.
- **Transport bags:** After inserting one bag into another, tie the corners sufficiently to prevent the fish from getting trapped. Fill one-third of the bag with water and then release the fry. The rest of the bag should be filled with oxygen, and the top should be securely tied. If transporting multiple bags simultaneously, place them in a thermally insulated box for increased safety.



Figure 109. Packaging fry in plastic transport bags with oxygen.

- **Oxygen consumption:** Fasting fry consumes about 1 to 1.5 g of oxygen/kg/hour. You will likely need about 2 L of oxygen/kg/hour in plastic bags (Figure 109).
- **Water renewal:** During long journeys renewing the water is recommended to prevent ammonia build-up and bacterial proliferation.

- **Ice:** Using ice during transport – at a ratio of 10 g/L of loaded water – can effectively reduce water temperature, which helps decrease oxygen consumption and other stress factors for the fish.

15 GROWTH PHASE

With the ponds prepared, the fattening phase commences. Well-executed grass carp fattening in earthen ponds can represent a promising practice, combining productive efficiency with environmental sustainability and animal welfare.

Successfully fattening grass carp in earthen ponds demands an integrated management approach, including proper water quality control, careful monitoring of soil conditions, and implementing sustainable management practices.

15.1 Stocking

Choosing the proper stocking process is a fundamental pillar for aquaculture success, and in the specific case of grass carp (*Ctenopharyngodon idella*) in earthen ponds, this step is crucial. In this context, we explore strategies and essential considerations for adequate grass carp stocking, aiming not just for optimal fish growth but also to promote sustainability and ecological balance.

- **Golden rule:** When stocking fish larvae, the golden rule is to handle and release them with utmost care, including selecting protected locations and acclimating the larvae to prevent thermal shock.
- **Temperature, key considerations:**
 - Ideally, the water temperature in transport tanks and ponds should be the same when releasing the fish, which is especially important for younger fish groups. Various technical devices, such as flexible tubes or ramps, should be used for gentle fish release.
 - The temperature difference between the water in the pond and the transportation containers should not exceed 3°C during stocking. However, some species used in polyculture systems are even more sensitive. For instance, pikeperch fry can quickly die if released into a pond where the water is about 1–2°C colder than the water they were transported in. Therefore, it is essential to know the characteristics of each species to be used.
- **Transfer:** It is advisable to introduce fry/juveniles into fattening ponds 10–14 days after the pond preparation, stabilising environmental conditions and thus ensuring ideal conditions for healthy fish growth.
- **Stocking:** Careful transport and release are also very important when stocking larger, older fish. If mishandled, fish can lose scales and the mucous layer covering their bodies. Their

organs can also be injured. Such wounds can quickly become infected, causing slower growth or widespread infection and, subsequently, even death.

- **Transport boxes:** The transfer from transport boxes to the ponds should be done to ensure the welfare of the fish and avoid unnecessary injuries. Fry/juveniles should not be dumped directly from a transport box into the pond. This method can cause stress and potential injuries to the fry due to direct impact on the water or transport structures. A more careful and recommended approach involves using a net attached to the transport box outlet, ensuring that the fry are transferred to the pond in a gentle and controlled way. This method minimises the risk of injuries and stress, ensuring the fish have a healthy lifecycle in their new environment (Figure 110).



Figure 110. The correct method is not to open the transport tank and pour the fry into the pond but to use a net attached to the water outlet of the transport box so that the animals do not suffer injuries when transferred to the fattening ponds.

- **Transport bags:** When transporting fry/juveniles in plastic bags with oxygen, the fish need careful acclimation before being transferred to the ponds. The goal is to adapt them to the pond water temperature before release, ensuring their health and maximising their survival rate. After the water temperature in the transport bags has balanced with the ambient temperature, the recommended method is to open the bags and slowly mix the inside and outside waters to allow gradual fish acclimation to the pond conditions (Figure 111).



Figure 111. Bags containing fry should be left in contact with the pond water for temperature acclimation before the fish are released.

15.2 Aeration

15.2.1 Molecules and gases in water

The sun, our planet's primary energy source, plays a crucial role in ponds. Sunlight penetrating the water provides energy for the photosynthesis process conducted by aquatic plants and algae. Through photosynthesis, these organisms consume CO_2 (carbon dioxide) and produce O_2 (oxygen), creating a healthier and more balanced environment for the fish. The issue is that during the night the respiration of microalgae, organisms and microorganisms present in the soil and water of the pond and the cultivated fish can deplete O_2 reserves and generate high concentrations of CO_2 , toxic to the fish.

Ponds are veritable chemical cauldrons, filled with molecules and gases beyond O_2 and CO_2 , directly influencing fish health. They include:

Ammonia (NH_3) and Nitrite (NO_2^-) result from the decomposition of organic waste and indicate water quality.

Methane (CH_4) and Hydrogen sulphide (H_2S): generated in oxygen-poor and organic-rich zones, which should be avoided.

Imbalances and high concentrations of CO_2 , NH_3 , NO_2^- , CH_4 and H_2S can harm or even kill the fish. Under these conditions, fish often cluster at the top of the water column, where water quality is better and dissolved oxygen concentrations are higher (Figure 112).

As essential as light and water chemistry is the aeration of cultivation ponds. A sound aeration system ensures that oxygen is evenly distributed throughout the pond. Oxygen-rich and poor zones can coexist in the same pond, but with proper aeration we provide an environment conducive to the fish.

Aeration also allows the farmer to stock a higher number of fish, increasing the productivity of the venture. It also minimises and even eliminates the formation of anoxic (no O_2) zones, avoiding the production of harmful gases.

Understanding these processes prepares the farmer to face the challenges of cultivation and ensure the fish grow in a healthy and well-balanced environment.

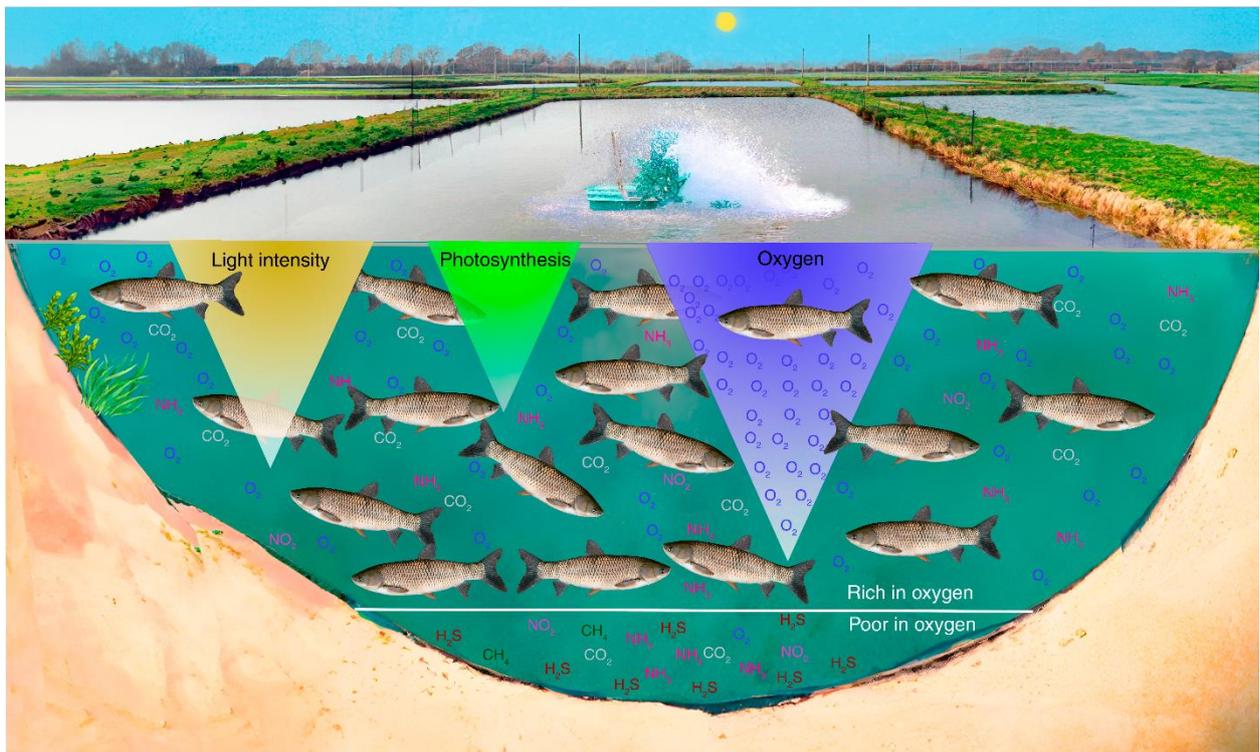
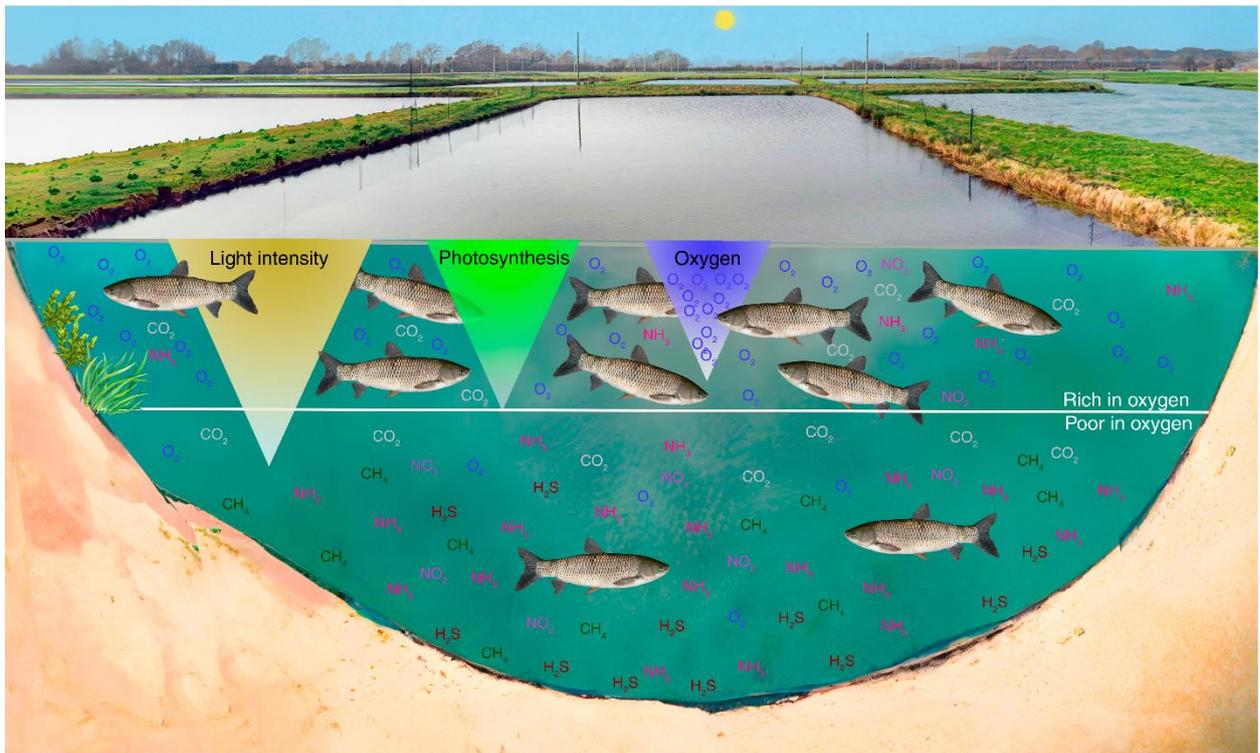


Figure 112. Aeration allows the farmer to improve water quality, increase the safety and welfare of the cultivated fish, and stock more fish in the ponds.

15.2.2 Diurnal variations in dissolved oxygen concentrations

Dissolved oxygen concentrations in water can vary significantly throughout the day. Typically, these levels are higher during the day due to photosynthesis by aquatic plants and microalgae and lower at night when plants and fish respire O_2 , but no photosynthesis occurs to replenish this O_2 . The lowest concentrations usually occur just before dawn.

Moreover, water temperature, fish density and decomposing organic matter can also influence DO concentrations. For example, warmer waters have a reduced capacity to hold oxygen. Therefore, greater attention should be paid to dissolved oxygen during summer or in areas with higher temperatures.

Regular and frequent monitoring of dissolved oxygen concentrations is necessary. Instruments like oximeters can provide real-time data, helping the farmer make informed decisions about when to use aerators to increase oxygen concentrations. This natural diurnal cycle is a vital technical consideration for effectively managing pond aquaculture systems.

15.2.3 Types of aerators

There are various types of aerators used in aquaculture, and the choice often depends on the specific needs of the pond and local conditions. Therefore, before purchasing an aerator, understand your property's particular needs.

Generally, concentrations above 5 mg/L are considered safe for most carp species. When choosing an aerator, consider several factors, such as the depth and size of the pond, fish stocking density, and local environmental conditions. The combination of the right equipment and management practices ensures fish health and production efficiency.

- **Paddlewheel:** These are perhaps the most common in many aquaculture systems. They feature large paddles that rotate and mix the water, promoting circulation and aeration. They are very effective in terms of the volume of water they can move (Figure 113).

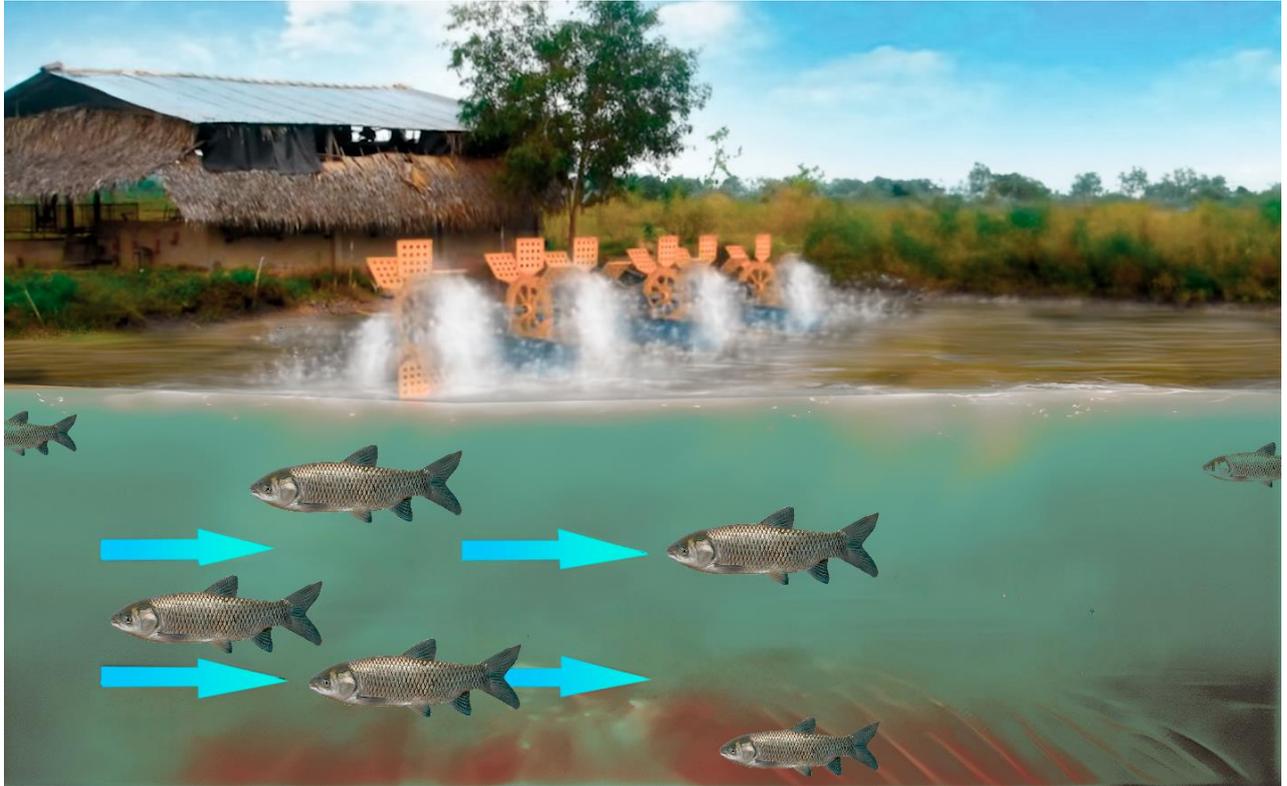


Figure 113. Besides increasing dissolved oxygen concentration in the water, paddlewheel aerators promote water circulation, ensuring a more homogeneous water quality throughout the water column and better environmental conditions for the fish.

- **Fountain or spray aerators:** These aerators throw water into the air as a fountain or spray. By doing this, they increase the water surface exposed to air, which promotes gas exchange and oxygen entry (Figure 114).



Figure 114. Fountain-type aerator.

- **Tornado-aspirating/impeller aerator:** This type of aerator uses an aspiration system to pull ambient air into the water. The air is then dispersed as fine bubbles in the water, increasing the contact area between air oxygen and water, promoting better oxygenation (Figure 115).



Figure 115. Impeller aerator.

- **Propeller-type aerators:** These use a propeller to move and circulate the water, promoting the entry of air containing oxygen (Figure 116).



Figure 116. Propeller-type aerator.

15.3 Water renewal

Frequent renewals, about 5–10 cm of the water level in the pond, conducted every 3–5 days, are recommended to maintain water quality. However, like stocking density, water renewal should not be seen as a fixed rule or a 'one-size-fits-all' solution but as a management practice defined by a careful daily water quality monitoring and control programme.

It is important for each producer to understand their ponds and how they react to the management practices adopted on the property.

The objective is to avoid unnecessary water renewal, optimise the use of water, energy, fertilisers and equipment, and maintain water quality at suitable levels for optimising the productive process of cultivated fish.

15.3.1 Basic rules for pond water renewal

- The most efficient method is first to remove the water to be renewed and then promote the entry of new water into the pond.
- Water can be drained from the pond during the day, and at night the outlet gate can be closed to recover the level, thus ensuring clean water entry into the pond during critical times.
- Always drain water from the bottom and the side opposite the inlet gate.
- Never renew if the water from the supply canal or adduction canal is of lower quality than the water already in the ponds.

15.4 Biometrics

- **What is it?** Biometrics is a procedure where a representative number of fish from the ponds are captured, analysed and weighed to obtain data on fish growth rate, total biomass and general health conditions.
- **Importance:** Biometrics are an essential step in fish cultivation, including for grass carp. Weighing the animals makes it possible to estimate the total biomass in the pond. This calculation is crucial to check if the amount of feed the producer applies is appropriate. Higher biomass may require more food. If it is noticed that the feed is not being fully consumed or that the fish appear undernourished, it may be time to reprogramme the quantity of feed to be provided. Biometrics also enable the farmer to determine if the fish have reached the ideal size for harvesting, ensuring that harvesting is done at the right time. Lastly, closely monitoring fish health allows for early diagnosis of potential diseases and necessary measures for treatment, thus ensuring the welfare of the fish and the quality of the cultivation.

15.4.1 Sampling method

- **Sampling nets:** Using a seine or trawl net is ideal for sampling. However, if these nets are unavailable a cast net can also sample a small pond. The sampled fish should be captured from different pond points in this case.
- **Seine or trawl net use:** After proper preparation, the seine or trawl net should be pulled to capture 90% or more of the fish on the first attempt. Under no circumstances should the net be pulled more than twice, as the stress experienced by the fish from a single effort in the pond requires at least one or two days for the fish to recover.
- **Fish transfer:** After transferring the selected fish for biometry to a hapa, the remaining fish captured in the net should be immediately returned to the pond.
- **Sampling accuracy:** At least 5–10% of the total stocked fish should be captured for accurate sampling results.
- **Minimum sampling:** If sampling 5–10% of the fish is impossible, 30–40 fish should be sampled.
- **Size inclusion:** Fish of all sizes, from small to large, stocked in a pond should be included in the sampling.
- **Weighing fish:** The weight of the fish should be measured with a field scale, preferably with the fish weighed in a net or mesh, so it cannot struggle and become overly stressed or injured during the procedure.
- **Physical examination:** The physical condition of the animals, the different external structures, and even the mucus present on the body of the captured fish should be carefully examined. The fish should also be checked for spots, lesions or parasites adhering to the body.
- **Species weight calculation:** The fish of each species should be weighed separately, and the average individual weight of the particular species should be multiplied by the total number of stocked fish, subtracting the number of fish of that species up accounted for as dead to that point. Thus, the biomass per species should be estimated and added to all stocked species to obtain the total biomass of the pond.
- **Speed of sampling and biometry:** The sampling and biometry should be completed as quickly as possible.
- **Gentle return:** Immediately after sampling, the fish should be returned gently to the pond.
- **Sampling schedule:** Sampling should begin no later than one month after stocking the pond, i.e., when the fish have grown a bit more, and it should continue at least once a month until the end of cultivation.

15.5 Recommendations for grass carp growth phase

15.5.1 Environmental indicators

Table 53. Environmental indicators for juveniles and adults of grass carp.

Indicators	Level	Reference values
Temperature (°C) during spawning and maintenance	Optimum	20–33
	Acceptable	16–19 or 34–37
pH	Optimum	7.0–8.5
	Acceptable	6.0–6.9 or 8.6–9.0
Transparency (cm)	Optimum	30–60
	Acceptable	20–29 or 61–70
Dissolved oxygen (% saturation)	Optimum	≥ 49
	Acceptable	43–48
Salinity (ups)	Optimum	0.0–0.7
	Acceptable	0.8–8.0
Un-ionised ammonia (mg/L of NH ₃)	Optimum	0.000–0.025
	Acceptable	0.026–0.059
Nitrite (mg/L of NO ₂ ⁻)	Optimum	0.0–0.9
	Acceptable	1.0–1.5
Alkalinity (mg/L of CaCO ₃)	Optimum	25–100
	Acceptable	≥ 101
Terrestrial predators	Optimum	Absence
	Acceptable	Controlled presence
Predators and other aquatic interspecific inhabitants	Optimum	Absence
	Acceptable	Controlled presence

15.5.2 Health indicators

Table 54. Health indicators for juveniles and adults of grass carp.

