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Field guide to the culture of tambaqui (*Colossoma macropomum*, Cuvier, 1816)






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by

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This Technical Paper is one of the outputs from the Food and Agriculture Organization of the United Nations (FAO) Technical Cooperation Programme (TCP) project "Promotion of Small Scale Aquaculture in Guyana for Food Security and Rural Development" (TCP/GUY/3501 (D). The main objective of the project, which was carried out in the period 2014–2017, was to reduce poverty and food insecurity, both of which are still prevalent in large parts of Guyana, particularly in some hinterland and coastal areas. The project provided technical assistance with a view to building capacity among small-scale farming households through simple production methods, and introduce new technology for fish reproduction. Farmers were trained in simple applicable hatchery, nursery, and feeding techniques for tambaqui (*Colossoma macropomum*, Cuvier, 1816).

As outlined in the project document, the project focused on tambaqui because it is an ideal species for producing cheap animal protein: as a fish species that is relatively easy to grow, it helps to improve food security. The fish is omnivore, and requires a comparatively low protein content in its feed. When farming tambaqui it is possible to make use of low-cost feed and feed ingredients such as forest fruits, the seeds of leguminous plants, cassava, etc. As a species it not only tolerates soft- and acid waters well, but also can grow in water with relatively high salinity, up to about 10 ppt. Tambaqui is a hardy fish and quickly grows to a large size, which means that even seasonal waters can be suitable for producing them. For these reasons, the aquaculture production of tambaqui has continued to increase – from 13 thousand tonnes in 2000 to 142 thousand tonnes in 2016 – and there is huge potential for its cultivation on both a large and small scale.

Tambaqui originates from the Amazon and Orinoco river basins in South America, and is currently farmed across South and Central America, as well as in some countries in Asia.

This technical paper contains a field guide developed in order to provide practical guidance on the culture of tambaqui. It was prepared to assist small- and large-scale aquaculture farmers with practical advice on how to grow and breed tambaqui, with the aim of further increasing the production of this important fish species, which is vital for food security. This guide combines all the immediately applicable practical information on the culture of tambaqui with the updated findings and conclusions of an earlier technical guide on the artificial propagation of tambaqui and related species, published by FONDEPES in 1998[1].

This technical paper was prepared by Mr András Woynárovich, Aquaculture Consultant, with the support of Mr Raymon van Anrooy, FAO Fisheries and Aquaculture Officer for the Caribbean. The document reflects the provisions of the FAO Code of Conduct for Responsible Fisheries in relation to aquaculture development and the management of tambaqui. During the development of this paper, specific aspects of genetic diversity and its practical consequences in the field were overseen by Ms Edit Eszterbauer, a Biologist at MTA ATK AOTI.[2] The authors also received comments from Aquaculture Officers from the Fisheries and Aquaculture Department at FAO.

The text was edited by Edward Fortes. The formatting, design and publication were further supported by Ms Chorouk Benkabbour and Ms Marianne Guyonnet of the FAO Fisheries and Aquaculture Department.

[1] Woynárovich, A. & Woynárovich, E. 1998. *Reproduccion artificial de las especies colossoma y piaractus – Una guia detallada para la produccion de alevinos de gamitana, paco y caraña*, Lima, Peru, Fondo Nacional de Desarrollo pesquero (FONDEPES).

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Abstract

Following a short introduction to the species and its closest commercially viable related species, namely pirapatinga (*Piaractus brachypomus*) and pacu (*Piaractus mesopotamicus*), this field guide provides practical information on the culture and reproduction of tambaqui (*Colossoma macropomum*).

As a field guide it aims to support the understanding and dissemination of applicable technologies for the culture and reproduction of tambaqui, i.e. what should be done – as well as when and how it should be done – in order to achieve success in the artificial propagation as well as the fingerling and table fish production stages.

The concise technical descriptions in this guide are accompanied by self-explanatory illustrations and a reader-friendly glossary of technical terms, which is important for tambaqui aquaculture farmers.

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1. INTRODUCTION

Tambaqui is a ***Neotropical**** fish of major cultural and economic importance in artisanal fisheries and commercial aquaculture (Wood *et al.*, 2017); it is one of the most important commercial fish species among the scaly fishes of the Amazon river basin, and highly sought after by the local consumers for its meat. Tambaqui is the second-largest scaly fish after pirarucu (*Arapaima gigas*) in the Solimões–Amazon River. Out of the three largest species of the Serrasalmidae family (i.e. tambaqui, pirapatinga and pacu)[1] it is the most economically important in terms of its commercial value in the Americas (FishStat, 2018). Tambaqui sustains thousands of professional fishermen, providing animal protein for the inhabitants of the Amazon river basin. Tambaqui is widely accepted as a fish for consumption in Brazil, Colombia, Peru and Venezuela (Bolivarian Republic of) (Amaya, 1992), and its economic importance in the Orinoco river and its tributaries is also significant (Golding, 1981).

Due to the intensive commercial fishing of mature, propagation-ready adult tambaqui in the period from 1976 to 1996, the population level has decreased considerably in the Amazon river basin (Goulding, 1981; Arauja-Lima and Goulding, 1997; Batista and Petrere Júnior, 2003). Therein one of the reasons why, in the late 1970s and early 1980s, efforts were increased to develop reliable, large-scale propagation techniques for the production of tambaqui fry and fingerlings, both for restocking and aquaculture.

From 1983 onwards, large-scale ***artificial propagation**** technology was adapted and widely introduced for warmwater fish species, including tambaqui, in northeast Brazil. This led to an increase in the popularity of tambaqui among farmers with water reservoirs and fish ponds. This increase was principally due to the following:

- Tambaqui can be handled easily at all ages and require neither special culture techniques nor special conditions to obtain good results;
- Tambaqui is an omnivorous fish and consumes a wide range of ***natural foods****; it also flourishes on many different types of ***feeds**** under culture conditions, and grows well if it can obtain enough natural food and/or feeds.
- Tambaqui is suitable for ***fish ranching**** (i.e. culture-based fishery (***CBF***)*), and fits well into the ***polyculture**** of fish pond production systems.

Today, a wide range of valuable publications are available on tambaqui, designed to help seasonal, full- or part-time farmers deal with the culture of tambaqui. This field guide is intended as a complement to some of those existing publications by offering information on practical, simple and low-cost technologies, methods and culture practices.

In order to provide information that is both practical and immediately applicable, this technical paper is divided into three main parts:

- Chapters 2, 3 and 4 provide some of the background information on tambaqui that has previously been overlooked, including the valuable species that are closely related to it within the Serrasalmidae family.
- Chapter 5 provides a step-by-step introduction to the artificial propagation of tambaqui, taking the entire technology from induced breeding to the rearing of advanced fry.
- Chapters 6, 7 and 8 discuss the techniques to be adopted for fingerling and table fish production, as well the rearing and keeping of brood stock.

[1] The name "pirapitinga" is also widely used; in this paper the same common name employed in FAO statistics "pirapatinga" is used.

- Chapter 9 provides and overview of the fish diseases that tambaqui may contract during culture.

The chapters listed are supported by four annexes in which an inventory and a discussion of production conditions, equipment, tools and production materials are provided.

To facilitate the easy use and interpretation of the descriptions and explanations provided, not only illustrations and photos are employed, but definitions of all the relevant technical terms are included in a glossary. Glossary terms appear in bold italic text when used for the first term, and are marked with an asterisk (*), to indicate a glossary entry.

2. TAMBAQUI AND ITS CLOSE RELATIVES USED IN AQUACULTURE

Tambaqui has two close relatives which are also widely used in aquaculture. All three belong to the same Serrasalmidae family. Within this family tambaqui (*Colossoma macropomum* Cuvier, 1816) belongs to the *Colossoma genus*. According to present systematization, tambaqui is the only species in the Colossoma genus, but the two other commercially important, closely related species pirapatinga (P*iaractus brachypomus* Cuvier, 1818) and pacu (*Piaractus mesopotamicus* Holmberg, 1887) were previously classified in this same genus, with scientific names *Colossoma brachypomus* and *Colossoma mitrei.*

The first two species are native to the Amazonas (Solimões, Amazonas) and Orinoco rivers and their tributaries. Pacu, endemic in the Paraná-Paraguay basin includes central and western Brazil, northeastern Paraguay, eastern Bolivia and northern Argentina. (Calcagnotto and DeSalle, 2009).

These species are so similar that tambaqui and pirapatinga occasionally mix in the wild (Araujo-Lima and Goulding, 1997) and their interspecific hybrids have been widely produced on purpose (Box 2-1). Moreover, hybrids of tambaqui and pacu appear to have good early survival rates and are most likely fertile (Bartley *et al*., 2001).

TABLE 1-1
Distinct characteristics of the three species formerly part of the *Colossoma genus*

Characteristics	Tambaqui	Pirapatinga	Pacu
Teeth on maxilla	In two rows	1–3	1–2
Filtering spines ("bristles") on first gill arch	84–107	33–37	30–34
Scales on lateral line	78–84	88–98	108–128
Scales above lateral line	23–27	37–42	50–60
Scales below lateral line	20–22	27–34	49–56

Source: Goulding (1980), CEPTA (1986).

BOX 2-1
Interspecific hybrids of tambaqui

Sterile progeny or superb performance are among the objectives when producing interspecific hybrids of fishes such as "tambacu" (hybrid of tambaqui ♀ and pacu ♂) and "tambatinga" (hybrid of tambaqui ♀ and pirapatinga ♂). As the juvenile stages of these hybrids are morphologically indistinguishable, there is a risk of pure species and hybrids mixing and fertile hybrids escaping; this reduces production and can have adverse impacts on native populations. For these reasons molecular markers are characterized to identify hybrid lineages, a technique which enables the admixing of pure stocks and their various hybrids to be identified.

Source: Hashimoto *et al.*, 2011.

FIGURE 2-1
Tambaqui (*Colossoma macropomum*)

FIGURE 2-2
Pirapatinga (*Piaractus brachypomus*)

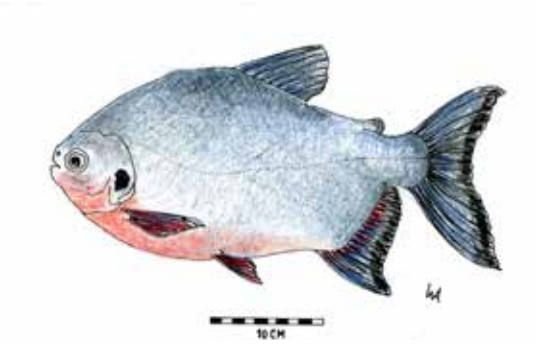


FIGURE 2-3
Pacu (*Piaractus mesopotamicus*)

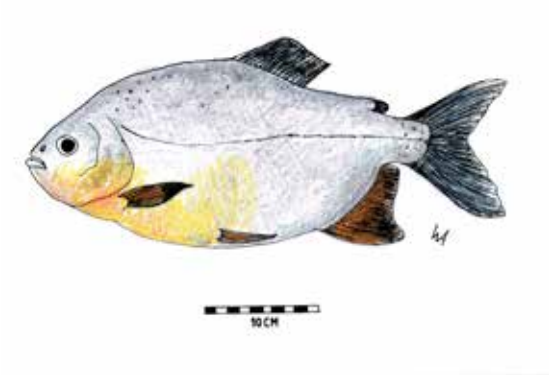


FIGURE 2-4
Fry of (A) tambaqui, (B) pirapatinga and (C) pacu

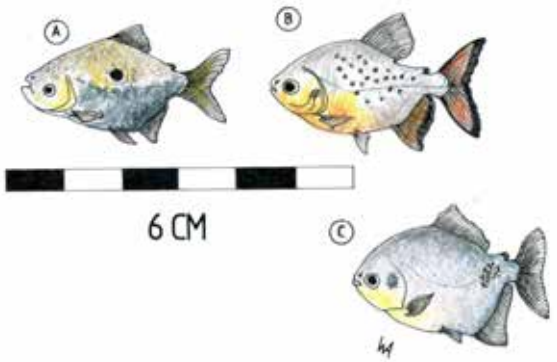
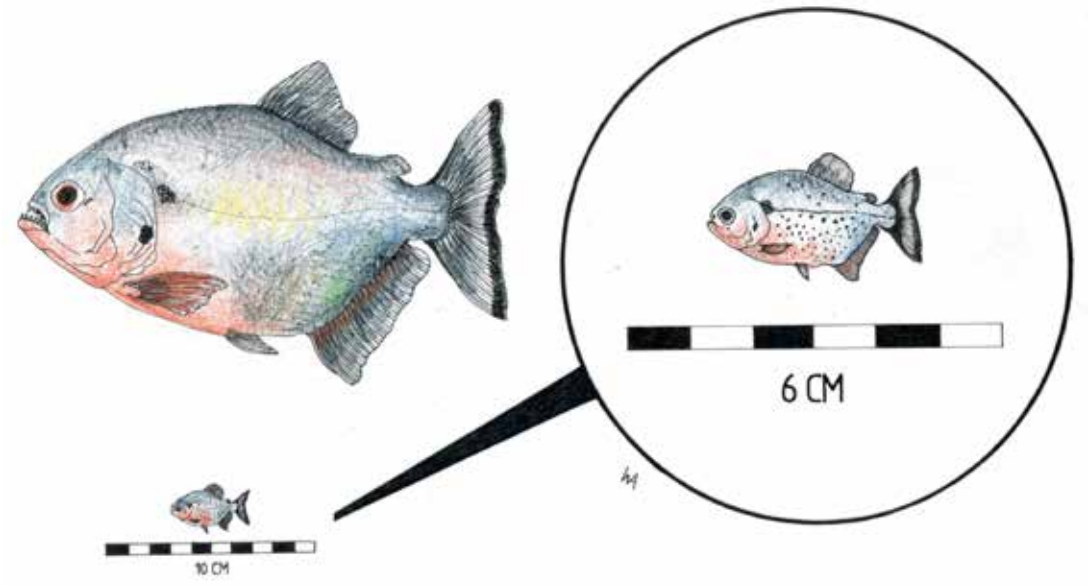


FIGURE 2-5
Piranha (*Serrasalmus* sp.)



Both the experimental culture phase of the 1970s and the subsequent rapid increase in commercial production in the 1980s proved that these three fish species are excellent for fish culture. The large-scale artificial propagation of these fishes ensured a steady fry and fingerling supply, which made these species popular among large numbers of farmers.

As advanced fry, pacu are especially appealing in shape and colour, as well as being a popular species for ornamental purposes. Many fish farms therefore propagate and sell them as aquarium (ornamental) fish.

Tambaqui (Figure 2-1) is a truly tropical fish, which dies if water temperature remains lower than 15 °C for several days. Advanced fry and fingerlings under 5 g in weight show high mortality in waters that are under 18 °C (CEPTA, 1986). These temperature limitations reduce the natural spread and establishment of the species in waters where the water temperature drops below 15–18 °C during colder seasons. The relative insensitivity of tambaqui to low pH proves its acid water tolerance, which occurrs naturally in ***black water**** habitats (Aride *et al.,* 2007). Tambaqui also tolerates salinity up to 10 ppt (Fiúza *et al.,* 2015).

Similarly to tambaqui, pirapatinga is also a tropical fish and cannot survive if water temperatures go below 15 °C. It is a rather flat, silvery-white fish (see Figure 2-2), and the weakest and least sturdy of the three species mentioned. The length-to-height ratio of pirapatinga is between 2.5 to 3.5 in the case of adult fish. It can grow to about 85 cm as a ***standard length (SL)**** and about 20 kg in weight (Goulding, 1980).

Pirapatinga has a black spot (an "eye") on the bony *operculum*. With this mimicry, young fish resemble the most ferocious piranhas, which have a similar black spot on the dorsal area above the lateral line, behind the *operculum*. Young pirapatinga show further similarities in the colour of the pectoral, abdominal and anal fins, as well as the breast, which are yellow, red or dark red respectively. A vivid red colour appears on the pectoral part of the fish during the spawning period. The most ferocious piranhas also have a vivid red colour on the pectoral area throughout their life cycle (see Figure 2-5).

The pirapatinga's adipose fin is fleshy without fin rays. On the first gill arch there are 33 to 37 filtering spines. The maxilla and lower maxilla (mandible) have sharper molar-shaped teeth. Pirapatinga is an omnivorous fish with a preferred diet of fruit, seeds and green leaves that fall into the water from the forest (Goulding, 1980). Pirapatinga's growth in its natural habitat is significantly slower than that of tambaqui. The reason for this is that pirapatinga reaches sexual maturity one year earlier, is a less aggressive feeder and has a more restricted spectrum of natural foods for its diet than tambaqui. When fed properly pirapatinga also grows very well, although not as part of polyculture with tambaqui, where food competition limits growth.

Pacu is also a tropical fish, but survives in waters below 15 °C in temperature, most probably surviving in waters as cold as about 10 °C. This fish is dark grey on its dorsal parts and golden yellow in the abdominal (ventral) area. Its length-to-height coefficient is between 1.5 and 2.5 in the case of adult fish. This reveals that the body of pacu is rather robust. It reaches 40.5 cm (SL) and about 20 kg in weight (FishBase, 2018).

Young pacu have red fins but the colour fades and disappears quickly with age. This appearance may well shelter young fish from being attacked by other fish.

Pacu is a somewhat aggressive feeder and its aggressiveness increases in higher water temperatures. However, it is a much less aggressive feeder than tambaqui. Pacu is omnivorous with preference for water-based insects and worms. Occasionally it grabs and eats vertebrates as well as considerably smaller fish.

Pacu grows well if it can feed properly; it may grow to about 1.5 kg in weight in fish ponds within one year when fed well.

According to Goulding (1981), the significant difference between tambaqui and the two other species is that neither pirapatinga nor pacu has the same type of filtering apparatus with fine "bristles", nor the wide muscular operculum. These features make tambaqui a special and unique omnivorous feeder of an especially wide range of natural foods, as discussed in Chapter 4.2.

3. NATURAL AND WORLDWIDE DISTRIBUTION OF TAMBAQUI

As Araujo-Lima and Goulding (1997) have described, in geographical terms tambaqui is found in Brazil, Venezuela (Bolivarian Republic of), Columbia, Peru and Bolivia (Plurinational State of) – but within these countries the species' native natural habitat is restricted to the Amazon and Orinoco Basins. The main capture fisheries production of tambaqui (reported as cachama in the FAO FishStatJ database) takes place in Brazil and Peru. The annual capture fisheries production reported to FAO for the species in these two countries has been relatively stable since 2001, at around 4 000 tonnes per year. The capture fisheries production of pirapatinga in Brazil and Peru over the same period (2001–2015) is around 2 500 tonnes annually.

Tambaqui was also introduced in regions outside of its native range (see Box 3-1) in the 1980s, which has led to an increase in production since that period. Currently the production of tambaqui and other species belonging to the Serrasalmidae family is much higher in aquaculture than in inland capture fisheries. Aquaculture production of tambaqui and other species of the Serrasalmidae family has spread to most countries in South and Central America, some Caribbean countries, and various countries in Asia: particularly China, Indonesia, Malaysia, Myanmar and Viet Nam.

BOX 3-1

Introduction of tambaqui to the North-East region of Brazil

According to Lopez (1982) the DNOCS (Departamento Nacional de Obras Contra as Secas) received 24 and 74 fingerlings of tambaqui from different regions of the Amazonian basin in 1966 and 1972 respectively. Between 1974 and 1977, the first sexually mature adults reared from the fingerlings were propagated with a sequence of four hypophysis injections.

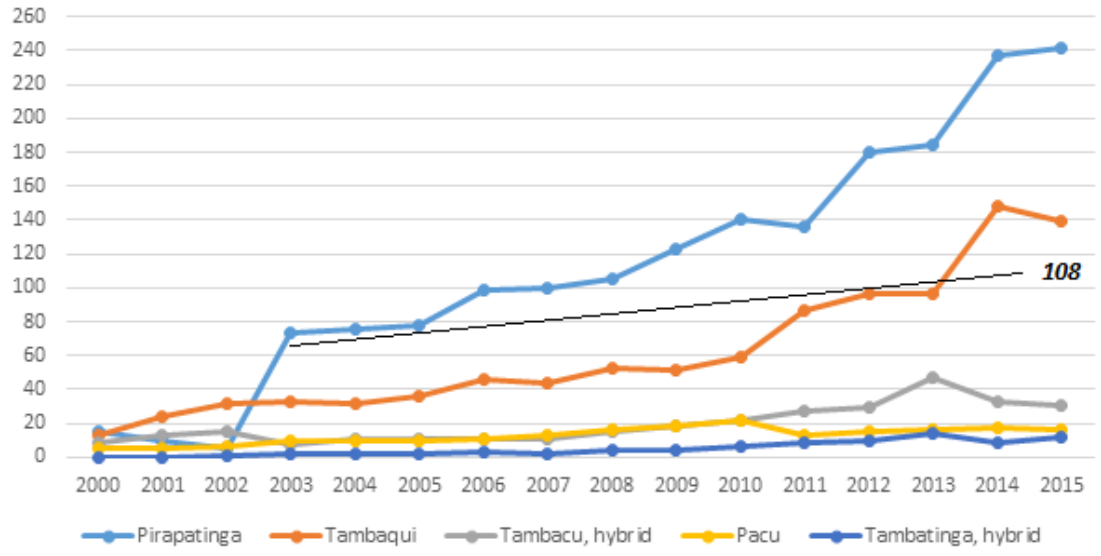
After these initial successes the same, rather experimental, technique was used in Brazil until about 1983.

In 1980 and 1982 two fish culture stations of CODEVASF (Compania de Desenvolvimento do Rio São Francisco), Itiuba (State of Alagoas) and Betume (State of Sergipe), received 3–5 cm long tambaqui fingerlings from DNOCS at Pentecoste Ceará in order to create brood stocks.

Stocks introduced in 1980 became sexually mature by late 1983 and early 1984, when suitable females and males were successfully propagated on both fish farms (Pinheiro *et al.*, 1988). On the basis of the results obtained, the first technical description of this artificial propagation technique for tambaqui was compiled in late 1984. Since then, the same or very similar large-scale artificial propagation technology discussed in this paper has been used extensively for tambaqui.

Alongside tambaqui, even in early 1984 pirapatinga was also propagated in the same way as it is today on the CODEVASF fish farm in Itiuba.

FIGURE 3-1
Aquaculture production of tambaqui and related species in thousand tons over the period 2000–2015



The black line shows the production of pirapatinga in China in the 2003–2015 period, which was 108 874 tons in 2015.
Source: FishStat (2018).

TABLE 3-1
Aquaculture production of tambaqui and its hybrids in 2016

Countries	Tambaqui		Tambacu		Tambatinga		Total	
	Tonnes	*%*	*Tonnes*	*%*	*Tonnes*	*%*	*Tonnes*	*%*
Bolivia (Plurinational State of)	660	0.5	0	0.0	0	0.0	660	0.3
Brazil	137 000	96.4	36 900	90.8	8 000	99.9	181 900	95.3
Colombia	1 700	1.2	0	0	0	0	1 700	0.9
Dominican Republic	10	0	0	0	0	0	10	0.0
Guyana	202	0.1	0	0	0	0	202	0.1
Panama	5	0	0	0	0	0	5	0.0
Peru	1 863	1.3	0	0	11	0.1	1 874	1.0
Suriname	71	0	0	0	0	0	71	0.0
Venezuela (Bolivarian Republic of)	624	0.4	3 751	9.2	0	0	4 375	2.3
Total	142 135	100.0	40 651	100.0	8 011	100	190 797	100.0

Source: FAO FishStatJ (2018).

The aquaculture production of tambaqui and other species of the Serrasalmidae family has increased exponentially in recent decades. The average production in aquaculture was just 397 tonnes in the 1980s, increasing to an average of 13 827 tonnes annually in the 1990s, before reaching 129 602 tonnes of annual production in the first decade of this millennium. The production doubled again in the period from 2009 to 2015, from 216 thousand tonnes to 438 thousand tonnes annually.

Figure 3-1 presents the production trends of tambaqui and other species of the Serrasalmidae family in aquaculture since 2000. It shows that pirapatinga production surpassed tambaqui production in 2003 and has since increased to 242 000 tonnes in 2015. The outstanding production figures for pirapatinga are due to the species' introduction in China, where its production has increased steadily and accounts for an

almost 45 percent share of global production. Tambaqui production continued to grow steadily until 2014, when production levels reached 148 000 tonnes, before a slight reduction to 138 000 tonnes in 2015.

The total tambaqui aquaculture production in 2016, according to available data from FAO FishStatJ (2018), amounted to 142 135 tonnes, of which 96.4 percent of was produced by Brazil, with 137 000 tonnes estimated in 2016. The other countries that reported tambaqui production to FAO included Colombia and Peru with 1 700 and 1 863 tonnes respectively in the same year. Other countries produced less than 1 000 tonnes and their contribution to overall production was less than one percent. Table 3-1 provides an overview of the countries producing tambaqui and its hybrids in 2016.

The international dissemination of tambaqui as an ornamental fish – often under the name of piranha – took place as early as the 1960s. However, aside from Latin American countries such as Costa Rica, Honduras, Jamaica, Panamá and Guyana (FishBase, 2017) deliberate introductions into nature or escapes from fish farms have not led to stocks in the wild outside of the native range. In several of the countries above, as well as the United States of America, only discarded or escaped specimens appeared in tropical waters, but no firm establishment of tambaqui stocks has been reported (Nico and Neilson, 2017).

4. TAMBAQUI IN THE NATURE

4.1 APPEARANCE, SIZE AND LIFE CYCLE

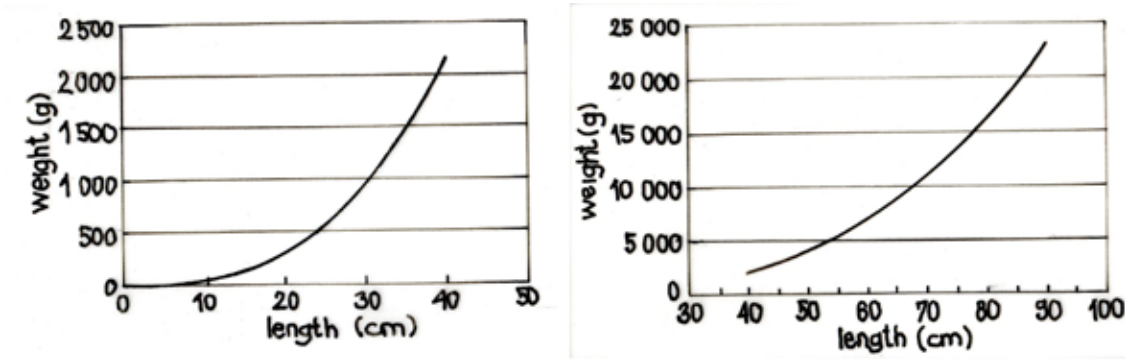
Tambaqui is a very sturdy fish. Common length is around 70 cm ***total length (TL)****, and a maximum weight of about 40 kg (FishBase, 2017). Figure 4-1 and Figure 4-2, as well as Table 4-1 and Table 4-2, show the correlation between the length and weight of fish.

Juvenile and pre-adult tambaqui are roundish rhomboid in shape, while the adults become to some extent elongated with age (Figure 4-2). The length to height coefficient, which is the ***standard length (SL)**** divided by the maximum height of the body, is between 2 to 3 in the case of adult fish. Advanced fry (young specimens up to approximately 40 days of age) have a black spot – an "eye" – above the lateral line, roughly in the middle on both sides. These disappear slowly with age. This spot makes the small fish look bigger, and resemble certain predators (e.g. piranhas). This is ecological mimicry, and its purpose is to avoid attacks. Such examples are demonstrated in Figure 2-4, although Araujo-Lima and Goulding (1997) question the efficiency of ecological mimicry in turbid waters where visibility is near to zero.

After reaching the size of about 30 cm the body becomes elongated, a distinct countershading develops and remains. Their colour depends on the water type in which the fish lives, and can change within days. In black water rivers it can become very dark, and countershading is minimal; while in relatively clear waters the dorsal side is olive green, and the ventral one remains dark green to black. In muddy waters tambaqui remain more yellowish and lighter in colour (Araujo-Lima and Goulding, 1997). In fish pond conditions the dorsal part of its body is a combination of dark yellow and grey; the ventral part is a mixture of yellow and white, which becomes black towards the anal and caudal fins, which remain black (Figure 4-2). However, when tambaqui is grown in ponds it generally has an olive green front head, a light to darkish yellow back and dorsal fin, while the abdomen is almost white, veering to black toward the anal fin. Besides the anal fin, the pectoral, pelvic and caudal fins are black.