Indicators	Level	Reference values
Eyes	Optimum	Normal and healthy appearance
	Acceptable	Bleeding, unilateral exophthalmos or traumatic injury
Jaws/lips	Optimum	Normal and healthy appearance
	Acceptable	Mild bleeding, redness, injury, or deformity (without affecting eating)
Operculum	Optimum	Normal and healthy appearance
	Acceptable	Absence of tissue (<25%)
Skin	Optimum	Normal and healthy appearance
	Acceptable	Scar tissue, punctual loss of scales, ulcers, or superficial lesions < 1 cm ²
Fins	Optimum	Normal and healthy appearance
	Acceptable	Light lesions or splitting
Gills	Optimum	Normal and healthy appearance
	Acceptable	Injury, mild necrosis, splitting or thickening
Abdomen	Optimum	Normal and healthy appearance
	Acceptable	Discreet hollow distension, redness
Anus	Optimum	Normal and healthy appearance
	Acceptable	Faecal residue, swelling and redness
Mortality (%)	Optimum	≤ 10
	Acceptable	11-24

15.5.3 Behaviour indicators

Table 55. Behaviour indicators juveniles and adults of grass carp.

Procedure	Indicators	Level	Reference values
Acclimatization Classification Transfers	Respiratory frequency (opercular rate/min)	Optimum	40–70
		Acceptable	20–39 or 71–90
Vaccination hormonal induction gamete extrusion	Anaesthesia – surgical stage (reduction of opercular rate, absence of swimming)	Optimum	Induction or recovery in 2–4 min
		Acceptable	Induction or recovery ≤ 1 or ≥ 5 min
Massive capture (partial or total)	Swimming behaviour	Optimum	Regular swimming, no or few body parts on the surface
		Acceptable	Restless swimming behaviour, swimming in different directions and or jumping
Stunning during slaughter	Reflexes	Optimum	Instantaneous loss of EQ, TGR, VER, OB
		Acceptable	Instantaneous loss of EQ and TGR, progressive loss of VER and BO in ≤ 30s

15.5.4 Nutritional indicators

Table 56. Nutritional indicators for juveniles and adults of grass carp.

Indicators	Level	Juveniles 2.1-150 g	Adults > 150 g
Crude Protein (%)	Optimum	33-44	28-35
	Acceptable	29-32	25-27
Formulated feed amount (% biomass)	Optimum	3.0-6.0	2.0-3.0
	Acceptable	1.0-2.9	1.0-1.9
Natural feed amount (% biomass)	Optimum	30-44	100-200
	Acceptable	10-29	50-99
Feeding frequency (times/day)	Optimum	≥ 3	≥ 2
	Acceptable	2	1
Food distribution (% of surface area reach)	Optimum	> 75 of surface area	
	Acceptable	50-75 of surface area	
Feed seizure (minutes)	Optimum	3-5	
	Acceptable	2-3 or 5-7	

The welfare and healthy development of grass carp in captivity are deeply influenced by proper nutrition. A well-balanced diet, offered according to recommended feeding practices, affects not only the fish's disease resistance but also its growth and the meat quality. Conversely, errors or omissions in nutrition can compromise the animals' welfare and harm both the efficiency of production and the economic viability of the fish farming venture.

The grass carp is a fish noted for its adaptable feeding behaviour. Although its diet is predominantly herbivorous, it is not exclusively restricted to plants. In its natural habitat, the species primarily feeds on algae and various parts of submerged plants, such as roots, shoots and fruits. Aquatic plants provide essential nutrients and also contribute to digestion, thanks to their fibre content, which aids the fish's digestive process.

However, grass carp can diversify their diet when aquatic plants are scarce or inaccessible. It can consume organisms such as zooplankton, aquatic insects, earthworms and other small invertebrates. This dietary flexibility is one factor that makes grass carp a resilient species, adaptable to various environments.

In cultivation systems, the diet of grass carp can be even more varied. It can include fresh aquatic plants, residues from other crops (including animal by-products), terrestrial grasses, and specially formulated feeds for the species. However, it is imperative to stress that just because the fish feeds on a particular plant product or residue does not mean it is adequately nourished. Table 57 shows how many kilograms of food items grass carp need to ingest to produce 1 kg of meat, the so-called feed conversion factor.

Table 57. Feed conversion rate for some feeds used in grass carp production.

Feed item	FCR (Feed Conversion Rate)
Rice bran/wheat bran	4–6:1
Peanut and soya bean cake	3–4.5:1
Silkworm pupae	1.1:1
Silkworm faeces	17:1
Sugarcane leaves	40:1
Mixed vegetables	33:1
Pig dung	45:1
Duck dung	43:1

In this context, using dry feeds brings several advantages to the producer, from ease of handling and storage to more precise nutritional control. The feeds should be specially formulated to meet the dietary requirements of each stage of the grass carp's life cycle, ensuring an adequate balance of proteins, amino acids, minerals and vitamins. When supplemented with fresh green vegetables, dry feeds facilitate digestion and allow for more efficient nutrient absorption, contributing to faster and healthier fish growth.

Besides being directly related to fish health and welfare, nutrition also has economic and environmental implications. It can represent the largest share of the production costs of a fish farm and, if poorly managed, can lead to environmental impacts such as water pollution.

Therefore, implementing good feeding practices and nutritional welfare protocols is not just an ethical issue but also a fundamental strategy for the sustainable success of the venture.

15.6 Feeding habits

Grass carp change their feeding habits throughout their life cycle (Figure 117), from the post-larva to the adult stage.

- **Larva:** After hatching, the larvae rely exclusively on their yolk reserves (yolk sac) to grow. Growth is rapid, and the larvae go from 5.5 mm to 9.0 mm in 3–5 days at temperatures between 24 and 32°C.
- **Post-larval stage:** When they reach a size of approximately 9 mm, the post-larvae enter a crucial dietary change phase, starting to consume zooplankton. Feeding begins with smaller zooplankton organisms, such as rotifers and protozoans, and evolves into larger forms, such as cladocerans and copepods. In cultivation conditions, post-larvae already adapt well to feeding on feeds, provided they are of a suitable size for their mouths. When they reach about

11.7 mm, the post-larvae are more agile and begin to include algae and detritus in their diet. Zooplankton, however, still make up the bulk of their diet.

- **Fry:** After 15 to 20 days, when they reach almost 3 cm in total length, the pharyngeal teeth begin to develop, and the intestine increases in length and begins to coil. At this stage the fish changes its diet to one based on vegetables and detritus (decaying organic matter). However, fry are flexible in their diet and can consume various types of food, such as small aquatic plants and even other fish larvae.
- **Juveniles:** As they grow, the mouth opening and pharyngeal teeth evolve, gradually improving their cutting ability. Thus, despite still being able to feed on zooplankton, they increasingly show interest in plants and aquatic algae. Juveniles above 10 cm in length already consume the same diet as adults, although their preferred plants may differ.
- **Adults:** In adulthood, grass carp become predominantly herbivorous, feeding mainly on aquatic plants and algae in their habitats. Their mouths are adapted to scrape and grind plant materials, enabling them to extract the necessary nutrients from vegetables.

Significant alterations in the carp's digestive system accompany this change in feeding habits. It starts with sharp and curved pharyngeal denticles, suitable for feeding on zooplankton, and gradually changes to a more serrated and grooved form, adapted for grinding fibrous aquatic plants. The intestine also undergoes three distinct stages of development, adapting to different diets throughout the fish's growth.

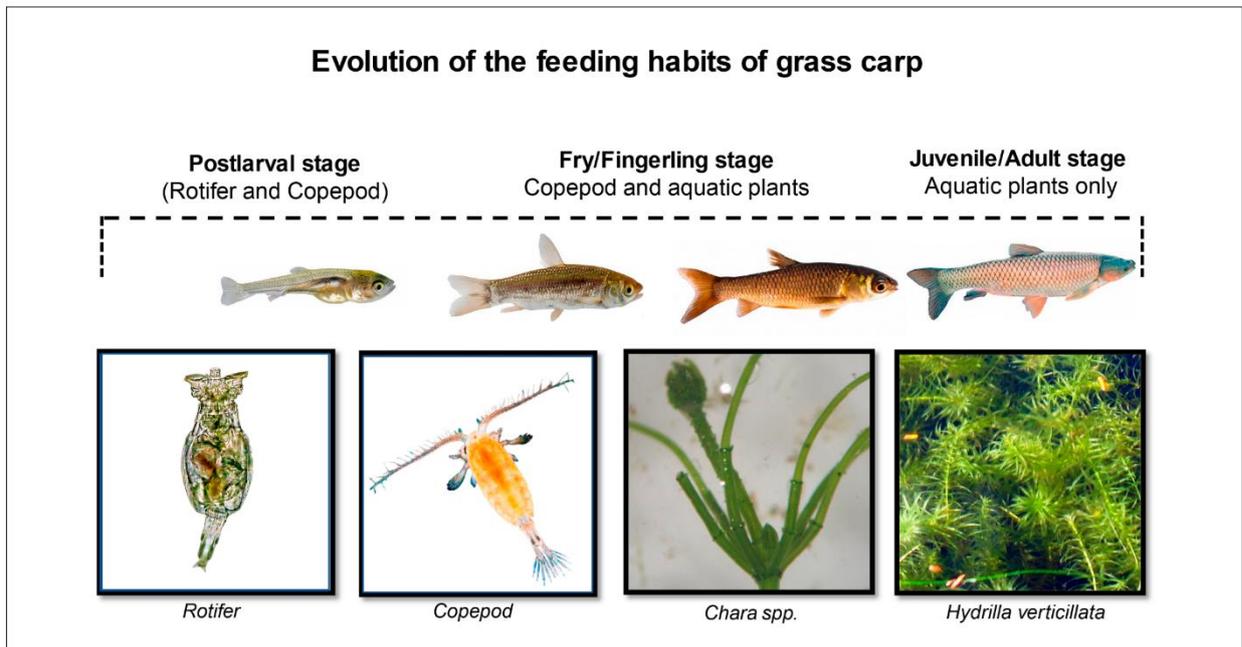


Figure 117. Dietary evolution of grass carp from an initial diet of zooplankton in the post-larval and juvenile phase to a predominantly herbivorous diet in adulthood, based on aquatic plants and algae.

15.7 Feeding behaviour

- **Food intake:** Grass carp do not have real teeth or chewing functions. After ingesting their food, they swallow it directly through the oesophagus. If the piece of food is too large, the carp regurgitates it and then consumes a suitably sized portion.
- **Digestion and absorption:** Grass carp do not possess a stomach. The digestion of their food primarily depends on digestive enzymes secreted by the liver, pancreas and intestines. Moreover, most digestion and nutrient absorption occur in the small intestine. In this region, nutrients from food are broken down and absorbed by the intestinal villi.
- **Temperature:** Feeding behaviour depends on temperature and the life stage of the fish. Optimal temperature ranges vary from 22–29°C during the larviculture phase, 20–33°C in the fingerling phase, 20–33°C in the fattening phase, and 20–30°C in the breeding phase.
- **Feeding intensity and food intake:** These factors are also affected by the type of diet. For example, grass carp fed with vegetable food, such as duckweed or elodea, are provided with food continuously throughout the day. Those fed a dry diet have shorter meals for about a quarter of the diurnal cycle.
- **Dissolved oxygen:** Feeding behaviour is also affected by dissolved oxygen concentrations (DO). Feeding ceases when DO concentration falls below 3 mg/L. The optimal range extends from 5–8 mg/L or greater than 49% saturation.
- **Feeding training:** Grass carp quickly learn to head towards the feeder at mealtimes. This behaviour is taught through sound stimuli to attract the fish, like tapping on the feed bucket or the noise of the automatic feeder.

15.8 Energy needs

- **Energy vs nutrients:** Energy is not the same as nutrients. Nutrients are substances necessary to grow, maintain and repair body tissues. Meanwhile, energy is a property that nutrients possess and is measured in caloric units, such as kilocalories (kcal) or megajoules (MJ).
- **Fish vs mammals:** The energy needs of fish are much lower than those of terrestrial mammals. The reasons for this are:
 - **Cold-blooded:** Fish are cold-blooded animals; they do not spend energy maintaining a specific body temperature that is different from that of their environment.
 - **Fluctuation:** Fish spend little or no energy to maintain their position in the water column, as they have mechanisms to maintain their neutral buoyancy in water.

- **Excretion:** Fish spend little energy in eliminating their nitrogenous waste, i.e., they excrete nitrogen wastes in the form of ammonia, generating a low caloric increase (3 to 5% in fish compared to 30% or more in mammals excreting urea).
- **Energy needs:** Nevertheless, like all living beings, grass carp need to accumulate and consume energy to sustain their vital activities, such as growth, reproduction and locomotion, as well as to maintain their metabolic processes.

Some of the effects of inadequate energy quantity in fish feeds include:

- **Growth delay:** Energy is essential for fish growth. Fish may grow slower than desired if the diet lacks sufficient energy, resulting in smaller sizes and lower productive yield.
- **Weight loss:** A lack of energy can lead to fish weight loss, which is especially problematic in commercial production systems.
- **Abnormal development:** Energy is also vital for proper development of organs and tissues. Lack of energy can lead to abnormal development, affecting fish health and quality.
- **Immunosuppression:** Energy is crucial to keep the fish's immune system functioning correctly. An energy deficiency can weaken the immune response, making fish more susceptible to diseases.
- **Reproductive problems:** Inadequate energy can negatively affect fish reproduction. It can result in reduced egg production, lower fertilisation rates and decreased larval survival.
- **Stress:** A lack of energy can cause metabolic stress in fish, leading to health issues such as the accumulation of metabolic toxins in the body.
- **Poor feed conversion:** Inadequate energy in the fish's diet can lead to poor feed conversion, meaning fish may require more food to obtain the necessary energy and nutrients, resulting in higher production costs.
- **Mortality:** In extreme cases fish may not obtain sufficient energy to maintain vital functions and may even die.

Generally, the energy requirements of fish like grass carp are determined by the amount of metabolisable energy (ME) the fish needs to perform its vital functions, growth and regular activities. The requirements for gross energy decrease slightly as the grass carp grows. The reason for this could be due to improved metabolic efficiency in older fish or perhaps their slower growth rate compared to earlier stages:

- **Fry phase:** The feed should contain 16 MJ/kg. In this initial phase, grass carp require a diet rich in proteins and energy to support rapid growth. Energy is essential for the development of tissues and muscles.

- **Growth phase:** Energy requirements drop slightly to 15.6 MJ/kg. A balanced diet containing proteins, lipids and carbohydrates is essential for healthy growth.
- **Adult/reproductive phase:** If the animals are not reproductive, the energy needed drops to 15.2 MJ/kg. However, this value can increase significantly if the fish are used as breeders, as gonadal maturation and egg production require a lot of energy, especially in females.

Environmental factors such as water temperature can also affect energy requirements. In colder temperatures the fish's metabolism may decrease, resulting in lower energy needs.

15.9 Nutritional requirements

Nutrients play vital roles in maintaining health and proper functioning of the body. Macronutrients are those required in more significant amounts. They provide the energy needed to sustain daily activities and are essential for growing and maintaining fish tissues. The primary macronutrients are proteins, carbohydrates and lipids.

About 40% of the grass carp diet should be composed of carbohydrates to provide the necessary energy, 8% cellulose to assist digestion, 4% crude fat for other nutritional needs, and the specific amount of protein appropriate to the fish's life stage.

Ensuring a balanced diet is very important for the success of a fish farm, as confined fish have much more restricted access to food than wild animals. Proper nutritional and feeding management will help:

- Strengthen the fish's immune system and enable them to resist diseases and parasites;
- Maintain physiological and metabolic processes;
- Reduce competition among fish for food, avoiding skin and fin injuries and batch unevenness;
- Promote adequate growth levels and more efficient feed conversion rates;
- Maintain water quality;
- Increase fish tolerance to handling and transportation;
- Improve reproductive performance;
- Optimise revenue and profit in fish farming;
- Prevent health problems such as malnutrition or obesity.

However, ensuring the best nutrition for grass carp cannot be approached as a 'one-size-fits-all' solution. The amount of macronutrients, micronutrients and energy to be provided – that is, the nutritional needs of farmed carp – can be influenced by a series of factors such as diet consumption, energy density of the feed, level and interaction of feed nutrients, nutrient availability to the fish, and presence and level of additives. It can also vary depending on factors external to the diet, such as age and size of the fish, density used, temperature, other chemical and physical characteristics of the water, presence of infectious pathogens, and the expected carcass characteristics for the produced animal.

15.9.1 Proteins and amino acids

All nutrients are relevant for fish, but proteins often receive special attention from fish farmers, as they are the most expensive ingredient in the feed and are directly linked to the development of cultivated organisms. They are crucial in forming muscles, internal organs, the brain, nerves and skin, and repairing and creating new tissues.

15.9.1.1 Protein requirements

Protein requirements change throughout fish life, and grass carp are no exception. For instance, larvae undergo rapid growth and development shortly after hatching. During this initial stage, they experience significant transformations in their morphology, physiology and digestive systems to adapt to the aquatic environment. This rapid growth requires substantial nutrients, especially proteins, essential for constructing tissues and organs. Due to these factors, diets for grass carp larvae are formulated with higher protein levels than those for adult fish (Figure 118).

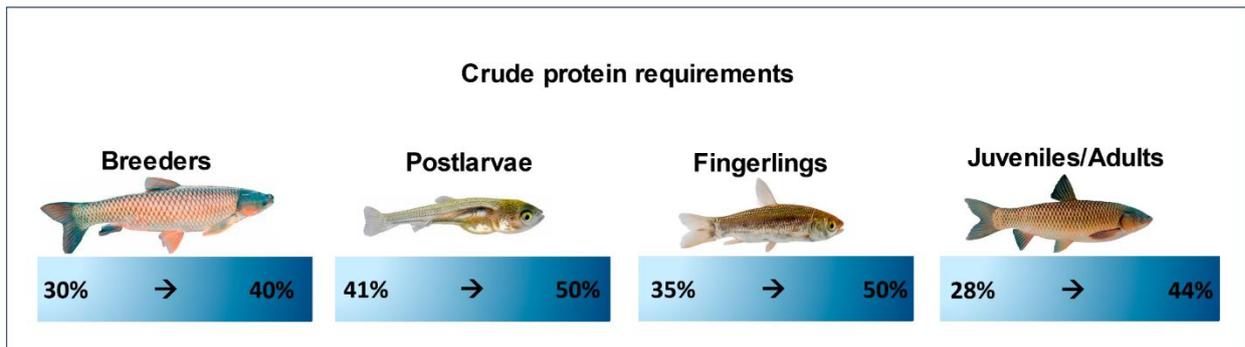


Figure 118. Recommended levels of crude protein throughout the life stages of grass carp.

Like other species, post-larvae and fingerlings of grass carp require more protein, lipids and energy than larger fish. For post-larvae, the need varies from approximately 41 to 50% in the diet. Meanwhile, fish with an initial weight of 990 g require between 35 and 45%. However, as the grass carp grows and reaches adulthood it starts to follow a more herbivorous diet. This implies that the protein need falls to around 28%. If the animals are used as breeders, however, the protein requirements can reach and even exceed 40%.

15.9.1.2 Protein quality

Focusing only on the gross amount of protein offered is not enough. The quality and digestibility of these nutrients are equally crucial. High-quality proteins are more easily absorbed, resulting in greater feed efficiency and less waste, factors which are essential in terms of water quality and sustainability in aquacultural systems.

Proteins are made up of 'building blocks' of amino acids. Some of them (essential amino acids) are not produced by fish metabolism and must be provided through the feed. Therefore, it is crucial to adjust the diet according to the amino acid requirements for the healthy and efficient development of the fish. Any deficiency can limit growth and compromise the animal's welfare.

The producer must adopt well-calibrated feeding management practices to ensure healthy growth and fish production with superior meat quality and low-fat content. This feeding practice includes

constant monitoring and adjustments of cultivation conditions, aiming to maximise the quality and well-being of the farmed fish.

15.9.1.3 Amino acid requirements

For grass carps in the initial juvenile stage, the needs are:

- Arginine: 1.7%
- Histidine: 0.7%
- Threonine: 1.0%
- Valine: 1.4%
- Isoleucine: 1.2%
- Leucine: 2.0%
- Phenylalanine: 1.1%
- Tryptophan: 0.2%

For grass carps in the final fattening stage and breeders, the needs are:

- Arginine: 1.4%
- Histidine: 0.56%
- Threonine: 0.84%
- Valine: 1.13%
- Isoleucine: 0.98%
- Leucine: 1.85%
- Phenylalanine: 0.91%
- Tryptophan: 0.2%

15.9.2 Lipids

In addition to providing energy, lipids also play other roles in fish metabolism and physiology. They lubricate the gastrointestinal tract, enhance the palatability of food, and even act as binders in the feed.

However, caution is needed: an excess of lipids can be harmful. Elevated fat levels in the diet can lead to liver failure and fat accumulation in the fish, which is detrimental to their health and can also negatively affect their commercial quality.

Therefore, the quality and quantity of lipids in the grass carp diet must be carefully balanced to meet the specific needs of each life stage, always considering the various roles that lipids play in the health and well-being of the fish.

Lipids are essential in the fingerling stage, making up between 6% and 8% of the diet. They are fundamental for rapid growth and healthy development, providing energy and absorbing fat-soluble vitamins such as A, D, E, and K.

As the grass carp becomes a juvenile, the need for lipids decreases slightly, to between 4% and 7%. Lipids continue to be necessary for growth, but at this stage the fish starts developing the capacity to process a broader range of nutrients.

In the adult stage the need for lipids remains similar to that of juveniles, between 4% and 7%. Besides providing energy, lipids also play a vital role in reproduction.

If the 'building blocks' that make up proteins are amino acids, then fatty acids are the 'building blocks' that make up lipids. Polyunsaturated fatty acids, such as omega-3 and omega-6, are especially noteworthy because the fish use them to produce their hormones and prostaglandins.³

15.9.3 Carbohydrates and fibre

Carbohydrates, including starches and sugars, are the primary 'fuel' for grass carp. They provide the energy the fish needs to grow, move and perform all its vital functions. Carbohydrates also have an economic advantage: they are generally cheaper than proteins. Corn bran, for example, is widely used as an economical and efficient source of carbohydrates in grass carp feeds.

On the other hand we have fibre, which is a type of indigestible carbohydrate. Although fibres do not directly provide energy, they play a highly important role in the digestion of grass carp. Fibres help regulate the digestive system and are crucial for intestinal motility, which means they help the fish process and eliminate waste efficiently.

So, how is this balanced in the diet of grass carp at different life stages? The need for crude fibre varies according to the age and size of the fish. For instance, fingerlings need up to 3% fibre in their diet, while early-stage juveniles require up to 8%. For final-stage juveniles and breeding fish, this need rises to up to 12%. This adjustment is due to changes in the fish's digestive system as it grows and to different needs related to intestinal motility and digestion.

15.9.4 Other nutrients

Besides these, some other nutrients stand out in the dietary requirements of grass carp; for example, the needs for vitamin E and K3 are 200 mg/kg and 1.9 mg/kg of diet respectively. The dietary needs for calcium and phosphorus vary between 0.5 and 0.8% and 1.4–1.6% of the diet. Additionally, administering 300 mg of iron per kg diet improves weight gain and provides better feed conversion, enhanced protein efficiency ratio, and increased protein retention. The recommendation for manganese in the diet is approximately 15 mg/kg.

³ Prostaglandins are a group of lipids that play a significant role in various biological processes. They function almost like hormones, although they are not stored but produced at the site where they are needed and used almost immediately. They are generated from essential fatty acids. In fish, prostaglandins are involved in multiple aspects, ranging from the regulation of inflammatory processes to reproductive function. Although these lipids are produced in small quantities and act locally, their influence is vast and spans various biological systems. In grass carp, prostaglandins are particularly important for ovulation and other functions related to reproduction. They also play a role in modulating the immune response, helping the fish combat infections and other pathogenic agents.

15.10 Forages

As predominantly herbivorous fish, fingerlings and juveniles consume only the leaves in their natural environment, but as they mature they begin to consume both the leaves and stems of plants. This dietary habit allows them to be cultivated using only fresh vegetation as a food source (Figure 119). This characteristic facilitates sustainable and low-cost production, given that fresh vegetation are more readily available and have a lesser environmental impact than other protein sources.

The green food must be fresh and tender, without signs of decomposition or fermentation. To prevent risks to fish health it is imperative to avoid using forage stored for long periods that may have deteriorated.

Young and tender plants tend to be easier to digest, as well as being rich in nutrients. It is important to keep an eye on the feeding behaviour of the fish; if they prefer certain types of plants, try to provide them in greater quantity. Wilted or deteriorated plants should not be used, to avoid the risk of diseases.

In adequate warm waters (above 20°C), an adult grass carp can consume its body weight in hydrilla daily. Large animals can consume up to 45 kg of plant material in a single day but can only digest half of that amount. Thus, the feed conversion rate of forages is usually meagre (between 1:5 and 1:6, meaning they need to ingest 5 to 6 kg of dry matter for each kilo of weight gain). The animals eliminate remnants of this ingested material and faeces into the water, which enriches it and can promote algal blooms that reduce water quality and decrease oxygen concentrations.



Figure 119. A grass carp feeding on plants.

15.10.1 Forage choice

Grass carp are recognised for their extensive feeding spectrum, as they consume various aquatic plants. However, a notable issue is that feeding grass carp with aquatic plants requires more plants to gain the same weight than for terrestrial forages. This increased requirement is because aquatic plants have a higher water content than terrestrial ones. Limiting the use of aquatic plants in the diet on a cultivation farm is therefore advisable.

15.10.1.1 Aquatic plants

The tip for better utilisation and nutrition of carp is to avoid forages with reticular veins and always observe the feeding preferences of the carp to adapt their diet over time, as fingerlings and juveniles have dietary preferences that are distinct from each other (Figure 120 and Figure 121). In the fingerling phase, grass carp have a small mouth and should not be fed with thick and hard forages. Juvenile fish have smaller mouths than adults and prefer softer plants like duckweed. As they grow, more fibrous plants can be introduced.

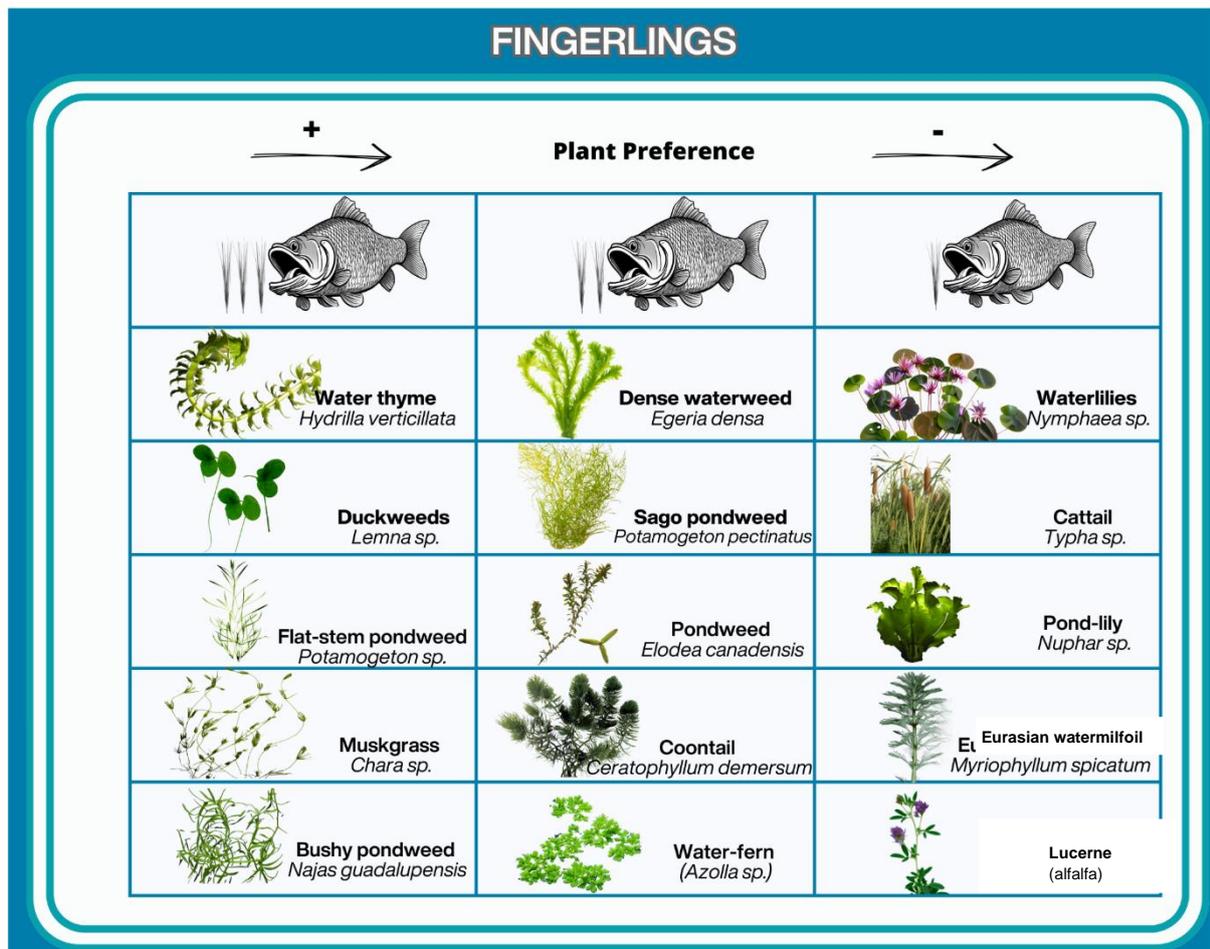


Figure 120. Order of preference of grass carp fingerlings for aquatic plants.

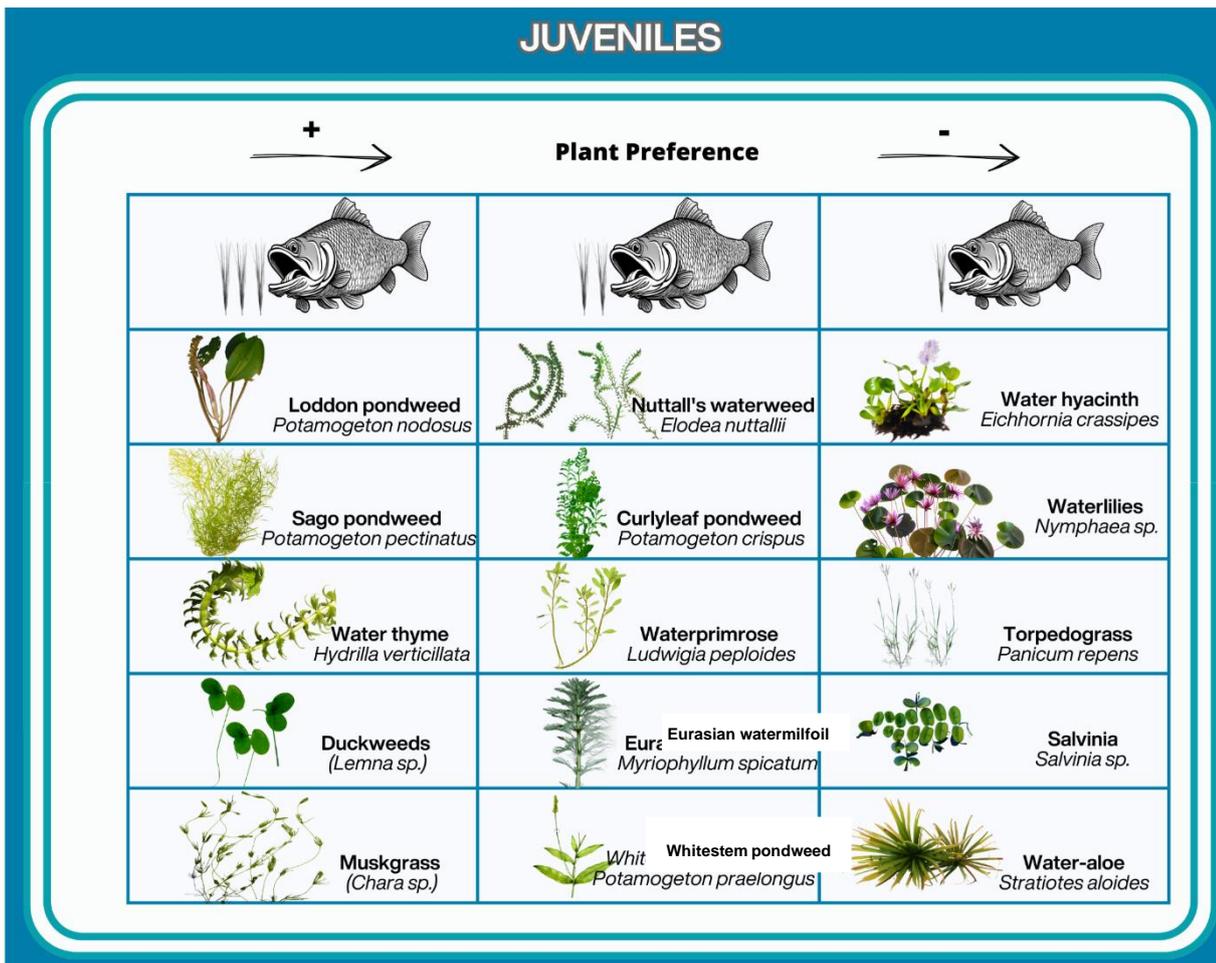


Figure 121. Order of preference of grass carp juveniles for aquatic plants.

15.10.1.2 Terrestrial plants

Although grass carp can consume aquatic plants, this is not the most economically efficient way to feed these fish in cultivation. On average, 60–80 kg of aquatic plants are needed to produce 1 kg of fish meat, while about 15–20 kg of terrestrial fodder would suffice. This proportion can be partly explained by the high moisture content in aquatic plants (which, in some cases, can reach 95%, while terrestrial plants usually stay below 80%).

Generally, it's perceived that grass carp prefer to feed on fresh and soft forages, such as ryegrass, Sudan grass and alfalfa. These plants have higher nutritional value and are more accessible for the fish to digest. However, more recent scientific studies show that things are not so simple and that there are some particularities regarding their preferences.

These studies indicate that the cellulose content in plants can be a decisive factor in the grass carp's dietary preferences. Plants with a higher cellulose content tend to be more consumed, probably because cellulose is a long-lasting energy source for the fish. On the other hand, plants that are toxic or deficient in nutrients can harm the fish's health. Therefore, seeking a balance between preferred

and nutritionally richer plants, even if only partially consumed, may be ideal for maintaining the fish's health and welfare.

Various non-aquatic plants can be used as fodder to diversify and complement the diet of grass carp. Plants and stems of Bermuda grass, rye, corn and even wheat are highly appreciated and provide the necessary fibre for proper digestion. The same applies to the tender stems and leaves of pumpkins, cucumbers and watermelons (Figure 122).

In Asia, phytotherapeutic additives are expected to be incorporated into the forage to stimulate the fish's appetite, strengthen the immune system and increase disease resistance. These additives are entirely natural, do not pollute the water and do not affect the quality of the meat. Examples of these phytotherapeutics include eleuthero (Siberian ginseng stem), liquorice root, ginkgo root (*Ginkgo biloba*), boneset herb, eucommia leaf, hawthorn berry, rhubarb root, horny goat weed (epimedium), dwarf lilyturf root (ophiopogon root), stemona root, poria mushroom and china root.

NON-AQUATIC PLANTS



Bermuda gras



grass Setaria grass



Asian rice



Wheat



Oat



Alfafa



Rye



Corn



Sorghum



Cucumber



Luffa



Watermelon



Melon



Pumpkin



Chayote

Figure 122. Non-aquatic plants whose leaves and stems can be used in feeding grass carp.

15.10.2 Removal of fodder residue

- **Fodder residue:** It is recommended to remove any uneaten fodder remnants daily to prevent the deterioration of water quality. The forage can be tied with ropes and introduced into the ponds during feeding to facilitate this removal. Afterwards, the ropes are pulled and the waste is appropriately disposed of.

15.11 Bio-floating beds

The quality and physiological condition of the fish meat are intrinsically linked to their cultivation environment. To optimise these aspects in grass carp cultivation, one of the effective strategies is the implementation of 'bio-floating beds'. These beds are essentially floating planters adorned with specific vegetation, as demonstrated in Figure 123 and Figure 124, where *Ipomoea aquatica*, popularly known as 'water spinach' or 'swamp cabbage', was used. However, it is worth noting that other plant species can also be used for similar purposes.

The integration of these beds into the cultivation system brings several benefits: it promotes an increase in grass carp muscle mass, raises the content of crude protein in the meat, significantly decreases crude fat levels, and gives the meat a firmer texture and a more pronounced flavour.

Bio-floating beds in cultivation ponds also improve water quality, minimising nitrite and ammonia concentrations. This improvement in aquatic conditions contributes significantly to the welfare and optimised growth of the cultivated fish.



Figure 123. Grass carp cultivation pond with planted floating beds.



Figure 124. Detail of floating beds planted with water spinach.

15.12 Feeding with raw grains

The quality of the fish meat is also influenced by what it eats. Therefore, it is common for producers to alternate or supplement feed-based nutrition using raw grains offered directly to the fish. This is possible because grass carp respond very well to dietary variation.

It is important to note that these grains should be introduced into the diet gradually and monitored to assess any adverse effects on the health or growth of the fish. The offering of grains must also be balanced with other nutritional elements to ensure a complete nutrient profile. Some of the grains that can be considered are:

- **Corn:** Corn is a rich source of carbohydrates and can be used to provide quick energy. However, it is poor in proteins and essential amino acids. Therefore, it should be complemented with other protein sources.
- **Soy:** Rich in proteins, soy is an excellent option, especially if treated to remove anti-nutritional factors (substances that, even in minimal amounts, reduce or prevent the

utilisation of a nutritional element). Caution should be exercised with the quantity provided, as soy also contains phytoestrogens, which can affect the hormonal system of fish.

- **Wheat:** Wheat grains or wheat bran can be helpful, mainly as a source of carbohydrates. They also contain some proteins, but like corn are deficient in essential amino acids.
- **Rice:** Rice is another rich carbohydrate source. However, its nutritional profile is not as complete as some formulated feeds. It is best used as a complement to a more balanced diet.
- **Oats:** Oats can be a good supplement, rich in fibre and some essential nutrients. However, they are relatively poor in proteins and essential amino acids.
- **Sorghum:** Although less common, sorghum is another carbohydrate-rich option. It should be used cautiously, as some varieties can contain significant levels of tannins, which are anti-nutrients.

Recently, **fava beans** have been used in the diet of grass carp. This leguminous grain is a reasonably good source of proteins and also contains a variety of nutrients, including minerals and vitamins. However, there are some important considerations to weigh up when using raw fava beans in the diet. The positive points include:

- **Protein source:** Fava beans contain many proteins, vital for growth and tissue repair in fish.
- **Minerals and vitamins:** They are also a source of vitamins such as B1, B5, and B6 and minerals like magnesium, phosphorus, and iron, which are necessary for various biological functions.
- **Accessibility:** In some regions, fava beans may be more accessible or economical than formulated commercial feeds.

On the other hand, the fish farmer needs to note the following about fava beans:

- **Anti-nutritional factors:** Like other legumes, fava beans contain anti-nutrients like phytates and lectins, which can interfere with the absorption of other nutrients. Proper preparation may be necessary to minimise these effects, such as the inclusion of enzymes in the diet for example.
- **Diet balancing:** Raw fava beans do not provide a complete nutritional profile for grass carp. Therefore, they should be offered in conjunction with other foods that meet the dietary needs of these fish.
- **Digestibility:** Depending on the size and life stage of the carp, it may be necessary to crush or grind the fava beans to increase the grass carp's ability to use their nutrients.

- **Monitoring:** As with any change in diet, the introduction of raw fava beans should be carefully monitored to ensure there are no adverse effects on the growth or well-being of the fish.

When using raw grains in the diet of grass carp, it is crucial to ensure that they are properly cleaned and free from contaminants. Minimal processing, such as grinding, may also be necessary to facilitate digestibility, depending on the grain. And, of course, the inclusion of grains should take into account the overall nutritional balance required for the welfare and healthy growth of the fish.

15.13 Dry feeds

Grass carp adapt very well to artificial diets, composed of specially formulated and balanced feeds to meet their nutritional need (Figure 125).

A quality feed provides healthy growth and proper development and ensures the health of the fish. Thus, the essential characteristics of a good feed are:

- Being nutritionally complete;
- Having appropriate granulometry and texture;
- Being palatable and digestible;
- Being stable in water.

Several other points should also be considered for the correct choice of feed to be used:

- **Protein:** It should contain a higher amount of animal protein if the fish are cultivated in environments with little natural food or in recirculation systems;
- **Feed supplier:** The feed supplier should be carefully chosen based on the quality of the product they sell and possibly the assistance they offer to the client, and not just on the price they charge for the feed;
- **Feed choice:** Consultation with a specialist or even your feed supplier should be done whenever possible to identify the feed available in the regional market that is most suitable for the existing cultivation system on your property and the life stage of your fish;
- **Communication:** Information should be exchanged with other fish farmers who use the feed you intend to use to find out the results that are being achieved.



Figure 125. A grass carp feeding on extruded feed.

15.14 Extruded vs. pelleted feed

Extruded and pelleted feeds can be used in grass carp cultivation, as the fish can feed both on the surface and at the bottom of the pond. What matters most in this case is not the manufacturing process but the nutritional suitability of each type of feed. Nevertheless, each type of feed has its specific features and advantages:

15.14.1 Extruded feed

- **Advantages:** Extrusion is a process that exposes the ingredients to high temperatures and pressures for a short period. The heat during the extrusion process improves the digestibility of the feed, reduces anti-nutrients and makes the feed float, allowing the fish farmer to observe how much the fish are eating and adjust the quantities provided if necessary (Figure 126).
- **Disadvantages:** Generally, extruded feeds are more expensive due to the more complex manufacturing process. In addition, floating feed may not be appropriate for all stages of grass carp development or in all cultivation systems.

15.14.2 Pelleted feed

- **Advantage:** It is more common and usually cheaper than extruded feed.
- **Disadvantage:** Pelleted feed may disintegrate more quickly in water, leading to nutritional losses and potentially worsening water quality if not consumed quickly.

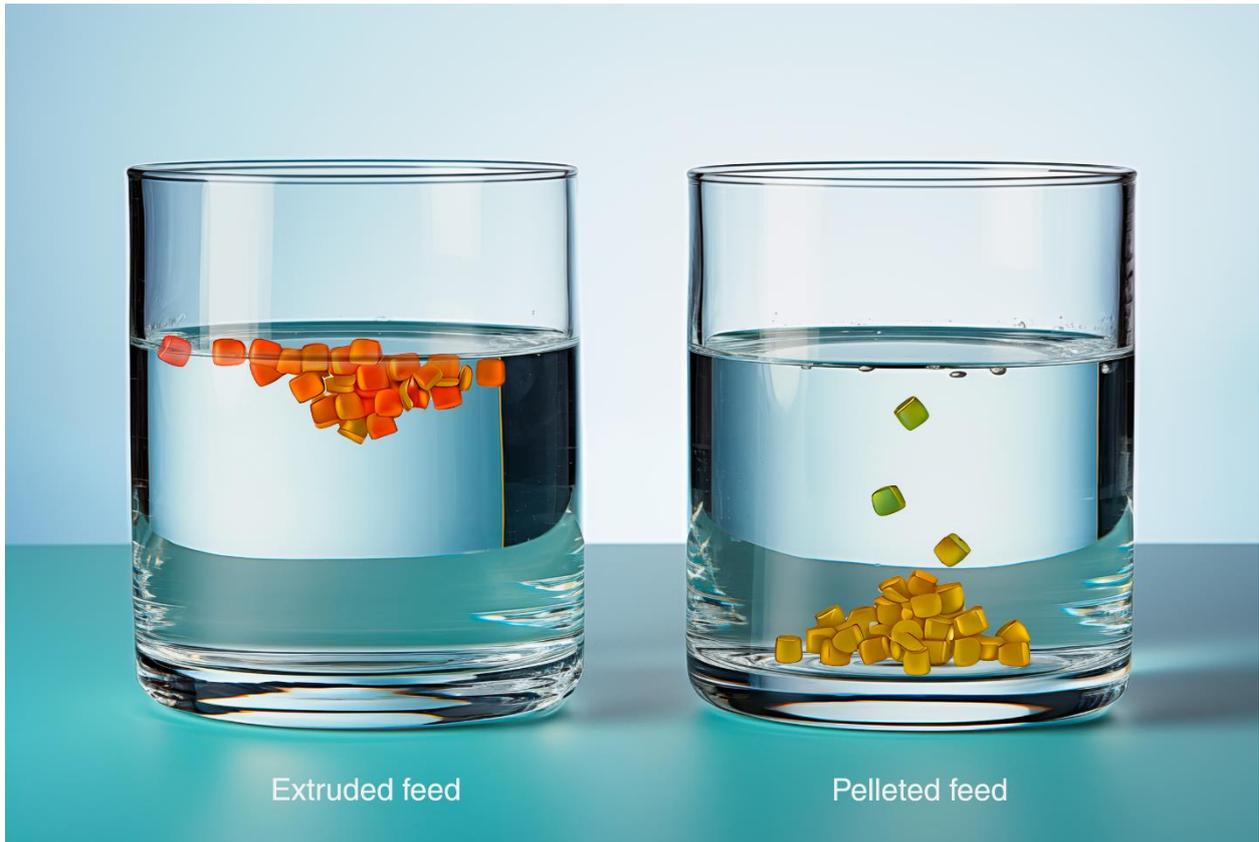


Figure 126. Extruded feeds float, allowing the fish farmer to observe and record consumption. Pelleted feeds sink, making it harder to identify leftovers.