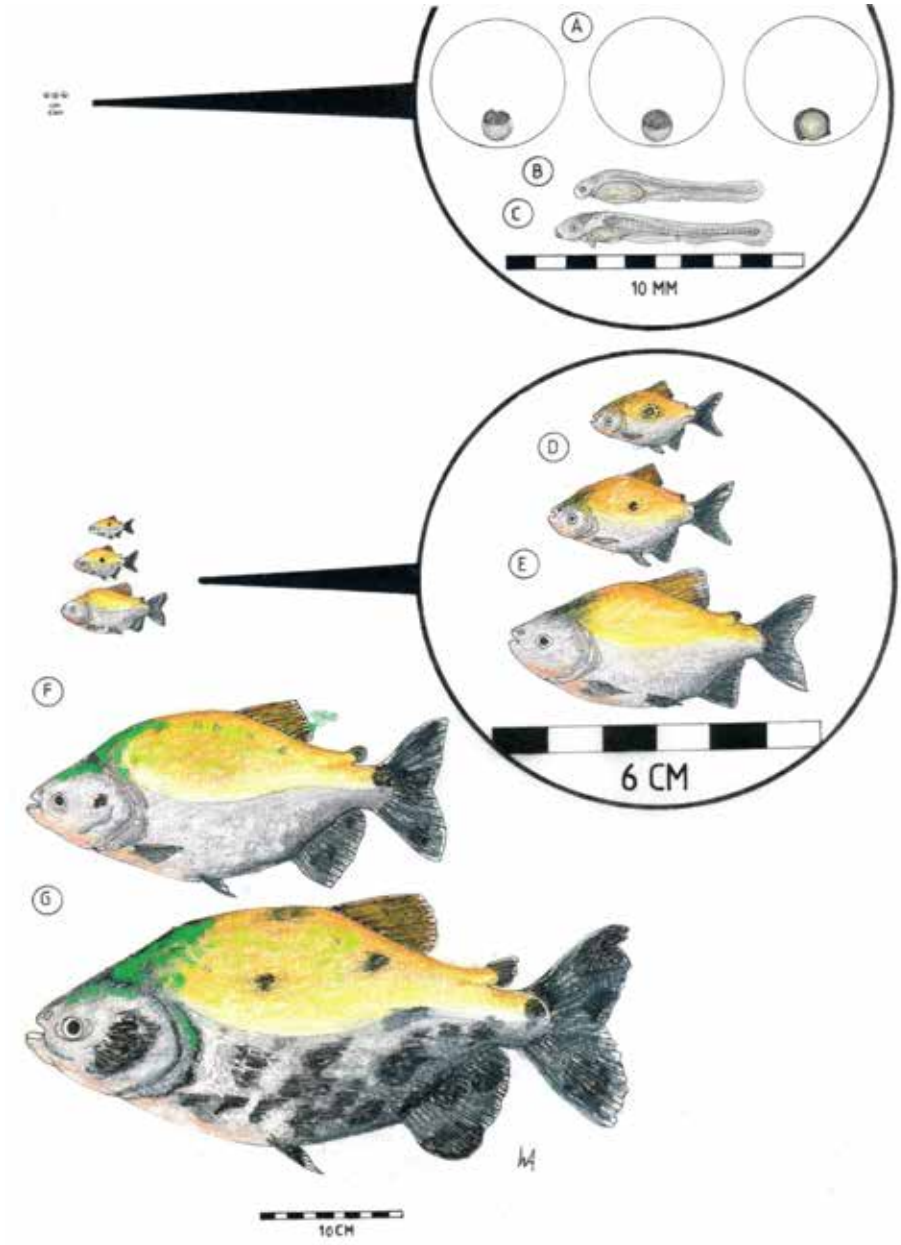
The adipose fin of tambaqui is bony, as the fin contains rays; its scales are relatively small, but they are firmly fixed into the skin; the ventral edge (abdominal line) is sharp

FIGURE 4-1
Correlation between weight and length of tambaqui



(A) The weight and length correlation of tambaqui fingerlings and juvenile fish up to 40 cm or approximately 2 kg. (B) The same correlation for adult fish.
Source: Araujo-Lima and Goulding (1997).

FIGURE 4-2
Development stages of tambaqui



(A) fertile egg and embryo at different stages of development; (B) developing larvae; (C) feeding larvae; (D) fry of different sizes; (E) fingerlings; (F) pre-adult; (G) adult fish.

with V-shaped scales. These characteristics enable tambaqui to adapt well to the habitat and allows for coexistence with the piranhas and pirambebas in their original habitat in the Amazon and Orinoco river basins. Ferocious species of piranhas can attack fish with loose scales and a soft belly more easily because they can bite into them.

4.2 FOOD SPECTRUM AND FEEDING HABITS

Special teeth and the a filtering apparatus allow tambaqui to ingest and make effective use of an exceptionally wide range of natural foods.

By the pre-adult phase, the maxilla and lower maxilla (mandible) of developing tambaqui has molar-like teeth (i.e. similar to the grinding teeth of mammals), with two

rows of teeth on each jaw. Of these the second row grows continuously and replaces the first one once worn out (Goulding, 1980). With these teeth, operated by extremely strong jaw muscles, a range of hard foods such as fruits, nuts, grains and snails can be crushed easily provided they are bite-sized.

Tambaqui is the only species in the Serrasalmidae family that has aberrant gill rakers. These increase in number as the fish grows. This highly developed ***gill raker structure**** allows the fish to filter zooplankton and planktonic particles (Araujo-Lima and Goulding, 1997).

The tambaqui's intestine is long, about 2 to 2.5 times longer than the standard length of the fish. It has a well-developed stomach, which is relatively long and has the shape of an elbow. The number of ***pylorus**** appendages varies between 43 and 75 (Honda, 1974). Such a high number of appendages facilitates an efficient absorption of all nutriments digested in the stomach.

As shown in Table 4-1 and Table 4-2, the food spectrum of tambaqui in its natural habitat changes according to size and season. The natural food of tambaqui varies with the high water (i.e. flood) and low water seasons. During the flood season the trees and shrubs of the inundated forest provide great quantities and varieties of fruits, nuts and grains. When the flood season is over and the water subsides into the oxbow lakes – the so-called marginal lakes – and natural canals of the flood plain, plankton is the most important natural food.

In the inundated forests of the Amazon and Orinoco rivers and their tributaries, the most important natural foods for tambaqui are the fruits and grains of trees, shrubs and creeper plants listed in Table 4-3. Zooplankton is another very important natural food

TABLE 4-1
Principal foods eaten by tambaqui in its natural habitat (size from 1 cm to 20 cm)

Length (cm)	1–2	2.1–4	7–10	16–20	
Weight (g)	0.1–0.3	0.4–3	14–40	150–300	
Season	Low water	Low water	High water	Low water	High water
Floodplain	Amazon	Amazon	Amazon	Amazon	Amazon
Zooplankton (%)	73	45	20	60	50
Insects (%)	23	20	4	-	-
Wild rice (%)	0	13	22	30	31
Fruits/seeds (%)	0	0	0	4	15
Algae (%)	1	0	54	-	-
Others (%)	10	20	0	5	2

Source: Araujo-Lima and Goulding (1997).

TABLE 4-2
Principal foods eaten by tambaqui in the wild (size from 20 cm to 40 cm)

Length (cm)	**20–36**			**> 40**			
Weight (g)	**300–1 600**			**2 100**			
Season	Low w.	High w.	High w.	High w.	Low w.	Low w.	High w.
Floodplain	Amazon	Amazon	Madeira	Amazon	Amazon	Madeira	Madeira
Zooplankton (%)	58	32	42	18	55	25	< 1
Insects (%)	-	-	-	-	-	12	< 1
Wild rice (%)	8	32	NA	7	6	< 1	-
Fruits/seeds (%)	< 1	34	42	71	71	10	94
Algae (%)	-	-	-	-	-	-	
Others (%)	34	2	18	4	38	52	5

Source: Araujo-Lima and Goulding (1997).

source. Plankton crustaceans, most frequently found in the stomachs of fish captured in the wild, are listed in Table 4-4.

Tambaqui has one of the widest natural food diets among cultured fish species. This means that it may feed on filamentous algae, parts of water plants (both fresh and decomposing), zooplankton, larger water and terrestrial insects and their larvae – as well as snails, molluscs, dry and fleshy fruits, hard and soft grains, and nuts. Tambaqui also occasionally eats much smaller fish: as an aggressive feeder, it finds and takes all available food resources in wild. The only thing that tambaqui does not do is dig into the muddy riverbed in search of food, as common carp does.

Tambaqui grows rapidly in its natural habitat. Young and pre-adult fish remain in floodplain lakes and flooded forests, while adults that are ready for propagation migrate seasonally between spawning grounds upstream and feeding grounds, such as flooded forests and floodplain lakes.

TABLE 4-3
List of fruits, grains, shrubs and creeper plants consumed by tambaqui

Tabebuia barbata **(Bignoniaceae),** ***Mabea*** **sp. (Euphorbiaceae),** ***Cecropia*** **sp. (Moraceae),** ***Vitex cynosa*** **(Verbenaceae), wild rice (*** ***Oryza perennis*** **), rubber tree (*** ***Hevea brasiliensis*** **) and** ***Hevea spruceana*** **(Euphorbiaceae), fruit of the palm tree (*** ***Astrocaqrium javary*** **),** ***Neolabatia*** **sp. (Sapotaceae),** ***Genipa americana*** **(Rubiaceae), water hyacinth (*** ***Eichornia crassipes*** **).**

Source: Goulding (1980).

TABLE 4-4
List of plankton and small crustaceans frequently found in the stomachs of tambaqui

Daphnia gessneri, Ceriodaphnia reticulata and ***C. cornuta, Moina reticulata, Notodiaptomus amazonicus, Diaphanosoma*** **sp.,** ***Bosmina*** **sp., Chydoridaea, Macrothricidae, Cyclopidae,** ***Ostracoda, Conchostraca,*** **Shrimp,** ***Acarina*** **and** ***Insecta.***

Source: Goulding (1980).

4.3 PROPAGATION IN NATURE

Tambaqui is a river spawner, ***piracema**** fish. When the environmental conditions are favourable (i.e. water levels in rivers rise due to rain in the catchment area of rivers), sexually mature fish school and migrate upstream so as to spawn in groups.

The smallest reported size of female to spawn in the wild was 45 cm (about 3.5 kg). According to extensive research by Villacorta-Correa and Saint-Paul (1999) 50 percent of the female fish population reach maturity at 58 cm SL, when fish are about 6.3 kg in weight (see Figure 4-3).

Water temperature at spawning is about 27 °C. Goulding (1980) describes the sites where tambaqui usually spawn as littoral areas of the river, near the natural canals of floodplain lakes through which these inundated areas and marginal lakes are connected to the river (Box 4-1). The actual spawning environment on the site can be woody shore areas, along levees with floating grasses, or under floating meadows of plants with large leaves (Goulding 1980; Araujo-Lima and Goulding, 1997).

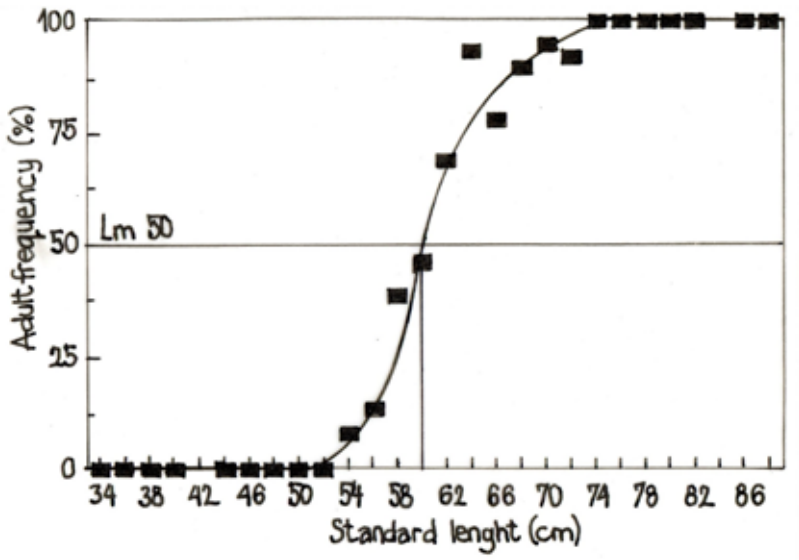
Tambaqui begins spawning when rivers are more than half full, the inundation of nearby floodplains begins and weather continues to be rainy. The spawning period lasts between two and five months, up to the middle of annual floods (SUDEPE 1981).

Brood fish, which accumulate and migrate in large schools in the river, are sexually mature, and prepare themselves for spawning. When sexually mature tambaqui migrate upstream they hardly find any food in the river, meaning they practically

BOX 4-1
The parental care of piracema fishes

The selection of spawning grounds in the river is a form of instinctive foresight: the passive parental care of brood fish is evident by the securing of a potentially propitious place for the incubation of floating eggs, hatching and larvae survival, as well as a suitable feeding and enemy-free living space for the offspring.

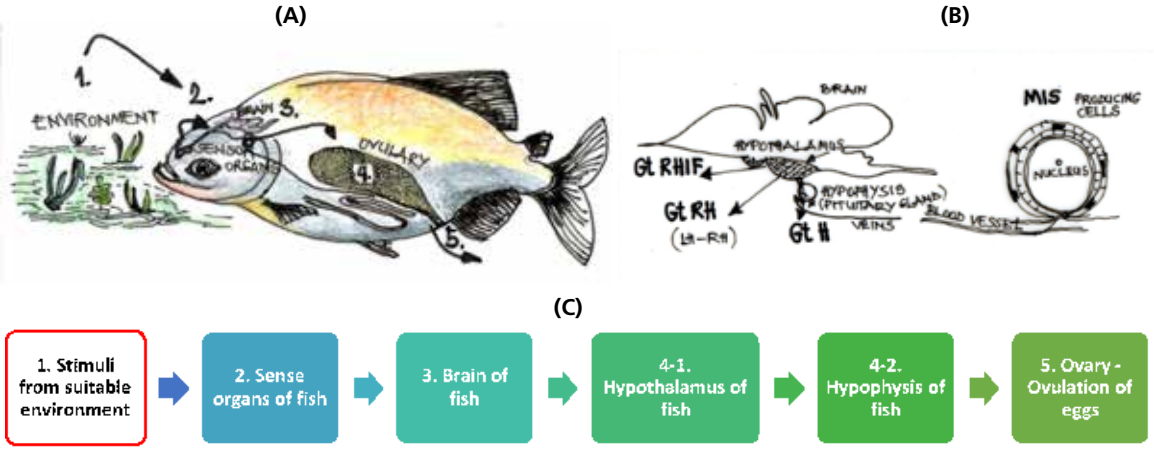
FIGURE 4-3
Distribution of accumulated frequency of *C. macropomum* matured females by SL class



Source: Villacorta-Correa and Saint-Paul (1999).

starve during the whole period of river migration. When they begin migrating, the female's ovaries have not yet developed, but all materials (***precursors****) necessary to the ovarian development (***vitellogenesis****) of eggs are already stored in the liver and other organs, as well as in abdominal fat reserves (Castelo *et al.*, 1980). Ovary development occurs at a very rapid pace, about one month before spawning. However, the belly of the females grows in size and becomes soft in consistency only a few hours before actual spawning, which is the result of a neurohormonal process in the females, as

FIGURE 4-4
Flow chart of propagation of fish in nature



Neurohormonal process (A) and control (B) of spawning in nature. (C) Nature ensures that neurohormonal control of spawning allows brood fish to find the best possible spawning sites. By the season in which spawning takes place the vitellogenesis of eggs in the ovary is completed; the eggs reach a "dormant" stage and remain in it. This means that eggs are ready. Only the ***final maturation**** and ***ovulation**** of the eggs is missing, which is completed only when/if environmental conditions are favourable – i.e. fish find suitable spawning ground. When suitable spawning ground is found the above-illustrated neurohormonal message arrives to the ovary, where the eggs complete final maturation and ovulate. This is the guarantee that females are successful in releasing fertile eggs at the most suitable time and at a location where the environmental conditions are favourable for the development of the eggs once fertilized.
Adapted from: Woynárovich (1992).

shown in Figure 4-4. The reason for this "last-minute" change in volume of the ovary is that an enlarged soft belly may make the female vulnerable to attack by piranhas.

Fertilized eggs and hatched non-feeding larvae float downstream and, because the river is flooded, are transported by the current through the natural canals into the floodplain lakes along the river. Spawning in turbid but fertile ***white waters**** gives floating eggs and developing larvae a better chance of survival (Goulding, 1980). By the time developing larvae start external feeding they have drifted to floodplain waters rich in suitable natural foods (i.e. zooplankton).

During their growing phase, young fish remain in the floodplain up to the time of sexual maturity. They are surfeited with natural food during the rainy seasons, but surviving the crowded foodless depressions and floodplain lakes during dry seasons (Goulding 1981).

After spawning adult fish search the inundated grounds of forests adjacent to all types of rivers, where their natural foods are abundant (see Table 4-3 and Table 4-4). There they remain, taking every opportunity to feed themselves so as to rebuild their strength and gain the weight that they had before, in a period of 4 to 7 months (Goulding and Carvalho, 1982).

5. THE ARTIFICIAL PROPAGATION OF TAMBAQUI

In the past young piracema fishes were collected from their natural habitat (i.e. in the marginal pools of the floodplains of rivers) and stocked in water reservoirs and ponds. As tambaqui is also a piracema fish, it does not spawn in standing water but migrates to spawn upstream of flooding rivers. To obtain sufficient quantities of young fish for stocking purposes, there was no other way but to develop artificial propagation.

The ***history of artificial propagation**** of piracema fishes dates back to 1932 (Fontenele, 1982). Since then, both the concept and techniques of artificial propagation have progressed considerably, in which tambaqui had a significant role. The techniques practised today are highly developed and allow the safe, programmed supply of (advanced) fry of practically all widely cultured freshwater fish species, including tambaqui.

The artificial propagation of fishes is the procedure that begins with obtaining eggs from brood fish and results in the production of 4- to 5-week-old advanced fry. As illustrated in Figure 5-1, artificial propagation of fishes includes four steps. Out of these four steps three are completed in the hatchery, while the last is usually completed in nursery ponds or large tanks. A general overview of these four subsequent steps is provided in Table 5-1.

5.1 HATCHERY OPERATIONS

Hatchery operations discussed in this chapter include:

- obtaining fertilized eggs
- incubation of eggs
- rearing non-feeding larvae.

5.1.1 Obtaining fertilized eggs

Good propagation results may be expected only from those sexually mature females that carry dormant eggs ready for final maturation and ovulation. Consequently, farmers should select females that are at this advanced stage of ***gonadal**** development. Though in nature tambaqui reaches this stage only during the propagation season, during culture in ponds, this stage of gonadal development may be attained at any period of the year, providing that the physical, biological and rearing conditions are favourable for developing dormant eggs.

As illustrated in Figure 5-2, during the selection of females for induced ovulation it is important to recognize the direct and indirect signs that the gonadal development has reached the dormant stage. In the case of tambaqui these "signs" or "marks" are rather obvious and easy to verify:

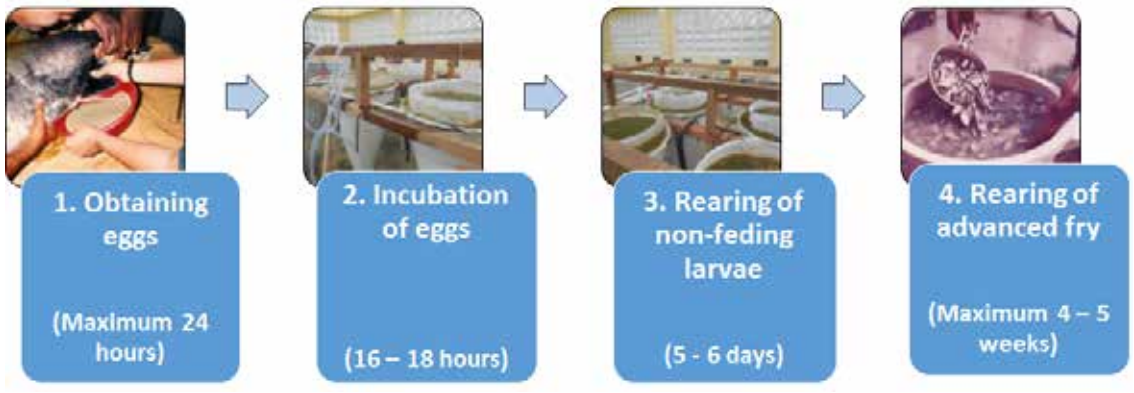
- the lower and rear part of the abdomen is bulky and often soft. The ovaries can be easily felt with the fingers.
- ***Sexual pocket**** of females is half or fully open.
- Sexual opening of the females is dark red, reddish or rose colour, emerging and distended.

Males are more slender than females, and therefore easily selected. One unmistakeable sign that they are ready for artificial propagation is when males release a few drops of milt (i.e. sperm) after gentle pressure on the abdominal part, near to the urogenital opening.

TABLE 5-1
Development stages of warmwater fish, as demonstrated by tambaqui

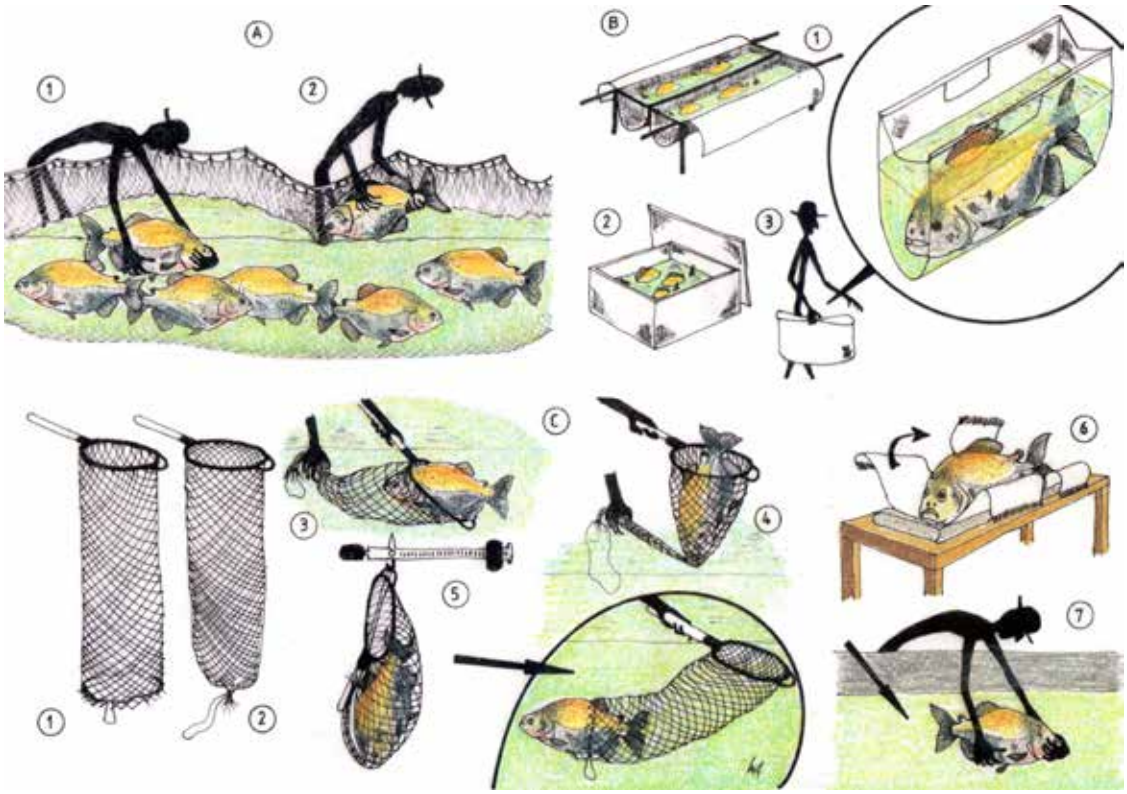
Development stages	Description and expected duration
Developing eggs or embryos	This stage starts with the fertilization of the eggs and finishes with the hatching of fish embryos from the eggs. Duration of this stage: 13–18 hours.
Fish larvae	Hatched fish embryos are called "fish larvae" or simply "larvae". This development stage is from hatching until they live off the yolk sac. For practical reasons, "non-feeding" and "feeding larvae" should be distinguished within this development stage.
Non-feeding larvae	The "non-feeding larvae" stage begins with the hatching of embryos and finishes when the ***endogenous feeding of larvae**** ends. This is the period when the digestive tract of fish larvae is fully developed. This allows larvae to start hunting for suitable food i.e. ***zooplankton****. Duration: 5–6 days.
Mixed feeding of larvae	There is a phase when larvae have mixed, both endogenous and ***exogenous feeding****. This period gives time to learn hunting for zooplankton; in other words, to adjust to feeding from the surrounding water. Duration: 1–2 days.
Feeding larvae	This term covers the stage when larvae start exogenous feeding. This is the time when they should be stocked into nursery ponds for the rearing of advanced fry.
Fry and advanced fry	Biologists dealing with populations of fish in nature use the term "fry". For practical reasons fish culturists of warmwater species use both the term "fry" and "advanced fry". The term "fry" is used for the period between starting exogenous feeding until they lose the appearance of fish larvae (and therefore start to resemble a fish). Duration: 10–15 days. The second stage of the fry period begins when the developing young fish start to look like real fish; this stage ends when the development of the eyes, respiratory system, digestive tract and ***somatic determination of sex**** is complete. By this time advanced fry has a standard length, between 1.5 and 2.5 cm. Duration: 10–20 days. The entire duration of the fry period, depending on water temperature and feeding conditions, is between 20 and 35 days.
Fingerling	This is a practical term for young fish older and larger than advanced fry. This term may often indicate a young fish stocked for rearing table fish. "Fingerling" therefore covers a wide range of sizes varying from 1–2 g up to 100 g. Duration: 1–3 months.
Table fish	***Juvenile**** is a term often employed in literature for a developing young fish, while "table fish" is a practical term used for any size of cultured fish sold for human consumption. Depending on countries, each species has its conventional ranges of sizes accepted or demanded by consumers. In the case of cultured tambaqui this range is between 750 g and 3 kg. Duration: 0.5–2.5 years.
Brood fish	Fish are sexually mature when females and males are able to produce fertilizable eggs and fertile sperm. Sexually mature fish is an adult fish, regardless of its actual size. In fish culture, sexually mature adult fish used for propagation are called "brood fish". Duration: 3–4 years under farm conditions.

FIGURE 5-1
Subsequent steps of the artificial propagation of tambaqui



Photographs: ©FAO/András Woynárovich.

FIGURE 5-2
Works of selection, transfer and weighing of brood fish



(A) Belly of brood fish should be checked gently, without lifting fish out of the water (A1). Those found ready for propagation should be lifted out of the water and examined gently (A2).
(B) Brood fish should be transported in a suitable container.
(C) The both-end-open scoop net has a string with which it can be temporarily closed (C1 and C2). This scoop net is used for capturing (C3 and C4) and weighing (C5) brood fish before they are tagged (C6) and taken (C7) into the hatchery tank.

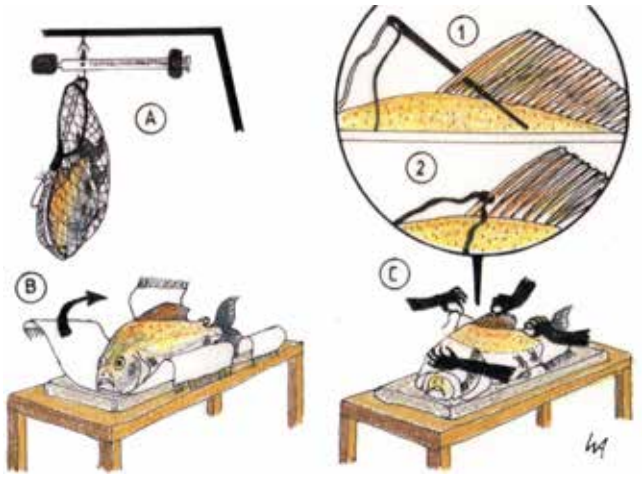
Although tambaqui is not a very sensitive fish, they should be handled with great care and caution during capture, examination, transport and treatment in the interest of successful propagation.

Fertilized eggs can be obtained from migratory river spawning fishes either by ***induced spawning**** or by induced ovulation. In both cases suitable females and males are treated with hormone suspension. With this treatment artificial or natural hormones replaces the fishes' own ***endocrine hormone**** within the neurohormonal process. The hormones induce the final maturation and ovulation of eggs in females and sperm production in males. As demonstrated in Figure 5-4 there are two options (B) and (C) for intervention and inducing final maturation and ovulation of eggs.

Out of the two main options (B and C) presented in Figure 5-4, the injection of preserved ***acetone-dried carp pituitary**** (i.e. carp hypophyses)[2] is by far the most extensively applied technique. Carp pituitary has a rather high market price, which led to the development of less expensive GnRH-analog artificial hormones for fish propagation, including Ovaprim, Dagin, Ovopel, Aquaspawn, Conceptal and Ovudal (Carvalho 2016; Mojica 2016; Aquaculture Innovation, 2018). Each of

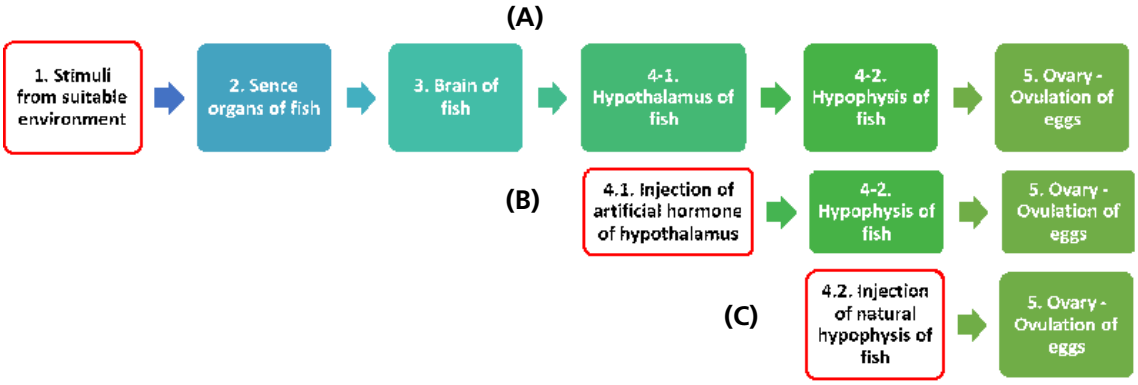
[2] Also called carp pituitary extract (CPE).

FIGURE 5-3
Weighing and tagging brood fish



(A) Weighing, (B) Rapping, (C) Tagging brood fish according to their weight with different coloured strings (1 and 2). Readings of weights of both females and males should be rounded up to kilogram.

FIGURE 5-4
Flow chart of propagation process of fish in the nature and in the hatchery



(A) Process of propagation in nature (see details in Figure 4-4). In the hatchery the natural conditions that could stimulate females to complete this process are missing, therefore requiring the use of hormones.
(B) Use of ***GnRH-analog**** (Ovaprim, Dagin, Ovopel, Aquaspawn, Conceptal, Ovudal, etc.) activates hypophysis, which induces the final maturation and ovulation of eggs.
(C) Instead of triggering the fish's own hypophysis to stimulate the ovulation process, ***preserved pituitary glands**** are injected into female which induce the final maturation and ovulation of eggs.

these hormones has a specific dosing and protocol to be followed, as prescribed by its producer.

Out of the different artificial hormones, Ovopel was deliberately developed to act as a substitute for acetone-dry carp pituitary glands: this and carp pituitary are used in the same way, and according to the same timing, with certain attention to details listed in Table 5-2.

TABLE 5-2
Comparative notes on the use of carp pituitary and Ovopel

Aspects	Acetone-dried carp pituitary	Ovopel
Size of a unit	Usually a gland is 3–3.5 ± 0.5 mg	1 pellet is equal in strength to a carp pituitary of about 3.5 mg
Efficiency	Similar for both hormones	
Programming injection	Similar for both hormones	
Number of doses	Two doses: the first dose is called priming; the second dose is called the decisive dose.	
Preparedness of females	Very important	Extremely important
Stress sensitivity of females	Stressful handling and disturbance, such as noise or a sudden change in water temperature, may stop final maturation and ovulation of eggs.	Stressful handling and disturbance, such as noise or a sudden change in water temperature, will stop final maturation and ovulation of eggs.
Programming ***stripping****	Similar for both hormones	

Both hormones (i.e. acetone-dry carp pituitary and Ovopel) are to be suspended in sterile fish physiological solution (FPS), which is a 0.65 percent NaCl solution. Earlier, this solution was made from distilled or well boiled water and iodine-free salt solution. These days, there is a safe and inexpensive way to achieve the FPS, which is the dilution of the human physiological solution (HPS),[3] available in all pharmacies. Calculations on how to dilute this 0.9 percent NaCl solution into 0.65 percent are demonstrated in Table 5-3. In order to replace the exact volume in the container of HPS, a large syringe of 10 ml or 20 ml or a measuring tube should be used.

TABLE 5-3
Steps to prepare the fish physiological solution from a human physiological solution

Type of saline solution	Volume in the container	Total NaCl in the container (gr)	NaCl (%)
Original human physiological saline solution (HPS)	500	4.50	0.90
Step 1: Take 139 ml out of the 500 ml HPS	361	3.25	0.90
Step 2: Add 139 ml sterile distilled water to the above 361 ml HPS will result the 500 ml FPS.	500	3.25	0.65

The actual quantities of hormone to be given as the first (priming) and second (decisive) doses are outlined in Table 5-4. The exact weight of the doses should be calculated using the "Fish larvae production form" shown in Figure 5-16.

TABLE 5-4
Doses of widely used hormones for male and female tambaqui

Carp pituitary	Females		Males	
	mg/kg BW	*ml/kg BW*	*mg/kg BW*	*ml/kg BW*
1st Injection	0.5	0.5	0.5	0.5
2nd Injection (for fish ≤ 5 kg)	5.0	0.5	2.5	0.5
2nd Injection (for fish ≥ 6 kg)	5.5	0.5	3.0	0.5
Ovopel	*No. of pellet/ kg BW*	*ml/kg BW*	*No. of pellet/ kg BW*	*ml/kg BW*
1st Injection	0.14	0.5	0.14	0.5
2nd Injection (for fish ≤ 5 kg)	1.4	0.5	0.7	0.5
2nd Injection (for fish ≥ 6 kg)	1.6	0.5	0.8	0.5

[3] Also called salt solution.

BOX 5-1

Deviation from the two hormone injection technology

If females are very ripe, one decisive injection is given instead of two. In this case the interval between injection and stripping will change considerably. This interval cannot be predicted, it has to be tested.

Other technologies may suggest the administration of 3 to 4 doses for brood fish to achieve ovulation. However, extensive experience indicates that females that do not react positively and do not ovulate following the described two doses will only be eligible for propagation later, potentially after one or two months.

As presented in Table 5-4, usually two doses of hormone are administrated to both males and females. With these two doses the final maturation and ovulation of eggs can be induced safely. Deviating from this technique is not recommended (Box 5-1).

Because dry hypophysis glands may be destroyed by fungus if they exposed to moisture, and in order to avoid mites attacking the contents, it is important not only to fill the vial (i.e. a small glass bottle) with fine dry cotton over the glands, but also to keep the hermetically closed vial in a sealed plastic bag.

The materials needed for the hormone treatment, shown in Figure 5-6, are simple, cheap and can all be made or purchased locally.

FIGURE 5-5

Calculation of hormone doses with the help of the "Fish larvae production form"

(A)

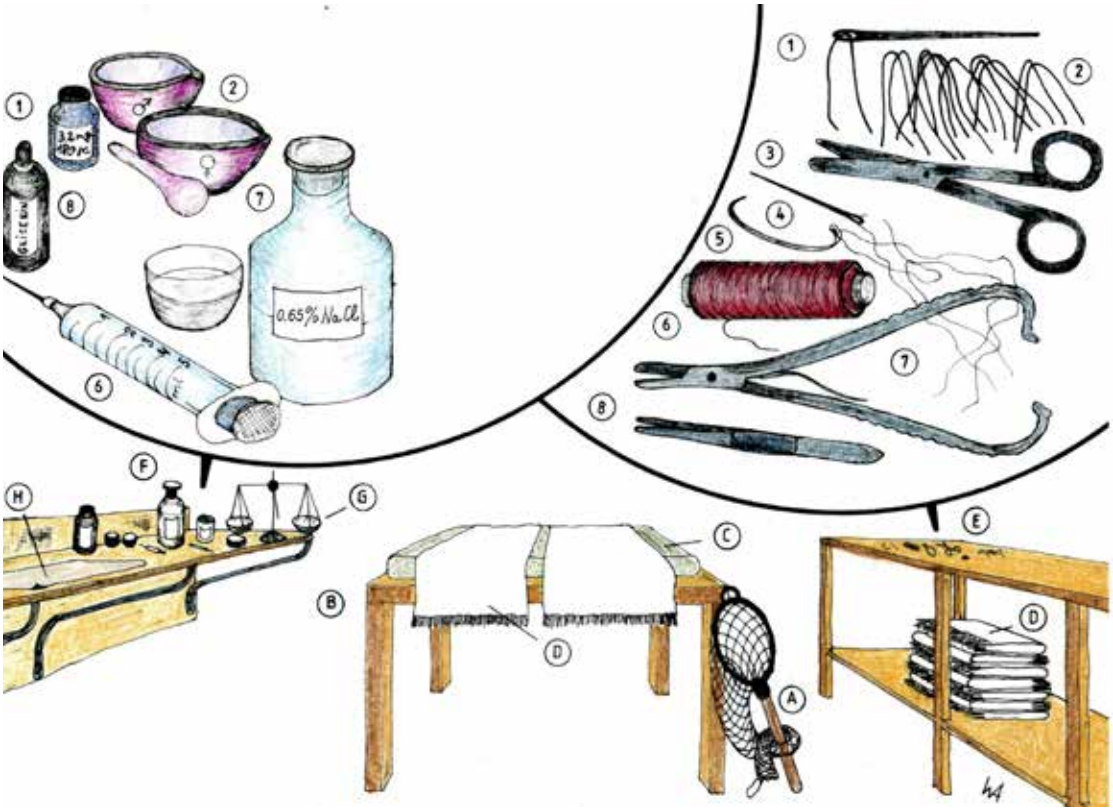
Species: TAMBAQUI		Brood fish		1st Injection		2nd Injection	
		Color of label	Weight (kg)	mg	ml	mg	ml
Females	1	-	4.0	2.0	2.0	20.0	2.0
	2	-	4.0	2.0	2.0	20.0	2.0
	3	Red	4.5	2.5	2.5	25.0	2.5
	4						
	5						
		Total	12.5	6.5	6.5	65.0	6.5
		+ 10 %	~ 1.5	0.5	0.5	5.0	0.5
		Grand total	14.0	7.0	7.0	70.0	7.0
Time: date/hours =>				24/04/17 - 14:30		25/04/17 - 07:15	
Males	1		4.0	2.0	2.0	10.0	2.0
	2		4.0	2.0	2.0	10.0	2.0
	3		4.0	2.0	2.0	10.0	2.0
	4						
	5						
		Total	12.0	6.0	6.0	30.0	6.0
		+ 10 %	~ 1.0	0.5	0.5	3.0	0.5
		Grand total	13.0	6.5	6.5	33.0	6.5

(B)

Species: TAMBAQUI		Brood fish		1st Injection		2nd Injection	
		Color of label	Weight (kg)	No.	ml	No.	ml
Females	1	-	4.0	0.6	2.0	6.0	2.0
	2	-	4.0	0.6	2.0	6.0	2.0
	3	Red	4.5	0.7	2.5	7.0	2.5
	4						
	5						
		Total	12.5	1.9	6.5	19.0	6.5
		+ 10 %	~ 1.5	0.2	0.5	2.0	0.5
		Grand total	14.0	2.0	7.0	21.0	7.0
Time: date/hours =>				24/04/17 - 14:30		25/04/17 - 07:15	
Males	1		4.0	0.6	2.0	3.0	2.0
	2		4.0	0.6	2.0	3.0	2.0
	3		4.0	0.6	2.0	3.0	2.0
	4						
	5						
		Total	12.0	1.8	6.0	9.0	6.0
		+ 10 %	~ 1.0	0.2	0.5	1.0	0.5
		Grand total	13.0	2.0	6.5	10.0	6.5

On the form presented in Figure 5-16: (A) Carp pituitary is calculated by weight, while in case of Ovopel (B) the number of pellets is counted. The effect of 1 pellet of Ovopel is equal to a pituitary gland of 3.5 mg. This allows to prepare the suspension of both hormones in the same way.

FIGURE 5-6
Equipment and materials required for the hormone treatment of fish



(A) Scoop net, (B) stripping table, (C) foam mat, (D) towels, (E1) and (E2) sewing needles with large holes and threads in different colour for tagging, (E3) scissors, (E4, E5 and E6) surgical or sewing needles and soft but strong cotton thread for closing the genital opening. (E7) Needle pincers (pliers, grippers or hook removers used by anglers is also serve the purpose), (E8) pincers, (F6) syringes, (F7) fish physiological solution (0.65–0.7% of NaCl sterile solution), (F8) glycerine, (H) propagation diary, (F1) hypophysis glands in a vial, (G) precise set of scales. Fish tranquillizer may also be useful in case a suitably tested and affordable product is available on the market.

Figure 5-7 demonstrates how to establish the exact quantity of dry hypophyses or the number of Ovopel pellets. The same figure also shows and explains how to prepare the hypophysis solution step by step – this procedure is the same for the Ovopel solution.

Based on several decades of experience with the artificial propagation of fish, injections are administered intra-peritoneally. The idea of injecting hormones into the dorsal muscle or the caudal peduncle was dropped because these techniques are more complicated and often result in losses of the injected solution. The administration of the injection into the abdominal cavity below the pelvic (abdominal) fins has numerous advantages:

- A stronger needle can be used with a wider hole: this way the needle will not become blocked and the solution will never flow back – which is very often the case with intramuscular injection.
- Contrary to intramuscular injections, it is not necessary to massage the injected area.
- The physiological solution can be larger in volume than if injections are applied intramuscularly.

Hypophysis doses for both males and females have to be prepared at the same time, but in separate china mortars, approximately 10 to 15 minutes ahead of the planned injection. For the capture of brood fish, scoop nets with both ends open should be used and the injection should be administered quickly and carefully on a table covered with soft sponge mat. This table will also facilitate the suturing (i.e. stitching) of the female genital pocket as well as the stripping of both males and females (see Figure 5-6).

BOX 5-2

Weighing, tagging and administration of the first injection with a single capture

Knowing the approximate weight of females allows the preparation of the first dose of hypophysis before the fish are captured. However, the doses should only be injected after proper weighing and tagging of females as illustrated in Figure 5-3. This way another capture and handling of the brood fish can be avoided, thereby reducing stress. In this case, the calculation and administration of the first hypophysis injection is done as follows: prepare as much hypophysis suspension as the estimate kg of fish to be selected for propagation. Then, once the females are actually weighed and tagged it is possible to administer the exact quantity of the hormone.

The time interval between the two injections should be 12 to 14 hours at least, but it is better if the interval is 18 to 22 hours. The former timeframe can be used when females look very well prepared: the latter if they do not, in order to ensure safer and more effective ovulation. Figure 5-8 shows the most practical timing of injections, while Figure 5-9 summarizes how its relevant tasks should be completed.

The calculation and administration of the first dose can be done as described in Box 5-2, providing those performing the task have the skills required. Otherwise, the first dose should be calculated and prepared only when the exact weight of both males and females is known. The second dose can be calculated and prepared on the basis of the already known exact weight of females.

Prior to the administration of the second dose, the females' genital pocket should be sealed, as illustrated in Figure 5-9. At the time of – and during – the hormone treatment of brood fish, a number of problems may occur as summarized in Table 5-5.

TABLE 5-5

Frequent problems during hormone treatment of tambaqui

Problem	Explanation
Females ovulate partially or totally well before their expected time.	Females were relatively or absolutely overdosed. – The gonadal development of some females may be extremely advanced; this means they react more quickly than others. This is called relative overdosing of the hormone. – Absolute overdosing: females received more hormone than they should have in accordance with the technology.
Delay in ovulation.	– An increase of the effects of stress during hormone treatment may result in the increased timeframe in which ovulation occurs. In extreme cases the entire process may be disturbed.
Stripped eggs did not swell.	– The eggs ovulated, but were not stripped in time and therefore overripened in the ovaries. – The physiological process of final maturation and ovulation of the eggs was disturbed.
There are a lot of white eggs among good eggs.	– Resorption of eggs developed in the previous propagation season was not completed; they ovulated and were stripped with the ripened good eggs.
Pieces of ovary tissue among stripped eggs	– A typical sign of an overdosing of hypophysis. With this, the female was forced to ovulate before the eggs reached the dormant stage. – Strongly forced stripping.
Eggs swell in a normal way but their development stops at the first cleavage divisions during the second or fourth stage.	– Sperm comes into contact with water too early and may lose its fertilizing capacity. – Either the eggs were overripe but still capable for swelling, or the ***microphyle**** closed before the sperm entered into them.

TABLE 5-6

Temperatures and DO content of water during hormone treatment of tambaqui

Parameters	Min.	Opt.	Max.
Water temperature (°C)	21–22	25–27	30–31
Dissolved oxygen (mg/l)	4–5	6–8	---

It is important that males and females are placed in separate tanks in the hatchery where water flow is continuous. The basic criteria required for brood fish tanks are summarized in Annex 1. The water in the tanks should have the right temperature and contain enough dissolved oxygen (DO) (see Table 5-6 and Box 5-3). If DO contents of water in brood fish tanks is lower than 6–8 mg/l, then diffused air or pure oxygen should be used.

BOX 5-3
Equipment for measuring dissolved oxygen

There are visual DO test kits developed for aquarists and field surveys. Many of these tests are cheap and reliable, and can be used in fish farms.

The speed of final maturation and ovulation depends on water temperature. Higher water temperature accelerates the process, while lower water temperature slows it down. Knowing the ***hour-degree (°H)**** makes it possible to calculate the time when ovulation (i.e. stripping of the eggs) can be expected.

For the sake of proper and timely handling, a maximum of four to five females and four to five males of medium size (about 5 kg each) should be injected at one time. Even with a conservative estimate, the expected quantity of stripped eggs could be as high as 1.2–2.2 million.

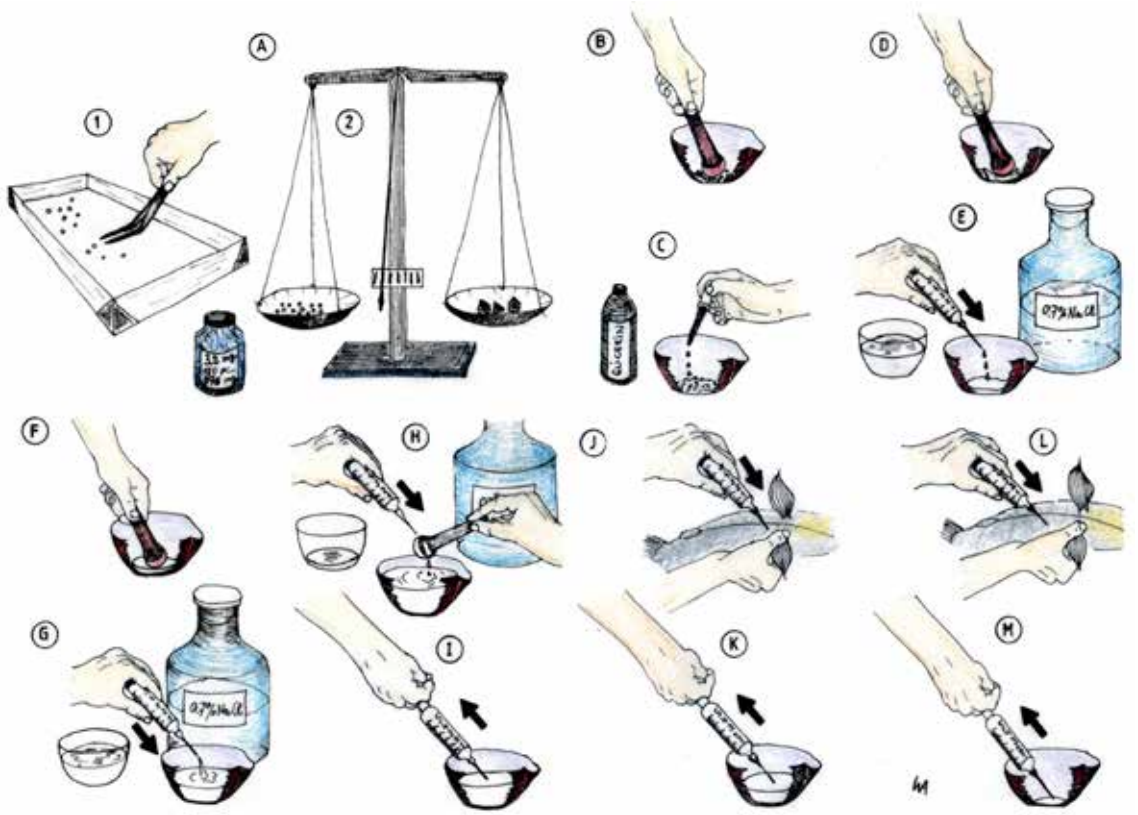
The stripping time can be expected when the hourly water temperature measurements are summed up and reach 260; in other words, ovulation of tambaqui occurs around 260 °H, when the water temperature ranges from 26 to 29 °C. If the average water temperature is between 24 and 26 °C, more is needed – about 270-290 °H. With an average water temperature of 29 °C the hour-degree will be lower, around 230 to 250. This calculation, together with observation of females' behaviour, allows for an accurate start to the stripping. Figure 5-16, the "Fish larvae production form" includes a section where this calculation may be completed.

Water temperature does not usually change rapidly in a fish hatchery, and its daily fluctuation can be predicted through daily routine. This allows an approximate forecast of the due stripping time in advance. Work in the hatchery can be well organized as a consequence. Irrespective of this, one or half an hour before the onset of the calculated time the females should be observed and monitored frequently for signs of ovulation.

Tambaqui often performs typical movements in the hatchery tank when it has ovulated; but because of the sutured genital pocket, females cannot scatter eggs in the tank. Typical movements start about one hour before total ovulation. The females, that have been extremely calm, start to rotate slowly. Fish swim in opposite directions. During this "rotation" they lift up and wind around their dorsal fins. Shortly before total ovulation, slowly moving females dash forwards and start "trembling". This "trembling" is a sure indication that the eggs are ovulated and stripping should start. These movements can also be observed if the fish are alone in a tank. If the suturing is not too taut, a few eggs may be released into the water (another very evident sign that stripping should start). The equipment and materials required for the stripping and fertilization of eggs are shown in Figure 5-11.

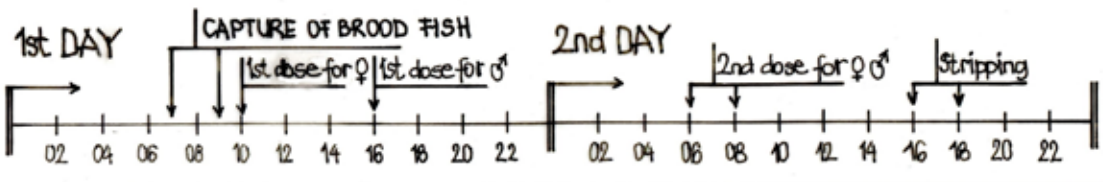
Tambaqui are calm and tranquil, and can therefore be handled (sutured or stripped) without anaesthetic. However, once females have grown larger and stronger, they start to jump around and can be difficult to handle. Therefore, it is advisable to tranquillize them before suturing or stripping. For this purpose, the readily available and inexpensive clove oil (eugenol) can be used, as it acts as an anaesthetic for fish. According to Roubach and colleagues (2005) around 65 mg/l of eugenol is needed to tranquillise tambaqui – interestingly, this is much higher than the quantity needed to achieve the same result with Chinese Major carp or common carp: for these species

FIGURE 5-7
Step-by-step preparation of hypophysis suspension



(A)–(A1) Determining the quantity of hypophysis required by counting on a paper tray or (A2) weighing the pituitary glands. (B) Crashing (pulverization) of the glands in the mortar. (C) Adding a few drops of glycerine. (D) Mixing and smearing the hypophysis with glycerine until it is smooth. (E) Adding a few drops of fish physiological solution (FPS). (F) Mixing and smoothening out the hypophysis with FPS. (G) Adding more FPS while mixing continuously. (H) Washing off the mortar stick (pistil). (I, K and M) Using only as much hypophysis suspension as the needed dose demands. (J and L) Administering the hypophysis.

FIGURE 5-8
Programming of hormone treatment of tambaqui (water temperature: 26–29 °C)

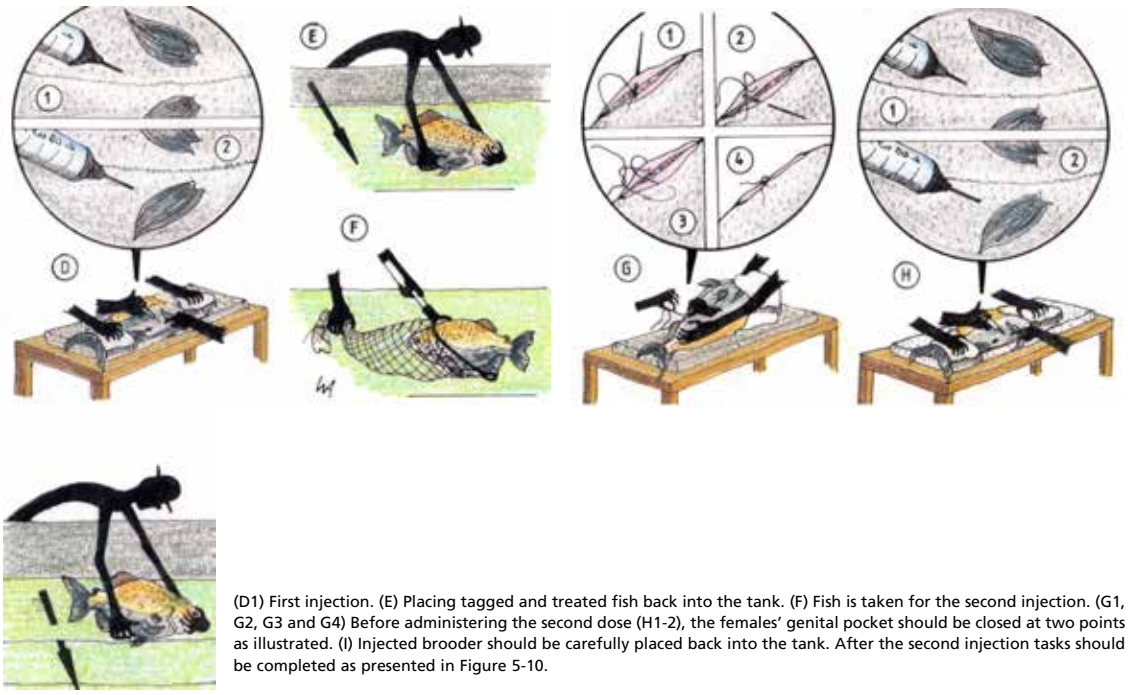


As there is no often reliable hatchery infrastructure – or skilled and conscientious inspection – especially at night, the most critical and fragile period of hormone treatment that starts after the second injection is programmed for the daytime. As this time the movement in the hatchery may be more intensive: a stress-free environment, even with reduced light, should therefore be ensured in and around the brood fish tanks.
Source: Woynárovich (1984)

only 0.5–1 ml/10 l is needed to tranquilize their brood fish. In order to make savings on the tranquilizer brood fish should be transferred into a smaller, approximately 40–50 litre mobile tank or fish carries, as per the examples in Annex 1.

Ovulation concludes in tambaqui females within 15 to 20 minutes. Stripping should therefore not be delayed and must be performed soon after ovulation. This is because ovulated eggs which slip from the follicle and fall into the ovarian cavity do not remain there unchanged whether they are to be stripped or not. Within about 30 minutes to

FIGURE 5-9
Steps in hormone treatment



(D1) First injection. (E) Placing tagged and treated fish back into the tank. (F) Fish is taken for the second injection. (G1, G2, G3 and G4) Before administering the second dose (H1-2), the females' genital pocket should be closed at two points as illustrated. (I) Injected brooder should be carefully placed back into the tank. After the second injection tasks should be completed as presented in Figure 5-10.

1 hour, eggs in the ovary become "overripe" and cannot be fertilized. Eggs that remain in the ovary for 1 to 2 hours even lose their swelling ability.

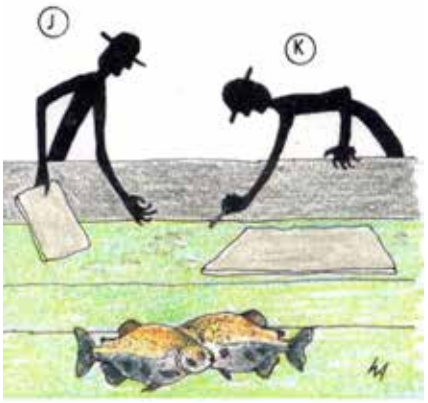
Stripping of tambaqui eggs is marginally different to the stripping of other fish species. The abdomen should be pressed behind the pectoral fins and the eggs are released quickly, in a thick jet. Of course, stitches must to be removed from the genital pocket before stripping.

A female may produce as much as 5 to 20 percent of its body weight (BW) in eggs – which means at least a few hundred grams. In the case of larger females, 0.7 to 1.5 kg or even larger quantities may be stripped. For this reason, it is advisable to divide the mass of eggs into batches of about 200 to 300 g, which can be fertilized in separate plastic bowls. In other words, it is not advisable to use large bowls and handle a lot of eggs together; fertilization will be easier and more effective the other way around. Losses will also be reduced, in the event of any mistakes.

The procedures for stripping eggs and milt (sperm) are shown in Figure 5-12. Ovulated eggs and milt are stripped into a bowl without water, before being mixed and fertilized artificially with hatchery water. The rate of fertilization, when executed properly, may easily reach 95–98 percent.

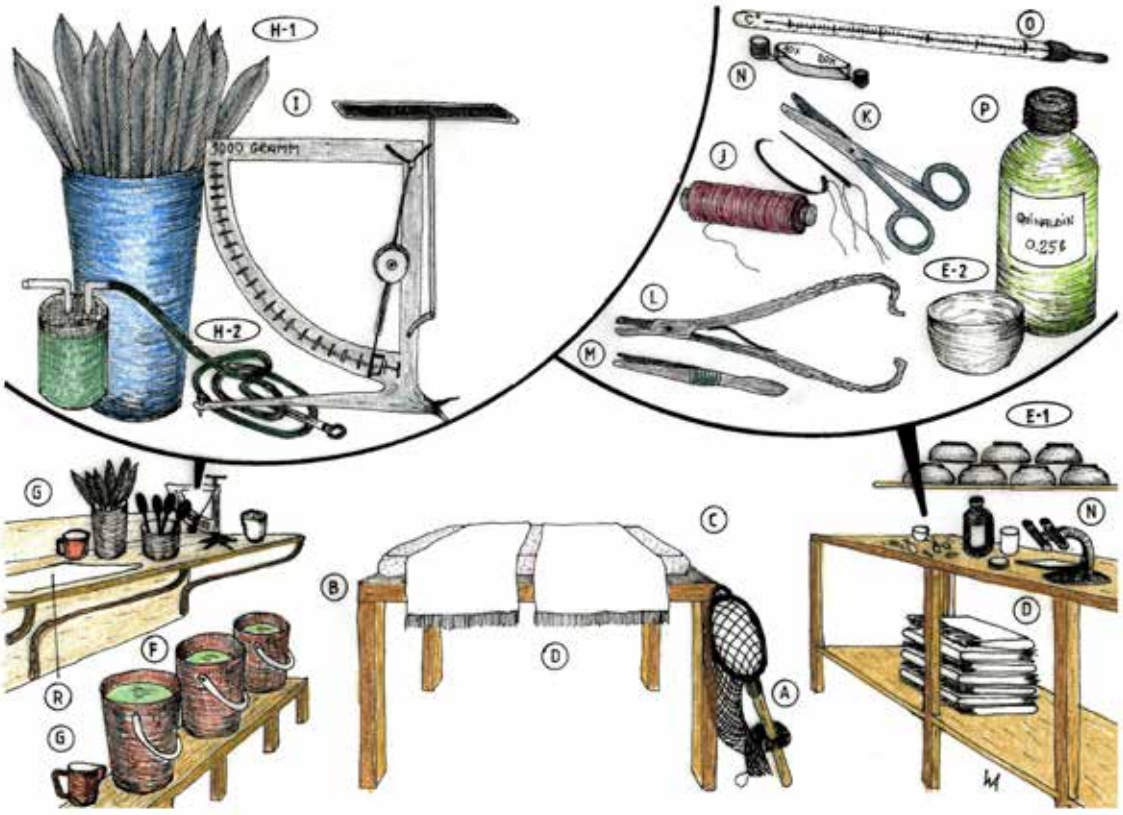
The fertilization of eggs is performed in two steps. First, the milt is added to dry eggs; it is then stirred gently with a strong and dry duck, goose, or cockerel feather. Thereafter, water is added as the eggs are gently but continuously stirred (see Figure 5-13).