15.15 Homemade vs commercial feeds

In Asia it is not uncommon for grass carp to be produced based on homemade feeds. However, formulating a suitable feed for fish is a complex task involving various technical, nutritional and economic factors.

One should not be misled into thinking that the fish farmer will easily be able to produce a good quality, balanced and low-cost feed from the inputs available on their property, as this is usually not possible. As explained here, fish require a large and complex series of nutrients, which are unlikely to be adequately supplied and balanced in homemade feeds.

Feed that is not correctly formulated can negatively affect the fish's growth, health and well-being, resulting in higher production costs and negative environmental impact. Therefore, it is vital to address these issues carefully and based on technical knowledge. Some of the difficulties a producer may encounter if they wish to formulate their feed instead of buying a commercial feed are:

- **Lack of knowledge:** Feed formulation requires in-depth knowledge about the nutritional needs of fish. Ignoring this can lead to dietary deficiencies or a waste of resources.
- **Species variety:** Different species have distinct nutritional needs. A feed suitable for one species may not be suitable for another.
- **Nutrient availability:** The ideal ingredients may not always be available in sufficient quantity and quality.
- **Nutritional variability:** The nutritional composition of natural ingredients can vary, affecting the final quality of the feed.
- **Equipment and technology:** The homogeneity of the mixture and the granulometry of the ingredients are crucial. Inadequate equipment can result in a non-uniform mixture or improperly ground ingredients.
- **Granulometry:** The size of the feed pellets must be appropriate for the species and life stage of the fish.
- **Costs:** Some high-quality ingredients can be expensive, especially in small quantities, making the feed less affordable.
- **Logistics:** The costs related to transporting the ingredients can affect the total cost of the feed.
- **Regulatory and environmental standards and guidelines:** Compliance with local and international regulations on using ingredients and additives is mandatory.
- **Sustainability:** The pressure to use more sustainable ingredients complicates feed formulation.
- **Adaptation to change:** Fish have different nutritional needs at different life stages. The formulation must be adjusted according to each stage and promote a gradual adaptation to the feed intended for the new life stage.

15.15.1 Fish feed formulas

All ingredients commonly used in aquaculture feeds can be applied in grass carp feeding. Formulations often contain fish meal, soybean meal, rapeseed meal, rice bran, corn, wheat, and other agricultural by-products. Vitamins and minerals are added, usually representing 2 to 5% of the total feed.

As an illustration (Figure 127), we present some formulation possibilities that allow for obtaining excellent results in grass carp cultivation. At the same time, we emphasise that each producer must assess the availability and costs of the indicated ingredients so that the final cost of their feed is not even higher than the cost of commercial feeds available in the regional market.

15.15.1.1 Feed 1

- Bean meal: 150 kg;
- Rapeseed meal: 200 kg;
- Cottonseed meal: 150 kg;
- Wheat bran: 145 kg;
- Subflour: 125 kg;
- Rice bran: 150 kg;
- Fish meal: 44 kg;
- Stone powder: 18 kg;
- Salt: 3 kg;
- Cactus powder: 5 kg;
- Aloe vera powder: 4 kg;
- Garlic powder: 2 kg;
- *Bacillus subtilis*: 4 kg.

Note: Dry cactus powder and aloe vera powder are natural additives that strengthen the immune system of grass carp and may help prevent gastrointestinal diseases. Dry garlic powder has anti-inflammatory and antimicrobial properties, contributing to disease resistance.

15.15.1.2 Feed 2

- Fava beans: 650 kg
- Fish meal: 20 kg
- Subflour: 88.5 kg
- Rice bran: 59 kg
- Rapeseed meal: 150 kg
- Betaine: 3 kg
- Choline chloride: 5 kg
- Dicalcium phosphate: 7 kg
- Enzyme preparation: 1.5 kg (1 kg of phytase and 0.5 kg of composite enzyme)
- Multivitamins: 6 kg
- Compound mineral salts: 10 kg

15.15.1.3 Feed 3

- Fava beans: 720 kg
- Fish meal: 10 kg
- Subflour: 25 kg
- Rice bran: 68 kg
- Rapeseed meal: 150 kg
- Betaine: 2 kg
- Choline chloride: 3 kg
- Dicalcium phosphate: 6 kg

- Enzyme preparation: 1 kg (0.5 kg of phytase and 0.5 kg of composite enzyme)
- Multivitamins: 5 kg
- Compound mineral salts: 10 kg

15.15.2 Preparation methodology

- Use a grinder to turn the ingredients not already in powder form into powder.
- Use a scale to weigh all the ingredients according to the provided proportions.
- Place all the ingredients in a mixer and mix until the composition is homogeneous.
- Add some water and fish oil to the mixture to aid in pelletising. The amount may vary, but you are trying to achieve a paste-like consistency.
- Use a grinder or a press to form the pellets.
- The pellets must be dried (at about 50–60°C) to ensure more extended durability.
- Once the pellets are dry store them in a cool, dry place, preferably in airtight packaging, to prevent moisture ingress and extend durability.
- If desired, add vitamin and mineral supplements to the pellets.
- Keep an eye on the behaviour and growth of the carps to adjust the recipe, as necessary.

Notes:

- You can replace the 5 kg dry palm powder with 125 kg fresh palm leaves.
- Similarly, the 4 kg of dry aloe vera powder can be replaced by 100 kg of crushed fresh aloe vera leaves.



Figure 127. Different formulations and diameters of fish feed pellets.

15.15.3 Reception and storage of feed

Feed, the most expensive input in captive fish production, must always be kept in optimal storage conditions to avoid deterioration or contamination, especially by fungi or chemicals. Here are some tips on properly storing the feed until it is provided to the fish.

- **Feed assessment:** The feed must be assessed as soon as each batch arrives on the property.
- **Acceptance criteria:** The producer should not accept the batch of feed if the amount of fines (dust) in pelleted or extruded feeds is high (more than 2%), as the fish seldom consume the dust. The dust is generated by friction between the feed grains, and too much dust will lead to a waste of feed, harming water quality. To measure the amount of dust, sift the feed from a feed bag using a mesh aperture smaller than the size of the pellet and separate the dust. Then, weigh it. In this example, the dust in a 25 kg feed bag cannot weigh more than 0.5 kg.
- **Moisture and mould:** The feed should also not be accepted if it shows signs of moisture or mould (fungi).
- **Floatability test:** If floating feed is used, it is recommended to evaluate its floatability, which should be over 85% (depending on size and protein level). A bucket or basin of water can be used to measure this. Place 100 pellets in the water, wait 10 minutes and count how many sank. In this example, the number must be less than 15.

- **Storage space:** The feed should be stored in a space exclusively for it, without the presence of any other agricultural input in the same location.
- **Dry conditions:** The storage location must always be dry to prevent the oxidation of fats and the growth of fungi in the feed.
- **Infrastructure:** The walls and floor of this location must be waterproof. There should be enough space for adequate ventilation and good lighting, but without allowing direct sunlight to enter.
- **Pest control:** The facility needs protection mechanisms against rodents and insects.
- **Hygiene:** Any feed that falls on the floor should be immediately collected to avoid attracting insects and rodents.
- **Cleanliness:** The area around the feed storage location should always be kept clean.
- **Inventory management:** The batches of feed should be separated in such a way as to ensure that the oldest is always used first to avoid loss of nutrients.
- **Pallet storage:** The feed bags must be kept on pallets (not directly on the floor) and arranged to allow good air circulation between them (Figure 128).
- **Visual inspections:** Frequent visual inspections should be conducted on stored bags to identify changes (moisture, torn bags, rodent, or bird droppings).
- **Feed storage:** It is recommended that the feed is not stored for more than 90 days and that it always be used within the validity period defined by the manufacturer.



Figure 128. Storage of feed bags on pallets is crucial, ensuring they do not directly touch the floor. Stacks should not be too high; intermediate pallets are advised to prevent the crushing of grains in the lower bags. Adequate spacing between stacks is essential.

15.15.4 Feeding management

Proper feeding management is vital for maximising the economic potential of grass carp cultivation. The grass carp requires careful attention to prevent overfeeding. It is known for its virtually insatiable appetite. Overfeeding can lead to health issues, such as enteritis, or an inefficient cycle where the fish excretes while eating and vice versa, leading to feed wastage and reduced production efficiency.

15.15.4.1 Feeding frequency

- **Frequent feeding:** The grass carp, renowned for its voracious appetite and capacity to consume large quantities of food, requires careful monitoring to avoid overfeeding, which results in food waste, water pollution and health issues for the fish.
- **Feed type:** Feeding frequency should be adjusted based on the type of feed. When fed macrophytes like duckweed (Figure 129), grass carp eat almost continuously over 24 hours. However, when fed pelleted feeds, they dedicate only about a quarter of their daily cycle to feeding.
- **Daily feeding instances:**

- The recommended daily feedings should be planned according to the fish's life stage (Figure 130). Generally, it's advised to divide the daily feed amount into several portions throughout the day. However, with vegetable feeds, it is important to note that grass carp take about two hours to digest a grass-based meal, which means more frequent feeding intervals are ineffective.



Figure 129. A vegetable-based complementary diet is crucial, even if the fish are regularly fed pellets.

- Ideally, fish in the fattening phase should be fed three or more times daily. However, feeding should be reduced or suspended during extreme heat, cold or heavy rain to avoid water quality issues.
- Feeding frequency thus varies with the seasons. In high-temperature seasons (22–30°C), grass carp should be fed more than three times a day. One or two daily feedings are more suitable in cooler temperatures (15–21°C).

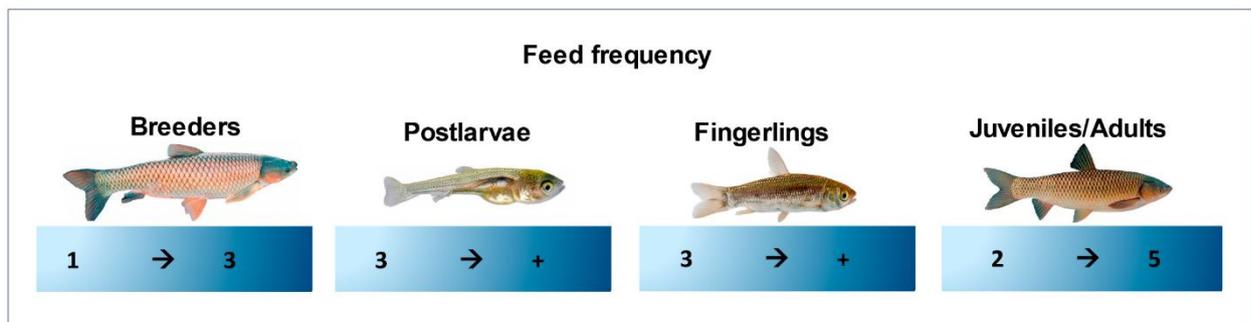


Figure 130. Recommended daily feeding numbers for grass carp at different life stages.

15.15.4.2 Feeding times

- **Feeding pattern:** The feeding pattern of grass carp varies throughout the day. They eat less in the morning than in the afternoon, with their peak feeding occurring after dusk, but they usually stop feeding from midnight until the early morning. This behaviour is linked to the concentration of dissolved oxygen in the water.
- **Summer feeding:** During high temperatures in summer, it is advisable to avoid feeding the carp at night as this can increase oxygen consumption and cause hypoxia.
- **Morning feeding check:** Before proceeding with the morning feeding of the fish, it is essential to check that there is an adequate concentration of dissolved oxygen in the water. Only start distributing the feed if the saturation exceeds 49%. Low levels of dissolved oxygen can lead to reduced appetite in fish and compromise their ability to assimilate the feed, which delays growth and affects water quality and feed efficiency.
- **Practical approach:** In practice, fish farmers often feed the fish with plant material in the morning and with feed in the afternoon (a procedure known as 'coarse and fine'). This method has proven effective in increasing profit margins.

15.15.4.3 Quantity and quality

- **Meat quality:** It is believed that the quality of grass carp meat can be improved through combined feeding, using, for example, a diet consisting of 40% feed and 60% macrophytes.
- **Manual feeding technique:** If the feed is provided manually, avoid dispensing it immediately. Initially, when only a few fish gather at the feeding area, offer a limited amount of feed slowly. As the majority of the fish group in the feeding region, increase the speed and distribute the feed more broadly. After a few minutes, when about 50% to 60% of the fish have fed sufficiently, gradually reduce the amount of feed. Stop feeding entirely when 80% to 85% of the fish are satisfied. Repeat this procedure at different points in the pond. This method optimises feeding costs, promotes uniform growth, prevents the dominance of larger fish over smaller ones, reduces the risk of injuries from excessive contact, lowers the likelihood of diseases and maximises economic benefits.
- **Feeding quantity:** The amount of feed used daily should be proportional to the biomass of the grass carp to be fed. This value can range from 2% in finishing phase animals to 9% per day for post-larvae (Figure 131).
- **Avoiding overfeeding:** Given the voracious and greedy nature of grass carp, it is crucial to avoid overfeeding, as the fish will not use the food provided effectively. This could also compromise the financial viability of the venture.
- **Feeding practice:** The fish should be fed about 80% of the amount that would lead to satiety. To determine this amount, observe the fish during meals to assess how they respond to

feeding. If the food is consumed quickly and the fish still seem hungry, the quantity offered can be slightly increased. If the fish reduce their feeding impetus, you should stop supplying.

- **Feeding duration:** It is also recommended that the duration of feeding management not be less than 45 minutes, allowing all the carp to feed adequately.
- **Preventing impaction:** Avoid giving large quantities of feed at once to prevent impaction. The term impaction refers to the phenomenon where the fish's digestive tract becomes 'impacted' or obstructed due to excessive consumption of fibres and cellulose, often found in vegetables and other plants. This problem occurs because grass carp have a digestive system adapted to degrading plant materials, but excess substances can hinder digestion and nutrient absorption. Impaction can lead to growth and health problems and, in extreme cases, even fish death. Therefore, it is crucial to offer a well-balanced diet that meets the specific nutritional needs of the fish.

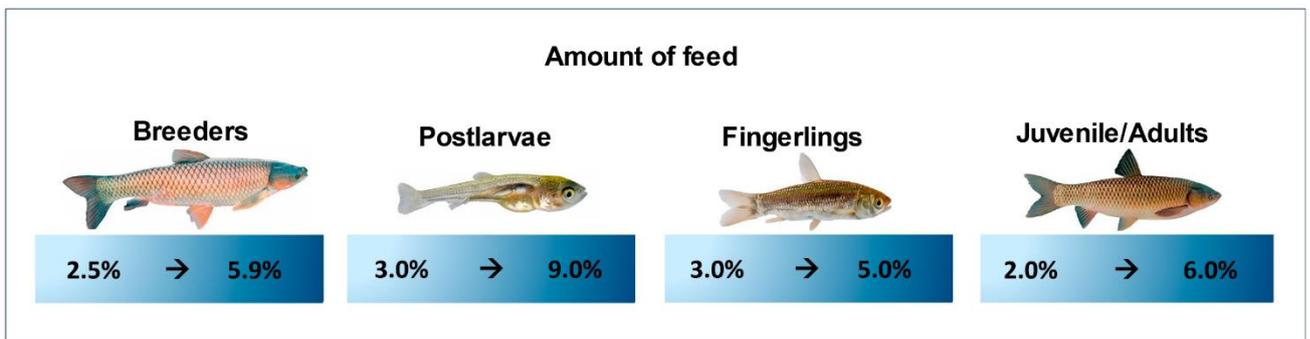


Figure 131. Recommended limits for the quantities of dry feed to be used for grass carp.

15.15.4.4 Pellet granulometry

A critical aspect to consider is the granulometry (dimensions) of the pellets in the feeds provided to fish. Fish need to consume the feed as quickly as possible, as it contains nutrients that dissolve in water and may be lost before consumption.

Table 58 shows the relationships between the size and weight of grass carp post-larvae and their mouth size, which is vital for determining the appropriate pellet size.

Table 58. Mouth width, body length and body weight of grass carp post-larvae.

Total Length (mm)	Body Weight (g)	Mouth Gape (mm)
45	0.8	2.0 x 3.0
50	1.7	2.5 x 3.5
55	2.4	3.5 x 4.0
60	3.1	3.7 x 4.5
65	3.6	4.0 x 5.0
70	5.5	4.3 x 5.4
75	7.2	4.5 x 6.0
80	10.5	5.0 x 6.5

The ‘rule’ for ensuring rapid ingestion is that the pellet size should not exceed 30% of the fish's maximum mouth gape. It is important to note that the ideal pellet size changes as the fish grows, as illustrated in Figure 132.

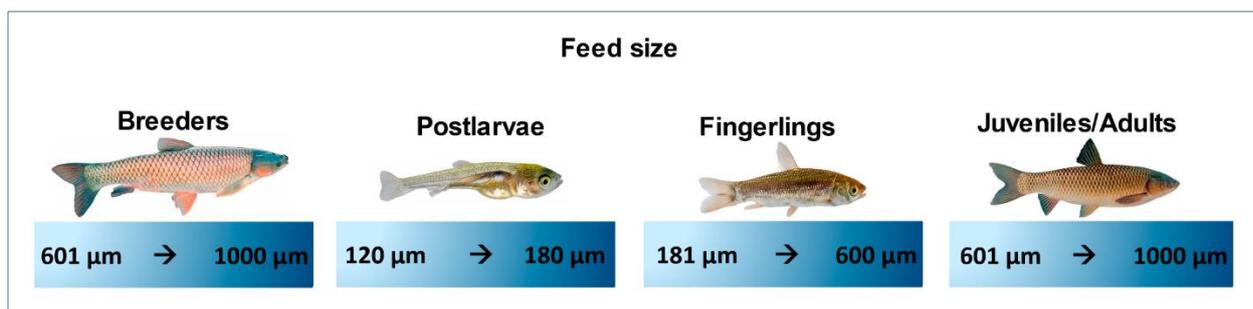


Figure 132. Recommended pellet sizes for different life stages of grass carp.

15.15.4.5 Feeding sites

- **Uniformity:** Feeding should be regular, with sufficient and well-distributed amounts of feed across the tank or culture pond. This ensures all fish have access to food, promoting uniform and healthy growth.
- **Energy conservation:** Evenly distributing feed minimises the fish's need to search for food, reducing stress and conserving energy for growth.
- **Wind:** Always feed in the direction of the wind, ensuring pellets are directed into the pond.
- **Pond exits:** Avoid feeding near water exits to prevent feed wastage.
- **Routine:** Maintain consistency in the feeding location and schedule. Familiarity with this routine helps reduce fish anxiety and aggression.

15.15.4.6 Feeding methods

Manual feeding (broadcast)

- **Direct observation:** As observed in Figure 133, a significant advantage of manual feeding is the ability to observe fish during feeding, which allows for the quick identification of any signs of stress, illness or changes in appetite.
- **Immediate adjustment:** If the food is consumed too quickly or there are excessive leftovers, quantities can be adjusted immediately to avoid waste and optimise feed conversion rates.
- **Dietary flexibility:** Manual feeding allows easy adaptation or changes in diet.
- **Lower initial cost:** Compared to mechanised or automated systems, manual feeding has a lower initial cost, making it accessible for small-scale producers.
- **Labour intensive:** Manual feeding can be time-consuming, especially for large tanks or ponds.
- **Inconsistency:** Manual feed broadcasting can lead to uneven distribution, potentially causing uneven growth within the same tank.
- **Risk of over- or underfeeding:** Without careful attention, manual feeding can lead to excess or insufficient feeding, both detrimental to fish welfare and production efficiency.
- **Constant monitoring:** Manual feeding requires someone to be present during feeding times, which can be challenging with limited staff.



Figure 133. Manual broadcast feeding.

Automatic feeder

- **Automatic feeders:** Programmed for specific feeding times, automatic feeders (Figure 134) aid in feeding regularity. Feeding intervals can be adjusted based on variables like climate and water quality.



Figure 134. Control panel and automatic fish feeder on a pier.

- **Installation:** Position automatic feeders strategically within the pond.
- **Operation:** Automatic feeders, such as a remote switch, should be installed for remote activation or deactivation. Once programmed, feeders distribute feed automatically.
- **Coverage area:** Typically, one automatic feeder is suitable for about 0.7 ha of pond area.
- **Demand feeders:** Beneficial for fish welfare as they provide food constantly and up to satiety, following the natural rhythm of the animals. They simplify daily tasks, reduce competition for food, minimise accidental biting and fish stress, strengthen their immune system, and improve feed efficiency. They also produce more uniform oxygen demand and ammonia production throughout the day. However, it's important to note that fish may learn to 'play' with feeders, potentially leading to increased feed wastage (Figure 135 and Figure 136).



Figure 135. Demand fish feeders.

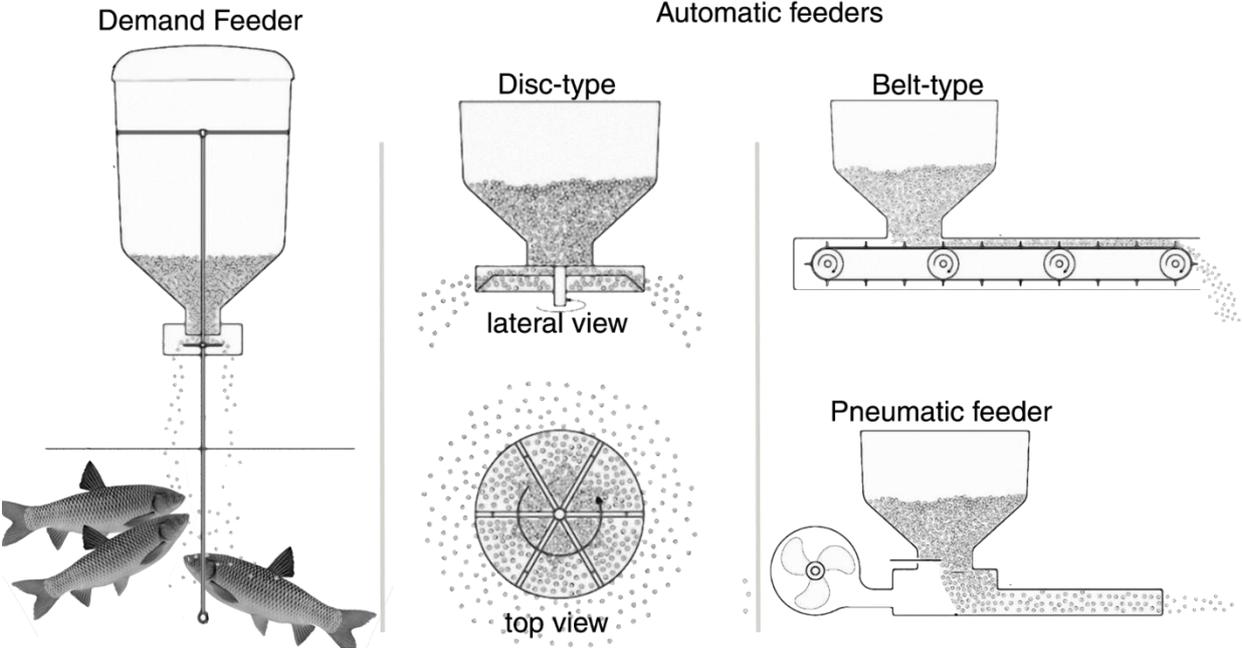


Figure 136. Demand and automatic fish feeders.