FIGURE 5-10
Tasks after second injection



(J) Placing floating stiropor covers on the water surface will reduce stresses and help fish find hidings, (K) Taking water temperature hourly is a precondition of determining exact stripping time.

FIGURE 5-11
Equipment and materials needed for stripping and fertilization of eggs



(A) Scoop net. (B) Stripping table. (C) Rubber foam mat. (D) Towels. (E1) Plastic bowls for the stripped eggs. (E2) Glass or plastic cup for the stripped sperm. (E3) Sperm taking device. (F) Clean hatchery water for fertilization of the eggs. (G) Plastic cups for dosing water. (H2) Feathers for stirring eggs. (I) Letter (or kitchen) scales for weighing the stripped eggs. (J) Needles and string for closing the genital opening. (K) Scissors. (L) Needle pincers. (M) Tweezers. (N) Loupe or microscope for observing the quality and development of the fertilized eggs. (O) Thermometer. (P) Tranquillizer.

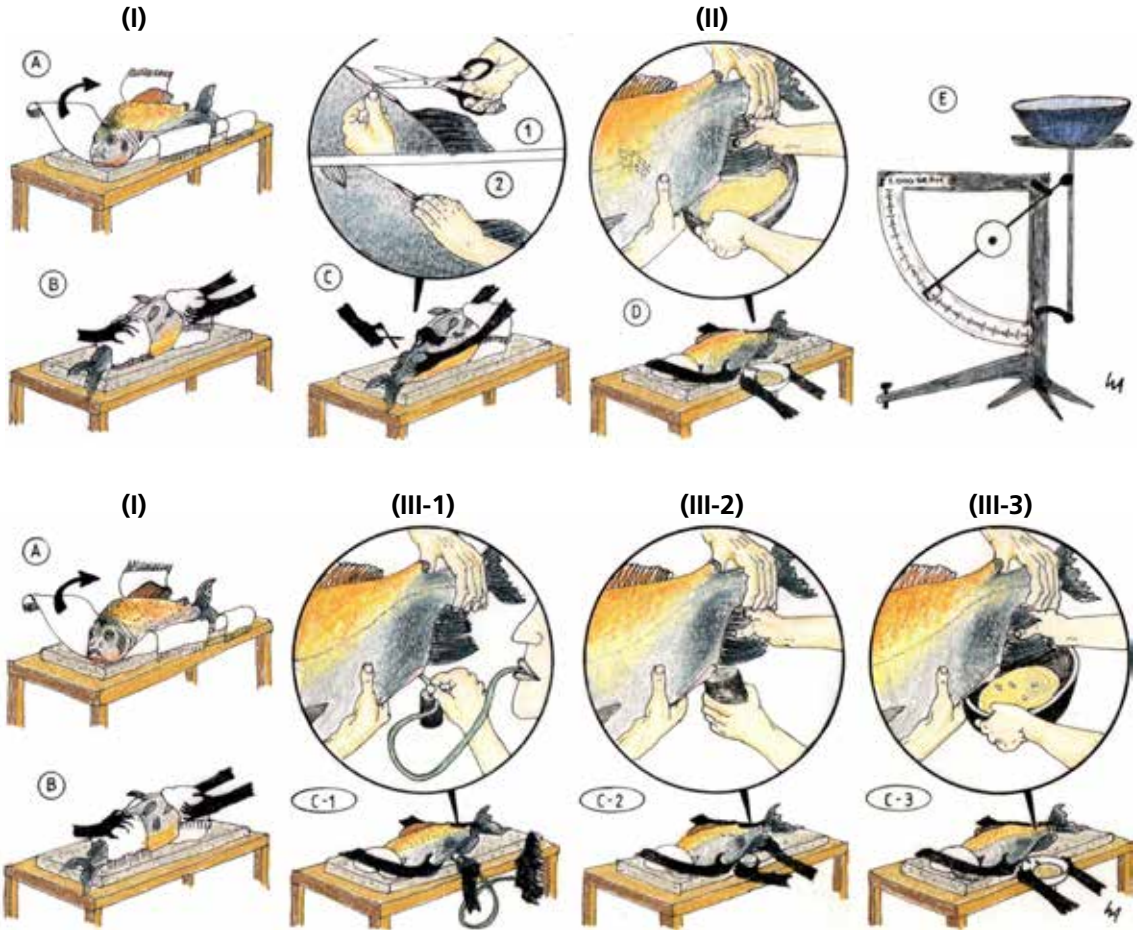
One very important detail: both bowls into which the eggs are stripped should not come into contact with any water at all, otherwise the eggs will start swelling before they are fertilized. For this reason, it cannot be overemphasized that eggs and sperms should be stripped "dry". Both fish and hands should be wiped in order to avoid water dropping onto the eggs and/or milt. Even a few drops may initiate early motion of sperm or the swelling of eggs in which the microphyle closes before sperm enters into the egg.

Males produce sperms (milt) in parts, meaning they can be stripped more than once, but stripping should be done gently, without applying excess force. One male may produce 2–5 ml milt per kg BW. Males can be stripped directly onto already stripped dry eggs, though this requires some practice, otherwise male fish may cause already stripped eggs to splash out of the bowl, or eggs may come into contact with water because of drops dripping from fish. The safer method is to use a sperm collector from which sperm is poured onto the surface of the dry eggs (see Figure 5-13). A sperm collector can be a plastic vial or flask with a mouth of about 4–5 cm in diameter.

Should the volume of milt collected be insufficient, it can be diluted with 10–20 cm^3 of physiological salt solution and immediately (within seconds) distributed over the eggs.

If sperm is not stripped gently, the colour of milt may be pinkish or reddish instead of white. This is because rough stripping causes a haemorrhage in the testicle. Though

FIGURE 5-12
Stripping eggs and milt step by step



Above: **I and II Stripping of eggs:** (A) Covering the head and tail of the female with dry towels. (B) Gently drying the genital opening. (C) Cutting off the thread from the genital pocket (C1) and closing it with fingers (C2) in order to stop jetting out eggs. (D) Stripping of eggs. (E) Weighing eggs. Below: **I and III Stripping of milt (sperm):** (A) Covering the head and tail of the male with towels. (B) Gently drying the genital opening. (III-1) Taking the sperm with a collector, (III-2) stripping milt into a small cup or (III-3) stripping milt directly onto eggs.

bloody milt may contain enough good sperm cells, it is advisable to reduce pressure or if possible wait for half an hour in order to allow the release of a new batch of sperm.

Milt sometimes becomes short, especially when too much of it has been taken for the fertilization of the first stripped eggs. In this case one or two males may be sacrificed by removing their testicles and putting these into a dense dry sieve (plankton net material) in cut pieces: they can then be wrung over the dry eggs.

Testicles can be stored with good fertilizing results for about one hour at room temperature and for several hours in the refrigerator.

After a few minutes of fertilization, any sperm floating over eggs should be washed off with clean hatchery water. After a few minutes the eggs should be placed into the incubator jars so as to let them swell there in very gently flowing water. This is because eggs are sensitive to mechanical effects (shaking, shocks, jerks, etc.) during the first stage of their development. When an egg swells, water penetrates through its semi-permeable egg shell; the complete swelling of tambaqui eggs takes about 30 to 40 minutes after they have come into contact with water.

Eggs which are not "overripe" start to swell independently whether they are fertilized or not. With the degree of "overripening" the swelling is diminishing.

Eggs should be weighed prior to fertilization in order to calculate the number of stripped eggs. The guiding figures for this are presented in Table 5-7.

TABLE 5-7
Some important parameters of tambaqui eggs

Number of eggs in 1 gr of stripped eggs	~ 1 000
Number of dry eggs in 1 ml	~ 1 350
Diameter of eggs prior to swelling (mm)	0.9 – 1
Diameter of vitelline (yolk) globe (mm)	0.8 – 0.9
Diameter of swollen eggs (mm)	4.0 – 4.3

BOX 5-4
Fecundity indicators of female fish

It is important to measure the fecundity of the brood fish from time to time, as this will indicate the reproductive potential of the brood fish and the health of the fish.
Fecundity i.e. performance indicators of female brood fish are:
- absolute fecundity expressed by total weight and/or number of eggs gained per female;
- relative fecundity expressed in weight or number of eggs gained from 1 kg body weight (BW) of a female.

The total dry weight and number of stripped eggs are the starting figures for estimating the quantity of feeding larvae expected to be produced in the hatchery. The way to calculate this is explained in later chapters, whereas Figure 5-16 shows the actual calculations made in the "Fish larvae production form". The same form helps to calculate fecundity indicators of females presented in Box 5-4.

In the case of partial ovulation, although the ovulation lasts for several hours the eggs do not ovulate at the same time. Females with partially ovulated eggs should be put together with males and allowed to spawn in the hatchery tank in order to release ovulated eggs.

5.1.2 Incubation of eggs

Tambaqui eggs are non-adhesive, and belong to the floating variety. Their ***specific gravity****, when swollen, is slightly higher than that of fresh water. They therefore float downstream in the water column of a river but sink down in still waters.

FIGURE 5-13
Step-by-step fertilization of eggs

(A) Distribution of sperm onto dry eggs. (B) Mixing dry eggs and sperm with a feather. (C) Adding a little hatchery water while stirring. (D) Adding more water after about a minute. (E) Pouring off used water from eggs. (F) Adding fresh water to eggs. (G) Placing eggs into the hatchery jar.

Figure 5-14 presents the developmental stages of incubating eggs and growing embryos, showing the swelling, cleavage divisions, morula stages and embryo development stages.

The requirements and conditions for the healthy development of fish embryos are not identical during the course of egg development, and they also differ from fish species to fish species.

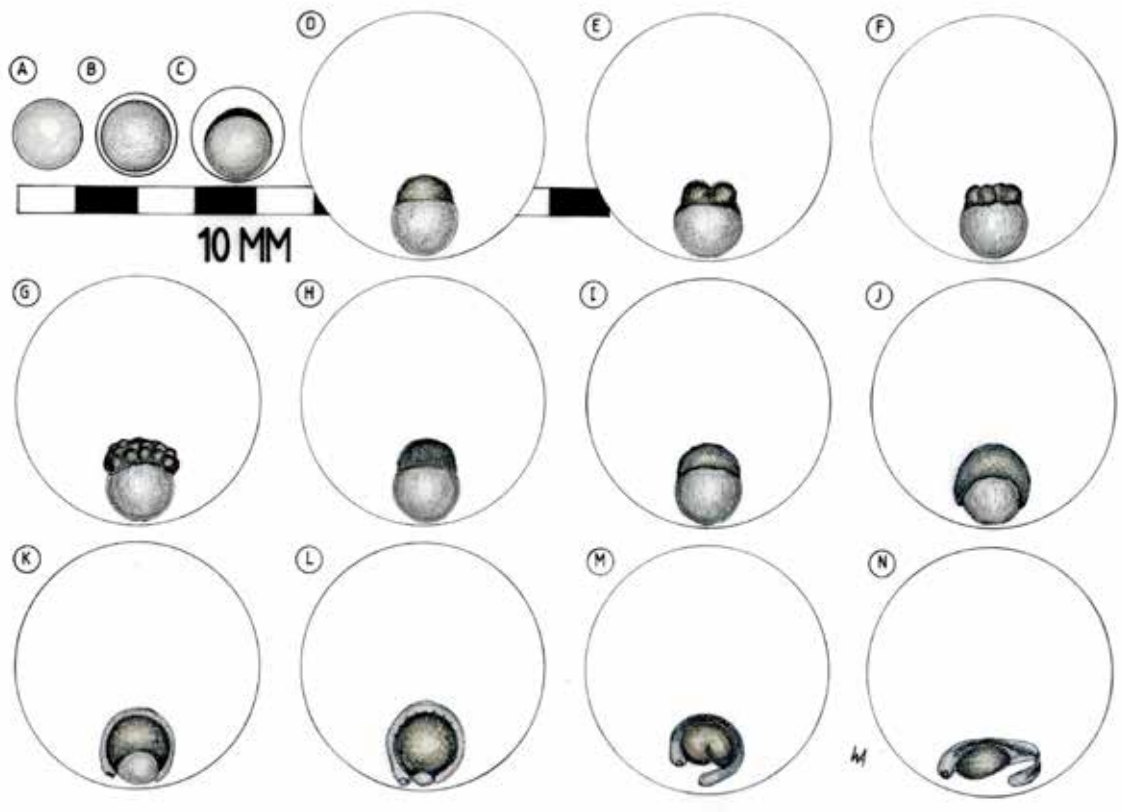
However, there are some essential considerations which are important to know in order to ensure proper development of eggs and non-feeding larvae in the incubation jars:

- adequate water temperature
- adequate levels of dissolved oxygen in the water
- no toxic metabolic wastes and/or harmful microorganisms present in the water
- water free from egg-damaging or predatory animals
- mechanical shock and jerk free incubation of eggs.

The actual duration of the incubation period depends on the water temperature (Table 5-8). Water temperatures that are either too high or too low may put the development of the eggs in danger and increase mortality rates. Temperatures exceeding 31 °C are fatal for eggs of almost all cultivated fish species. High temperatures are especially lethal for eggs in their initial development phases.

In general, the eggs' demand for oxygen is low in the first stages of development. However, as the embryo develops, so its demand for oxygen grows exponentially. Because of their rapid development, tambaqui eggs require high oxygen content in the water from the very beginning of incubation. Otherwise, a lot of crippled embryos will develop, which later results in an increased mortality rate. When oxygen content is 7 mg/l in the incubators' inflowing water, it transports about 2.100 mg oxygen per hour into a 200-litre incubator (inflowing water 5 litres/min). This is sufficient for 200–300 thousand eggs. Keeping in mind that respiration for eggs and larvae takes place either by diffusion through the egg's shell or the larva's skin respectively, the differences in concentration influence the speed of oxygen diffusion into the developing eggs,

FIGURE 5-14
Development stages of incubating eggs and developing embryo



(A) Stripped dry egg. (B) Egg just after fertilization. (C) Swelling of the fertilized egg. (D) Swollen fertilized egg. (E) First cleavage. (F) 4 cells stage. (G) Morula (early stage). (H) Morula (later stage). (I) Blastula. (J) Gastrula. (K and L) Closing of the blastopore. (M) Development of the embryo. (N) Embryo before hatching.

TABLE 5-8
Correlation between water temperature and duration of incubation for a tambaqui embryo

Water temperature °C	26.0	26.5	27.0	27.5	28.0	28.5	29.0
Hours	17.8	16.7	15.9	15.0	14.4	13.5	12.8

embryo and larvae. It is important to make sure that the dissolved oxygen is kept at a level of at least 5–6 mg/litre and that the level is monitored frequently.

There are several types of organisms – such as bacteria, single-cell animals, ***plankton crustaceans**** and decaying organic matter – which, when too many are present in the incubator, may consume large quantities of oxygen. This should also be taken into consideration, and if possible clean water should be used for incubating eggs and rearing larvae.

Abnormal embryo developments and malformations can have two principal causes: 1) an insufficient oxygen supply at the early stages of embryogenesis, or 2) a lack of essential materials in the diet of the female fish which may affect the vitelline (yolk) composition of the eggs. Deformed embryos can vary in shape: some lack the head or tail, or the tail is curved or short; with others, the head or tail are not properly developed. There is an almost limitless variation in the nature of deformed bodies; incomplete development depends on when, at which stage of development, the relative oxygen deficiency occurred. Low oxygen content in the water may also lead to premature hatching. There are cases when oxygen shortage is so acute that hatched larvae die.

In the course of the embryos and non-feeding larvae's development, metabolic wastes are produced, and the resultant excretion of ammonia is toxic if it accumulates in the water.

Bacteria and fungus are microorganisms which usually develop on dead eggs and decaying organic particles; together with toxic metabolic wastes, these may accumulate in the incubation jars. It is therefore important to remove the dead eggs.

Water that is free of egg and larvae-damaging animals such as cyclops, mites or harmful insect larvae, is also a criterion for good quality incubation water.

Tambaqui eggs are more sensitive to mechanical injuries than eggs of other fish species that have thicker and stronger shells. During the course of incubation it can happen that eggs are "stripped" and fatally damaged as a result of the high pressure of water in the incubator. One solution to this problem is for the quantity and pressure of the incubator's water supply to be correctly measured to reduce water pressure and strong currents. The design criteria of good incubation jars are included in Annex 1. The right design of incubation jar will protect eggs (and larvae) from undesirable shaking, shocks and jerks, or any other mechanical motion that may harm the developing eggs.

Because tambaqui eggs have a high demand for oxygen, the number of eggs to be placed in one incubator is limited. Approximately 2 ± 0.5 gr (or 2 000 ± 500 eggs) can be incubated per litre of water in an incubation jar with a volume of 40–200 litres.

The water quantity should be sufficient to ensure 2–3 exchanges within one hour. If the water floats gently in the incubator jars and the eggs are being moved without much agglomerating or settling unmoved in the corners of the jar are the sign of a proper water flow. If

BOX 5-5
Behaviour of tambaqui larvae in the hatchery jar

The behaviour of tambaqui larvae is rather peculiar and worth describing. Freshly hatched larvae swim vertically towards the surface and fall downward afterwards. This alternate swimming up and falling down continues almost uninterrupted for about 2 to 3 days. As the larvae develop, the "swimming up" becomes less frequent, and then the "lie down" period starts, which lasts for about 1 day. After that the larvae swim diagonally upwards again.

eggs start to agglomerate this can lead to mortality rates as high as 80 percent (Araujo-Lima and Goulding, 1997). The water supply is gauged in the incubators by measuring the volume of outflowing water.

5.1.3 Rearing non-feeding larvae

Once all the demands have been met, the development of tambaqui eggs is extremely rapid. Providing the water temperature is not lower than 26 ºC, the whole process lasts less than 24 hours. Developed embryos that are ready for hatching revolve in the egg shell with increasing intensity, while the enzyme produced dissolves the shell. The larvae then break the egg shell and hatch.

It is essential to know how many eggs were fertilized. The rate of fertilized eggs can be determined using a simple technique: a sample of developing eggs should be collected from each incubation jar in a petri dish; the number of live and dead eggs are then counted. With experience, a rough estimate can also serve the purpose. This is usually done in the final phase of germ development, when the germ goes through the gastrula stages; or in the early embryo development stage (see Figure 5-14).

When the fertilization rate is below 40%, it is more practical to dissolve the egg shells artificially with ***alkalic protease enzyme****. The reason for doing so is that egg shells and dead eggs are good substrates for bacteria and fungus (Saprolegnia) which not only destroy healthy larvae, but also consume plenty of oxygen when they are in abundance. Removing them is therefore an important task.

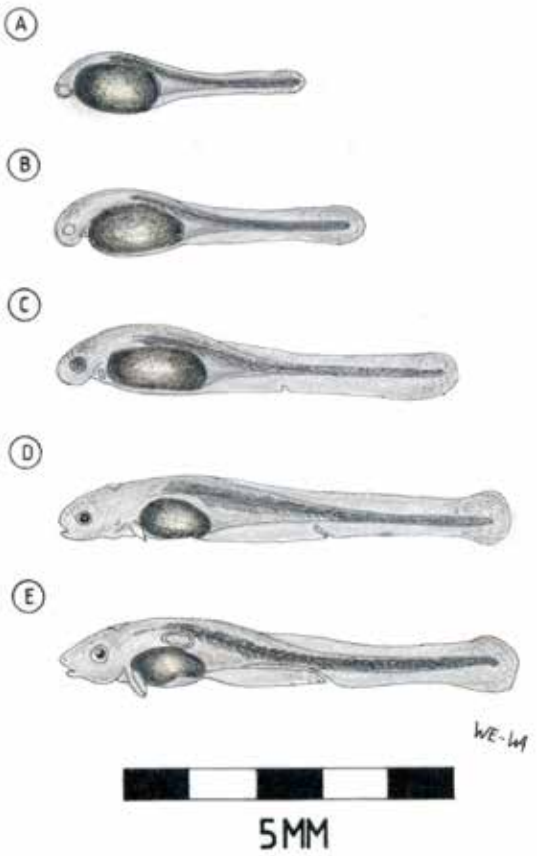
The rearing of non-feeding larvae takes place in the same jars as the incubation of eggs. In order to rear healthy and well-developed larvae, it is important to provide all the necessary conditions in the hatchery jars. These conditions are similar to those necessary during the incubation of eggs. The differences are as follows:

- The increased demand for dissolved oxygen in the water should be met with a 3 to 4-fold hourly exchange of water in the hatchery jar, as well as the removal of increasingly produced metabolic wastes such as CO_2 and NH_3
- To remove egg shells, bad eggs and other hatchery debris, positive phototropism of larvae should be used. Negative phototropism happens when direct sunlight illuminates the jars. Debris, egg shells and bad eggs can be removed in the first hours of darkness following the hatching of an entire batch of eggs. For this, the surface of the jar should be slightly illuminated with an electric bulb or lamp while the inflow of water is stopped. The result will be that for approximately five minutes debris such as dead eggs and egg shells will sediment. This sediment can then be syphoned out with the help of a flexible tube and let into a bucket, as demonstrated in Annex 1. Occasionally, a few larvae may also escape into the bucket. However, if the bucket is filled up with water the larvae will swim up to the surface and can be decanted. This step can be repeated as long as there are viable larvae in the sipped debris. Usually, only the crippled larvae remain in the debris. To save the healthy larvae that might still be caught in the bucket, the remaining debris should be poured into the already prepared nursery (advance fry rearing) pond.

Larvae do not tend to be damaged by water motion in the same way as the eggs. As a result, they may have more inflow water with a stronger current. After the eggs have hatched the inflow of the water should be increased in the jars. This is because the demand for oxygen also increases. During the rather long period of larvae development of about 5 to 6 days they draw on their "food reserves", their yolk sacks (see Figure 5-15).

Tambaqui larvae, similar to the larvae of other bony fish, do not have only the initial development stages of mouth, gills, digestive tube, anus, air bladder, to mention

FIGURE 5-15
Development stages of tambaqui non-feeding larvae



(A) Larvae at hatching. (B) Larvae 8 hours after hatching. (C) Larvae 36 hours after hatching. (D) Larvae 60 hours after hatching. (E) Larvae 84 hours after hatching.

BOX 5-6
Preparation of micro-capsulated chicken eggs

Raw poultry eggs contain the growth inhibitor ovidine; it is important that this must be deactivated by heat before the eggs can be fed to fish. Eggs should be prepared as follows:

- beat the cracked chicken eggs vigorously with a fork or spoon (an electric blender may also be used to emulsify white and yolk);
- boil approximately 150 cm^3 of water for each egg used;
- add the boiling water to the homogenized eggs, while continuing to stir: a fine, opalescent suspension will be obtained;
- double or triple the volume with cold water;
- any unused micro-capsulated egg dispersion should be stored in the refrigerator (Chou, 1980).

only the most important organs. During the non-feeding larvae stage, after about 5 days of development, the larvae have a mouth, gills, a simple digestive tube, an anus and their air bladder is ready to fill up with air. Although they still have 30–40 percent of their yolk sack, when the air bladder is filled up they are able to take on food from their surrounding environment. It is this mixed feeding which allows larvae to learn how to hunt for food and feed on their own. In an additional 1–2 days, larvae consume the yolk sack and start to cover their needs exclusively from exogenous feeding. Larval development and growth is illustrated in Figure 5-15.

For the correct calculation of hatched larvae, the percentage of crippled embryos has to be estimated and noted in the "Fish larvae production form" (Figure 5-16).

As mentioned previously, once the listed organs have all developed, larvae swim up to the surface and take a small bubble of air into the mouth and press that into the air bladder. By doing so repeatedly they fill up their air bladder and so become able to swim in a fish-like manner and get food from the environment. In short, larvae become so-called "feeding larvae".

Shortly after their air bladder is filled with air and the larvae can swim properly, it is advisable to remove them from the hatchery jars and transfer them into nursery ponds. The best and safest option is to stock swimming larvae in a nursery pond that abounds in natural food suitable for feeding larvae. This technology will be discussed later, in Chapter 5.2.

Prior to stocking still in the hatchery jars, the feeding larvae may be offered the boiled yolk of chicken or duck eggs. A simple approach is to press the hard-boiled yolk of poultry eggs through a 60 micron plankton sieve. Another option is to prepare micro-capsulated chicken egg dispersion and use it to feed the fish larvae before stocking. Preparation is described in Box 5-6.

It is important to estimate and record the quantity of hatched larvae and stocked feeding larvae in the "Fish larvae production form" (see Figure 5-16). This form also helps to prepare

and complete hatchery tasks, because all the figures required can be properly calculated using this form.

The quantity of produced larvae provides the basis for rearing fry and is determined by deduction from the quantity of stripped eggs, as demonstrated in Figure 5-16. Recording data is not only useful, but essential.

Filling in this form, among others, enables hatchery staff to archive all data; this allows them to calculate the following, at any time:

- stocking density of brood fish in the preparation ponds (number of fish/1000 m²).
- efficiency of preparation of females (number of eggs/female, number of eggs/1 kg BW of female).
- efficiency of selection and hormone treatment of females (rate of ovulated females as a percentage).
- efficiency of stripping, fertilization and incubation of eggs and larvae rearing (percentage of fertilization, percentage and quantity of hatched larvae).
- Efficiency of larvae rearing (percentage of losses between hatching and external feeding of larvae, quantity of feeding larvae produced and quantity of feeding larvae stocked in ponds).

5.1.4 Programming of feeding larvae production and calculation of the final number produced

The stripping time, together with water temperature, determines the required stocking time for the feeding larvae that have been produced in the hatchery. About 5 days are needed from the time when eggs are stripped and fertilized (see Figure 5-17).

The way of calculating the number of feeding larvae produced in the hatchery (and stocked in ponds) is simple, especially if the form presented in Figure 5-16 is used:

- The weight of stripped dry eggs should be recorded, because it is the starting figure for further calculations.

FIGURE 5-16
Fish larvae production form

Species: TAMBAQUI

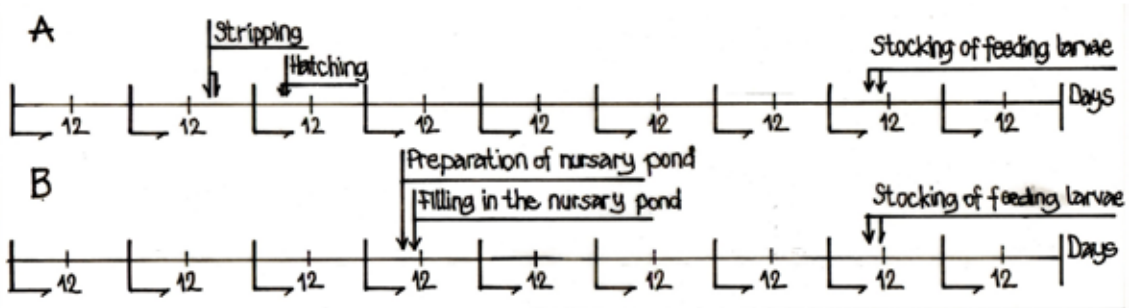
		Brood fish		1st Injection		2nd Injection		Stripped eggs		Fertilization		Hatching		Stocking	
		Color of label	Weight (kg)	mg	ml	mg	ml	kg	No.	%	No.	%	No.	%	No.
Females (+)	1	-	4.0	2.0	2.0	20.0	2.0	0.15	150,000			80	120,000	90	110,000
	2	-	4.0	2.0	2.0	20.0	2.0	0.25	250,000			60	150,000	90	140,000
	3	Red	4.5	2.5	2.5	25.0	2.5	-	-			-	-	-	-
	4														
	5														
		Total	13.0	6.5	6.5	65.0	6.5	0.4	400,000	Not checked		67	270,000	90	250,000
		+ 10 %		0.5	0.5	6.0	0.5	Observations: 1) The 4.5 fish was promising but remained to long while her genital opening was closed. 2) Both females fully ovulated. 3) 1 gland was 3.5 mg, equal to 1 pellet of Ovopel.							
		Grand total		7.0	7.0	71.0	7.0								
Time: date/hours =>				24/04/17 - 14:30		25/04/17 - 07:15		25/04/17 - 16:40		-		26/04/17 - 08:00		01/05/17 - AM	
Males (>)	1		4.0	0.4	2.0	10.0	2.0								
	2		4.0	0.4	2.0	10.0	2.0								
	3		4.0	0.4	2.0	10.0	2.0								
	4														
	5														
		Total	12.0	1.2	6.0	30.0	6.0								
		+ 10 %			0.5	3.0	0.5								

°H	-	27	54	81	108	135	162	189	216	243	~ 260						
°C	2nd injection	27	27	27	27	27	27	27	27	27	Stripping						
Hours	7:15	8:15	9:15	10:15	11:15	12:15	13:15	14:15	15:15	16:15	16:45						

- After the eggs pass the "morula" development stage and are in the blastula or gastrula stages, a sample of a few hundred eggs has to be taken from each incubator. The number of good and bad (white) eggs should be counted, from which the percentage of fertile eggs can be determined easily. A very accurate percentage of good to bad eggs can be calculated if the eggs are collected with a glass tube with an inner diameter of approximately 0.7-0.8 mm. For more accurate estimations, counting can be repeated three to four times with new tests. Another, less accurate, method is when egg samples are taken in a petri dish or in a small flat glass container.

Regardless of the method used for determining the fertility rate of the eggs, the result should be written in the "Fish larvae production form". If there are mortalities due to malformation or mass killing, relevant estimates should be made during hatching and incubation and recorded in the same form.

FIGURE 5-17
Programming of feeding larvae production and nursery pond preparation for tambaqui (water temperature: 26-29 °C)



(A) Expected time of stocking of the feeding larvae. (B) Programming pond preparation and stocking of feeding larvae. Mornings and afternoons of days are separated by indicating noon with '12'.
Adapted from: Woynárovich (1984).