- **Alternative feeders:** Other methods, such as tractor-driven air blowers, are also an option for larger ponds or farms.

15.15.4.7 Rainy periods

- In stormy periods, suspend or reduce feeding as environmental conditions can severely compromise water quality.

15.15.4.8 Feeding method

- Distribute food evenly throughout the pond to ensure all fish can access it. Adapt the quantities based on environmental factors such as climate and water quality.

15.15.4.9 Pesticides

- Avoid forage that may have been treated with pesticides or contain toxic agents.

15.15.4.10 Probiotics

- Probiotics are live microorganisms that confer health benefits to the fish when administered adequately. They are commonly used to improve gut health and strengthen the immune system. An easy way to provide these beneficial bacteria to grass carp is to mix them with multivitamins and administer them orally every 10 days, 3 to 5 times. This practice makes the fish's intestine more acidic, which helps in warding off undesirable bacteria. These good bacteria can also aid digestion, allowing the fish to absorb their food better.

15.16 Peculiarities of grass carp feeding

- **Cellulose:** There is evidence that grass carp do not digest cellulose alone. Researchers suggest that microorganisms in the fish's intestine play this role, allowing for more efficient nutrient absorption.
- **Feeding habits:** Grass carp prefer a plant-based diet but are not exclusively vegetarian. Without sufficient aquatic plants, they can consume zooplankton and benthic organisms to satisfy their nutritional requirements. Consuming these tiny aquatic organisms is particularly important because they offer essential nutrients and fatty acids not typically found in adequate quantities in a regular plant-based diet.
- **Preference:** The genes related to taste in grass carp reveal a distinct inclination towards detecting sweet flavours, suggesting a genetic predisposition for preferring carbohydrate-rich plants.

15.17 Tips

- **Suppliers:** Choose trusted feed suppliers. Ensure your supplier has a proven track record of quality and consistency.
- **Stability:** The stability of the feed plays a crucial role in the success of grass carp cultivation, impacting not only the health of the fish but also significantly influencing operational costs.
- **Check composition:** Check labels for protein, fat, fibre and other nutrient levels. The feed should contain 28–35% crude protein for grass carp.
- **Continuous monitoring:** Perform regular tests (floatability, stability, etc.) to ensure that the quality of the feed remains constant over time.
- **Type of feed:** Use specific pelleted feed for grass carp.
- **Quantity:** The daily feed amount should vary from 2–3% of the total weight of the fish, reaching up to 6% in the juvenile phase.

16 PRE-SLAUGHTER AND SLAUGHTER

Routine pre-slaughter activities such as harvesting, sorting, size-based weighing, and transferring or transporting fish are crucial in fish farming. The efficiency of these operations depends on the planning, team training, quality of equipment, and condition of the fish at the time of handling (Figure 137). Improper handling can lead to high mortality rates in fish, either at the farms themselves or after transportation. Therefore, it is crucial for all involved to be familiar with stress factors and best practices to mitigate them.

The moment of harvesting is one of the most critical in aquaculture, and when well planned and executed, it can positively impact not just the welfare of the fish but also the profitability of the activity.



Figure 137. Aerial view of harvesting one of the ponds of a large farm dedicated to the polyculture of grass carp , highlighting the infrastructure and operational logistics set-up. Visible are trucks for fish transport, the seine net used for fish capture, and aerators turned on to ensure adequate oxygen concentrations in the water.

- **What is harvesting?** Harvesting removes fish or other organisms from tanks, ponds or other cultivation systems when they reach the desired commercial size. This process begins once it is observed that they have reached this size, and should be planned to ensure maximum biomass removal with high quality. In polyculture, where multiple species are raised together, harvesting becomes a logistical and technical challenge as each species may have different requirements.

- **When to harvest?** The ideal time for harvesting depends on several factors, such as the life cycle, cultivated species, desired final size and market conditions. Typically, harvesting is done when fish reach a commercial size, which can vary between 1 and 2 kg or more for grass carp, depending on the market and the cultivation goal.

16.1 Harvest

16.1.1 Types of harvesting

- **Strategies:** There are two strategies for fish harvesting in a fish farm: partial and total. In Asian countries, the former is commonly carried out at the end of summer and during autumn, preferably in the early morning. This schedule is adopted mainly for two reasons. Firstly, the cooler temperatures of the early hours are less stressful for the fish, favouring their welfare. Secondly, synchronising harvesting with the opening hours of fish markets allows producers to offer a fresher product to consumers.
- **Partial harvesting:** In polyculture systems, partial harvesting is an essential technique to optimise production. This method involves the selective removal of fish that have reached commercial size, reducing population density in the pond (Figure 138). This practice minimises competition for resources, allowing smaller fish to grow more quickly, thus making the overall production cycle faster and more efficient. It also contributes to a less stressful harvesting process and a healthier and less stressful environment for the fish. Finally, the farmer ensures a more continuous income flow by moving ready fish for the market.



Figure 138. Selection of fish during a partial harvest.

- **Total harvesting:** Total harvest (Figure 139) is a process of harvesting or removing all fish from a pond at once, typically taking place when most fish reach the average weight suitable for market demand. The removal of the animals must also be thoroughly planned, considering factors such as the duration of the operation, weighing, and costs related to sales, among other factors. In this case, the water volume in the pond should be reduced to 20 to 30% of the total volume. After about 80% of the fish have been captured, the pond should be almost wholly drained to ensure a safe and efficient operation. Total harvest offers advantages like logistical and operational efficiency. This technique eliminates manual selection, saves time and allows for more thorough maintenance of the cultivation environment, which is crucial for disease control and water quality. Moreover, it simplifies planning for the sale or processing of the fish, contributing to a profitable operation. However, the procedure should be as brief as possible to avoid exposing the animals to light and air and overcrowding in the capture nets.



Figure 139. Total harvest of a small nursery.

16.1.2 Methods for fish harvesting

- **Seine netting:** This method is typically used in large ponds to capture a significant volume of fish. Preference should be given to knotless multifilament nylon drag nets. The net's dimensions should directly correlate with the width of each excavated pond. The ideal length of the net should be one and a half times the total width of the pond, allowing for the formation of a 'pocket' (as demonstrated in Figure 140). Regarding the net's height, it is recommended that it should be twice the depth of the pond. However, a taller net is advised when the pond is deeper and presents drainage difficulties. This procedure ensures the net reaches the bottom of the pond, preventing fish from escaping. The dragnet mesh selection should consider the fish's size. For juvenile fish, 5–8 mm meshes are recommended, while for adult fish ideal meshes are 10, 15 or 20 mm. It is important to note that this technique should not be employed more than twice a day in the same location to minimise stress on the fish. Smaller specimens are more prone to injury and should be promptly released after capture, while larger ones can be segregated for marketing.



Figure 140. Seine nets are used for harvesting in a grass carp cultivation pond.

- **Cast netting:** (Figure 141) may be the most suitable option if the fish population in the tank is not too large or if the pond/tank is small.

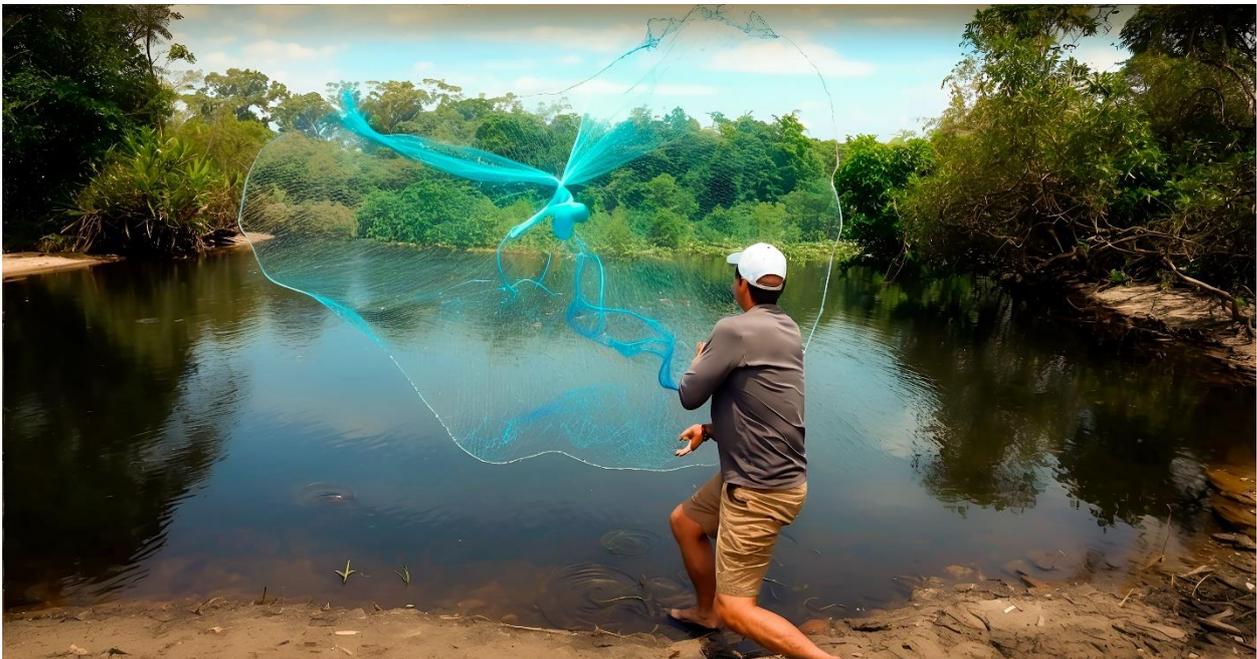


Figure 141. Use of a cast net for harvesting in a small pond.

- **Pond draining and fish collection at water exit:** Particularly effective for total harvesting, mainly in small ponds. Draining should be done gradually to reduce fish stress and allow them to adapt to the decreasing water volume. The draining speed should be controlled to avoid excessive turbulence and not be so slow as to unnecessarily prolong the process, as this could harm the quality of the remaining water, significantly reducing the dissolved oxygen concentration. Collection boxes or nets are strategically positioned at the water exit. These devices should be big enough to accommodate the expected quantity of fish and allow efficient collection without causing harm to the animals. The collection box should have an entrance wide enough for the fish to enter without injury and without creating a bottleneck that could cause stress and injuries. Once the fish are in the collection boxes or nets, they should be carefully and quickly transferred to their destination environment, whether a transport truck, tanks or other ponds. The remaining fish in the pond can be collected with a dragnet (Figure 142).

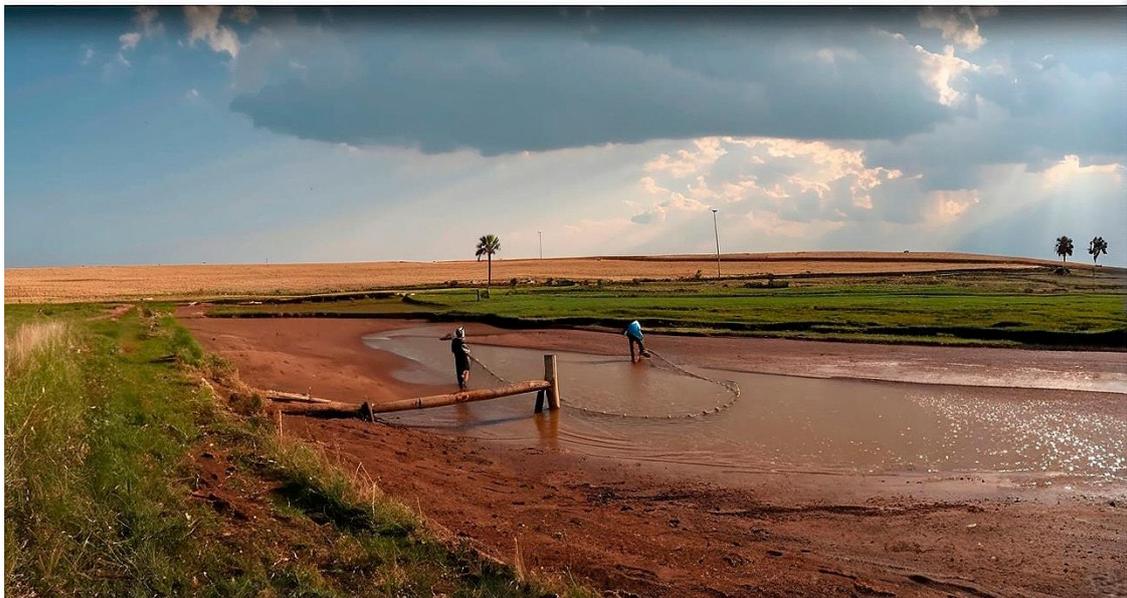


Figure 142. In smaller ponds, harvesting can be done by draining the water and collecting the fish in a mesh box positioned outside. The remaining fish in the pond can be managed with a dragnet.

- **Mechanised harvesting:** The so-called ‘fish pumps’, available in various models and systems, are designed to handle live fish. They are used for mechanised harvesting and offer an efficient and safe way to transfer fish from the pond to the next stage, whether a sorting system or a transport tank for processing or sale. The pumps operate in a manner that creates a continuous water flow, in which fish are gently enveloped and transported without the need for direct handling, minimising injuries or unnecessary stress (Figure 143). The pumps operate at low pressure to ensure the fish are not injured during transport. The diameter of the transfer tube is designed to accommodate the size of the fish, allowing them to move without restrictions and reducing the risk of injury. The pumps are made from materials that resist corrosion and are safe for fish, such as stainless steel or special plastics. It is also possible to regulate the water flow to suit different species and sizes of fish, ensuring safe transfer. These pumps can facilitate the rapid transfer of tonnes of fish per hour, optimising

harvesting time and reducing the need for manual labour. This is the best option for animal welfare, as the animals are not removed from the water throughout the process.



Figure 143. 'Fish pump', used for mechanised harvesting.

16.1.3 Planning and equipment

For an efficient and responsible harvesting operation (Figure 144), several aspects are fundamental and must always be considered:

- **Operation time:** Minimising the operation time is crucial, and rigorous planning is necessary to ensure that all resources, such as equipment, machinery and labour, are ready for immediate use.
- **Timing:** It is advisable to avoid the hottest hours of the day (12:00 pm–3:00 pm) to minimise animal stress and high temperatures for the workers.
- **Fish aspects:** The producer must know the fish's quantity, health and weight to avoid surprises during harvesting. A prior assessment carried out through the biometry of a sample helps ensure that the amount and quality of the fish meet the client's expectations.
- **Planning:** Define the route, location of the pond to be harvested, water depth, number of people and necessary equipment.
- **Personnel:** Inform in advance about each person's operation and role. Maintain a list of equipment to be used and assign a person to gather them.
- **Fasting:** It is crucial to stop feeding the fish at least one day before harvesting to avoid the stress of handling, which can be fatal for grass carp and compromise their marketability.
- **Water level:** Harvesting generally begins with reducing the water level in the pond to make fish capture easy and to reduce the intensity of the water flow at the gates during harvesting. In the case of total harvesting, it is recommended to lower the water level to 1/3 of the original volume. In the case of partial harvesting, the reduction should not be as significant.
- **Aerators:** Harvesting stirs up the bottom of the pond, resuspending organic matter and mineral sediments deposited at the bottom, all of which cause significant consumption of dissolved oxygen in the water. Therefore, keeping aerators on during harvesting is essential to maintain dissolved oxygen concentrations above 4 mg/L.
- **Personal protective equipment:** Use protective gear such as helmets, shin guards, rubber clothing and gloves to prevent accidents. Many of the species used in polyculture, including grass carp, are jumpers, and can cause injuries to the staff.
- **Harvesting materials:** Check for adequate nets, dip nets and plastic boxes for fish transport.
- **Harvesting pumps:** Specific pumps for harvesting are also used in certain operations. However, such pumps are not routinely used in polyculture involving grass carp.

- **Large-volume baskets:** In harvesting involving large quantities of fish, large-volume baskets capable of carrying 500 to 1,000 kg of fish at a time, with the help of a crane, may be employed.
- **Transport:** Ensure that vehicles, such as tractors or box trucks, are available and suitable for accommodating the volume of fish to be harvested. If a box truck is used, it should be cleaned beforehand.



Figure 144. Harvesting and slaughter structure on the farm. In this case, the fish are killed in ice without stunning. This method is not recommended, as it causes animal suffering and deterioration of meat quality.

16.1.4 The harvesting process

- **Time:** Harvesting should be conducted at night or early morning when water temperatures are lower. Running the harvest at these times is crucial to minimise stress on the fish and maintain meat quality.
- **Start:** It is advisable to start the dragging process in the deepest part of the pond. This approach is recommended because the bottom line of the net, containing the lead weight, should be carefully dragged across the entire pond bed. Using this technique increases the likelihood of successful capture.
- **Net dragging, key considerations:**

- Avoid pulling the net's ropes with hands or feet. Using hands or feet for this task requires more effort from those involved and can reduce process efficacy. To make the process easier and more efficient, tie a wooden pole about 1.5 m long at each end of the net (Figure 145). Pulling the net via the wooden pole saves effort and enhances harvesting efficiency.



Figure 145. A wooden rope is used to pull the surrounding net.

- If the net is prolonged or heavy, a rope can be attached to the wooden rope and tied to a tractor. However, a worker should still guide the net along the inner edge of the pond.
- **Net passage:** After capturing the fish with the initial drags, the next step involves emptying the pond to facilitate catching the remaining fish, which can be accomplished using a net or scoop nets. The process is simplified if the pond has a well-designed harvesting box (Figure 146). The remaining fish gather in this box, making their capture more efficient, less stressful for the animals and less labour-intensive.



Figure 146. Harvesting box.

- **Careful handling:** When removing fish, do so carefully to avoid stress or injury. Use holding tanks with aeration systems to keep the fish in optimal conditions until they are transported or processed.
- **Weighing and measuring:** The fish should be weighed and measured to facilitate sales once captured.
- **Fish welfare:** Throughout the entire process, prioritising the well-being of the fish is essential. This means avoiding practices that may subject them to excessive stress or cause physical harm. During capture issues such as fish exhaustion, lack of oxygen, exposure to air and light, excessive handling and the accumulation of organic waste from the decomposition of faeces and metabolites become more pronounced, thus intensifying stress. Sometimes, such stress can shorten the rigor mortis period, reducing the product's shelf life.

16.2 Transport and marketing

Maintaining fish during transportation and marketing live fish is critical in aquaculture production. There are at least two important aspects to consider:

Shelf-life extension: Maintaining the fish alive until the slaughter or marketing site is believed to extend the product's shelf life, which only begins when the animal is slaughtered.

Stress and discomfort: Keeping the fish alive involves additional steps that can cause animal stress and suffering, potentially compromising meat quality.

Furthermore, there are many different ways of transporting and maintaining live fish. Ideally, fish should be transported in tanks specifically designed for this purpose, allowing some control over water temperature and continuous oxygenation support until the slaughter or marketing site (Figure 147).



Figure 147. A truck with tanks for transporting live grass carp from the farm to the slaughterhouse.

16.2.1 Oxygen and water quality

The most urgent issue during transport is ensuring an adequate supply of DO. Regular monitoring of oxygen levels with an oximeter is necessary throughout the journey. When loading, the water in the transport box should have at least 6–8 mg of O₂/L. Remember, DO consumption is linked to the activity and metabolic rate of the fish, which tends to increase when several animals are confined in a minimal space. Therefore, regulate the DO concentration to increase concentration but avoid supersaturation, as this, combined with high temperatures, can cause embolism and be fatal to the fish.

16.2.2 Temperature and fish density

Ideally, the fish's metabolism should be reduced, which is achievable by lowering the transport water temperature using ice. This approach also reduces the production of toxic compounds like ammonia and CO₂. Maintaining the transport water temperature approximately 3 to 4°C lower than the original tank is recommended, as this minimises thermal stress and subsequent mortality. It is crucial to be able to monitor and adjust the temperature during the journey if necessary.

16.2.3 Additives and fasting

Adding salt to the water can be beneficial as it stimulates mucus production, reduces stress and has a bactericidal effect. The recommended dose is between 3 and 8 g/L. However, it is crucial to understand that the efficacy of this method can vary between different fish species, and in polyculture it is common for multiple species to be transported together. Also, pre-transport fasting (previously addressed) becomes particularly relevant, as it reduces water contamination with faeces and food regurgitated by the fish.

16.2.4 Equipment and alternatives

Use appropriate fish transport boxes and monitor the animals and water quality throughout the journey. If the option is to transport already slaughtered fish, use 20 kg capacity isothermal plastic boxes, commonly called monoblocks. These should be filled with high-quality ice to maintain the fish temperature around 5°C. Regarding ice, three critical factors must be observed: 1) Quantity: The amount of ice should be sufficient to keep the internal temperature of the slaughtered fish around 5°C, as measured in the centre of the fish. In practice, the average amount of ice needed is approximately 2 kg per kg of fish, although this may vary based on the ambient and body temperature at harvesting time. 2) Quality: The water used for ice production should be potable to avoid any risk of contaminating the fish. 3) Granularity: The ice size should be such that it does not cause physical damage to the fish, such as injuries, punctures or other lesions.

16.2.5 Marketing

Fish should be slaughtered as soon as possible after removal from the ponds. However, for cultural or socioeconomic reasons this is not the reality in the leading countries producing farmed fish. In these countries, fish are often marketed live directly to end consumers (Figure 148) and are not always kept in suitable conditions until slaughter.



Figure 148. Marketing live fish kept in inadequate conditions is a reality in many countries. The practices adopted often cause intense stress and discomfort to the animals.

16.3 Slaughter

With the advancement of aquaculture, more farmed fish are being slaughtered and processed. However, how these fish are killed is often a critical issue. The cheaper and simpler method is anoxia or suffocation, i.e., removing the fish from the aquatic environment until they die from lack of oxygen. This method is not only painful for the fish but can also compromise the quality and safety of the final product. In the suffocation process, the fish are removed from the water and left in an environment where they cannot breathe. In this situation, they undergo a period of intense stress and suffering. Unlike humans, who can hold their breath for a while and then quickly suffocate, fish can take a considerably more extended period to die from asphyxia. During this time the fish's physiological system is subjected to severe strain, increasing blood lactate and other stress metabolites, affecting meat quality.

After being harvested, farmed grass carp are often transported live to fish processing facilities. In Asia it is common for the fish to arrive alive at restaurants. Transport boxes with aeration and water temperature controlled by adding ice are used for this. In other regions, it is common for grass carp to be slaughtered after immersion in ice water – a method also not considered humane.

16.3.1 Issues related to fish slaughter

Grass carp slaughter must be carried out responsibly to minimise the risks associated with the practice:

- **Acts of cruelty:** The slaughter of fish should be carried out humanely to avoid unnecessary pain and suffering. In other words, the animals should be stunned beforehand, so they are not conscious and susceptible to pain.
- **Regulations and standards:** Many countries are updating their legislation to include humane slaughter standards for fish. These standards ensure that the methods used cause least possible amount of pain and suffering.
- **Technical expertise:** Fish slaughter must be carried out by trained individuals who understand humane slaughter methods and the needs of the fish.
- **Challenges:** Implementing large-scale humane slaughter practices, especially in aquaculture environments and, more specifically, in family-scale aquaculture, can present significant logistical challenges. However, the evolution of techniques and growing consumer demand for ethical practices are steering the market in this direction.
- **Public awareness:** As the public becomes more informed about animal welfare issues, there is an increasing demand for more humane slaughter practices. This new situation is driving the industry to adopt more ethical practices.

- **Meat quality:** Humane slaughter of fish not only prevents animal suffering but also enhances the quality of the fish. Proper bleeding is crucial for both purposes. Regarding meat quality, it is vital that bleeding removes most of the blood, preventing the multiplication of unwanted microorganisms. After the fish's death, the cessation of oxygen flow leads to anaerobic ATP production and lactic acid accumulation, resulting in a pH reduction that can delay bacterial growth. However, the pH in fish does not drop drastically, as it does in warm-blooded animals, generally remaining above 6.0. The eventual lack of ATP prevents calcium regulation, resulting in muscle contraction known as rigor mortis. After this phase, the 'resolution of rigor mortis' begins, involving muscle relaxation and meat tenderisation, termed 'meat maturation'. In other words, the meat starts to decompose. Therefore, slaughter directly affects meat quality and the product's shelf life.
- **Environmental impact:** Fish slaughter waste, such as scales, skin, blood, viscera and faeces, can contaminate the environment. Appropriate and safe disposal of generated waste, avoiding improper environmental disposal, is necessary for more sustainable aquaculture.
- **Human health:** Slaughter waste from fish can contain bacteria and other pathogens that can cause human diseases. Another reason to be concerned is proper waste disposal.

16.3.2 **Stunning and humane slaughter**

Fish are sentient beings, meaning they can feel pain and suffering. Furthermore, they exhibit behaviours that indicate emotional and cognitive experiences.

As previously described, scientific studies have shown that fish have complex nervous systems that allow them to experience emotions such as fear and anxiety. Thus, evisceration while the fish are still alive cannot be regarded as 'normal'; on the contrary, it is a cruel practice that causes prolonged pain and suffering to the fish and can also compromise meat quality.

Humane fish slaughter aims to minimise the suffering of these animals during the pre-slaughter and slaughter stages. For this, the fish must be stunned before being effectively slaughtered. The methods applied in this stunning need to ensure that the fish's loss of consciousness is rapid and irreversible (as shown in Figure 149). [Table 59](#) shows the main methods used for stunning and slaughtering farmed fish.

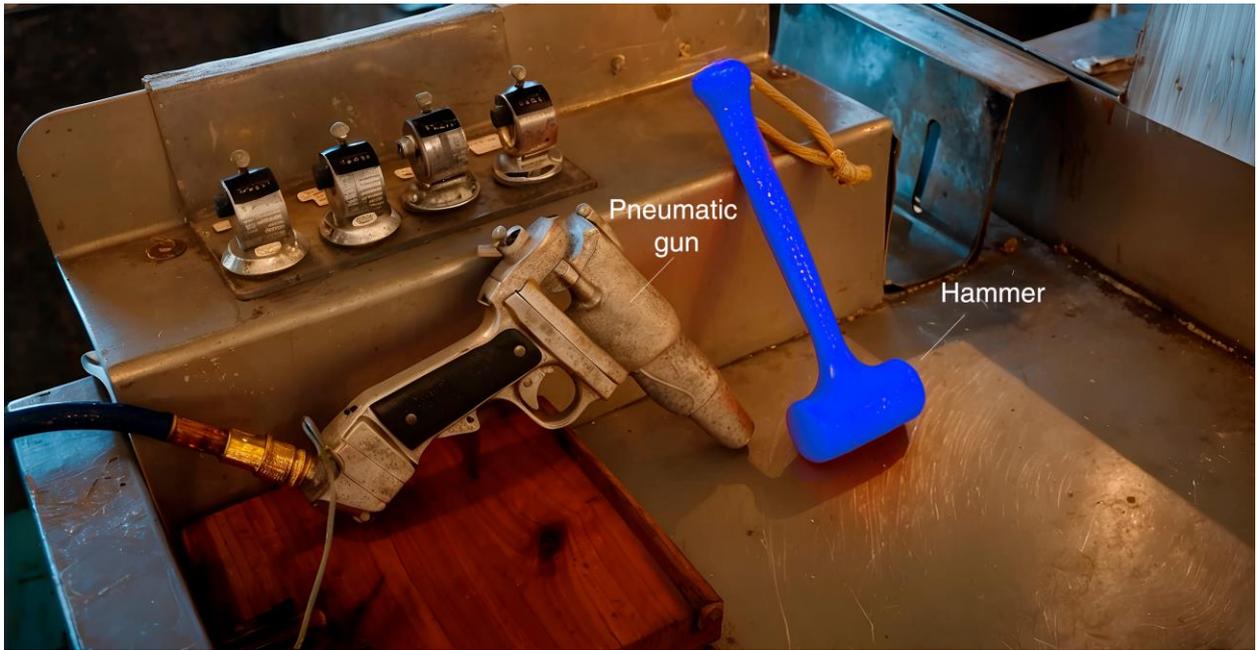


Figure 149. Equipment used for stunning fish: pneumatic gun and hammer.

Table 59. Advantages and disadvantages of methods used for stunning and slaughtering fish.

Rank	Stunning and Slaughter Methods	Principles	Causes desensitisation?	Causes death?	Advantages	Disadvantages
7	Suffocation (Asphyxia)	Fish are exposed to air and die of suffocation as gill filaments collapse	No	Yes	<ul style="list-style-type: none"> • No cost 	<ul style="list-style-type: none"> • Causes prolonged pain and stress to animals • Compromises meat quality • Accelerates decomposition • Reduces shelf life
6	Thermal Shock	Water and melting ice in a 1:1 ratio	No	Yes	<ul style="list-style-type: none"> • Low cost • Allows slaughtering of large volumes of fish at the same time 	<ul style="list-style-type: none"> • Not a stunning method • Temperature may vary during the slaughter process • Causes intensive suffering to animals • Reduces shelf life
5	Bleeding	Cutting of major blood vessels to cause death by blood loss	No	Yes	<ul style="list-style-type: none"> • Low cost • Simple and easy to apply • The same process is applicable for any species or size of fish • Excellent when combined with a stunning method • Traditionally accepted and easy-to-understand method 	<ul style="list-style-type: none"> • Desensitisation occurs slowly due to blood loss • Not humane if used alone • Requires a prior stunning method
4	Spinal destruction (pithing)	Mechanical destruction of the spinal cord	Yes	Yes	<ul style="list-style-type: none"> • Low cost • Rapid loss of consciousness, if correctly applied 	<ul style="list-style-type: none"> • Labour-intensive, especially with small fish • Requires skill and precision • Risk of cross-contamination

Rank	Stunning and Slaughter Methods	Principles	Causes desensitisation?	Causes death?	Advantages	Disadvantages
					<ul style="list-style-type: none"> • Stunning and slaughter combined 	<ul style="list-style-type: none"> • Ideally, it should be combined with a prior stunning method
3	Ikejimi	Destruction of the brain and spinal cord, followed by bleeding	Yes	Yes	<ul style="list-style-type: none"> • Low cost • Immediate stunning • Quick cessation of movements, including involuntary • Preserves high levels of ATP in muscle, prolonging meat shelf life 	<ul style="list-style-type: none"> • Requires trained personnel • Quite labour-intensive, especially with small fish or large volumes • It can be challenging to apply in large fish
2	Concussion	Severe mechanical shock to the head causing brain injury	Yes	Yes	<ul style="list-style-type: none"> • Immediate and irreversible stunning, if well applied • Death results from the action • Easy control in small operations • Suitable for large fish 	<ul style="list-style-type: none"> • There is a higher chance of human error, leading to ineffective stunning • Labour-intensive, especially with small fish or large quantities
1	Electrical stunning	Electrical current in a saline fluid medium	Yes	It may or may not, depending on equipment settings	<ul style="list-style-type: none"> • Less stress • Adaptable to different sizes and species of fish • Scalable for large-volume operations • It is highly effective in stunning • It does not require the removal of fish from the aquatic environment • Preserves high levels of ATP in muscle, prolonging meat shelf life 	<ul style="list-style-type: none"> • Need for subsequent cutting of blood vessels • Requires specific equipment and structures • Regular maintenance and calibration needed • The higher initial cost for system implementation

16.3.2.1 Suffocation

This method of slaughter is considered inhumane, as it causes a prolonged and potentially painful death to fish when they are removed from water and exposed to air. This exposure interrupts the animals' ability to perform gas exchanges – an essential fish survival process that usually takes place in water through the gills.

Fish gills comprise delicate structures called gill filaments, which contain many blood vessels and are optimised to extract dissolved oxygen from the water. When fish are removed from their aquatic environment these filaments can no longer capture oxygen, as air does not diffuse directly through the gills as it does when the animals are in water.

Moreover, without water support the gill filaments tend to collapse. They lose their structure and fall onto themselves, sticking to each other, effectively preventing residual gas exchange. This collapse further contributes to the fish's inability to breathe, leading to asphyxia, which is the lack of oxygen, and ultimately to death (Figure 150).



Figure 150. Carp slaughtered by suffocation (asphyxiation) without any form of stunning.

16.3.2.2 Thermal shock

Thermal shock, which involves the abrupt exposure of fish to extreme temperature changes compared to their acclimatised environment, is not considered a method of stunning or desensitisation, let alone a humane method for fish slaughter. Technically, this is due to various reasons involving the animal's welfare and biological and physiological aspects.

Firstly, it is crucial to understand that fish are ectothermic animals, meaning their body temperature varies according to the environment. Sudden changes in water temperature can cause thermal shock, leading to an acute stress response in the animal. Instead of stunning, this stress can result in pain and suffering as the fish tries to adapt to the new temperature, a process involving complex physiological adjustments that can lead to tissue damage.

Biologically, thermal shock can induce severe metabolic changes. In the case of a sudden temperature increase, the fish's metabolism rapidly accelerates, potentially leading to oxygen consumption that exceeds the blood supply capacity, resulting in hypoxia and suffering. If the temperature drops abruptly, metabolic processes can be slowed, impeding normal vital functions, and causing muscle paralysis, stress and suffering.

Grass carp can withstand being frozen and then 'return' to life if transferred to water at the ideal temperature for the species. However, this does not mean the fish did not experience pain or acute suffering during this process.

Furthermore, thermal shock does not meet the criteria for efficient desensitisation, which should be rapid, irreversible and possibly cause minor pain and suffering. On the contrary, exposure to extreme temperatures can be an extremely uncomfortable and painful experience for the fish without ensuring desensitisation.

Fish slaughter by thermal shock is typically done by immersing the fish in ice water or covering them directly with ice as soon as they are harvested (Figure 151).

The cause of death for fish slaughtered in ice water is typically a combination of hypothermia and asphyxiation. When fish are submerged in ice water, the extreme cold causes a thermal shock that rapidly lowers the animal's body temperature. This thermal shock decreases metabolic rate and reduces their capacity to capture oxygen, resulting in asphyxiation. In addition, the low temperature causes a progressive weakening of the fish's vital functions, leading to inactivity until death occurs from hypothermia.

When fish are slaughtered directly on ice without the presence of water, the leading cause of death is hypothermia. In this process, fish are removed from their aquatic environment and immediately placed on a surface covered with ice. Direct contact with the ice rapidly reduces the fish's body temperature, slowing their metabolism and ceasing vital functions. Hypothermia occurs because ice removes heat from the fish's body much more quickly than water. As fish do not have efficient mechanisms to generate internal heat, they are particularly susceptible to abrupt temperature changes. When exposed to ice, the circulatory system begins to slow down, neuromuscular activity is reduced, and critical functions such as heart pumping and brain activity progressively slow down and eventually stop altogether.



Figure 151. Fish are slaughtered by thermal shock in icy (top pictures) water or directly on ice (bottom pictures).

16.3.2.3 Bleeding

Although it is a traditional method used in various cultures and production systems, direct bleeding by cutting the gill arches on at least one side of the fish's head (Figure 152) without the animal being previously stunned or desensitised not truly a humane slaughter practice. The fish remains conscious, as the central nervous system is still active, and the animal can feel pain, fear and stress. Therefore, it is essential that bleeding is carried out with a technique that causes prior stunning of the fish.

In many countries the practice of direct bleeding is falling out of use or is discouraged by animal welfare guidelines, being replaced by methods that ensure the fish is desensitised before bleeding.

Besides ethical and welfare considerations, there are also considerations for the final product quality, as stress can affect the quality of the fish meat. Fish that are slaughtered without prior stunning may release hormones like cortisol and adrenaline, which can lead to a lower post-mortem muscle pH, resulting in lower-quality meat with undesirable flavour and texture.

Effective bleeding is a process where, after the animal is stunned, most of the blood is removed, usually facilitated by a combination of precise cuts and immersion in running water, which helps remove blood from the fish's body.

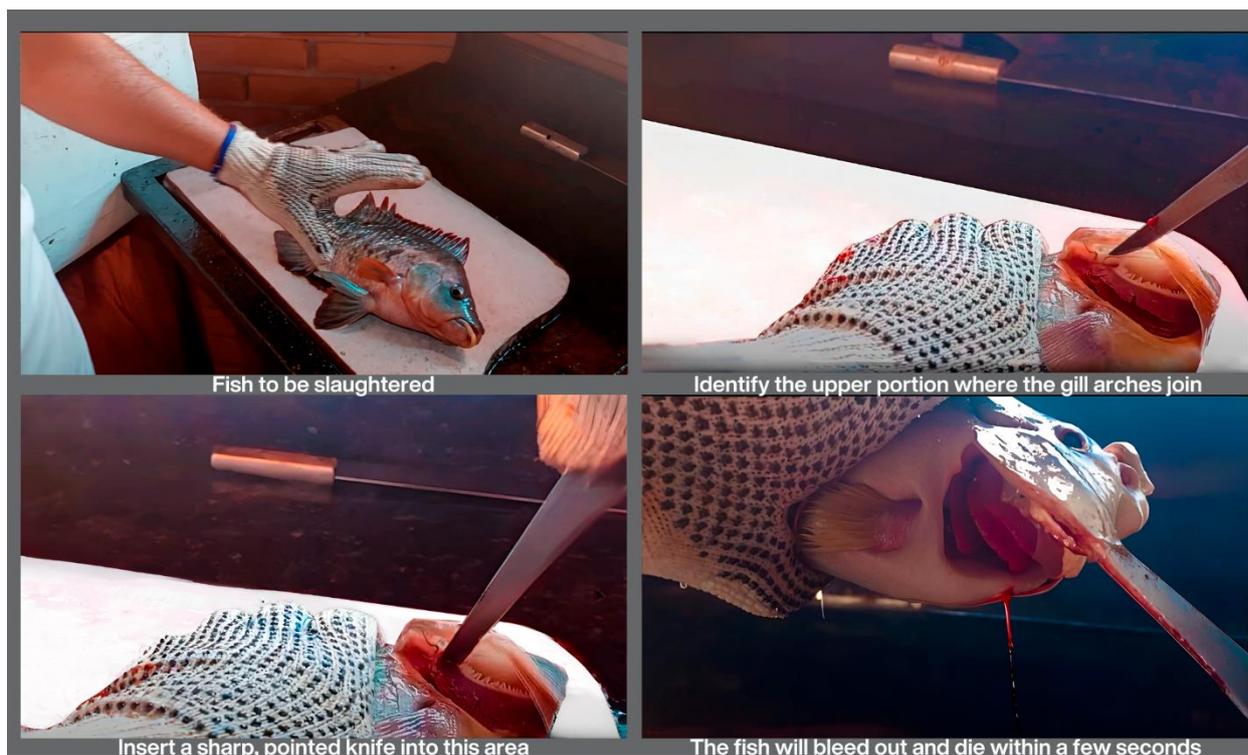


Figure 152. Depiction of the bleeding method using a tilapia as an example.

16.3.2.4 Cervical spinal cord section

Cervical spinal cord section (Figure 153) achieves the rapid interruption of brain functions through an injury to the spinal cord in the nape region, immediately behind the skull. To carry out this technique efficiently and humanely, the executor requires knowledge of the fish's anatomy, as well as dexterity and precision.

The procedure is as follows: the fish is held firmly, minimising its ability to struggle, and a sharp, appropriate instrument is used to make a quick, firm transverse cut in the cervical region, just behind the gills and above the spinal column. This cut must be deep enough to reach the spinal cord but without compromising the gills or the front part of the fish's body. Immediately after this incision the fish should stop moving, indicating that the spinal cord has been effectively sectioned, and the animal is no longer conscious.

This stunning method is invasive, so the operator needs to be experienced to achieve accurate application. Moreover, the animal does not lose consciousness immediately and may take more than 1 minute. Thus, this stunning method is not ideal, as fish can bleed while still conscious.

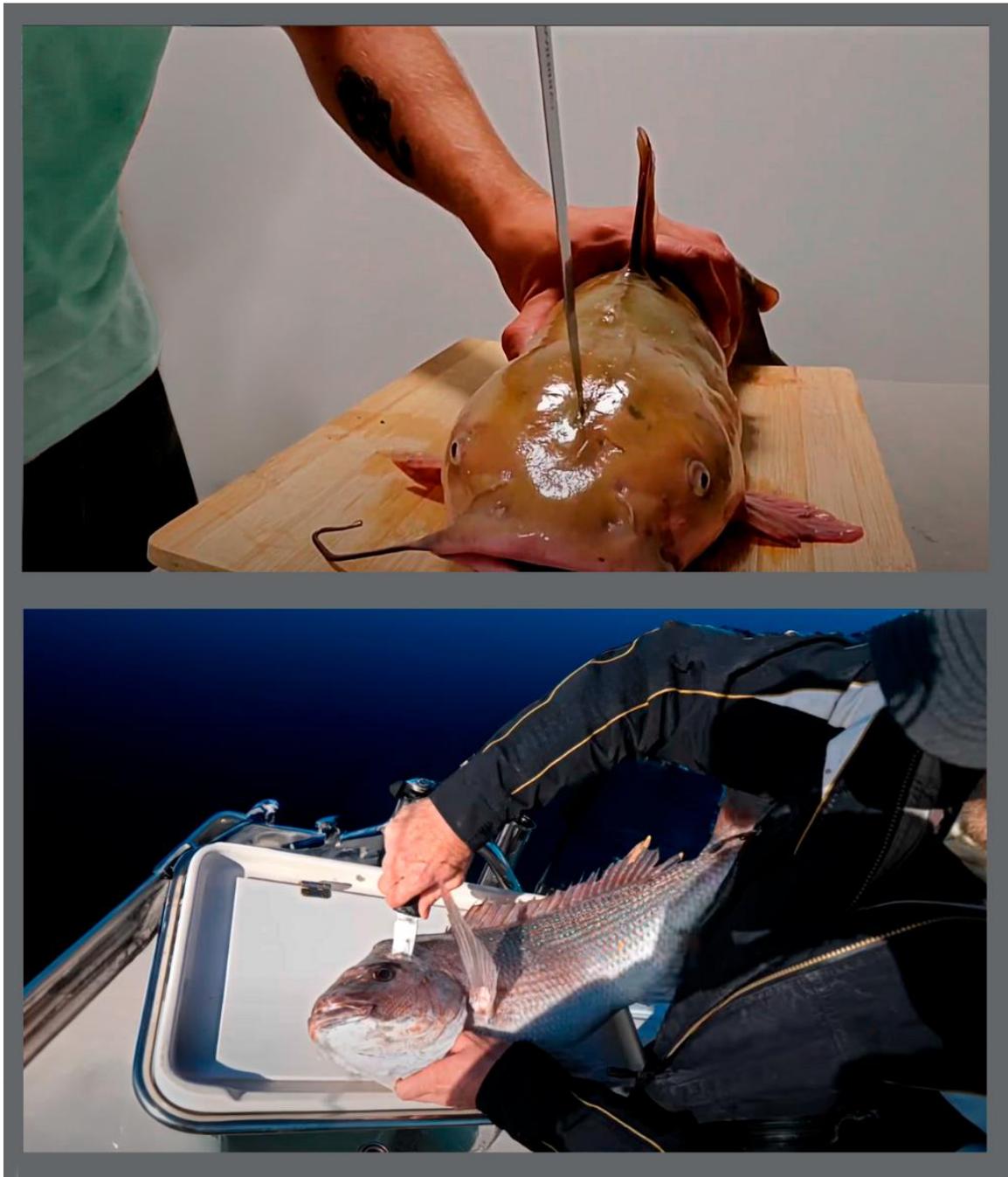


Figure 153. Stunning of fish through cervical spinal cord section.

16.3.2.5 Ikejime method

The Ikejime method is an ancient Japanese fish slaughtering technique, refined over the years to enhance meat quality and ensure that the slaughter causes minimal stress and pain to the animal. Its relevance from an ethical perspective is that animal welfare impacts the final product quality.