5.2 REARING ADVANCED FRY IN PONDS

The objective of fry rearing is to produce 2–3 cm (ST) fish in a large quantity within a period of about 3–4 weeks.

Out of the three ***main culture systems of fish****, the rearing of advanced fry is conducted in nursery ponds or large outdoor tanks, as that is the most cost-effective way of producing this tambaqui age group. In this case the essential ***natural fish food**** or ***simple fish food**** can be easily and cost-effectively produced in the pond where the feeding larvae are stocked. This advanced fry rearing method is discussed in detail, while other options are summarised in Box 5-7.

5.2.1 Basic concepts and overview of rearing advanced fry in ponds

When larvae begin external feeding there are still some yolk reserves: these ensure the larvae's survival while adjusting from internal to external feeding. These reserves function as a backup in case there is limited food available or accessible. During this mixed feeding period the larvae learn to catch pray from the plankton. As noted by Araujo-Lima and Goulding (1997) the mouth gape is 0.4 mm and larvae can swallow any food items smaller than 0.5 mm. This allows for the consumption of a wide range of zooplankton. The first foods consumed by tambaqui larvae are selected by size. In this group are ***rotifers**** and the smaller young development stages of ***cladocerans**** and ***copepods**** (Nagy, 1998). Developing larvae of about 7–8 mm start to feed on cladocerans, small copepods and chironomid fly larvae, which measure about 0.4–1.0 mm in length. They can also eat larger chironomid larvae (2–5 mm long and 0.2–0.5 mm

BOX 5-7

Rearing tambaqui advanced fry in indoor troughs and tanks

Advanced fry of expensive predator and ornamental fish species are often reared in indoor troughs and tanks. The same can be done with tambaqui. There are three different ways to do this. The devices and densities (300–1 000/100 l) are the same for all three methods. Another similarity of these indoor methods is that all of them are labour-intensive.

The first method involves collecting live zooplankton and feeding it with the growing fry. This is the least expensive, but most labour intensive indoor solution.

The second method involves hatching Artemia and feeding it with the growing fry, while in the third method fully balanced artificial micro feed (also called artificial plankton) is used as a starter, which is later replaced, in the second half of the rearing period, with very high-quality balanced feed. Though the survival rate of these rearing methods are high the price of Artemia cysts (brine shrimp eggs) and their hatching – as well as the fully balanced industrial dry feeds – are usually too expensive to ensure the profitable production of advanced tambaqui fry.

in diameter). The latter are most probably "manipulated" in their mouth before they are swallowed (Lima and Goulding, 1997).

The concept of rearing advanced fry in nursery ponds is simple, and hence widely applicable. If the few key rearing conditions are ensured and the basic aspects followed, good results can be expected. These key rearing conditions and aspects include proper preparation of the ponds, as well as stocking and feeding in proportion to the pond conditions and to the number of stocked larvae.

5.2.2 Pond preparation

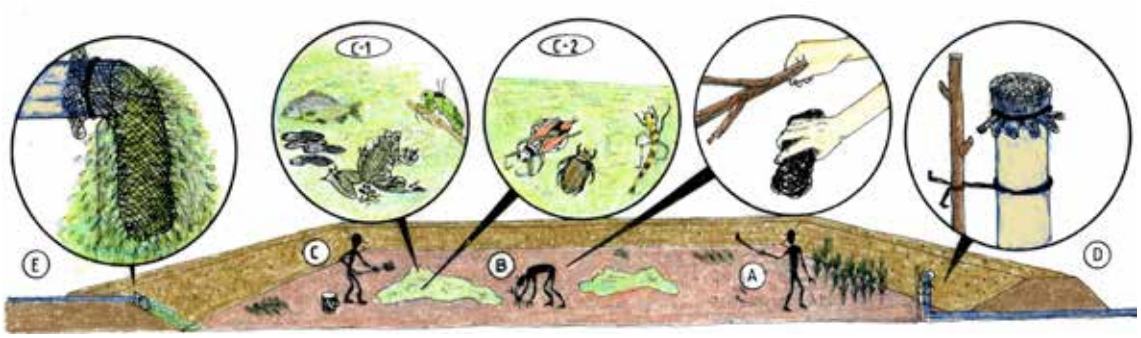
Feeding larvae should be stocked in a well-prepared pond that is free of predators and full of natural food suitable for the developing young fish. To fulfil these criteria, the procedures illustrated in Figures 5-18 and 5-19 should be followed.

Tambaqui nursery ponds can be used for fry rearing about 6 to 10 times a year. It is therefore very important to dry out the nursery pond for about 3 to 7 days between each shift. Consequently, total drainage of the pond and drying its bottom (as much as possible) is the first step of pond preparation. Parasites and the few remaining fish will die in the dry mud.

It is unavoidable that a few small fish remain in some undrained pools at the bottom of the pond, and impossible to stop water insects and frogs from breeding in the remaining pools. However, if these fish, tadpoles, aquatic insects, etc. remain in the pond, they will cause considerable damage to the newly stocked feeding larvae. To prevent this, quicklime should be applied about a day before the planned inundation time of the nursery pond. Treatment of the remaining and undrained pools on the pond bottom (with about 15 to 30 kg quicklime to every 1 000 m^3 of final volume of pond water) ensures that all remaining fish, water insects and larvae are killed, together with any parasites which may have developed and accumulated in the previous nursery period.

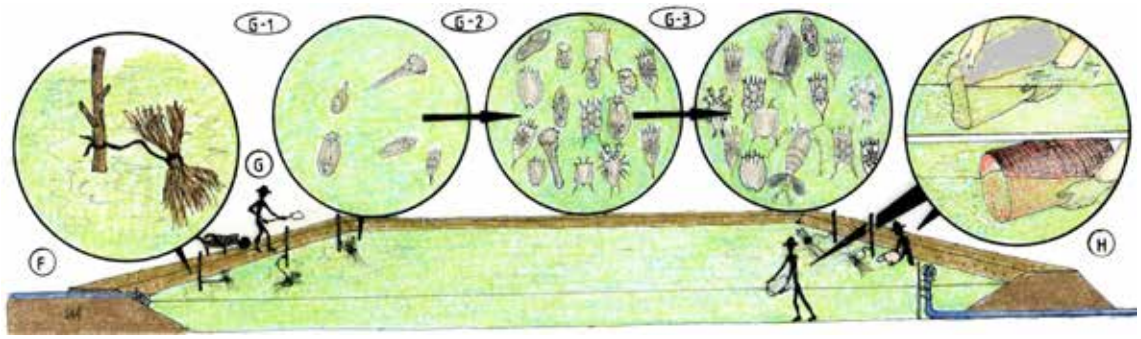
When the preparation of the nursery pond is delayed, the stocked larvae will not find enough suitable food. By contrast, if the preparation of the nursery is done too far ahead of stocking, the suitable plankton succession will have passed and the larvae will find only food that are too large to feed on (e.g. Copepods and Cladocerans). In addition, aquatic insects and their larvae that pray on the tambaqui larvae and fry will populate the pond by the time the feeding larvae are stocked (see Figure 5-20). For these reasons, preparation of nursery ponds and hatchery operations (i.e. larvae production) should be synchronized as demonstrated in Figure 5-17.

FIGURE 5-18
Step-by-step preparation of a nursery pond: cleaning and inundation



(A). Slash the vegetation in the dry pond. (B) Remove stones, branches and cut vegetation from the pond. (C) Apply lime in order to eliminate eggs of frogs, tadpoles, fish (C1) and water insects from the pools of the pond bottom. (D) Close the drainage structure of the pond, in this case by sealing off the pipe. (E) Inundate the pond using a strong sack made out of mosquito net at the inlet. This will prevent unwanted fish of different sizes and ages entering the fish pond. (This technique is necessary only if there is no central filter.)

FIGURE 5-19
Increase of natural food by manuring and stocking of feeding larvae



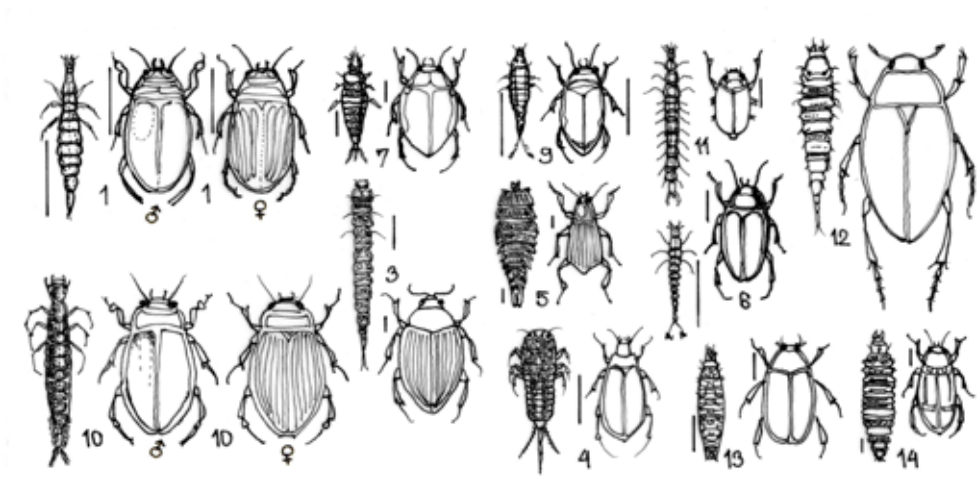
(F) Place bangles of straw into the water to advance the development of zooplankton in the pond water. (G) Apply manure after the pond is covered with water. It is very important to distribute the manure proportionally all over the pond. Because of manuring, the population of zooplankton will develop quickly in the originally "empty" pond water (G1). In the first days the protozoa, rotifers and the young development forms of cladocerans and copepods will occupy the water (G2). After this time only a few adult (i.e. fully grown) plankton crustaceans can be found in the pond water (G3). (H) One or two days after filling up the pond the feeding larvae should be stocked. Parallel with the feeding larvae, population of plankton crustaceans will grow, which ensures natural food for the growing fry.

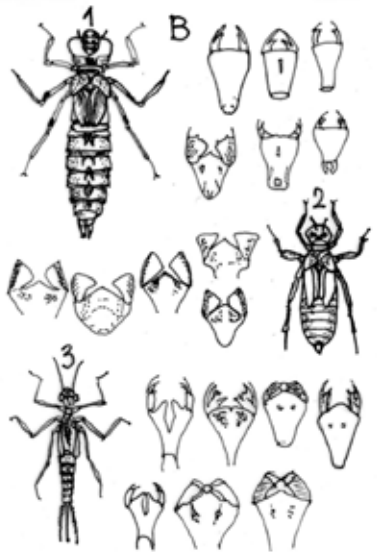
A prepared (i.e. dried, cleaned and limed) nursery pond should be inundated about one day after the larvae hatch in the incubator jars (see Figure 5-17). Considering that any type and size of fish is unwanted in a pond where feeding larvae will be stocked, the water inflow should pass through a mosquito net as demonstrated in Figure 5-18. First, the pond should be filled up as fast as possible to a minimum of 50–60 cm depth, then the water should be immediately manured. Table 5-9 shows the relevant quantities of the most frequently employed manures. When a nursery pond is used in "chain", the quantity of manure may be decreased by 20 to 50 percent. This is because the pond bottom becomes saturated with organic materials, and lower quantities of manure are therefore sufficient to develop rich plankton populations.

Alongside manuring, bangles of dry hay or rice (paddy) straw should be fixed with sticks around the dam (i.e. pond sides) about 5 to 10 meters apart, altogether 20 to 25 kg/1 000 m3. The hay or straw is very useful for the quantitative and qualitative development of protozoa and rotifer populations, because they increase the nursing capacity of ponds.

According to Ray DeWandel personal communication (2017) the even distribution of a few kilograms of dry rice husk over the water surface provides shelter and generates micro-plankton production in the water.

FIGURE 5-20
Water beetles, their larvae and dragonfly fly nymphs





Above: 1) *Acilius* sp. A relative to great diving beetle. 3) *Haliplus* sp. Larvae feeds on *filamentous algae*. 4) Screech beetle (*Hygrobia* sp.). 5) *Helmis* sp. larvae found under stones. 6) Agabus sp. common in waters. 7) *Hypydrus* sp. 9) *Ilybus* sp. 10) Great diving beetle (*Dytiscus* sp.). 11) Whirling beetle (*Gyrinus* sp.). 12) Great silver beetle (*Hydrophilus* sp.). 13) *Hydrobius* sp. 14) *Laccobius* sp. (Adapted from: Clegg, 1967)
Left (B): 1) Long-bodied dragonfly nymphs and their distinctive masks. 2) Short-bodied dragonfly nymphs and their distinctive masks. 3) Slender-bodied damsel nymphs and their distinctive masks. (Clegg, 1967)
Source: Woynárovich *et al.* (2017).

5.2.3 Stocking

The actual length of advanced fry rearing depends on water temperature, as well as on the quality and quantity of food available to the fry.

The quantity of feeding larvae stocked in a nursery pond may differ depending on the conditions farmers are able to provide in their nursery ponds. If a fish pond is well

Manure should not be applied in the nursery ponds during the entire fry rearing period.

TABLE 5-9
Recommended quantities of the different manures to be used in nursery ponds

Layer hen or duck or chicken	Pig	Cattle
80–120 kg/1 000 m³	120–150 kg/1 000 m³	200–300 kg/1 000 m³

Observation: In case of dry or washed out manure the higher figure, and in case of fresh manure the lower figure should be calculated.

prepared and fertile, and the feeds provided are both qualitatively and quantitatively adequate, then the stocking of the feeding larvae of warmwater fish species such as tambaqui may be intensive. However, if some of the determining conditions demonstrated in Figure 5-19 cannot be ensured, the stocking density should be reduced, as shown in Table 5-10. As a general rule, 100 feeding larvae stocked per 1 m^3 of pond water is a good semi-intensive level of advanced fry production.

5.2.4 Feeding

One of the key factors of fry rearing is to provide proper food for the developing fry, because young fish have to grow to several hundred times their weight within a period of three to four weeks.

Most feeding larvae of cultivated warmwater fish species eat zooplankton as their first food in the period up to 15 to 25 days of age. After this period, they switch to the natural food that is typical of the species. Young tambaqui consume zooplankton for a longer time which, complemented with feeds of plant and fruit origin, may remain their main natural food for some years.

TABLE 5-10
Intensity of stocking density of feeding larvae of major Latin American characids (number of fish/1 000 m^3)

Extensive	Semi-intensive	Intensive	Super intensive
25 000–75 000	75 000–150 000	150 000–225 000	225 000–300 000

Observation: Within the above indicated ranges pond/rearing conditions determine whether the actual number of stocked feeding larvae is nearer to the lower or to the higher indicated value.

Rotifers and smaller specimens of developing plankton crustaceans, such as cladocerans and copepods, which are more rich in protein, are the first natural food of tambaqui larvae (Nagy, 1998). These should be plentiful in the water following manuring because they will later be important in the diet of young fish. These natural foods supplemented with feeds, called ***supplementary feeds****, will ensure adequate nutritional resources for the rapid growth of fry: this is because when natural food is consumed together with supplementary feeds, the nutritive effect will be highly superior to those cases when fish receive only natural food or feed alone.

The basic aspects of feeding tambaqui fry are the same as those applicable to other fish species:

- The size of the feed particles must be smaller than the mouth of the young fish. The first supplementary feed should therefore be offered in powder, followed by a fine meal form. During the culture process, the feed particles can become larger, but still proportional to the actual mouth gape.
- To achieve good growth rates, young fish need feeds with a high protein content (at least 40 percent), and the quality of feed used should be almost the same during the entire nursery time (see Table 5-11). According to many authors, in the first few days it is difficult to detect feed in the digestive tract of larvae (Kubitza, 2004). Distribution of feed from the day of stocking remains important, as it will nourish the system (i.e. zooplankton).
- The quantity of feed should be increased during the nursing period. The quantity of applied feed is proportional to the number of stocked feeding larvae. Table 5-12 shows the applicable daily quantity for 100 000 feeding larvae of tambaqui.

Based on extensive experience it can be concluded that only relatively modest survival rates will be obtained if pond preparation and feeding is not correct; alternatively, in the event of proper preparations and proportionate feeding, stocking intensification will be returned with excellent results in an almost linear character.

When the procedures are followed properly (explained in Figure 5-21) advanced fry will be uniform in size. Large differences in the size of the advanced fry produced

indicate a shortage of natural food and/or supplementary feeds. Therefore, in extreme cases, considerable differences in size of advanced fry may occur.[4]

TABLE 5-11
A simple formulated feed for rearing advanced fry

Contents	First option (%)	Second option (%)
Rice	25	-
Maize	-	25
Soya meal	25	25
Fish meal	25	25
Meat/blood meal	25	25

Adapted from: Horváth and Tamás (1981)

TABLE 5-12
Recommended quantity of feed for 100 000 of stocked feeding larvae

Week	Quantity (kg)		Quantity (kg/week)	Quality consistency of the feed
	Morning	Afternoon		
1	~ 0.25 *	~ 0.25 *	~ 3.5	powder **
2	~ 0.5	~ 0.5	~ 7	powder then very fine
3 ***	~ 1.0	~ 1.0	~ 14	fine
4	~ 1.5	~ 1.5	~ 21	fine
5	~ 2.0	~ 2.0	~ 28	fine

Source: Woynárovich (1984).

Observation: * This is so as to adapt to the artificial feeds. ** Feed mixture after milling should be sieved with the bigger particles separated and given to bigger fish. *** From the third week onwards the actual number of fry should be estimated and the quantity of feed adjusted accordingly, because feeding should be done on the basis of day-to-day checking whether given feed is consumed.

During feeding the development of young fish can be observed well. When feed is distributed into a nursery pond containing tambaqui fry that are only a few weeks old, the water surface will "boil" because of the hungry young fish grabbing for the supplied feed. A simple farm-made mixture of widely available feeds presented in Table 5-11 and Figure A3-8 are satisfactory for nursing advanced tambaqui fry in ponds. The quantities and frequency of feeding are presented in Table 5-12.

5.2.5 Follow-up advanced fry production

An integral part of the work at the fish nursery is the observation of developing fry at least twice a day. Their behaviour, and eagerness to feed (congregation at the feeding place when the feed is given) provide valuable information about the actual state and size of reared young fish. It is important to check the growth weekly by measuring a minimum of 10 to 20 fish on the same day of each week during the rearing period.

The quality and quantity of plankton should also be observed frequently, as explained and demonstrated in Chapter 1.2.1 of Annex 3. There is another group of organisms that should be checked in tambaqui nursery ponds, especially in the first half of the fry rearing period. These are the predators of developing fry which may cause considerable losses if they are present in large numbers. The most common and frequent predators of feeding larvae are presented in Figure 5-20. These include aquatic insects, different dragonfly nymphs, odonata, the water boatman, backswimmers and the tadpoles of some frogs. Out of these listed fish larvae predators odonata can be

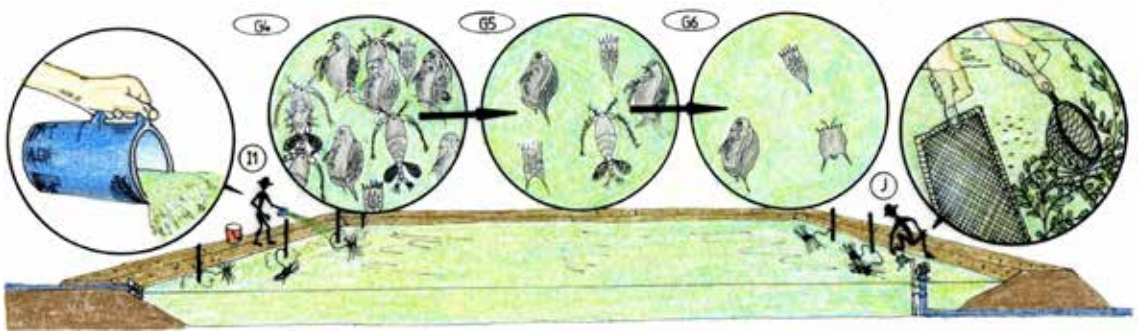
[4] The outstandingly big specimens are usually "cannibals" which eat other weaker, wounded or dead fry.

the most dangerous in nursery ponds that are left filled with water long before larvae stocking. Odonata and many of others can be killed with an insecticide called Folidol in a concentration of 0.25–0.5 ppm (CODEVASF, 1986; Galvao and Franca, 1986).[5]

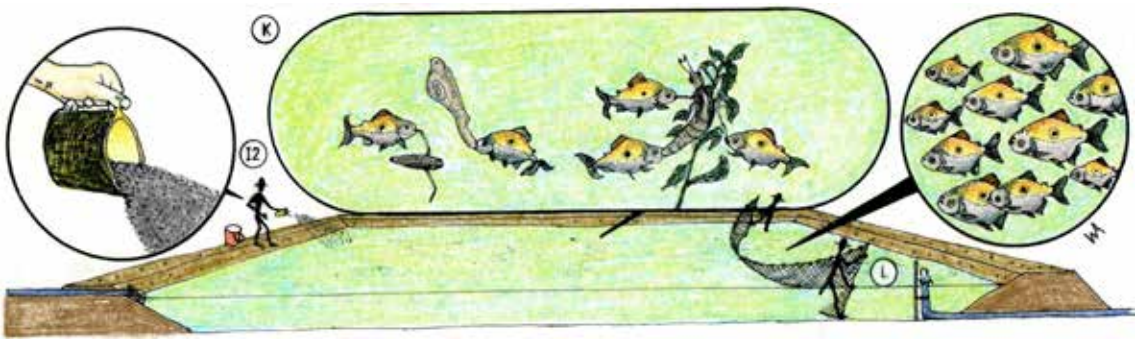
Predators are less harmful when they gradate in large numbers during the second half of the fry rearing period. By this stage tambaqui fry are able to escape from them, and some of the fry can even grab and eat them (see Figure 5-21).

There are a wide range of insecticides under same or similar brand names, but with different strengths and effects. Testing them before they are actually used is therefore strongly recommended. It is also important to use only officially approved products with due protection for human health and the environment.

FIGURE 5-21
Feeding and monitoring the growth of developing fry



After the first week of inundation of the nursery pond, the plankton crustaceans will grow in number (G4); this will reach a peak (G5), then the quickly growing fry will consume them. Therefore, the pond water loses natural foods towards the end of the fry rearing period (G6).
(I1 and I2) In the first ten days the developing fry should be fed with liquid feed. This helps distribute the smaller quantities of feed properly. Later the feed may be distributed dry.
(J) It is important to check both the developing fry and the aquatic insects and their larvae, which live on and among the water weeds. This enables the detection of odonata and other unwanted insect predators (see Figure 5–20) so that the necessary measures can be taken in time.
(K) Predator insects and their larvae are especially dangerous at the early stage of fry development. When fries are bigger, they attack and feed on their earlier predators.
(L) When fries grow bigger than 2 cm (SL) harvesting should start.



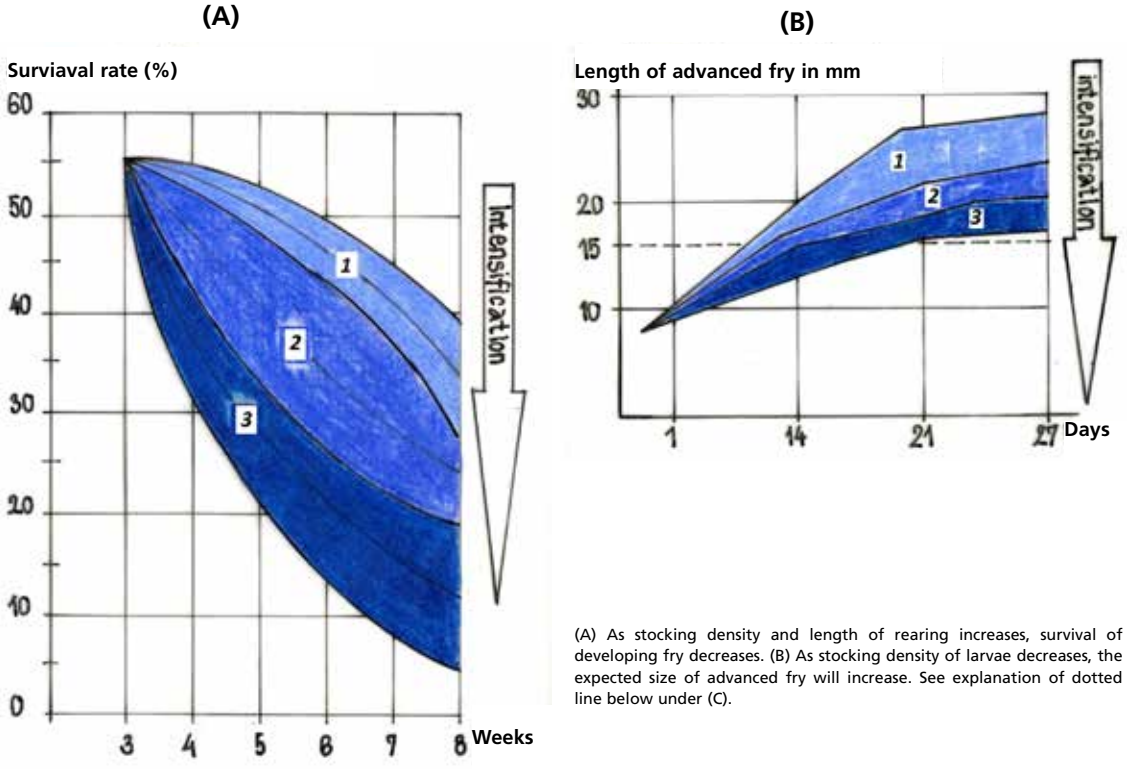
[5] The concentration of Folidol - Emulsion 60 should be 0.2–0.4 ppm (parts per million). This means that 0.2–0.4 litres of chemical should be applied into 1 000 m3 of pond water. (The lower concentration kills slowly, within 24–30 hours.)

5.2.6 Survival rate and harvesting

Survival rates up to the size of advanced fry may vary from 30 to 70 percent,[6] giving an average of about 55 percent, providing that the management is proper and the rearing does not last longer than about 30 days. After this time, if advanced fry are not removed from the pond, their mortality will increase rapidly and the survival rate will decrease to about 20 percent, especially in the event of a pond more crowded with advanced fry (see Figure 5-22).

Due to the lack of natural food, mortality rates increase dramatically if the tambaqui fry are left in the same density for more than 30 to 35 days. Even the best supplementary feed cannot substitute the natural food that is consumed by this time. The physical condition of produced fry becomes worse and worse when the natural food decreases in the pond, and cannibalism among the fry also increases. As a consequence, the fry are not able to endure fishing, handling and transportation. In other words, when

FIGURE 5-22
Schematic correlation between stocking density and the harvested size and number of advanced fry produced



(A) As stocking density and length of rearing increases, survival of developing fry decreases. (B) As stocking density of larvae decreases, the expected size of advanced fry will increase. See explanation of dotted line below under (C).

(C)

SL (mm)	13	14	15	16	17	18	19	20	21	22	23	24	25
Weight (mg)	50	52	54	110	130	160	190	240	270	310	350	410	500
Weight (gr)	0.05	0.05	0.05	0.11	0.13	0.16	0.19	0.24	0.27	0.31	0.35	0.41	0.50

In figure (A) and (B) the intensity of fry rearing marked as 1) extensive, 2) semi-intensive and 3) intensive.
(C) Correlation between length and weight of developing and advanced tambaqui fry. About 16 mm SL is the smallest size to capture advanced fry safely.

[6] Low stocking density results in a higher survival rate; higher stocking density may result in a lower survival rate, especially if the intensity of stocking is not proportionate to the conditions and the quality of management.

the stock of fry is weak and undernourished, parasite infections and diseases usually appear. This may result in a high mortality rate, and even the whole stock may die on occasion.

A frequent mistake, demonstrated in Figure 5-22, is that some farmers prefer to wait until fry grow to a greater length. However, fry do not grow in length, but weight. Extending the time that fry are kept in the nursery pond can easily result in considerable quantitative and qualitative losses to the stock. The reason for these losses is the same as that discussed earlier. The solution to this problem is for the fry already produced to be sold, or stocked in other ponds in lower densities if the farmer needs advanced fry of larger sizes.

When the stock of advanced fry produced in a nursery pond is reduced by 40 to 60 percent through partial harvesting, without decreasing the water level, the remaining stock may be left in the same pond for an additional 1 to 2 weeks, if this is for some reason unavoidable.

The catching of advanced fry should be conducted with a net of a suitable mesh size. If a net with a bigger mesh is used, a high number of fry get caught in the net by their gills and can be fatally injured. The mesh size for the recommended net for fishing is from 1 to 3 mm for small advanced fry, and 5 to 7 mm for bigger fry. The length of the net may vary from 5 to 10 m, while the height of the net should be about 1.5 to 2 m. Otherwise there will be no pocket of the net in which captured young fish can be concentrated. Soft, flexible but strong mosquito nets are especially suitable for making fry nets (see in Annex 4).

The quality of the net is also important when advanced fry is fished. Netting materials which have knots are not suitable for capturing either advanced fry or any other age groups of fish intended to be reared further, because knots will injure fish. These often minor injuries will be the entry points for disease, which will cause an increase in mortality. In many countries where it is difficult to purchase fine netting materials normal curtain material is used for making nets. Figures in Annex 4 show the most important characteristics of nets to be used for capturing advanced fry.

Catch of some thousands of advanced fry is usually achieved when attracting them by feeding. The other way of catching advanced fry is attracting them with fresh water. Fresh in-flowing water attracts fish and since they have accumulated they can be easily captured. With these techniques there is no need to drain or lower pond water.

Draining of a nursery pond is permitted only after a large part of the advanced fry have already been removed from the pond and it is no longer possible to catch large numbers anymore, or when at least 50 to 60 percent of the stock is to be removed on the same day.

It is important to drain nursery ponds slowly. If done quickly, advanced fry will remain in the deeper pools and remaining puddles, as well as between and under water plants.

After decreasing the water level, it has to be increased again because keeping fry in shallow water will result in substantial predation by birds and other fish predators. While refilling, the pond sieve must be used to prevent the entry of unwanted fish.

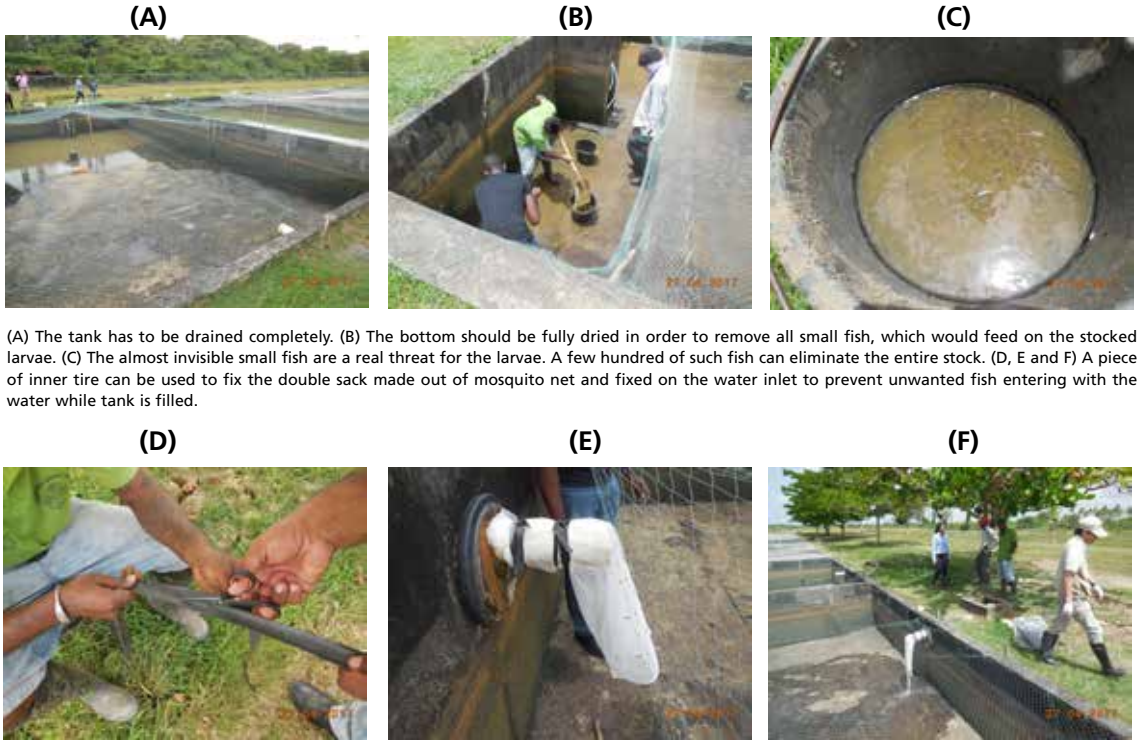
Captured fry should be transferred as quickly as possible into the conditioning tanks, where they are given continuously flowing water and will remain until they are transferred to another pond, or are sold.

It is strongly recommended that a diary or logbook is kept, where all the important production data can be registered. Figure 5-24 provides an example of how to organize this work.

5.3 REARING ADVANCED FRY IN LARGE CONCRETE TANKS AND LINED EARTHEN PONDS

Using large concrete tanks or lined earthen ponds as nursery ponds is also frequent on farms where there is no suitable soil for constructing earthen nursery ponds. The necessary technology for how to prepare such tanks is presented in Figure 5-23.

FIGURE 5-23
Steps in the preparation of a concrete tank used as nursery pond



(A) The tank has to be drained completely. (B) The bottom should be fully dried in order to remove all small fish, which would feed on the stocked larvae. (C) The almost invisible small fish are a real threat for the larvae. A few hundred of such fish can eliminate the entire stock. (D, E and F) A piece of inner tire can be used to fix the double sack made out of mosquito net and fixed on the water inlet to prevent unwanted fish entering with the water while tank is filled.

Photographs: ©FAO/András Woynárovich.

FIGURE 5-24
Form of stocking, transfer and sale of fish

DATE	TRANSFER (Write pond No.)		NAME OF FISH	QUANTITY OF FISH (No.)				INDIVIDUAL SIZE OF FISH		TOTAL WEIGHT OF FISH	TOTAL PRICE OF FISH
	From	To		Feeding larvae	Adv. fry	Fingerling	Grower	cm	gr.	(kg)	($)
Total of page											
Total of month											

Each batch of fish transferred within the farm or sold from the farm should be written in a separate row. When larvae come from the hatchery, no pond number should be written in the "from" column but an "H". In case of sale an "S" should be written in the column "To". When the page is filled it should be summed up. At the end of the month all pages within the period should be summed up – this will facilitate an easy and swift overview and evaluation of production.

In this type of tanks or lined ponds less manure should be applied than in earthen ponds. The way how to do this is simple: placed loosely in an agricultural sack used for keeping grain or fertilizer, the manure should be washed evenly into the previously filled tank or lined earth pond. When this technique is used, the large particles of manure will remain in the sack, while particles small enough to solve in the water will ensure the expected result, i.e. fertile water in which zooplankton develops.

5.4 MONITORING AND EVALUATION OF ARTIFICIAL PROPAGATION

The overall aim of the artificial propagation of fish is the production of advanced fry. After presenting the elements of artificial propagation, the recording of important data is essential both for monitoring and evaluating the efficiency of the work. The analysis of recorded data not only avoids errors being repeated, but also helps to develop the technology by detecting unknown or little-known elements and optimize the entire production cycle. Filling out the "Stocking, transfer and sale of fish" form (see Figure 5-24) is recommended in order to monitor the fry rearing stage. All the production figures needed can be calculated using this form:

- the stocking density of feeding larvae (number per unit area), survival rate (percentage), and the total number of harvested and sold fish;
- length of advanced fry production (days);
- average size of harvested fish (cm or g).[7]

By also maintaining a feed register, the utilization of feeds applied both in the brood stock and the fry rearing ponds can be calculated at every stage of the production cycle, as well as the ***feed conversion ratio (FCR)****. Optimizing the feed supply and feed conversion rate is important for the growth of the fish, as well as the economic feasibility of the farm, as feed costs are a major component of overall production costs.

When designing the recommended form, its designers were aware that record-keeping and administration is not popular a task among fish farmers. The intention was to make this work as simple as possible, while still making sure that all the important data for the calculation of the overall results of artificial propagation (i.e. its physical and financial efficiency) can be recorded.

In conclusion, the data previously analysed and recorded, together with the information archived on the forms presented in Figure 5-16 and Figure 5-24 provides useful reference and guidance when planning the number of feeding larvae to be stocked, the expected number and size of advanced fry produced, or to estimate the duration of the rearing period.

[7] The growth of feeding larvae can be measured and noted, and the growth curve calculated later from these data. Another method involves taking a sample of growing feeding larvae and leaving it to dry on a "mm" paper. In this way, not only can the measurement of samples be performed automatically, but the samples can also be easily visualised.

6. REARING TAMBAQUI FINGERLINGS

Young fish weighing between 10 and 100 g are called fingerlings. They are usually reared from advanced fry within a period of about 3–4 months, either in ponds, tanks or cages.

Fingerlings are stocked in table fish producing ponds; however, this age and size group could also be stocked in larger water bodies where there are predators.

6.1 REARING FINGERLINGS IN PONDS

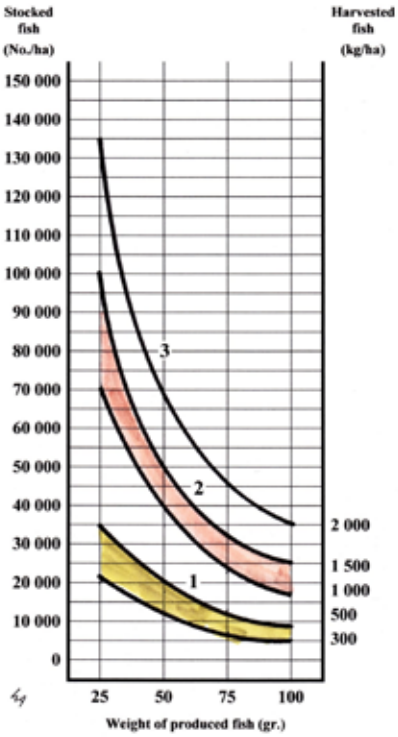
Similarly to other omnivorous warmwater fish species such as common carp, the fingerlings' growth depends on the stocking density, which should always be proportional to the intensity of production (see Figure 6-1).

The production of tambaqui fingerlings in ***monoculture**** does not require the sorting of species after harvest; however, this approach does not utilize pond resources as efficiently as it does when fingerlings are produced in polyculture with other farm-cultured species that are neither feed competitors nor harmful to the growing fish. In the case of predator species only smaller age groups may be reared with growing fingerlings, to avoid possible predation.

In the event of extensive production, some supplementary feed should be provided; equally, as the intensity of production increases, so the volume of protein and energy in the supplementary feeds should be increased accordingly. Volume and dietary requirements are described in the relevant sections and tables of Annex 3.

One frequent problem is that the expected levels of large fingerlings do not materialise at harvest time. It is because without appropriate manuring and the provision of good quality and adequate levels of feed, it is not possible to reach the production outcomes desired.

FIGURE 6-1
Schematic correlation between number of stocked fish and the size / total weight of fingerlings produced in ponds



(1) Extensive, (2) semi-intensive and (3) intensive culture.
Size of stocked fish: 0.5–1.0 g.
Length of rearing period: 3–4 months
Expected survival rate: about 50–70 %.

Source: Woynárovich *et al.* (2011).

6.2 REARING FINGERLINGS IN TANKS AND CAGES

Fingerlings should be reared in tanks or cages for the same period as in ponds i.e. 3–4 months. If it takes longer, the reasons for slower growth should be investigated. Inadequate rearing conditions frequently include a permanent DO deficiency, or insufficient or unsuitable feed. The success of rearing tambaqui fingerlings in tanks or cages depends on the quality of feeds supplied. The feed should be ***biologically balanced**** with the requirements of fingerlings. This means that a protein content of about 40 percent is required at the beginning of rearing period; in the last month this can be reduced to 32–35 percent.

In tanks a continuous exchange of water is vital to supporting DO supply, as well as the removal of faeces and waste. If cages are used, a steady but not excessive movement

of water between the cages, and an exchange with the fresh water around the cages is necessary, as well as movement of water within each cage. The size of cages for fingerling production should not exceed a few cubic meters and they should be made out of fine mesh nets. Cages made out of wire covered with a UV-resistant plastic coating are preferred.

Some 150–250 advanced fry should be stocked per 1 m^3 of tank or cage; from these, the expected yield is about 120–200 large fingerlings of 50–100 g each, giving a total of 6–12 kg/m^3.

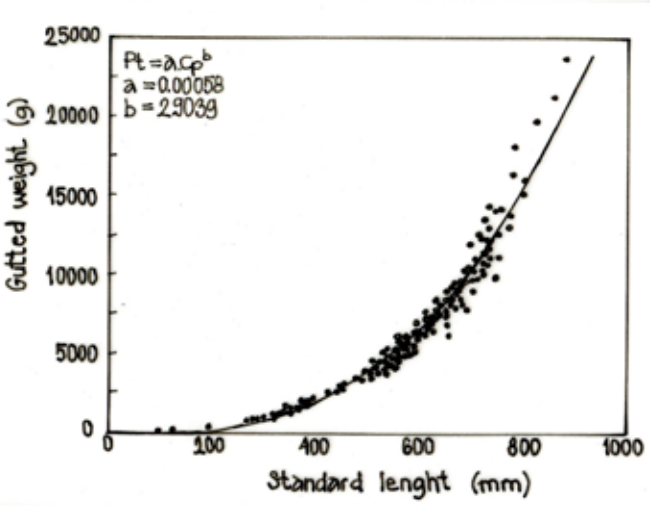
7. TABLE FISH PRODUCTION OF TAMBAQUI

Araujo-Lima and Goulding (1997) concluded that under fish farm conditions tambaqui can grow to 3.5 kg within 2 years, but commercial farmers have also reported that the same size can be achieved within 18 months. The same experts reported that tambaqui of over 50 cm (i.e. above 2 kg in weight) is considered a good size for table fish in Brazil. In Central America however, smaller "plate-sized" fish are preferred by consumers, as demonstrated by Van Anrooy *et al.* (1996). There appears to be good market demand for farmed tambaqui of between 700 g and 1 kg in Central America.

In the authors' experience in Venezuela (Bolivarian Republic of), Brazil and Costa Rica, when tambaqui is fed adequately, large specimens of 100 g can grow to 1 kg within 3 months. Of course, such rapid growth may not be the most economical under all circumstances.

In most household and commercial farms the growth of large tambaqui fingerlings to 1 kg fish generally takes place in periods of about 4–6 months. Scientific and technical publications, in addition to the authors' own field experience, suggest that there are many different ways to produce table fish.

FIGURE 7-1
Correlation between gutted weight and standard length of marketed tambaqui



Source: Villacorta-Correa and Saint-Paul (1999).

In order to support readers with a practical approach to deciding on the culture system and production intensity most suited to their physical, financial and market conditions, the options for producing table fish outlined below are discussed in the following chapters:

- production of tambaqui in water reservoirs
- production of table fish in ponds
- production of table fish in cages.

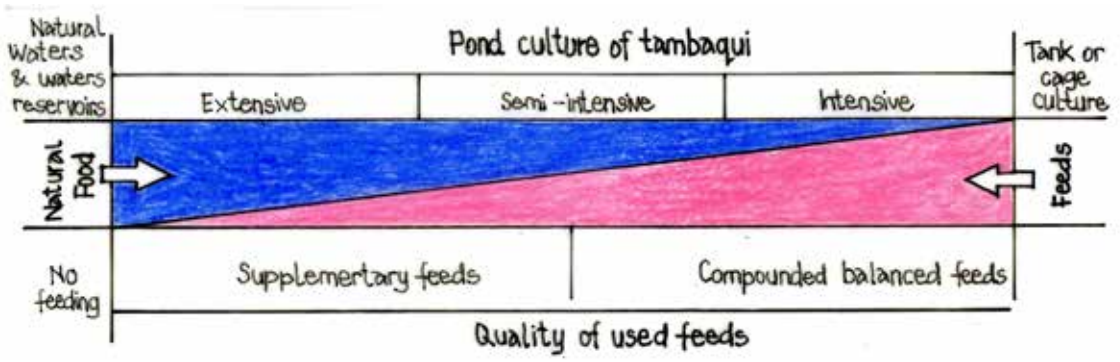
Though the relevant information is discussed in detail in Annex 3, Figure 7-2 provides a schematic summary of the correlation between the quality of feed used and the varying intensity of culture systems.

7.1 TABLE FISH PRODUCTION IN WATER RESERVOIRS

Public and private water reservoirs that are used for irrigation and drinking water for livestock are the ideal water bodies to produce table fish under natural conditions. This is often called culture-based fishery (CBF) or simple fish ranching, when the natural fish food production capacity of a water body is estimated and fingerlings of different species, grown in aquaculture, are stocked accordingly.

According to the principles of CBF neither manuring nor feeding are practised. Stocked fish will grow only on the natural food available in the water. Consequently,

FIGURE 7-2
Schematic correlation between the quality of feeds used and the varying intensity of different tambaqui culture systems



(A) In the natural waters and large water reservoirs where culture-based fisheries (CBF) are practised there is no additional feeding: the fish only feed on natural food. (B) Pond culture can be extensive, semi-intensive and intensive, where the role – as well as the quality and quantity – of feed changes as the intensity of production increases. Accordingly from a nature like starting point described above fish receive an increasingly higher quality of feed, both to supplement natural food in the later stages with ***compounded balanced feeds****. (C) In the event of cage or tank culture natural food has only a minimal role, if any at all, which is why ***biologically complete balanced feeds**** are used when fish are reared in these culture systems.

the more eutrophic a water body is, the more fish will grow. This may be as much as 100 kg fish/ha/year, in the event that the proportion of species and the number of stocked fingerlings are well estimated. Different age groups are often stocked together, ensuring a continuous presence of marketable sizes. However, there are some cases each year when a certain number of stocked fish grow large in one year and are harvested in one time, after which the waters are restocked again. As these kind of waters may be full of predators, larger fingerlings should be stocked.

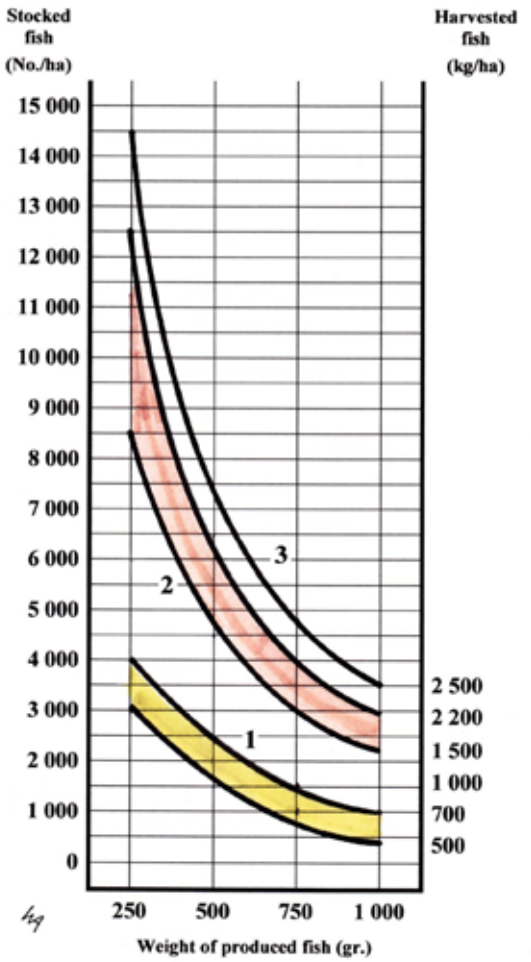
FIGURE 7-3
Packing tambaqui fingerlings in order to stock them in the water reservoir of a private farm in northeastern Brazil

Photographs: ©FAO/András Woynárovich.

7.2 TABLE FISH PRODUCTION IN PONDS

On intensive commercial farms table fish production of tambaqui is done in monoculture with the help of biologically balanced, compounded pellet feeds. However, on traditional fish farms, pond polyculture production is less cost-intensive and makes of more readily available resources. The production of large table fish from fingerlings should be done in two subsequent steps, where fish are restocked – at a lower density – in the second phase. Each production period lasts about 5–6 months.

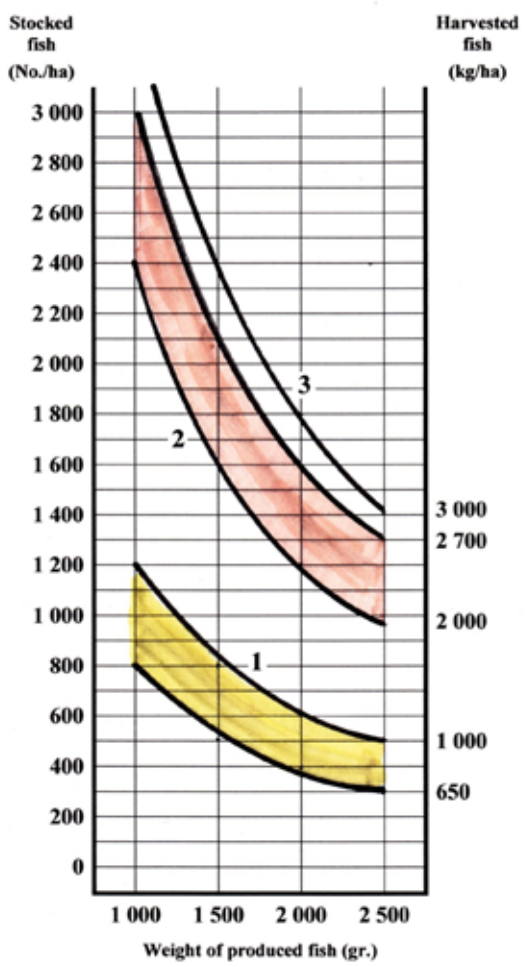
FIGURE 7-4
Schematic correlation between the number of stocked fish, and size and total weight of small table fish produced in ponds



(1) Extensive (2) semi-intensive and (3) intensive culture.
Size of stocked fish: 25–50 g
Length of rearing period: 5–6 months
Expected survival rate: about 60–80 %

Source: Woynárovich *et al.* (2011).

FIGURE 7-5
Schematic correlation between number of stocked fish, and the size and total weight of large table fish produced in ponds



(1) Extensive (2) semi-intensive and (3) intensive culture.
Size of stocked fish: 250–500 g
Length of rearing period: 5-6 months
Expected survival rate: about 80–90 %

Source: Woynárovich *et al.* (2011).

The results to be expected are presented in Figures 7-4 and 7-5. Nevertheless, various experiments (see Table 7-1 and Box 7-1) suggest that even better results than those shown in Figures 7-4 and 7-5 can be expected.

TABLE 7-1
Intensive production of small tambaqui table fish in polyculture

Name of species and their proportion (%) in the polyculture		Stocking			Harvest			Net production (kg/ha)
		No/ha	*g/fish*	*kg/ha*	*No/ha*	*g/fish*	*kg/ha*	
Tambaqui	48	6 100	63	380	4 600	490	2 250	1 870
Curimata	25	3 100	46	140	2 300	350	810	660
Grass carp	27	3 500	57	200	3 000	426	1 280	1 080
Total	**100**	**12 700**	**-**	**720**	**9 900**	**-**	**4 340**	**3 610**

Source: Hancz (1993)

BOX 7-1

Results of super-intensive pond polyculture of tambaqui

The experiment summarised in Table 7-1, which took place in large earth ponds of several thousand m^2, included a production season of 6 months. The ponds received 100 kg fresh manure every week, the grass carp was fed with fresh chopped grass *ad libitum* and the tambaqui received a simple mixture shown in Table A3-16 as feed A01 and A02. Feed A01 was given during the first 100 days, while feed A02 (with a higher protein content) was provided until the end of the experiment (days 101 to 180). The daily ration of feed was 3 percent of estimated tambaqui ***biomass****.

Results suggest a very efficient use of pond resources, which can easily be adopted and replicated. Apparent conversion of grass was 5.5 kg/kg BW of grass carp; an average 7.7 kg of manure produced 1 kg of fish biomass, while the Feed Conversion Rate (FCR) of the feeds given to tambaqui was 1.13 (after Hancz 1993).

FIGURE 7-6

Integration of commercial household animal husbandry with fish culture in northeastern Brazil

Photographs: ©FAO/András Woynárovich.

Integration of tambaqui pond culture with poultry or pig production is also widespread in various tropical countries such as Brazil, Venezuela (Bolivarian Republic of), Bangladesh, China, Indonesia and Viet Nam (see Figure 7-6). In these farm systems, the uneaten feed of the farm animals is utilized by fish and there are no – or very small – labour costs involved in the transport and distribution of manure. However, on the other hand, these systems may need greater pond maintenance. The number of animals to be integrated into the system should be limited to those producing a maximum of 120 kg dry matter daily per hectare (Hepher and Pruginin, 1981). Relevant guiding figures are listed in Table A3-2.

7.3 TABLE FISH PRODUCTION IN CAGES

The cage culture of tambaqui can also be a profitable activity. A precondition for a successful cage culture system is the continuous exchange of water in the rearing space, in addition to the use of compounded, biologically balanced feed (or a wide range of different feeds given together to ensure the same feed quality, i.e. a biologically complete and balanced diet). Feeding is essential, because the natural food found in the rearing water is insufficient for the density of fish grown in the cages.

The growth rate of fish in cages should be similar to that in ponds, i.e. the same timespan should yield the same size of fish if sufficient feed of adequate quality is provided. In addition, it is important to keep and rear approximately the same size of fish to avoid unnecessary hierarchy as a result of uneven sizes. Grading may therefore be required once or twice during the growth period.

Different figures circulate among specialists. These can be better interpreted and adopted if (1) initial size, (2) number of stocked fish, (3) expected final weight of fish and, (4) duration of rearing period are presented together. As a rule of thumb, a production output of more than 20–30 kg/m³ will need extra attention and care in addition to high quality feeds.

Experiments published by Gomes and his colleagues in 2006 prove the technological and financial feasibility of cage culture for this species. Key results and summary are presented in Table 7-2 and Box 7-2.

TABLE 7-2
Cage culture of small tambaqui table fish in cages

Cages	Stocked fish (rounded figures)			Harvested fish (rounded figures)			Net production (kg/m³)	FCR
	No/m³	*g/fish*	*kg/m³*	*No/m³*	*g/fish*	*kg/m³*		
1st group (3 cages, 6 m³ each)	20	55	1	20	1 000	20	**19**	**2.85**
2nd group (3 cages, 6 m³ each)	30	55	2	30	840	25	**23**	**2.50**
3rd group (3 cages, 6 m³ each)	40	55	2	39	870	34	**32**	**2.07**
4th group (3 cages, 6 m³ each)	50	55	3	49	890	43	**40**	**1.88**

Source: Gomes *et al.* (2006)

BOX 7-2

Results of the experimental cage culture of tambaqui

In the experiments summarised in Table 7-2 the production season lasted 8 months. Twelve cages of 6 m³ (2 m × 2 m × 1.5 m) were used. Each experiment (i.e. stocking densities of 20, 30, 40 and 50 fish per m3) was repeated three times. Fish were fed with two types of feeds. First an extruded feed with 34 percent Crude Protein (CP) was fed for a period of two months. After this an extruded feed with 28 percent CP was fed until the end of trial. With both types of feed, the fish were fed to apparent saturation twice a day, 6 days per week.

The experiment also demonstrated that the stocking densities tested are far from the upper limitations (Gomes *et al.*, 2006).

8. REARING AND KEEPING MATURED BROOD FISH OF TAMBAQUI

8.1 REARING BROOD FISH

The rearing of brood fish is not part of artificial propagation. However, the precondition for successful propagation is to have adequate numbers of good quality, sexually mature female and male fish.

Tambaqui becomes sexually mature very late compared to pirapatinga or pacu – or indeed compared to other widely cultured fish species such as common carp and tilapia. Table 8-1 compares age and conditions under which tambaqui and some other selected fish species reach sexual maturation. Tambaqui males, as with most other fish species, become sexually mature earlier than females. However, not all the females in a stock of brood fish become mature at the same time (only about 40 to 60 percent of the whole stock), because their individual maturation depends on how well they are fed. A young, recently matured female will always produce fewer eggs than females that matured (developed eggs) during an earlier propagation season.

TABLE 8-1
Approximate size and age of sexual maturation for some widely cultured fish species

Tambaqui	Pirapatinga	Pacu	Common carp	Nile tilapia
In its natural environment				
5–7 kg	4–5 kg	3–4 kg	1.5–2.5 kg	0.5–1.5 kg
4–5 years	3–4 years	2–3 years	2–3 years	1 or 2 years
In ponds under tropical climate conditions		**In ponds under tropical and subtropical climatic conditions**		
4–5 kg	3–4 kg	2–3 kg	0.4–0.6 kg	0.05–0.1 kg
3–4 years	2–3 years	1.5–2.5 years	0.7–0.8 year	0.4–0.5 year

Source: Balarin (1979); Goulding (1980).

Determining the actual size of brood stock to be reared and kept on a fish farm – i.e. actual number of females and males – is based on:

- planned number of produced feeding larvae per month and per year;
- number and size of weekly propagated females and males;
- how often females and males are used in a year.

All the calculation figures noted in the "Fish larvae production from" presented in Figure 5-16 will serve well as source data.

In addition to the brood stock, the number of future brood fish should also be determined and reared. The general rule if females and males are propagated all year round is that the size of the stock of future brood fish should be about 10 to 20 percent of the current brood stock. This size of brood stock will not only allow for the replacement of losses, compensate for some overly large or old specimens, but also provide a safety reserve for future growth of production.

The selection and use of brood fish in fish farms where the propagation of tambaqui is conducted requires some important aspects to be considered. In many such fish farms

BOX 8-1

Aspects of genetics to be considered during the selection and use of a brood stock

The development of tambaqui brood stocks outside of its native range started from a few hundreds of fingerlings in the 1970s and 1980s. Though 5–8 kg large tambaqui females produce 0.5–1.5 million of eggs, in the event of repeated propagation of a small number of females and males and their progenies, the chance for inbreeding will increase. This may reduce the genetic diversity of the progeny produced, with likely consequences for increased susceptibility to diseases, reduced ability to perform well under atypical rearing conditions or having less hardy young fish.

Today, the extent of inbreeding in a given stock of brood fish can be verified through the ***genetic characterization**** of actual stocks, using ***microsatellite**** and lineage-specific ***genetic markers****. The genetic examination of brood stock in a representative sample size allows the close estimation of the rate of inbreeding (Eszterbauer *et al*., 2015).

In many cases, the reduced performance of progeny often "automatically" explained by genetic problems: replacing brood fish is therefore recommended. However in order to establish a reliable diagnosis as to whether declining performance is due to inbreeding or to poor rearing conditions, the determination of genetic diversity outlined above is particularly important – before an expensive replacement of brood stock is planned, for instance.

brood fish are usually selected from table fish production ponds and reared in a separate pond (or ponds) under natural conditions. Unfortunately, for reasons summarised in Box 8-1, this selection is usually unsuccessful from a genetical point of view.

Unless the selection of broodfish is done in cooperation with an advanced fish breeding station, which uses brood fish from different genetic origins, it is likely that inbreeding will occur. To avoid inbreeding, a sperm bank for wild tambaqui was established in 2001 in the north of Brazil. This sperm bank helps specialized propagation fish farms far from fish breeding stations with gene banks of wild tambaqui so as to ensure access to good quality semen. In this sperm bank, sterile test tubes are used to collect 6–12 ml of tambaqui semen per fish. The semen is then analysed for viscosity, motility and spermatozoid concentration, before dilution in a cryopreservation solution for freezing and stocking in liquid nitrogen at -196 °C (Suplicy, 2007; Pastrana, 2015).