The technique begins by securely restraining the fish, ensuring the operator's and the animal's safety. A quick and precise incision is then made in the fish's brain, usually with a metal spike, leading to instant death through brain destruction. This fast action is crucial to halt the fish's sensory perception and prevent suffering.

After this, bleeding is performed through incisions at the gills and bases of the tail section. This process helps drain the blood swiftly, preventing the build-up of substances that might adversely affect the flavour and preservation of the meat. Rapid exsanguination is essential in maintaining quality, as blood remaining in the fish's body can trigger processes that degrade muscle tissue.

Finally, a wire rigid enough to be inserted through the caudal incision and access the animal's spinal column can be used. The wire will destroy the spinal enervations and immediately stop the nerve impulses that cause muscle spasms and involuntary muscle movements, contributing to reduced metabolic stress and maintaining meat texture (Figure 154).

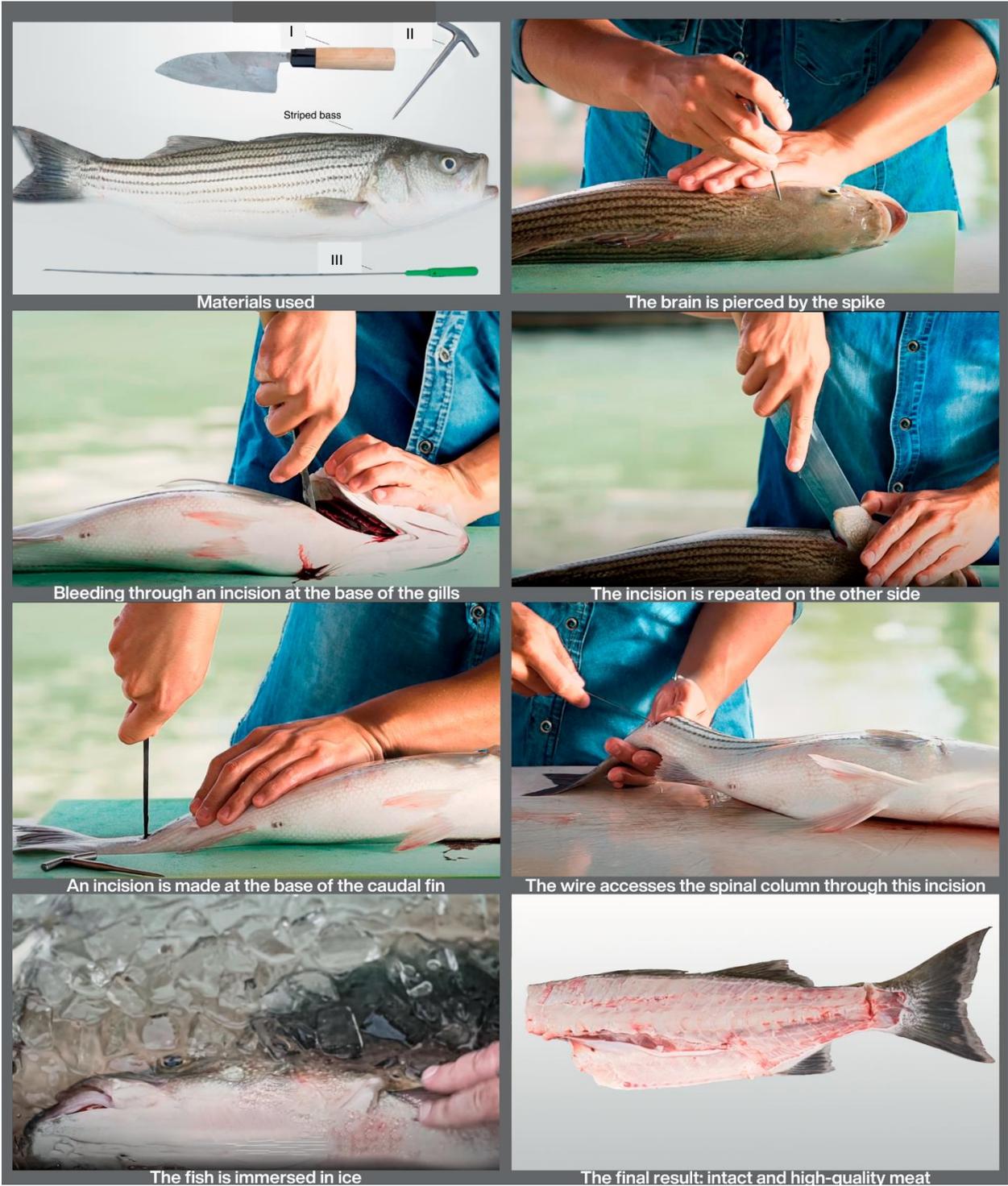


Figure 154. The sequence uses a striped bass to show the application of the Ikejime humane fish slaughtering method. Top picture left shows the equipment used: i) a knife, ii) a spike and iii) a wire.

The precise brain incision and subsequent bleeding allow for the rapid removal of blood from the fish's body, reducing the presence of microorganisms that initiate the decomposition process. This effective blood removal delays deterioration and ensures that the meat remains fresher and cleaner-looking for longer (Figure 155).

The sensory quality of the meat, including the umami flavour – that fifth basic taste deeply associated with high-quality fish meat – and texture, are also preserved in their purest state. The Ikejime method plays a crucial role in this preservation because it prevents the initiation of enzymatic and chemical reactions that could alter these characteristics. Breaking the spinal column also inhibits muscle spasms that would consume the energy (ATP) present in the muscle. This action helps to delay the onset of rigor mortis and the decomposition of the meat, thereby maintaining its texture and flavour for a more extended period.



Figure 155. Comparison between two fish slaughtered simultaneously, the first by the Ikejime method and the second by suffocation. The black circles show the excess blood in the meat that will accelerate the decomposition process of the fish killed by suffocation and reduce its shelf life.

16.3.2.6 Concussion

The practice of fish slaughter through concussion, when properly executed, is considered humane and effective. This method involves delivering a swift and decisive blow to the fish's head, specifically targeting the brain region, to cause immediate insensitivity and lead to a quick death with minimal stress and suffering.

How the fish is handled before slaughter is also crucial for the success of this method. Keeping the fish calm and minimising stress is essential, as this affects animal welfare and meat quality. Therefore, despite correct stunning methods, as depicted in Figure 156, it cannot be asserted that the fish did not experience stress and pain before being slaughtered on asphalt.

Understanding the fish's anatomy is necessary for concussion slaughter to be carried out correctly. The brain is located just above the eyes, and the blow should be struck here. The impact force must be strong enough to cause instant loss of consciousness without causing disfigurement or unnecessary harm to the animal. After a concussion, death should be confirmed by signs such as the absence of swimming, respiratory movements or ocular reflexes. Additional procedures like bleeding should then be carried out to complete the slaughter process.

The blow-causing concussion can be delivered using different tools suitable for the situation and size of the fish. Among the options are the use of a hammer or baton and pneumatic weapons.

- **Hammer or baton** (Figure 156): Using a hammer or baton is the most straightforward and direct concussion method. It involves a heavy and solid object that, when applied with force and precision to the fish's head, causes immediate desensitisation. This tool is suitable for smaller operations or individual fish slaughter, as it does not depend on sophisticated technology or electrical/pneumatic power.
- **Pneumatic weapon** (Figure 157): Pneumatic weapons use compressed air to propel a pin or rod against the fish's head, causing concussion. This system is more consistent and controllable than a hammer or baton and can be particularly useful for slaughtering larger fish or in larger-scale operations. The weapons provide a controlled and constant impact force, reducing dependence on the physical skill of the operator and increasing the likelihood of effective and humane slaughter. They are handy in situations where many fish need to be slaughtered quickly.



Grass carp being killed by concussion on the asphalt



Salmon being killed by concussion in a slaughterhouse



Fish stunned by concussion

Figure 156. Fish being slaughtered by concussion under different conditions. In the upper images, slaughter occurs under absolutely inadequate conditions despite the correct stunning method.

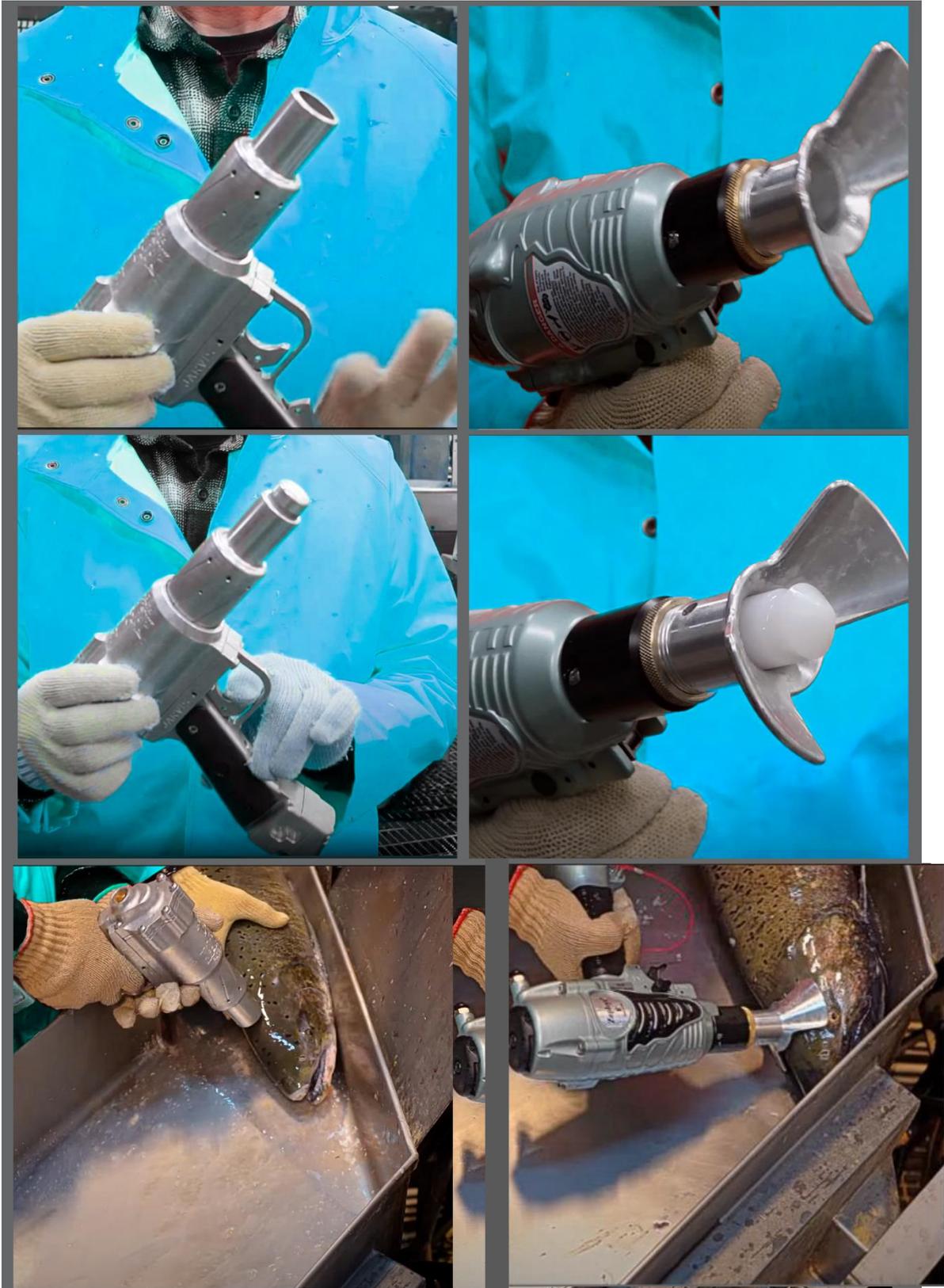


Figure 157. Pneumatic weapons are employed in fish slaughtering, as exemplified in the method used for killing salmon.

16.3.2.7 Electric shock/electronarcosis

This method applies an electric current (Figure 158) of specific parameters in terms of strength, duration and frequency. To be effective and safe, these parameters must be carefully calibrated according to the species in question and water conditions, such as its conductivity. Otherwise, the method may result in haemorrhages and bone fractures, compromising meat quality. Salt can be added to improve water conductivity. Most of these procedures require that, after the fish loses consciousness, its death is immediately induced. The final procedure can be done through methods like bleeding – which involves cutting the gills, leading to haemorrhage and consequent death – or by decapitation.



Figure 158. Tilapias subjected to electronarcosis.

16.4 Preservation

Fish meat is sensitive and can deteriorate rapidly. Therefore, appropriate handling is essential from its capture in the pond until it is fit for consumption. Different methods of fish preservation include:

- **Refrigeration:** Immediately after fishing and slaughter, the fish must be washed in water with chlorine at a concentration of 5 ppm. Then it should be stored in alternating layers of ice and fish, ensuring it is entirely covered by ice. The recommended ratio is 1.5 kg of ice for every kg of fish. Thus preserved, fresh fish can last up to 10 days. If the fish is washed, gutted and refrigerated in ice, this period extends to 20 days.
- **Freezing:** After it has been washed and gutted, freezing proves to be an excellent way to prolong the shelf life of the fish. The freezing process must be carried out quickly with appropriate equipment. Ideal storage occurs in cold chambers between -18°C and -30°C.
- **Salting:** This is one of the most traditional preservation methods. It is based on the addition of salt, which penetrates the fish tissues. At a concentration of 8% to 10%, salt can extract some of the water in the meat, preventing its deterioration. Usually, already gutted fish is used for this process.
- **Smoking:** Although widely used in antiquity as a preservation technique, nowadays smoking is more appreciated for imparting a particular aroma, flavour and colour to the fish, which consumers highly value.

17 REFERENCES CONSULTED

1. Ahmed, S. M., Jaffar R. S., Effect of salt stress on energy usage and growth in grass carp *Ctenopharyngodon idella* (Valenciennes. 1844). Iraqi J Aquac. 2013; 10(1): 1-24. Accessed October 22. 2021. <https://www.iasj.net/iasj/article/78636>
2. Ahmad, F., Fazili, K. M., Sofi, O. M., Sheikh, B. A., Sofi, T. A., Distribution, and pathology caused by *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidae): a review. Rev Vet. 2018; 29(2): 142-149.
3. Akar, M., Effects of stress on spawners reproductive performance in female Grass Carp (*Ctenopharyngodon idella*). J Arab Aquac Soc. 2011; 6(2): 171-180.
4. Alcaraz, G., Espina S., Effect of nitrite on the survival of grass carp *Ctenopharyngodon idella* (Val.) with relation to chloride. Bull Environ Contam Toxicol. 1994; 52(1): 74-79. doi:10.1007/BF00197360
5. Alcaraz, G., Espina S., Scope for growth of juvenile Grass Carp *Ctenopharyngodon idella* exposed to nitrite. Comp Biochem Physiol Part C Pharmacol Toxicol Endocrinol. 1997; 116(1): 85-88. doi:10.1016/S0742-8413(96)00131-4
6. Alcaraz, G., Rangel, L., Early Signs of Nitrite Toxicity in Juvenile Grass Carp *Ctenopharyngodon idella*. J World Aquac Soc. 2004; 35(1): 94-99. doi:10.1111/J.1749-7345.2004.TB01064.X
7. Appelbaum, S., Uland, B., Intensive rearing of grass carp larvae *Ctenopharyngodon idella* (Valenciennes 1844) under controlled conditions. Aquaculture. 1979; 17(2), 175-179. doi:10.1016/0044-8486(79)90051-6
8. Assis, G da C., Cultivo de peixes de água doce na Piscicultura Panamá. TCC (graduação em Engenharia de Aquicultura) - Universidade Federal de Santa Catarina, Florianópolis, 2008. 63p. Published online 2008. Accessed November 28. 2021. <https://repositorio.ufsc.br/handle/123456789/119706>
9. Bettoli, P. W., Neill, W. H., Kelsch, S. W., Temperature preference and heat resistance of grass carp *Ctenopharyngodon idella* (Valenciennes) bighead carp. *Hypophthalmichthys nobilis* (Gray. and their F1 hybrid). J Fish Biol. 1985; 27(3): 239-247. doi:10.1111/j.1095-8649.1985.tb04024.x
10. Bogutskaya, N., Jones, L. A., Mandrak, N. E., Cudmore, B., Annotated bibliography of grass carp (*Ctenopharyngodon idella*) from Russian-language literature; Advis. Sec. Res. Doc. 2017. iv 1-44. Accessed November 30. 2021. <http://www.dfo-mpo.gc.ca/csas-sccs/>
11. Boyd, C. E., Bottom soils, sediment, and pond aquaculture. Springer Science, Boston, MA, 1995. 348p. ISBN 0-412-06941-5.
12. Boyd, C. E., Tucker, C. S., Pond aquaculture water quality management. Springer Science & Business Media, Boston, MA, 2012. 685p. ISBN 978-1-4613-7469-5.
13. Cerva, C., Avaliação do cultivo em gaiolas e do beneficiamento da carpa-capim (*Ctenopharyngodon idella*). Dissertação (Programa de Pós-Graduação em Zootecnia) - Universidade Federal do Rio Grande do Sul. Faculdade de Agronomia, Porto Alegre, 2003, 126p. Published online 2003.
14. Chen, Y., Sun, H., Yang, W., Yang, Z., Oxidative stress responses of grass carp *Ctenopharyngodon idella* larvae exposed to purified microcystin under different ammonia concentrations. Fresenius Environ Bull. 2011; 20(11): 2869-2874. Accessed August 23. 2021. <https://www.cabdirect.org/cabdirect/abstract/20123029892>

15. Chitrakar, P., Parajuli, K., Length and weight relationship studies of alimentary canal compared to the total body weight of grass carp *Ctenopharyngodon idella* (Valenciennes 1844) at Balkhu live fish Market of Kathmandu. Nepal. Int J Fish Aquat Stud. 2017; 5(6):185-190. Accessed November 30, 2021. <https://www.fisheriesjournal.com/archives/?year=2017&vol=5&issue=6&part=C&ArticleId=1423>
16. Choi, Wai Ming, Culturing grass carp and grey mullet using food waste incorporated with traditional Chinese medicine, baker's yeast and enzymes. Tese (Biology) - Hong Kong Baptist University, Hong Kong, 2013, 271p.
17. Cudmore, B., Mandrak, N. E., Canada BONLR. Biological Synopsis of Grass Carp (*Ctenopharyngodon idella*). Vol 2705.; 2004. 144p.
18. Cui, Y., Chen, S., Wang, S., Effect of ration size on the growth and energy budget of the grass carp *Ctenopharyngodon idella* Val. Aquaculture. 1994;123(1-2): 95-107. doi:10.1016/0044-8486(94)90122-8
19. Cui, Y., Chen, S., Wang, S., Liu, X., Laboratory observations on the circadian feeding patterns in the grass carp (*Ctenopharyngodon idella* Val.) fed three different diets. Aquaculture. 1993;113(1-2):57-64. doi:10.1016/0044-8486(93)90340-5
20. Dabrowski, K., Protein requirements of grass carp fry (*Ctenopharyngodon idella* Val.). Aquaculture. 1977;12(1):63-73. doi:10.1016/0044-8486(77)90047-3
21. Davis, M. W., Fish stress and mortality can be predicted using reflex impairment. Fish Fish. 2010;11(1):1-11. doi:10.1111/j.1467-2979.2009.00331.x
22. De Silva, S. S., Weerakoon DEM. Growth, food intake and evacuation rates of grass carp. *Ctenopharyngodon idella* fry. Aquaculture. 1981;25(1):67-76. doi:10.1016/0044-8486(81)90100-9
23. Dias, ER de A., Maruyama L. S., Transportes de peixes. Problemas e soluções. Published 1998. Accessed November 29, 2021. <https://www.abrappesq.com.br/materia9.htm>
24. Díaz, F., Espina, S., Rodríguez, C., Soto, F., Preferred temperature of grass carp. *Ctenopharyngodon idella* (Valenciennes). and brema carp. *Megalobrama amblycephala* (Yih). (Pisces. Cyprinidae) in horizontal and vertical gradients. Aquac Res. 1998;29(9):643-648. doi:10.1046/j.1365-2109.1998.00254.x
25. Du Z-Y, Liu Y-J, Tian L-X, He J-G, Cao J-M, Liang G-Y, The influence of feeding rate on growth, feed efficiency and body composition of juvenile grass carp (*Ctenopharyngodon idella*). Aquac Int 2005 143. 2005;14(3):247-257. doi:10.1007/S10499-005-9029-7
26. Du Z-Y, Tian L-X, Liang G-Y, Liu Y-J, Effect of Dietary Energy to Protein Ratios on Growth Performance and Feed Efficiency of Juvenile Grass Carp (*Ctenopharyngodon idella*). Open Fish Sci J. 2009;2(1):25-31. doi:10.2174/1874401x00902010025
27. Edwards, D. J., Hine, P. M., Introduction, preliminary handling, and diseases of grass carp in New Zealand, New Zealand. J Mar Fresh Research, 1974;8(3): 441-454, doi: 10.1080/00288330.1974.9515518
28. El Gamal, R., Influence of photoperiod regime on hatchability, growth and ontogeny tract during the larval development of grass carp *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844). Glob Vet. 2009;3(1):204-215.
29. EL-Fiky, N., The influence of water pH on the development of grass carp. *Ctenopharyngodon idella*. Egypt J Aquat Biol Fish. 2002;6(3):233-261. doi:10.21608/ejabf.2002.1760
30. Espina, S., Alcaraz, G., Effect of nitrite on the respiratory response of grass carp *Ctenopharyngodon idella* (Val.) with relation to chloride. Comp Biochem Physiol Part C