In order to ensure natural conditions, the stocking density of would-be brood fish should be low, as summarized in Table 8-2. Would-be brood fish kept in extensively stocked fertile ponds should be fed regularly with a diet containing a minimum of 20 percent protein. However, it should be emphasized that natural food is best for future brood fish and it is therefore better to keep them in a pond, instead of a tank or cage. The stocking density may be increased if good quality feed – with a higher protein content – is applied regularly, in addition to the appropriate manuring. However, if the development of the would-be brood fish depends only on the natural productivity of a pond, which is not sufficiently increased by manuring, the stocking density should be less than is indicated in Table 8-2.

TABLE 8-2

Stocking density of would-be tambaqui brood fish

Approximate weight in grams/fish	Fish/1 000 m²
1.000	30–40
1.500	20–30
2.000	15–20
3.000	10–15

Observation: It is possible to stock other fish species in addition to tambaqui in the brood stock ponds – though no more than an additional 30 to 40 percent of the numbers above. The main rule is that these extra species should not compete with the tambaqui.

8.2 KEEPING BROOD FISH

The objective of preparing female and male fish for propagation is to produce the required quantity of good quality eggs and milt (sperm). Accordingly, the result of brood stock preparation is measured by the quantity and quality of eggs and milt produced. In practice, finding males for propagation with good quality milt is not a frequent problem. Farmers should thus concentrate most of their efforts on the preparation of females.

The preparation of females (and males) starts immediately after their propagation; this means that brood fish that have ovulated 5 to 20 percent of their bodyweight should recover afterwards in order to be able to develop a new batch of eggs.

To produce good quality eggs in the required quantity, it is important to feed the female brood fish properly. It is essential to provide as much natural food as possible: together with supplementary feeds, this will ensure the results desired. The increase of natural food availability in the pond should be achieved with manuring as discussed in Annex 3.

FIGURE 8-1
Brood fish harvest from a small pond

Photographs: courtesy of Aoyama.

Tambaqui are great consumers of water snails, which are the best, protein-rich natural food for them (they are species from the Ampullariidae family – apple snails Pomacea and Planorbidae). Once these snails are established on fish farms they can usually be collected in large volumes in the fry and fingerling rearing ponds on the same farm, where they breed and grow on the leftover feed given to the young fish. As such, these snails could be a pest in the nursery and other ponds with fish that do not eat them. However, the snails collected and fed to the larger tambaqui will become a useful component of their diet. When snails are not consumed they remain harmful in the pond because they compete with the stocked fish for the feeds. Collecting them after harvesting a pond, or whenever there is time to do so, is therefore essential.

If no compounded industrial or farm-made feeds are available, pellets fed to layer hens (or ducks) is generally the most easily procured supplementary feed available for tambaqui brood fish. The daily quantity should be no more than 3 percent, but preferably around 1.5–2 percent of the brood fishes' biomass. The latter ratio will force fish to search for natural food. When the stocking density of brood fish is low and they can collect enough natural food, the quantity (i.e. protein content) of daily supplementary feed may be reduced considerably, to the aforementioned 1.5–2 percent, which will make savings on production costs.

In addition to supplementary or balanced feeds, fresh fruit not used for human consumption (e.g. because of physical appearance) may also be introduced; and if done regularly, it can be a good source of vitamins and minerals. However, those giving the fruits and other supplementary feeds to fish should ensure they are not rotten, and do not contain biological and/or chemical ***pollutants****.

Prior to the propagation season, it is also worth feeding germinated maize or other cereals to the brood fish, in a quantity of about 0.5 percent of bodyweight, which will encourage the development of good quality eggs.

The optimum pond surface area per kg weight of a female breeder is about 10–15 m^2 (for males 7–10 m^2) depending on how productive the fish pond is. This pond area seems to be sufficient for the breeders to collect most of their required natural food, provided the management is good. If the pond area available for brood stock is limited, then the quality and volume of feed supplied should be increased. This means giving a biologically complete, balanced feed that can and will ensure the production of brood fish eggs when kept in a smaller pond (as shown in Figure 8-1).

Having the necessary feedstuff available before – and in the course of – vitellogenesis is essential for good egg production in the females. If feeding is inadequate, the individual egg production of females will differ considerably from one another, even in the same pond.

Following the propagation season, it is important to keep tambaqui brood fish in bigger ponds where there is enough space for them to search for natural food. If tambaqui brood fish are kept in larger ponds in polyculture (together with the brood fish of other species), these ponds should be fished in shifts of 2–3 months and the brood fish should be separated into smaller ponds from which they can be captured promptly and taken into the hatchery for propagation. In the ponds where brood fish with eggs at a dormant stage are kept, the quantity of feed (protein-rich supplementary or compounded feeds) should be decreased to the daily 1.0 to 1.2 percent of the biomass of the fed fish.

Experience, gained in Lower San Francisco region (Sergipe and Alagoas States of Brazil), suggests that the propagation season for tambaqui can be extended and the same brood fish can be successfully propagated two or even three times per year if the climatic conditions are favourable and with good feeding, (Pinheiro *et al.*, 1988). With a proper programme for the rotation of brood fish it is possible to propagate tambaqui throughout the year under tropical climate conditions, i.e. when water does not cool down below 24 °C for a prolonged period.

Where a definite propagation season exists, the recommended action is to collect all brood fish, and stock females and males into smaller ponds separately, even before season starts. The smaller ponds in which they are stocked should be freshly filled and manured. Fresh water and a new environment accelerate and stimulate egg (gonadal) development.

The separation of males and females allows an easier, more controlled use and rotation of the two sexes. Separating the fish by sex is also a good opportunity to examine the gonadal development of brood fish, as well as their general condition and health status, sex ratio and number of brood fish, which will also help improve the planning and programming of the propagation.

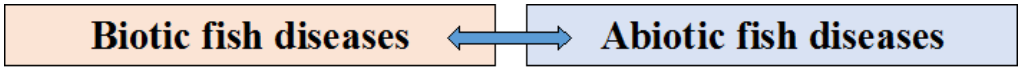
If there is enough available pond area, this separation of the sexes is recommended for the whole year. This is because it facilitates more economical feeding, as males do not need the same quantity and quality of food as females. Another reason for keeping males separately is a safer selection for hormonal treatment. This is because it can be difficult to distinguish males from females, especially when they are not absolutely ready for artificial propagation. In the event it is possible to keep males and females separate throughout the whole year, the stock monitoring and inventory mentioned previously – together with the examination of conditions and health, etc. – can also be done more efficiently.

9. NOTES ON POTENTIAL DISEASES OF TAMBAQUI[8]

9.1 NATURE AND TYPE OF FISH DISEASES

One of the practical classifications of fish diseases is dividing them according to their causes: these can be biotic and abiotic. Biotic fish diseases derive from a living organism, while abiotic diseases do not involve or derive from a living organism: they are related to water quality, poisonous materials or management problems (including incorrect feeding). As illustrated in Figure 9-1 these two main groups have a complex interrelationship.

FIGURE 9-1
Practical grouping of fish diseases



9.1.1 Biotic fish diseases

A wide range of organisms can cause fish diseases, when conditions are favourable for their development. The organisms are categorized in some major groups based on whether they are ***viruses****, bacteria, fungi, algae or animals. Of these, some groups of diseases known to farmers will help to identify them and call for professional assistance.

Diseases caused by fungi and algae are common in fish with low immunity. The most frequent fungus is called saprolegnia, which develops on dead eggs in the hatchery and lesions and wounds on fish. In the strictest sense, algae do not belong to ***pathogenic**** organisms – however they may lead to massive fish mortality for two reasons: 1) they produce toxic materials, or, 2) when they bloom the oxygen content in the water dangerously reduces.

Diseases caused by parasites – one of the largest groups of fish diseases is caused by parasitic organisms. In a pragmatic sense only animal organisms, both protozoans and metazoans (all animals, but protozoans and sponges), are real parasites. Some of the parasites live their entire life in the same host, while other parasites have more complex life cycles, including periods when they live and develop elsewhere. As well as the main host they have one or more intermediate hosts, in which they grow during their subsequent development stages. Of these hosts the main host, or final host, is that organism in which they reach sexual maturity**.** The following main groups of parasites of fish are distinguished:

- **Protozoan parasites** – Protozoan parasites are those single-celled microscopic animals which can be ***flagellate****, ***ciliate**** or ***coccidian****. Some of them are obligate parasites of fish, which means they cannot live without fish. Others are facultative parasites, because they can survive without fish – however, they also frequently cause infection and disease in fish. Most of the flagellate and ciliate species belong to this latter group. Growing on the fish body, these parasites cause changes in the fins, skin and gill; and frequently cause death. The majority of flagellate and ciliate parasites are ectoparasites i.e. they lives outside of the host.

[8] The entire chapter is based on information summarized from Molnár *et al.* (2019).

- **Myxosporeans** - These microscopic organisms propagate with ***spores****; they are are a common and pathogenic fish parasites.
- **Parasitic worms (Helminthes)** – Parasitic worms are the most common and pathogenic fish parasites. Some of them are ectoparasites, while others are endoparasites, i.e. they live inside of the host. One part of them infects fish in their adult phase; others however, are parasites of aquatic birds and mammals, and fish serve only as the intermediate hosts of their different developmental stages.
- **Parasitic larvae of molluscs (Glochidia)** – Certain species of freshwater bivalve molluscs use fish as hosts for developing their young larvae, which are known as glochidia. First, mature female freshwater mussels incubate their fertilized eggs within the shells. After the incubation period they release the hatched larvae, known as glochidia into the water. These young larvae attach themselves to the fins or gills of fish and remain parasitic for one or more months, while young mussels develop. Although fish are able to sustain low levels of glochidia invasion without apparent harm, heavy infestations, especially in the gills of young fish, can cause injury and even death.
- **Diseases caused by crustacean parasites** – The majority of aquatic crustaceans are free-living organisms. This means that they live independently, and not as a parasite. However, some species develop a parasitic lifestyle or a close association with fish. Many of them, such as Lernaea, are responsible for diseases, especially in farmed fish. They can be fatal in young fish while in larger fish they cause wounds, which reduces the market value of table fish.

9.1.2 Abiotic fish diseases

There is an ever-increasing knowledge of the diseases caused by virus, bacteria, fungi and parasitic organisms. Still, both in natural waters and pond polyculture, as well as in cage culture, greater harm is caused by environmental factors, including: oxygen shortage, low and high water temperature, accumulation of poisons in the water; and by human activities, including unsuitable or poorly implemented fish production technologies, wrong nutrition or rough handling. These abiotic fish diseases are listed in Table 9-1.

TABLE 9-1
Practical classification of the most frequent abiotic fish diseases

Diseases induced by physical and chemical qualities of water
– acute and/or chronic oxygen shortage
– gas-bubble disease (GBD)
– diseases caused by unfavourable water temperatures.
Poisoning
– Poison of industrial origin
– Poison of agriculture origin
– Poison of aquatic habitat origin.
Enteric inflammations caused by feeds
Rough handling

Adapted from: Molnár *et al.* (2019).

9.1.3 Fish tumours

Tumours are widely reported in many families of fish. Fortunately, tumours in cultured fish occur relatively rarely. This can be attributed to the fact that the rearing periods in culture systems do not allow the development of tumours which characteristically develop in older fish. Tumours thus appear more frequently in natural waters.

9.2 FIELD INSPECTION OF FISH HEALTH

The diagnosis of fish diseases is based on proper observation, sampling and examination of both the fish and their ***habitat****. The best practice is when on-site and laboratory examinations are subsequently performed.

9.2.1 Examinations on site

The relevant steps of field examinations are:

1. Inspect the water body
2. Take a sample of fish
3. Examine fish
4. Maintain a record of data and information.

Inspecting of the water body

The field examination starts with an inspection of the affected water body and the behaviour of fish.

Unlike healthy fish, sick ones neither feed nor hide. They vaguely swim up to the oxygen rich locations, such as the water surface or inflow.

Dead and severely sick fish often drift with the currents or float in unusual positions and jerk irregularly.

It is important to note that dead fish sink to the bottom and rise to the water surface only after a couple of days. The length of the period while dead fish remain sunk on the bottom depends on the water temperature. In colder water dead fish can appear on the surface after as much as a week, while in warmer water this happens faster, within two or three days.

Sampling of fish

After studying the behaviour of the fish stock, fish samples should be taken from different parts of the water body. In waters where fish are fed, some samples should be captured at feeding spots. Here mainly healthy or healthier specimens can be found. An examination of samples taken at inflows and outflows, together with fish captured at feeding points, will provide a more reliable overall picture of the health of the stock and any diseases therein.

Examination of fish

Examination of fish on site includes a close observation of the appearance of the fish; its body parts; and, after dissection, its organs.

Observation of body and body parts of fish

The appearance of fish that are not healthy (see Box 9-1) enables an initial diagnosis. The most frequent changes in the shape, colour and intactness of body are as follows:

The physical **body condition** can be estimated from the back of fish. In an abnormally thin and weak fish, the back resembles the blade of a knife. **Abnormal thinness** may indicate different diseases, including an inflammation of digestive track and an increased number of worms.

BOX 9-1

Appearance of a healthy fish

The body of a healthy fish is covered with a thin layer of mucus and is free of wounds, ulcers and parasites.

The scales fit tight into the dermis (skin) and their colour is characteristic of the species and age.

The fish's eyes are white and the pupil is black. The eye reflex is responsive, and its eyes also move when turning. The ***cornea**** on the eyes is tight, shiny and reflective.

The back of the fish is fleshy and rounded.

The gill covers are undamaged. Gill rakers are also intact and free of wounds and parasites. They are covered with a thin layer of mucus and their colour is deep red.

The fins are also undamaged and free of wounds and parasites. (Adapted from: Molnár *et al.*, 2019).

The **body surface** of the fish, as well as volume and quality of **mucus** on it, gives useful information about the possible cause of death. Poisons provoke a production of excess mucus in the skin, while with some diseases (such as saprolegniosis) the mucus disappears from the skin.

The **loss of scales** suggests mechanical injuries. However, these are also frequently worsened by bacterial and fungal infections.

Wounds, **ulcers** and **parasites** on the body and fins are clear evidence of a problem. Wounds can be caused by predators, but in the case of parasites these can be very often observed on the skin.

An **unusually large belly** can be a viral or bacterial infection. The enlargement of organs, the accumulation of secretions in the abdominal cavity, or the presence of parasites of large sizes and/or in great quantities can also cause an unusually large belly.

The protrusion of the anus indicates a possible inflammation, as well as a sign of fluid accumulation in the digestive tract.

Rough handling, as well certain parasites, cause **broken fins**. As fins regenerate rather quickly, such wounds indicate recent problems.

Whether the fish is dead or alive can be judged by the **eye reflex**. Stiff eyes are evidence of death. The white outer layer of the eyeball (sclera) of recently deceased, though healthy fish is tight, shiny, but the sclera of a dead sick fish is sunken, dim and wrinkled. In normal cases, the colour of the pupil is black. G**oggled, protruding eyes** can often be observed a result of different infections or parasites.

Mouth and **gill slits** are usually closed but in the event of suffocation they remain open. After death the gills become pale, and the tissues lose their structure. The presence of excess mucus on the gills suggests poisoning or suffocation, while erosions in the area can be symptoms of gill ***necrosis**** and the putrefaction of the gills.

Crippled or dwarfed fish are rare in nature. **Deformed spines** can be observed mostly on fish hatched and developed under unsuitable conditions during their larval stage, or as a result of inbreeding.

Observation of the organs of fish

Prior to dissection fish should be killed swiftly, as generally outlined in the relevant animal or fish welfare regulations at the national level.

Maintaining a record of data and information on fish health status, brood fish background and measures that have been taken is the third of the on-the-spot actions. This includes finding out:

- whether there were similar earlier incidents, and the date when mass mortality started,
- number of dead fish,
- whether action has been taken and if so, what.

Studying the management and situations on site gives useful information and data on:

- type and parameters of the water body
- water supply/source and whether there is any source of pollution or poisoning
- physical (temperature, transparency/turbidity colour), chemical (pH, oxygen content) and biological (waterweeds, number and size of the different fish species) parameters and qualities of water body
- dates of last stockings
- quantity and quality of feeds given if the fish stock was fed
- fishery and/or aquaculture management techniques applied
- location of nearby industrial and agricultural plants
- possible agricultural sprayings in the vicinity.

9.2.2 Taking and sending samples for laboratory examinations

In the event that there is no specialized professional available, or if a veterinary inspection does not result in diagnosis, fish should be sent to a laboratory. In the laboratory the dissection and parasitological, bacteriological, virological and histological examinations will support the establishment of exact diagnoses. As soon as the results of the tests are available, an official letter is generally issued by the laboratory with the outcomes.

If there is mass mortality, samples should be a minimum of 10 fishes. However, 5 fishes should generally be taken for a routine control examination. The sample should be accompanied by an order or a letter requesting examination signed by the owner of the farm or the veterinarian in charge. This order or letter should contain the data and information summarized in Box 9-2.

BOX 9-2
Data and information to be included on the examination order for fish in the laboratory

General information:
- Name and particulars of owner
- Name and particulars of veterinarian
- Name and address of payee of the examination.

Details for the sample of fish:
- Species:
- Age/age group:
- Number of specimen:
- Temperature of water (°C):
- Location (name):
- Location (code or GPS):
- Requested examination:
- Symptoms (yes/no):

For the above reasons, either recently deceased or dying live fish showing symptoms should be sent to laboratories. The best way to preserve these is to place the sick but still alive fish in a plastic sack of water with oxygen; alternatively, the fish may also be carried in water cooled with ice. If there is no ice available a third – though not ideal – solution is to pack the fish in fresh green plants.

If the cause of mortality is potentially environmental, water samples of one litre each should also be sent to the laboratory. If the container is a plastic mineral water bottle, it should not only be clean but also rinsed with the water from where the sample is taken. Sets of three samples from different points in the pond or tank – such as near the inflow, outflow and feeding place – should be taken. Each set of samples should be taken from at least three different depths (upper, middle and lower sections) of the water column.

If poisoning is suspected, an official person (veterinarian, notary, etc.) should be present when taking the samples; by closing and signing the bottles, the official person can later testify as to the origin of the water, if the case goes to court.

Sometimes it may also be necessary to transport tissue samples to a specialist laboratory. Such samples, if they are for virological examination, can be frozen. Samples requiring bacteriological examinations must not be frozen.

It is important to be aware that for laboratory examinations only live fish or fresh dead fish with undamaged organs can be sent. Decaying fish that died much earlier are unsuitable for laboratory examinations because their organs will show post-mortem changes. Fish captured alive, but transported dry, without water, are also unsuitable for examination as parasites dry and die. Even heavy infections of protozoans or monogeneans will remain undetected.

9.3 PREVENTING THE SPREAD OF FISH DISEASES

In most fish ponds, especially when production is extensive or semi-intensive, the prevention of disease is particularly important. This is because there is generally

BOX 9-3
Data and information for the label of the fish and water sample

Each sample of fish should be labelled as follows:
Name of farm or/and owner, address, phone or/and email, case history, number of fish pond or name of water body and species, number and age of sent fish.
Each water sample should be labelled as below:
Name of farm or/and owner, address, phone or/and email, case history, number of fish pond or name of water body from which the sample was taken.

In the case of different samples from the same water body, the exact location (based on Molnár *et al*., 2019).

It is important to fill the bottles in full without leaving air bubbles. The filled bottles should be properly labelled, as shown in Box 9-3

little or no scope for a full change of pond water. Moreover, applying multiple treatments is generally uneconomical.

In addition, in human and environmental concerns restrict the use of many chemicals in the treatment of fish diseases in numerous countries.

Prevention is therefore the best means to obtain and maintain disease-free stocks in natural waters and fish farms. This can be achieved with rigorous control in the trade of live fish, as well as with regular, scheduled monitoring and inspections. Prevention should include:

- administrative measures for preventing the spread of fish diseases between continents, watersheds and fish farms;
- practical measures for preventing the spread of fish diseases between continents, watersheds and fish farms; and
- practical measures for preventing the outbreak and spread of fish diseases within a fish farm.

Of the three areas above, fish farmers should be especially concerned by fish purchased for further rearing. This must be done from regularly inspected hatcheries, nurseries and other fish farms. All purchased fish should be accompanied with valid health certificates and documentation that the fish are disease-free.

9.4 TREATMENT OF FISH DISEASES

Diseased fish should be treated only with suitable, carefully selected products on the basis of a precise diagnosis. There are three different kinds of treatment:

- bath treatments
- oral treatments
- injections.

Bath treatments

In fish medicine, when the objective is to remove ectoparasites, or the healing of lesions or wounds, bath treatments are used in the majority of the cases.

Depending on the time of application, four different types of the bath treatments are used.

Flush bath or dipping – This method is used when the chemical is effective within a very short period of time, i.e. from half a minute to about two minutes. Flush techniques are used in culture systems where eggs or fish are kept in flow-through tanks, and the water is continuously renewed. Fish concentrated in a net and dipped into a concentrated solution of chemical also belongs to this technique.

Short bath – This is usually the cheapest, most effective and commonly used approach when using chemicals to cure fish diseases. The treatment takes 5 to 40 minutes in tanks where the drug is precisely dosed. The reaction and state of the fish can be checked and, if necessary, treatment can be modified or stopped by reducing concentration (in

other words by adding clean water). In practice, fish are placed into a tank containing the precisely dosed chemical and are kept in this solution for the suggested time.

During treatment the water must be aerated. At the end of the treatment fish are either removed or the solution is diluted with fresh water. The latter is less stressful for the fish. For shorter periods (5–10 minutes) the use of nets helps to remove fish from the solution in time.

Transit bath – This is used as a routine prevention of ectoparasites when fish are transferred from one pond to another during harvesting, storing or stocking. The method is particularly recommended when fish fry are transferred into a larger pond or prior to placing the fish into storing ponds.

If the fish are transferred, one or more tanks containing anti-parasitic or bactericide solution are used, before the fish is passed on to the next pond. The period of treatment takes between 30 minutes and 4 hours, depending the concentration of the solution and the actual time of the transfer.

Long bath – This kind of bath treatment is used in ponds and aquaria. With this method highly diluted drugs are applied for a period of between 24 and 48 hours. Prior to treatment, the water level is usually lowered by half, and the drug is added continuously to the inflowing water so that the drug solute can be evenly distributed in the pond. At the end of the application, the drug can be flushed out of the pond by opening the water inflow further. In order to save water – or when ponds are very large – some farmers prefer spraying the condensed drug solution from boats. Some drugs may lose their effect within one or two days and then there is no need to refresh the water.

In the past this method was used regularly to treat fish stocks with malachite green and organophosphates; however, it is now prohibited for consumption fish in many countries, as a number of health hazards related to the use of malachite green have been discovered. In storing ponds the long bath method can still be applied for treatments with formaldehyde solution.

Oral treatments

Oral treatments are a practical way of administrating fish medicine, but only for those species which take prepared feed. This treatment is therefore not suitable for treating fish which feed on plankton and vegetation.

This is a widely followed method for treating sick fish with antibiotics or anthelminthics (i.e. anti-parasitic drugs that expel parasitic worms). Before treatment farmers must check whether the medicated feed will be consumed by the fish: often sick fish do not feed, or eat less than needed for consuming the necessary dose of drug mixed into the feed, which makes this type of treatment unsuitable in some cases.

The concentration of the medicine in the feed also depends on the ingestion of fish. Therefore, the body mass index should be considered when calculating the concentration of the medicine needed. In some countries there are premixed drugs available, but in most cases farms prepare the medicated feeds themselves. The most frequent on-farm methods for preparing medicated fish feeds are: saturating wet grain

BOX 9-4

Use of certified and uncertified chemicals when treating fish diseases

There are some countries where almost all chemicals can be legally used for curing fish diseases, while in other countries there are restrictions that should be observed.

For this reason it is important to check official lists as to whether the selected chemical is licensed for treating fish disease, before it is applied.

In the event that the selected chemical is not licensed for fish but only for other animals then the product can generally be used, but only with certain restrictions and with caution: the minimum withdrawal period or permitted maximum residual level must be 500 days.

These days are calculated as follows:

The 500 days are divided by the average daily water temperature. Accordingly the withdrawal period will be 20 days where the daily average water temperature is 25 °C (Molnár *et al.*, 2019).

with the antibiotic solution; mixing the drug(s) into vet feed with a concrete-mixer; spraying the feed with antibiotics dissolved in alcohol and coated with cooking oil.

Injection

Injecting farm fish with drug(s) is not practised frequently. The costs involved in individually injecting all farmed fish are high. The use of injections is practically restricted to curing valuable warmwater brood fishes and some high-value ornamental fish, such as koi carp.

BOX 9-5

Testing a new product used for the treatment of fish disease

The steps for the testing of new products includes:

– Three batches of 10 fish should be treated separately, as instructed in the product description.

Once the treatment is finished, the fish should be kept and observed for 48 hours, before a decision can be made to treat the others that require treatment. If there is no mortality observed after 48 hours, the product can generally be used safely (Molnár *et al.*, 2019).

GLOSSARY

Acetone-dried carp pituitary glands – Most frequently chemically pure alcohol or acetone is used to preserve the extracted pituitary glands. The freshly removed pituitary gland is put into, and kept, in a small container with pure acetone. After eight hours the glands are placed into a second container with fresh acetone for another eight hours. After this second eight hours the pituitary glands are placed into a third container of clean acetone for a final eight hours. From here, the glands are placed onto dry and clean paper tissue (blotting or absorbent kitchen paper) where they dry. The acetone evaporates from the glands and they are packed hermetically into phials (small glass bottles) that are tightly closed. The phial should be labelled with the exact number, the total and individual weight of the glands.

Alkalic protease enzyme is used as an active material in the washing powder industry. When the bad eggs ratio is about 60–40 percent or lower it is advisable to use this enzyme. If 0.4–0.5 g of the enzyme is placed in the incubator, while maintaining the same water inflow helps to eliminate (dissolve) the bad eggs and egg shells.

Artificial propagation is the term used for the work process in the hatchery, which lasts for about 3–5 weeks, starting with the propagation of sexually matured brood fish and finishing with the harvest of advanced fry.

Biologically complete balanced feed is high quality farm-made or commercial feed which quantitatively, qualitatively and proportionally contains the necessary energy and proteins, vitamins and minerals, as well as digestive enzymes, which are needed for the proper development and growth of fish.

Biomass – The total weight of fish in a pond, rearing tank or cage is called biomass. This term allows to calculate the exact quantity of feeds, drugs etc. to be applied.

Black water – Rivers which drain the water of the virgin forests of the Amazon lowlands are referred to as "black". These rivers, also known as "blackwater rivers" have no basic nutrients, but a large amount of oxidized (dark) humic acids and detritus deriving from the rotten leaves and branches of the virgin forest. The pH of these waters is very low. The Rio Negro is the largest "black" river in the world.

BOD – Biochemical oxygen demand is a standard test to examine the oxygen-demanding strength of the waters. BOD only measures the amount of oxygen consumed by microbial oxidation and is the most relevant to waters rich in organic matter. It is important to understand that COD and BOD do not necessarily measure the same types of oxygen consumption.[9]

Chromosomes are threadlike nucleic bodies consisting of chromatin that carry the genes in a linear order; they are thus responsible for the mechanism of inheritance by which genetic characteristics are expressed in successive generations.[10]

COD – Chemical oxygen demand is a measure of the **water's** capacity to consume oxygen during the **decomposition** of organic **matter** and the oxidation of inorganic chemicals such as ammonia and nitrite. COD measurements are commonly made on samples of waste waters or of natural waters contaminated by domestic or industrial wastes. Chemical oxygen demand is measured as a standardized laboratory assay in which a closed water **sample** is incubated for a specific period of time with a strong chemical oxidant under precise **temperature** conditions.[11]

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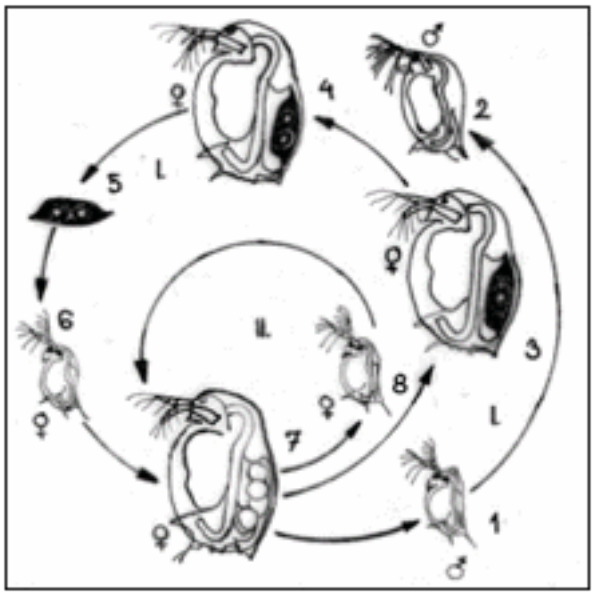
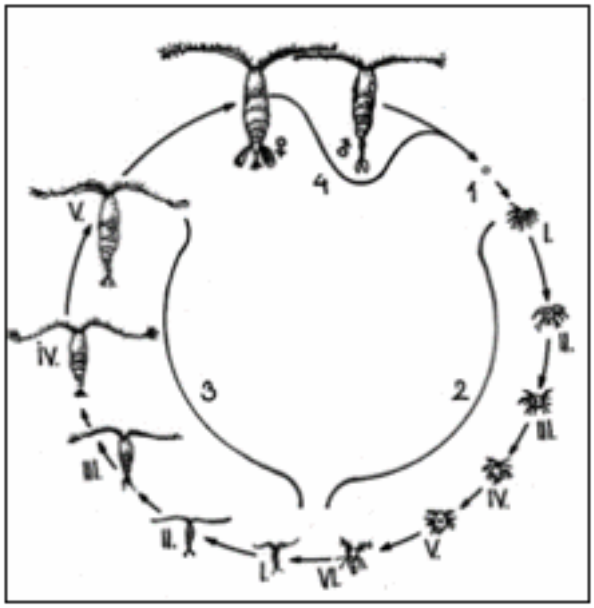
[9] Source: http://camblab.info/wp/index.php/272/
[10] Source: http://aquafind.com/articles/Fish-chromosomes.php
[11] Source: http://camblab.info/wp/index.php/272/

Cladocerans are plankton crustaceans that together with protozoans and rotifers can rapidly occupy pond water, provided there is enough food available that can be filtered. If environmental conditions are favourable, they propagate asexually; but when conditions change to unfavourable, they produce dormant eggs through mating. The life cycle of cladocerans is demonstrated with daphnia (Daphnia magna). I. The sexual cycle starts when environmental conditions become unfavourable. 1) At this time a parthenogenetic son is produced, 2) male matured to mate, 3) haploid egg is produced, 4) mating with male, 5) the sexual egg produced is dormant until conditions become favourable to hatch after diapause, 6) the female is produced, 7) When conditions are favourable the asexual cycle starts, while in the case of unfavourable environments male offspring is produced. II. Parthenogenetic cycle: 8) Parthenogenetic female offspring is produced. It continues until conditions are favourable.[12]



Compounded balanced feeds are those which are composed and blended from different protein- and energy-rich feeds in order to have the necessary chemical composition required for the age group of the fish being reared. These feeds are pressed into pellets, which ensures that each of them contains all blended ingredients.