- Pharmacol Toxicol Endocrinol. 1993;106(3):761-764. doi:10.1016/0742-8413(93)90239-H
31. Fan, Y., Rao, S., Zeng, L., Ma, J., Zhou, Y., Xu, J., Zhang, H., Identification and genomic characterization of a novel fish reovirus, Hubei grass carp disease reovirus, isolated in 2009 in China. *J General Virol.* 2013; 94(10):2266-2277.
 32. FAO, 2023: *Ctenopharyngodon idella*. Cultured Aquatic Species Information Programme. Text by Weimin, M. Fisheries and Aquaculture Division [online]. Rome. https://www.fao.org/fishery/en/culturedspecies/ctenopharyngodon_idella?lang=en
 33. FAO, 2021: Nutritional requirements. Aquaculture Feed and Fertiliser Resources Information System. Published 2021. Accessed October 31, 2021. <https://www.fao.org/fishery/affris/species-profiles/grass-carp/nutritional-requirements/en/>
 34. FAO, 2021: Natural food and feeding habits. Aquaculture Feed and Fertiliser Resources Information System. Accessed November 28, 2021. <https://www.fao.org/fishery/affris/species-profiles/grass-carp/natural-food-and-feeding-habits/en/>
 35. Faria RHS de. Morais M. Soranna MRG de S. Sallum WB. Manual de Criação de Peixes Em Viveiros. 2013. Brasília. Codevasf, 132p.
 36. FAWC. Five Freedoms First Written Report.; 1979. <http://www.fawc.org.uk>
 37. Froese, R., Pauly, D., Grass Carp (Diploid) *Ctenopharyngodon idella* Var. Diploid); 2017. FishBase. <http://fishbase.org/summary/Ctenopharyngodon-idella.html>
 38. Gan, L., Liu, Y. J., Tian, L. X., Effects of dissolved oxygen and dietary lysine levels on growth performance, feed conversion ratio and body composition of grass carp. *Ctenopharyngodon idella*. *Aquac Nutr.* 2013;19(6):860-869. doi:10.1111/anu.12030
 39. George, A. E., Chapman, D. C., Embryonic and larval development and early behavior in grass carp *Ctenopharyngodon idella*: implications for recruitment in rivers. *PLoS One.* 2015;10(3): e0119023. doi: 10.1371/JOURNAL.PONE.0119023
 40. Gidmark, N. J., Taylor, C., LoPresti, E., Brainerd, E., Functional morphology of durophagy in black carp, *Mylopharyngodon piceus*. *J Morpho* 2015; 276(1): 1422-1432.
 41. Glasser, F., Mikolajczyk, T., Jalabert, B., Baroiller, J. F., Breton, B., Temperature effects along the reproductive axis during spawning induction of grass carp (*Ctenopharyngodon idella*). *Gen Comp Endocrinol.* 2004;136(2):171-179. doi: 10.1016/j.ygcen.2003.12.009
 42. Gneiding, B., Efeitos de diferentes anestésicos sobre o bem-estar de carpa capim (*Ctenopharyngodon idella*); Dissertação (Pós-Graduação em Ciência Animal) – Pontifícia Universidade Católica do Paraná, Curitiba, 2017, 60p. <https://archivum.grupomarista.org.br/pergamumweb/vinculos/000072/000072a8.pdf>
 43. Hamackova, J., Kouril, J., Kozak, P., Stupka, Z., Clove Oil as an Anaesthetic for Different Freshwater Fish Species. *Bulg J Agric Sci.* 2006;12(1):185-194.
 44. Hamackova, J., Lepicova, A., Kozak, P., Kouril, J., The tolerance of early larvae of common carp, grass carp and silver carp to different levels pH. In: *Proceedings of the 4. Czech Ichthyological Conference. Vodnany (Czech Republic): Jihočeská Univ, 2000, 281p.*
 45. Hoole, D., Bucke, D., Burgess, P., Wellby, I., *Diseases of Carp and Other Cyprinid Fishes.* Wiley Blackwell; 2008. doi:10.1002/9780470999752
 46. Huang, S., Book review: *Aquaculture in China: Success stories and modern trends.* *Aquac Fish.* 2018;3(4):174-175. doi:10.1016/j.aaf.2018.07.001

47. Huisman, E. A., Valentijn, P., Conversion efficiencies in grass carp (*Ctenopharyngodon idella*. Val.) using a feed for commercial production. *Aquaculture*. 1981;22(C):279-288. doi:10.1016/0044-8486(81)90154-X
48. Ijaz, N., Iqbal, Z., Chaudhary, J. A., Effects of ammonia on growth of *Ctenopharyngodon idella* (Valenciennes) fingerlings. *Punjab Univ J Zool*. 2010;25(1-2):59-66.
49. Incardona, J. P., Collier, T. K., Scholz, N. L., Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol*. 2004;196(2):191-205. doi:10.1016/J.TAAP.2003.11.026
50. Incardona, J. P., Vines, C. A., Anulacion, B. F. et al., Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 Cosco Busan oil spill in San Francisco Bay. *Proc Natl Acad Sci U S A*. 2012;109(2):E51-E58. doi:10.1073/PNAS.1108884109/-/DCSUPPLEMENTAL
51. Jaafar, R. S., Ahmed, S. M., Effect of salt stress on osmoregulation and energy consumption in grass carp *Ctenopharyngodon idella* (Val.1844). *IRAQI J Aquac*. 2011;8(1):15-38. doi:10.21276/IJAQ.2011.8.1.2
52. Jeney, Z., Bekh, V., Technical manual on broodstock management of common carp and Chinese herbivorous fish. *FAO Fisheries and Aquaculture Circular*, 2020, I-67.
53. Jiang, Yulin., Hemorrhagic disease of grass carp: status of outbreaks, diagnosis, surveillance, and research. *Israeli Journal of Aquaculture-Bamidgeh*, v. 61, 2009. 11p. ISSN 0792 - 156X
54. Kestin, S. C., van de Vis, J. W., Robb, D. H. F., Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. *Vet Rec*. 2002;150(10):302-307. doi:10.1136/VR.150.10.302
55. Khan, M. A., Jafri, A. K. Chadha, N. K., Growth, reproductive performance, muscle and egg composition in grass carp *Ctenopharyngodon idella* (Valenciennes) fed hydrilla or formulated diets with varying protein levels. *Aquac Res*. 2004;35(13):1277-1285. doi:10.1111/j.1365-2109.2004.01150.x
56. Kilambi, R. V., Food consumption, growth and survival of grass carp *Ctenopharyngodon idella* at four salinities. *J Fish Biol*. 1980;17(6):613-618. doi:10.1111/J.1095-8649.1980.TB02794.X
57. Kilambi, R. V., Zdinak, A., The effects of acclimation on the salinity tolerance of grass carp. *Ctenopharyngodon idella* (Cuv. and Val.). *J Fish Biol*. 1980;16(2):171-175. doi:10.1111/J.1095-8649.1980.TB03696.X
58. Köprücü, K., Effects of dietary protein and lipid levels on growth, feed utilization and body composition of juvenile grass carp (*Ctenopharyngodon idella*). *J Fish*. 2012; 6(1): 243-251. Published online 2012. doi:10.3153/jfscom.2012028
59. Kristan, J., Blecha, M., Policar, T., Survival and growth rates of juvenile grass carp *Ctenopharyngodon idella* overwintered in ponds and recirculating aquaculture systems including a comparison of production economics. *Turkish J Fish Aquat Sci*. 2019;19(3):261-266. doi:10.4194/1303-2712-v19_03_09
60. Kubitzka, F., Manejo na produção de peixes. *Panor da Aquicultura*. 2009;108(11):14-15. Accessed November 29, 2021. <https://panoramadaaquicultura.com.br/manejo-na-producao-de-peixes-parte-4-manejo-nutricional-e-alimentar/>
61. Kubitzka, F., Qualidade do alimento, qualidade da água e manejo alimentar na produção de peixes. In: *SIMPÓSIO SOBRE MANEJO E NUTRIÇÃO DE PEIXES*; 1997:63-101

62. Lin W, Li L, Chen J. et al., Long-term crowding stress causes compromised nonspecific immunity and increases apoptosis of spleen in grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol. 2018;80(1):540-545. doi: 10.1016/j.fsi.2018.06.050
63. Ma, S., Feng, C., Han, M., Dong, C., Chen, W., Dou, J., Li, D., Liu, X., The structure changes of microbial food web during the culture of grass carp larvae. Aquaculture 2023; 570(1): 739421. doi.org/10.1016/j.aquaculture.2023.740419.
64. Maceina, M. J., Nordlie, F. G., Shireman, J. V., The influence of salinity on oxygen consumption and plasma electrolytes in grass carp. *Ctenopharyngodon idella* Val. J Fish Biol. 1980;16(6):613-619. doi:10.1111/j.1095-8649.1980.tb03740.x
65. Maceina, M. J., Shireman, J. V., Effects of salinity on vegetation consumption and growth in grass carp. Progress Fish-Culturist. 1980;42(1):50-53. doi:10.1577/1548-8659(1980)42[50:EOSOV]2.0.CO;2
66. Maceina, M. J., Shireman, J. V., Grass carp: Effects of salinity on survival, weight loss, and muscle tissue water content. Progress Fish-Culturist. 1979;41(2):69-73. doi:10.1577/1548-8659(1979)41[69:GCOSOS]2.0.CO;2
67. Makvandi, H., Khodadadi, M., Shokoh, S. K., Makvandi, Z. M., Effect of salinity stress on cortisol hormone and glucose in grass carp fingerlings (*Ctenopharyngodon idella*). J Aquat Anim Fish. 2012;85(42):77-84. Accessed August 23, 2021. <https://www.cabdirect.org/cabdirect/abstract/20133300723>
68. Makvandi, H., Kodadadi, M., Keyvanshokoh, S., Effect of salinity on growth and survival of grass carp (*Ctenopharyngodon idella*) fingerlings. J Wetl Ecobiol. 2010;1(4):51-57. <https://www.sid.ir/en/journal/ViewPaper.aspx?id=200104>
69. Mammen, T. A., Live fish transport using modified splashless carrier with compressed air aeration. In: Training Course on Live Fish Transport. 1962;5(1):1-126.
70. Mammen, T. A., Transport of fry and fingerlings of saltwater fishes. In: November 19th to 21st, 1962. Hyderabad, Fisheries Extension Unit, Government of India; 1962;4(1):1-68.
71. Marques, N. R., Hayashi, C., Sorza, S. R., De. Soares, T., Efeito de diferentes níveis de arraçoamento para alevinos de Carpa-Capim (*Ctenopharyngodon idella*) em condições experimentais. Bol do Inst Pesca. 2004;30(1):51-56. Accessed November 28, 2021. https://www.pesca.sp.gov.br/boletim/index.php/bip/article/view/Marques30_1
72. Masood ul Hassan MR. Induced Breeding of Grass Carp (*Ctenopharyngodon idella*) and Silver Carp (*Hypophthalmichthys molitrix*) Using Ovartide as Synthetic Hormone at National Fish Seed Farm (Nfsf) Manasbal, Kashmir. J&K. Fish Aquac J. 2014;05(04):1-4. doi:10.4172/2150-3508.10000110
73. Moher, D., Shamseer, L., Clarke M. et al., Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Rev Esp Nutr Humana y Diet. 2016;20(2):148-160. doi:10.1186/2046-4053-4-1/TABLES/4
74. Muelbert, J. R. E., Estágio Supervisionado De Engenharia De Aquicultura: Reprodução. Larvicultura E Alevinagem De Peixes De Água Doce. Published online June 29, 2012:37-39. Dissertação - Universidade Federal de Santa Catarina, Florianópolis, 2012, 44p. Accessed November 9, 2021. <https://repositorio.ufsc.br/handle/123456789/198001>
75. Murtaza, M. ul H., Zuberi, A., Ahmad, M., Amir, I., Kamran, M., Ahmad, M., Influence of early rearing environment on water-borne cortisol and expression of stress-related genes in grass carp (*Ctenopharyngodon idella*). Mol Biol Rep. 2020;47(7):5051-5060. doi:10.1007/s11033-020-05574-5
76. Nekorbin, H., Sudagar, M., Effects of feeding frequency on growth performance and survival rate of grass carp (*Ctenopharyngodon idella*). World Appl Sci J. 2012;17(8):1001-

1004. Accessed August 23, 2021. <https://www.cabdirect.org/cabdirect/abstract/20123365883>
77. Ni, D., Wang, J., Biology and Diseases of Grass Carp. Science Press.; 1999. Pp. 29-33.
78. Nie, D. S., Pan, J. P., Diseases of grass carp (*Ctenopharyngodon idellus* Valenciennes, 1844) in China, a review from 1953 to 1983. *Fish pathology*, 1985; 20(2-3): 323-330.
79. Noble, C., Gismervik, K., Iversen, M. H., Olarevic, J., Nilsson, J., Stien, L. H., Turnbull, J. F., Welfare Indicators for Farmed Atlantic Salmon: Tools for Assessing Fish Welfare. (Noble, C., Gismervik, K., Iversen, M. H. et al., eds.). Nofima; 2018, 452p. Accessed November 29, 2021. <http://dspace.stir.ac.uk/handle/1893/28435>
80. Olsen, R. E., Sundell, K., Mayhew, T. M., Myklebust, R., Ringø, E., Acute stress alters intestinal function of rainbow trout. *Oncorhynchus mykiss* (Walbaum). *Aquaculture*.2005;250(1-2):480-495. doi:10.1016/J.AQUACULTURE.2005.03.014
81. Ostrensky, A., Dal Pont, G., Geraldine, G., Westphal, C., Pedrazzani, A. S., Use of Clove, Mint and Camphor Essential Oils on Confinement of Clown Anemonefish *Amphiprion ocellaris* (Cuvier 1830): Anesthetic Effects and Influence on Water Quality. *J Aquac Res Dev*. 2016;7(11):1-7. doi:10.4172/2155-9546.1000457
82. Pedrazzani, A. S., Neto. A.O., The anaesthetic effect of camphor (*Cinnamomum camphora*), clove (*Syzygium aromaticum*) and mint (*Mentha arvensis*) essential oils on clown anemonefish, *Amphiprion ocellaris* (Cuvier 1830). *Aquac Res*. 2016;47(3):769-776. doi:10.1111/ARE.12535
83. Pedrazzani, A. S., Quintiliano, M. H., Bolfe, F., de O Sans, E. C., Molento, C. F. M., *Sci*. 2020;7(1):1-16. doi:10.3389/FVETS.2020.606388/BIBTEX
84. Pessi, Z. F., Evoy, R., Piscicultura de água doce: produção de alevinos; TCC (Curso de Engenharia de Aquicultura) – Universidade Federal de Santa Catarina, Florianópolis, 2003, 49p. Published online 2003.
85. Poli, A. T. B., Andreatta, E. R., Beltrame, E., *Aquicultura experiências brasileiras*, Vol. 1 Multitarefa, Florianópolis Editora, 2004, (pp. 369–406).
86. Prada, A. F., George, A. E., Stahlschmidt, B. H., Jackson, P. R., Chapman D. C., Tinoco, R. O., Influence of turbulence and in-stream structures on the transport and survival of grass carp eggs and larvae at varies developmental stages. *Aquat Sci*. 2020;82(1):1-16. doi:10.1007/S00027-019-0689-1/TABLES/2
87. Rao, Y., Su, J., Insights into the antiviral immunity against grass carp (*Ctenopharyngodon idella*) reovirus (GCRV) in grass carp. *J Immuno Research*, 2015; 1-18. doi.org/10.1155/2015/670437.
88. Ross, L. G., Ross, B., *Anaesthetic and Sedative Techniques for Aquatic Animals: Third Edition*. Blackwell; 2009. 218p. doi:10.1002/9781444302264
89. Rottmann, R. W., Shireman, J. V., *Hatchery Manual for Grass Carp and Other Riverine Cyprinids*.; 1990. Accessed October 29, 2021. <https://agris.fao.org/agris-search/search.do?recordID=US9172504>
90. Shaoqi, L., *Main Fish Diseases and their control Significance and Principles of Disease Control in China*. In. *Training Manual Integrated Fish Farming in China*. NACA/TR/85/11, Chapter 6. FAO, Bangkok, Thailand. September 1985. <https://www.fao.org/3/AC233E/AC233E06.htm#ch6>
91. Sharm, J. G., Chakrabarti, R., Effects of Different Stocking Densities on Survival and Growth of Grass Carp. *Ctenopharyngodon idella*. Larvae Using a Recirculating Culture System. *J Appl Aquac*. 1998;8(3):79-83. doi:10.1300/J028v08n03_08

92. Shireman, J., Smith, C., Synopsis of Biological Data on the Grass Carp. *Ctenopharyngodon idella* (Cuvier and Valenciennes. 1844). Vol 135.; 1983. Accessed November 16. 2021. [http://books.google.com/books?hl=en&lr=&id=xu8U1v_A5IMC&oi=fnd&pg=PA1&dq=Synopsis+of+biological+data+on+the+grass+carp.+Ctenopharyngodon+idella+\(Cuvier+and+Valenciennes.+1844\)&ots=1phEDx5QTD&sig=y6jmnY-0UvACf0bRuRA6Ba2Qp3M](http://books.google.com/books?hl=en&lr=&id=xu8U1v_A5IMC&oi=fnd&pg=PA1&dq=Synopsis+of+biological+data+on+the+grass+carp.+Ctenopharyngodon+idella+(Cuvier+and+Valenciennes.+1844)&ots=1phEDx5QTD&sig=y6jmnY-0UvACf0bRuRA6Ba2Qp3M)
93. Stanley, J. G., Reproduction of the Grass Carp (*Ctenopharyngodon idella*) Ortside Its Native Range. Fisheries. 1976;1(3):7-10. doi:10.1577/1548-8446(1976)001<0007:rotgcc>2.0.co;2
94. Stott, B., Cross, D. G., A note on the effect of lowered temperatures on the survival of eggs and fry of the grass carp *Ctenopharyngodon idella* (Valenciennes). J Fish Biol. 1973;5(6):649-658. doi:10.1111/j.1095-8649.1973.tb04501.x
95. Taher, M. M., Effects of fish density on growth and condition factor of grass carp *Ctenopharyngodon idella* cultivated in earthen ponds. MARSH Bull. 2021;15(2):62-72. Accessed October 22. 2021. https://www.researchgate.net/publication/351436127_Effects_of_fish_density_on_growth_and_condition_factor_of_grass_carp_Ctenopharyngodon_idella_cultivated_in_earthen_ponds
96. Tiwary, C., Pandey, V., Ali, F., Effect of pH on growth performance and survival rate of grass carp. academia.edu. Biolife original article, 2013; 1: 374-376. Accessed October 22. 2021. https://www.academia.edu/download/42696139/48_TIWARY_172-176.pdf
97. Towers, L., How to Farm Grass Carp - The Fish Site. Published 2010. Accessed October 22. 2021. <https://thefishsite.com/articles/cultured-aquatic-species-grass-carp>
98. Tranquilli, W. J., Thurmon, J. C., Grimm, A., Veterinary Anesthesia and Analgesia.; 2007. Accessed November 29, 2021. <https://books.google.com.br/books?hl=pt-BR&lr=&id=j3KpK4Pdgy8C&oi=fnd&pg=PT17&dq=Veterinary+Anesthesia+and+Analgesia&ots=0A9tvHIHm3&sig=M8lp96EnviVpppyL1V5VukYJG8E#v=onepage&q=Veterinary+Anesthesia+and+Analgesia&f=false>
99. Veiverberg, C. A., Desempenho e características de carcaça de juvenis de carpa capim (*Ctenopharyngodon idella*) em resposta a níveis e fontes de proteína da dieta. Published online February 6. 2009. Accessed November 1. 2021. <http://repositorio.ufsm.br/handle/1/10723>
100. Williams, E. M., Nelson, J. A., Heisler, N., Cardio-respiratory function in carp exposed to environmental nitrite. J Fish Biol. 1997;50(1):137-149. doi:10.1111/J.1095-8649.1997.TB01346.X
101. Woynarovich, A., Moth-Poulsen, T., Péteri, A., Carp polyculture in Central and Eastern Europe, the Caucasus and Central Asia: a manual. Food and Agriculture Organization of the United Nations, 2010.
102. Wright, R., Reeves, C., Using grass carp to control weeds in Alabama ponds. Alabama Cooperative Extension System, 2004. ANR-452, 1-4.
103. Wu, B., Huang, L., Chen, J., Zhang, Y., Chen, X., Wu, C., Deng, X., Gao, J., He, J., Effects of feeding frequency on growth performance. feed intake. metabolism and expression of fgf21 in grass carp (*Ctenopharyngodon idellus*). Aquaculture. 2021;545. 737196 doi: 10.1016/j.aquaculture.2021.737196
104. Xiao, C. Liu, Z., Li, D., Refaey, M. M., Tang, R., Li, L., Zhang, X., Acute nitrite exposure alters the metabolism of thyroid hormones in grass carp (*Ctenopharyngodon idellus*). Chemosphere. 2017;186(1):798-804. doi:10.1016/J.CHEMOSPHERE.2017.07.119

105. Xiao-qin; L. Xing-xing; L. Xiang-jun; L. Xian-min; L. Xi-cang; W. Jia-le L. Effect of different salinities on growth and flesh quality of *Ctenopharyngodon idellus*. J Fish China. 2007;31(1): 343-348. Published online 2007. Accessed October 22. 2021. https://en.cnki.com.cn/Article_en/CJFDTTotal-SCKX200703010.htm
106. Xu, J., Wu, P., Jiang, W. D. et al., Optimal dietary protein level improved growth, disease resistance, intestinal immune and physical barrier function of young grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol. 2016;55(1):64-87. doi: 10.1016/j.fsi.2016.05.021
107. Yavuzcan-Yıldız, H., Kırkavgaç-Uzbilek, M., The evaluation of secondary stress response of grass carp (*Ctenopharyngodon idella*, Val. 1844) after exposing to the saline water. Fish Physiol Biochem. 2001;25(4):287-290. doi:10.1023/A:1023279604975
108. Yu, H., Liang, H., Ren, M., et al., A study to explore the effects of low dietary protein levels on the growth performance and nutritional metabolism of grass carp (*Ctenopharyngodon idella*) fry. Aquaculture. 2022; 546:737324. doi: 10.1016/j.aquaculture.2021.737324
109. Zhao, Y., Zhang, L., Wang, C., Xie, C., Biology and Ecology of Grass Carp in China: A Review and Synthesis. North Am J Fish Manag. 2020;40(6):1379-1399. doi:10.1002/nafm.10512
110. Zhao, X. L., Jin, Z. H., Di, G. L., Li, L. I., Kong, X. H., Molecular characteristics, pathogenicity and medication regimen of *Aeromonas hydrophila* isolated from common carp (*Cyprinus carpio* L.). J Vet Medical Scie, 2019; 81(12): 1769-1775.
111. Zhao, Z., Dong, S., Xu, Q., Respiratory response of grass carp *Ctenopharyngodon idellus* to dissolved oxygen changes at three acclimation temperatures. Fish Physiol Biochem. 2018;44(1):63-71. doi:10.1007/s10695-017-0413-9
112. Zoccarato, I., Benatti, G., Calvi, S. L., Bianchini, M. L., Use of pig manure as fertiliser with and without supplement feed in pond carp production in Northern Italy. Aquaculture. 1995;129(1-4):387-390. doi:10.1016/0044-8486(94)00290-5