Copepods are plankton crustaceans which appear in pond waters after the protozoans, rotifers and cladocerans. The life cycle of copepods lasts about 3–6 days when the water temperature is about 20–25 °C. 1) Egg, 2) Nauplii stages, 3) Copepodic stages and, 4) Adult mating.[13]



Cornea is the transparent layer forming the front of the eye.

Culture-based fishery (CBF) – This technique, also called fish ranching, is widely used all over the world. In CBF, natural or man-made water bodies are stocked with young fish, proportional to the fish food productivity of the given water. The fish originally in the water bodies are generally removed before stocking to reduce competition and predation by these fish on the young fish introduced.

Diapausing egg is an egg capable of surviving an unfavourable period during which its development is suspended.

DNA is the abbreviation of deoxyribonucleic acid, a self-replicating material which is present in nearly all living organisms as the main constituent of chromosomes. It is the carrier of genetic information.[14]

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[12] Source: www.arofanatics.com

[13] Source: http://science.kennesaw.edu/

[14] Source: https://www.bing.com/search?q=DNA+definition&src=IE-TopResult&FORM=IETR02&conversationid=&pc=EUPP

Endocrine hormone is directly released into the blood or lymph.

Endogenous feeding (of fish larvae) means that the nutritional demands of the developing larvae are supplied by the yolk sack.

Exogenous feeding (of fish larvae) is when the fish larvae take their food from the water.

Feeds or **fish feeds** are any type of materials, plants, fruits, grains etc. given by the farmer and consumed by the fish. Additional terms help to provide more precise information on the type of fish feeds and are distinguished accordingly: energy and protein feeds; dry, wet and green feeds; supplementary and compounded pellet feeds. The latter, also defined in this glossary, can be further differentiated as farm-made and commercial feeds. Feeds are also often termed artificial food or artificial fish food, but these can be easily confused with natural or natural fish food, which is a key definition in the practice of pond fish farming. The official FAO definition (2014) is: Fodder intended for fish in aquaculture establishments, in any form and of any composition. Also defined as any material (single or multiple), whether processed, semi-processed or raw that is intended to be fed directly to aquatic animals.

Feed conversion ratio (FCR) is an important figure that indicates the efficiency of feeding in a farmed animal, including fish: it shows how much feed is needed to gain 1 kg of growth.

Final maturation is the process just prior to ovulation when cell division is completed in fish eggs (called meiosis).

Fish food is all materials and organisms, dead or alive, which are found and/or develop in waters and which fish consume. Also called "natural fish food" or simply, "food".

Fish ranching – see "culture-based fisheries".

Genetic characterization – In the agreed terminology of gene banks the term 'characterization' stands for the description of characteristics that are usually highly heritable, easily identified by the eye and expressed equally in all environments. In genetic terms, characterization refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors (Vincent *et al*., 2005).

Genetic markers can be defined as specific DNA sequences with a known location on a ***chromosome****. Genetic markers are essential tools for linkage and association studies, which are simply not feasible without the possibility of differentiating the origin of the recombinant chromosomes on the DNAs obtained from mapping crosses.[15]

Gill-raker structure is the set of very fine filtering spines or "bristles" present on the first gill arch. This filter apparatus allows even adult tambaqui to filter out the 0.2 mm-sized plankton and floating particles from the water. The gill-raker structure can be found between the mouth and branchial cavities (i.e. gill cavity). Fish can produce pressure and vacuum alternately in both cavities, thereby enhancing the efficiency and effective functioning of the filtering apparatus. The mouth cavity has two folds behind the teeth which close and hermetically seal the mouth when it is full of water. The big tongue, when lifted up, can produce pressure, and when it is lowered produces vacuum. The vacuum can be produced in the branchial cavity with the help of a wide muscular operculum on both sides (i.e. gill cover), which is connected to the bony operculum. When the bony operculum is open, the muscular operculum keeps the cavity closed. Here the pressure is produced simply by closing the bony operculum (Goulding, 1981; Goulding and Carvalho, 1982; Woynárovich, 1986).

GnRH-analog is the abbreviation of artificially made gonadotropin-releasing hormone of the hypothalamus.

Gonadal – that which relates to the gonads, i.e. ovary and testicle.

[15] *Source*: https://www.sciencedirect.com/topics/neuroscience/genetic-marker

History of artificial propagation of fish started in Brazil when, in 1905, a law was enacted that hydroelectric companies that constructed dams across rivers must construct suitable structures to ensure either: the migration of fish to spawning grounds, or the replacement of the missing progeny by stocking young fish in waters below or above the dams. For this reason, Rudolf Von Ihering developed a technique that induces spawning, whereby pituitary suspension of fish is administered into sexually mature fish. This technique used by DNOCS (Department National de Obras Contra Secas) in Brazil and was presented at a scientific congress in the Former USSR in 1931. After the 1930s, Soviet and Hungarian ichthyologists simplified the technique in order to adapt it to the large-scale artificial propagation of carps. Thereafter, this technology, developed for common and Chinese major carps, was introduced to Brazil on selected fish farms run by the Compania do Desenvolvimento do Vale do Rio San Francisco (CODEVASF) from 1983 onwards.[16] In the 1980s this facilitated a large-scale propagation of not only tambaqui, but also a wide range of other fish species, including pirapatinga (*Colossoma bidens*), pacu (*Colossoma mitrei*), curimata pacu (*Prochilodus margravii*), surubim (*Pseudoplatystoma* sps.), etc.

Hour-degree is the summed up (cumulative) value of the water temperature measured hourly. The hour-degree unit sign is ºH.

Induced spawning is a widely used technique for the artificial propagation of river spawning Chinese and Indian major carps. For the induced spawning of riverine fish species, the course of running water is imitated in a round or oval tank where females and males treated with a natural (pituitary) or artificial hormone perform synchronized spawning. Fertilized eggs obtained this way either remain in the same spawning tank or are collected and taken into hatchery jars for incubation.

Juvenile is the term widely used by specialists dealing with fish stocks in the wild. As per the FAO definition, it is the term used to refer to the young stage of animals, usually for fish from the post-larval stages until the time they attain sexual maturity, by which point they are generally hardy.

Main culture systems of fish – There are four basic fish culture systems widely practised worldwide. These include: natural-fish-food-based, culture-based fisheries (CBA), pond culture and their combination called pan culture; cage and tank cultures are based on either collected live feeds, a mixture of different feeds, and farm-made or industrial, biologically balanced, dry pellet feeds.

Microphyle is the opening in the egg through which the sperm enters and fertilizes the egg. The microphyle is a small aperture in the egg shell that closes very quickly, within 40–60 seconds, after the eggs come into contact with water. After this interval the spermatozoa are not able to enter into the egg protoplasm and achieve fertilization. Therefore, when the stripping of dry eggs finishes and the milt is well spread on the surface of the egg-mass, they should immediately be mixed thoroughly with a soft plastic spoon or, even better, with a dry strong feather.[17] Mixing should be performed rapidly but carefully, in a gentle and accurate manner, taking care that the eggs are neither damaged nor broken. After mixing eggs and milt, clean hatchery water of normal temperature should be poured on the egg mass in small, 5–10 ml, quantities and stirred with the eggs. This way, the water and eggs are gradually yet completely stirred in a short time. The amount of total water used for fertilization should equal about half the volume of eggs. Accordingly, about 100–150 ml of water should be used to fertilize about 200–300 g of eggs. Using more water is not advisable, as the spermatozoa become highly "diluted" and are therefore unable to fulfil the fertilization purpose during the short time available, when the microphyle is open: they would die before they could find their way into the eggs. Spermatozoa are motionless in the sperm-liquid – popularly known as "milt" – but if they come

[16] The technology was widely referred to as the "Technologia Hungara" during the 1980s in Brazil.

[17] The necessary quantity of milt for fertilising 200–300 g eggs is about 4–5 ml.

into contact with water, they start to move. Only mobile spermatozoa can penetrate the egg through the microphyle and thus achieve fertilization. The mobility of sperm does not extend beyond one minute. The artificial fertilization must be performed rapidly but carefully. For the sake of certainty, after one minute more water should be poured on to the eggs and stirred carefully for no longer than 2–3 minutes. Stirring the eggs for longer could damage the eggs.

Microsatellite is a section of DNA (i.e. deoxyribonucleic acid, a substance present in nearly in all living organisms as the carrier of genetic information) consisting of very short nucleotide sequences repeated many times; the number of repeats varies between members of the species. Used as a marker in determining genetic diversity, identifying important genetic traits and in forensics, population studies and paternity studies.[18]

Monoculture is when only one fish species is cultured in a pond, tank or cage.

Natural food or **natural fish food** is any type of material, plant or animal which develops or is found in the water and consumed by fish.

Neotropicalis is a zoological term that relates to or denotes a zoogeographical region comprising Central and South America and the Caribbean.

Ovulation is the discharge of eggs from the "wall" of the ovary, i.e. eggs in the ovary complete their final maturation, the follicle which held them to the ovary dissolves, and eggs are remaining free for release.

Pathogenic relating to any organism that can cause disease.

Piracema – This word, of indigenous origin, comes from two Indian words: "pira", meaning fish, and "cema", meaning rise. Long ago, the Indians observed the movement of fish in shoals upstream to mate and reproduce. Prior to this phenomenon nature emits signals, perceived by the fish, that the favourable season is on its way to arrive. Warmer days, frequent rains and more oxygenated water cause millions of male and female fish scattered across rivers to clump together in large schools to prepare for the "climb". Piracema fish, also known as migrators, need to make an intense physical effort to swim upstream and reach spawning grounds. Some of the species swim hundreds of miles in a few days. The rains increase the level of rivers, which overflow and supply water to marginal lakes and swampy lagoons, allowing the fish to reach these places or climb to the headwaters, where they find the adequate environmental conditions to spawn: warmer, oxygenated and turbid waters, which help protection against predators. When they reach these places, the adult fish are mature and ready to mate. The fertilization of the fish is external, and the great concentration of males and females increases the chances of fertilization in the aquatic environment. From there, hundreds of millions of fertilized eggs float downstream. After hatching, the larvae develop in the freshly inundated marginal lagoons and flood plains, which serve as "nurseries" for the fish.[19]

Plankton crustaceans are among others the cladocerans and copepods that occupy fertile waters and are an excellent natural food for the developing fry of practically all freshwater fish species.

Pollutants – harmful and poisonous materials, which endanger fish stocks in waters that are contaminated with them. There are industrial, agricultural and domestic pollutants.

Polyculture is when three or more fish species, which have partly or entirely different diets and feeding habits are grown together in the same pond. In a polyculture there is always a main fish species, which fish producers focus on, while the remaining species in the polyculture are additional, which make use of those areas of the pond not utilized by the main species. Of course, tambaqui should not be reared with one of its food and feed competitors unless their number is negligible. However having an either native or exotic species which is herbivorous and another which feeds on the pond floor can complement tambaqui without compromising them.

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[18] Source: http://www.dictionary.com/browse/microsatellite).

[19] Source: http://www.ief.mg.gov.br/pesca/piracema 2017

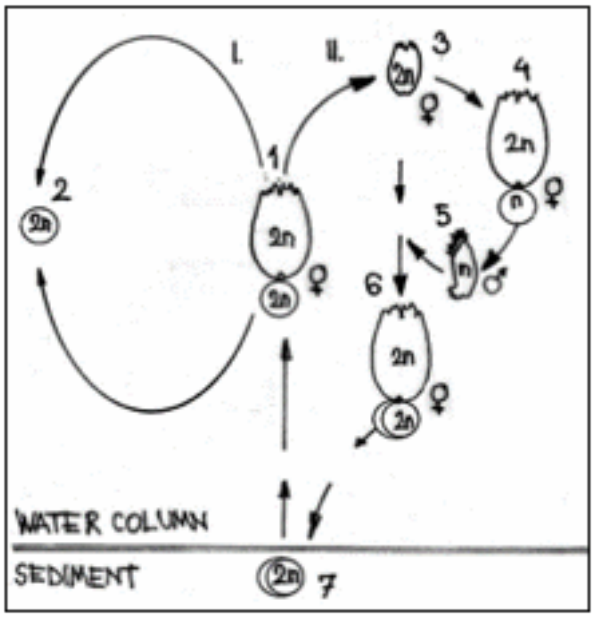
Precursors are substances from which another substance is formed, especially by metabolic reaction. In the case of eggs these include a variety of proteins from which the female builds up the yolk in the egg.

Preserved pituitary glands are glands collected for use in fish propagation, which should be conserved either with alcohol or acetone. This publication recommends the use of acetone dried pituitary glands, as explained in Annex 1.

Pylorus – The opening of the stomach into the duodenum (i.e. first section of the intestine).

RNA is the abbreviation of ribonucleic acid, a nucleic acid present in all living cells. Its principal role is to act as a messenger carrying instructions from the DNA to control the synthesis of proteins, although in some viruses RNA rather than DNA carries the genetic information.[20]

Rotifers are one of the first appearing types of zooplankton. The life cycle of a rotifer is presented through *Brachionus plicatilis*: I. It has an asexual phase, which lasts until environmental conditions are favourable II.; the sexual phase occurs when conditions become unfavourable. In this case, females produce males and their pair which result in the production of a ***diapausing egg****, which lies in the sediment until conditions are favourable for hatching and starting a new life cycle. (Tortajada *et al.,* 2013).



Sexual pocket is a typical feature of tambaqui, pirapatinga, and pacu, which encapsulates the anus, sexual opening, and urine aperture. Outside of the propagation season, and in specimens which are not yet sexually mature, it is usually closed. This pocket may be an adaptation to living together with fish such as piranhas, which could otherwise bite the soft body, that is now hidden by the sexual pocket.

Somatic determination of sex – Initial formation of ovaries or testicles.

Specific gravity or **relative density** is the ratio of the density of a substance or object to a standard density (usually that of water or air).

Spore – Typically a tiny single-cell asexual reproductive unit, characteristic of lower organisms.

Standard length (SL) is the length of fish measured in a straight line from the tip of the snout to the end of the caudal skeleton.

Stripping – Technical term is used for the action when ovulated eggs or milt (sperm) are taken from fish.

Supplementary feeds are those energy-rich grains and byproducts given to supplement the natural fish food grown in the pond.

Total length (TL) is the overall length of a fish measured in a straight line from the tip of the snout to the tip of the tail.

Vitellogenesis – The process before each reproductive season when the yolk builds into the eggs.

Viruses are extremely minute (maximum 300 nm) infectious agents which cannot survive and multiply outside the cells of host organisms. Though viruses are not considered living organisms, they are biological systems because they have ***DNA**** and ***RNA****. Therefore, for thematic as well as didactical reasons, they are discussed within the group of biotic agents responsible for causing diseases in fish.

[20] *Source*: https://www.thefreedictionary.com/RNA and http://www.dnaftb.org/25/

Whitewater – When a river transports large quantities of sediment it is usually rendered "muddy," a colour somewhat similar to coffee with milk. Major tributaries with headwaters in the Andes are all turbid because of the huge amount of loose material in the high mountain chain that is easily eroded and then carried downstream as far as the Atlantic Ocean. Locally, these rivers are often referred to as whitewater rivers, but this term should not be confused with its English interpretation that can also refer to the turbulent waters of rapids. These tributaries include the Madeira, Ucayali, Marañón, Putumayo-Içá and Caquetá-Japurá rivers. There are also several tributaries that do not have headwaters in the Andes but are also considered whitewater rivers because of their relatively high sediment loads, such as the Purus and Juruá. Whitewater rivers have a higher nutrient content than blackwater and clearwater rivers. The pH of water in turbid rivers is often near or above neutral (7.0). At elevations higher than approximately 2 000 m in the Andes, the pH of river water can be above 8.0.[21]

Zooplankton is the collective name for tiny worms and insects swimming passively or actively in the water. In other words, they are animal members of plankton (Thain and Hickman, 1980).

[21] http://amazonwaters.org/waters/river-types/whitewater-rivers/

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Annex 1
REQUIRED PRODUCTION CONDITIONS, DEVICES, EQUIPMENT AND MATERIALS USED IN A TAMBAQUI HATCHERY

1. DECIDING ON HATCHERY TYPE AND SIZE

Usually a fish hatchery is not used exclusively for propagating only one species, but the same hatchery devices are used in parallel or subsequently for producing larvae of different species. The same is generally true of tambaqui hatcheries. These hatcheries are called multispecies fish hatcheries.

The actual size of a fish hatchery is described by two figures. The first one is the maximum number of feeding fish larvae, which the hatchery can produce within one shift; the other figure is the total feeding larvae production capacity of the fish hatchery within a propagation season. In the case of tambaqui, this season may be the entire year.

During planning for the size of one shift, it is advised not to produce more larvae per shift than can be stocked and/or sold immediately, once the larvae start to feed. The size of one shift is further determined by the maximum number of brood fish, which staff can safely and efficiently handle and strip. Accordingly, there are smaller or larger hatcheries as demonstrated in Figure A1-1.

FIGURE A1-1
Multispecies fish hatcheries

(A) **(B)**

(A) A smaller fish hatchery, as demonstrated in Figure A1-3 – one block of the different hatchery devices allows the production of a maximum of about 3 000 000 feeding tambaqui larvae per shift and about one shift of this quantity per week. (B) A large fish hatchery with many blocks can produce more per shift or can increase the number of shifts per week.

2. BASIC CRITERIA FOR HATCHERY DESIGN

Similarly to other fish hatcheries, a multispecies fish hatchery for tambaqui should meet three basic criteria: 1) a secure, demand-driven, general water supply, 2) continuous drainage of effluent water, and 3) a safe and secure water supply system to all devices within the hatchery.

2.1 Water supply and drainage

There are three main options to supply water to the hatchery. These are shown in Figure A1-2.

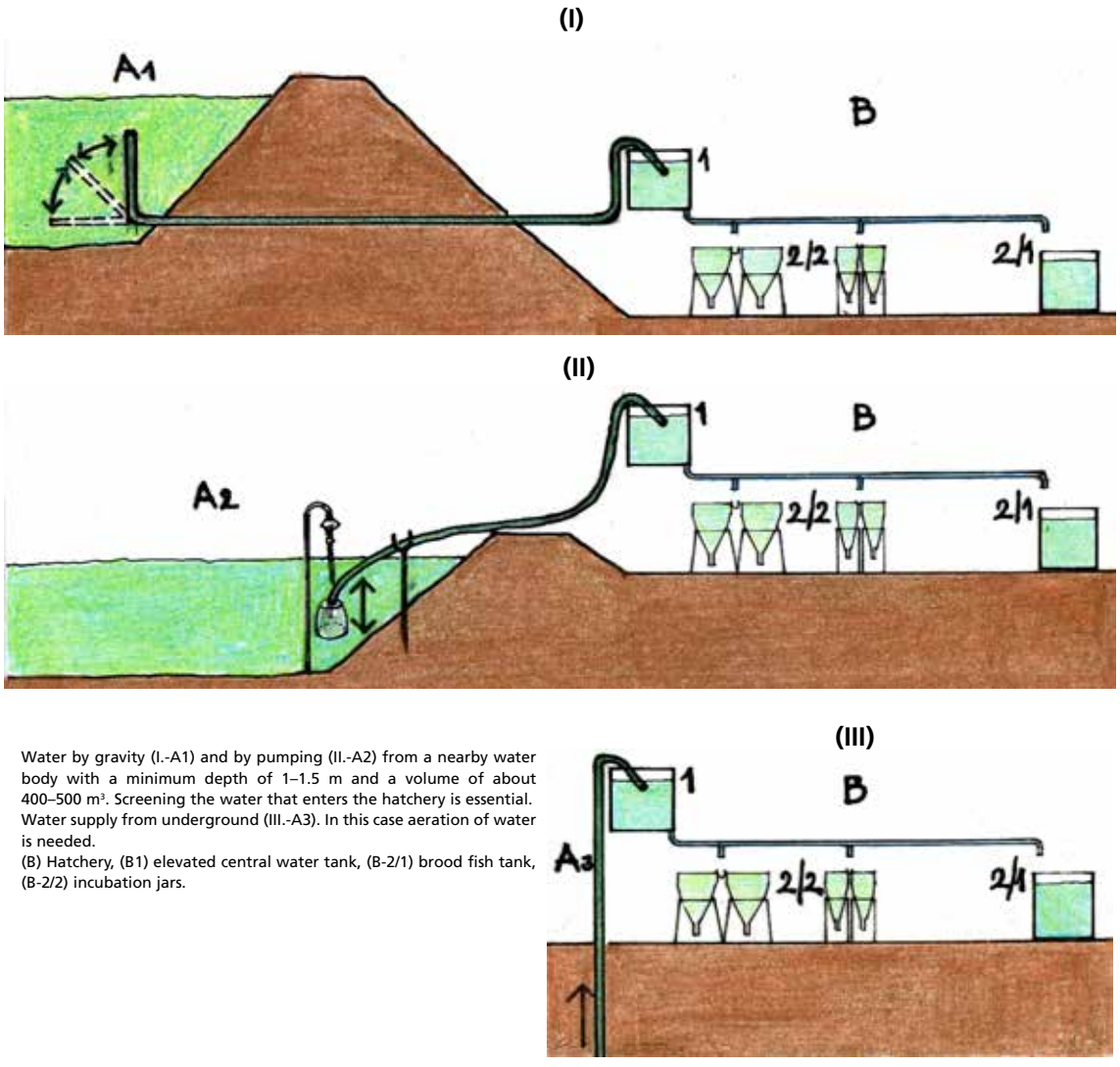
2.2 General layout and arrangement of devices

The arrangement of hatchery devices should ensure enough space for stripping brood fish, siphoning fish larvae from the devices, etc. as outlined in Figure A1-3. In the figure the water supply pipes marked with red should be of a suitable diameter and size to supply all devices with the maximum water required; the drainage system marked in blue should collect all effluent waters. If drained water from the hatchery is conducted into a small pond then accidentally escaping larvae can still survive and grow there.

The large cleanable central tank, of several cubic metres, is to ensure uniform water pressure in the hatchery devices and to facilitate screening space for the water if needed.

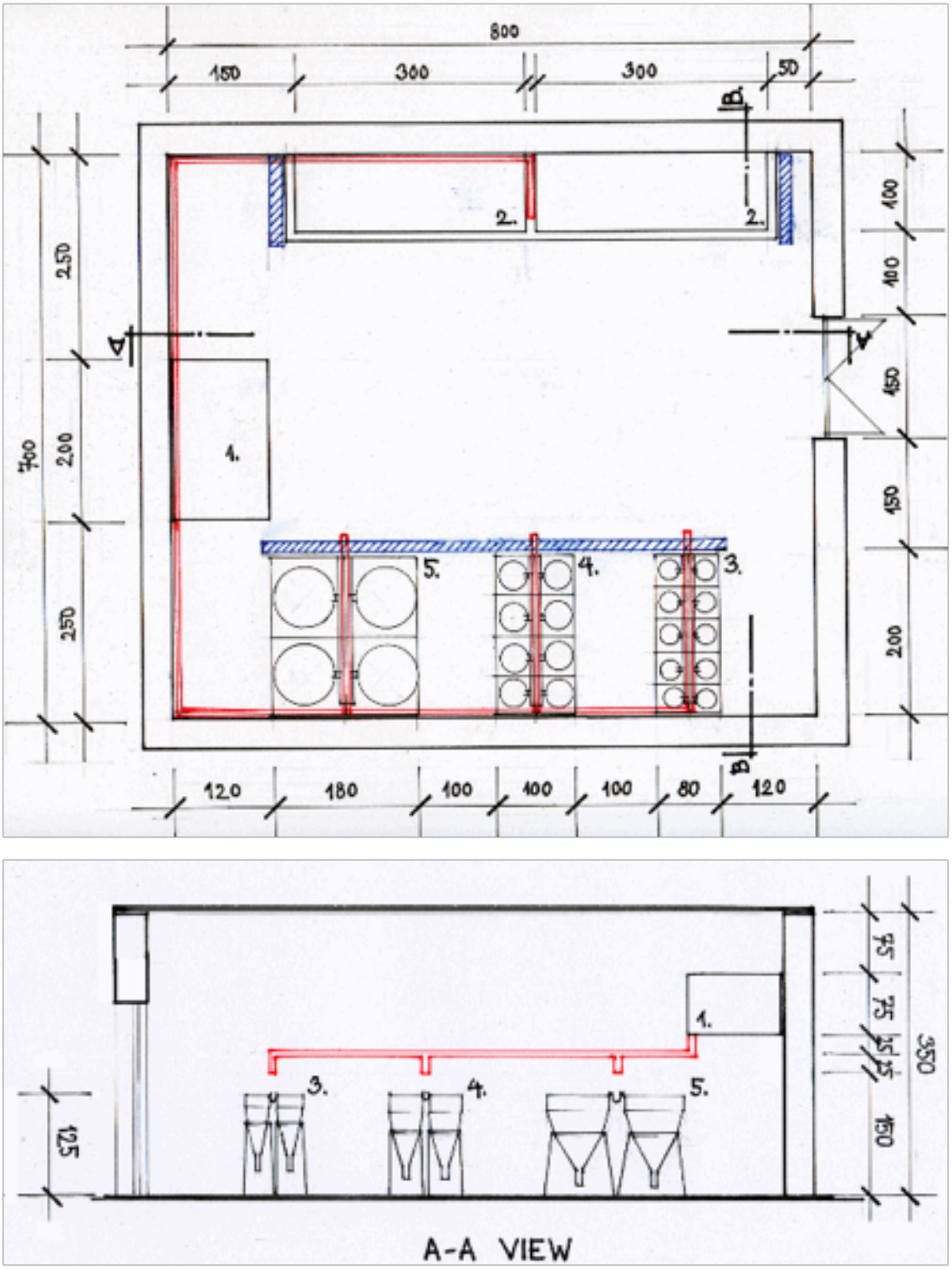
The water consumption of such small hatchery will be around 250–380 m^3 per day. More exact estimations are presented in Table A1-1.

FIGURE A1-2
Main options for water supply to a fish hatchery



Water by gravity (I.-A1) and by pumping (II.-A2) from a nearby water body with a minimum depth of 1–1.5 m and a volume of about 400–500 m^3. Screening the water that enters the hatchery is essential. Water supply from underground (III.-A3). In this case aeration of water is needed.
(B) Hatchery, (B1) elevated central water tank, (B-2/1) brood fish tank, (B-2/2) incubation jars.

FIGURE A1-3
General layout of a multispecies fish hatchery



(1) Elevated central water tank, (2) tanks for hormone treatment of brood fish, (3) block of small incubation jars (20 litres each), (4) block of medium incubation jars (60 litres each) and, (5) block of large incubation jars (200 litres each).

TABLE A1-1
Water consumption of a small multispecies fish hatchery

Devices		Water consumption (l/min)		No. of devices	Total of required water					
					l/min.		m³/hour		m³/day	
Name	Volume (l)	Avg.	Max.		Avg.	Max.	Avg.	Max.	Avg.	Max.
Large jar	200	10.0	15.0	4	40	60	2	4	58	86
Medium jar	60	3.0	4.5	8	24	36	1	2	35	52
Small jar	20	1.0	1.5	10	10	15	1	1	14	22
Brood fish tank	1500	50.0	75.0	2	100	150	6	9	144	216
Total of water consumption					**174**	**261**	**10**	**16**	**251**	**376**
Total with an additional 6–7 % water										**380**

It is important that all the hatchery equipment and jars are kept clean and dry when they are not in use. It is also advantageous to keep the water supply and drainage system of the hatchery dry whenever it is possible. Through keeping the equipment, jars and water pipes clean and dry, the development of microscopic parasites of eggs and larvae can be prevented. Another way of preventing infections in the hatchery is to disinfect the water supply pipes, equipment and jars regularly, using a 1–2 percent formaldehyde solution.

3. HATCHERY DEVICES

Brood fish tanks and the different incubation jars should suit their purpose.

3.1 Brood fish tanks

In brood fish tanks the male and female fish are kept separately during the entire hormone treatment. The supply of oxygen-rich water to these tanks is essential. This is ensured with a continuous flow/exchange of water. In cases, when a reduced quantity of water is available only temporarily, additional aeration or oxygenation should be provided for the water in these tanks (see Figure A1-4).

FIGURE A1-4
Tanks used for the hormone treatment of brood fish

(A) tanks made out of tarpaulin fixed on a stiff frame and covered with net to avoid brood fish jumping out of the tank. (B) A concrete tween tank with dividers. This setup allows the addition of temporary compartments where brood fish can smoothly be concentrated and captured.
These brood fish tanks are usually 2.5–5 m long, 1–2 m wide and 0.8–1 m deep. They are supplied with running water and drained through an elbow pipe of about at least 5 cm in diameter with which the water level in the tanks can be regulated. The depth of the water in these tanks is generally about 40–50 cm.

3.2 Incubation jars

Usually the same jars are suitable for the incubation of eggs and the rearing of non-feeding tambaqui larvae. These jars can be made out of different materials such as

stainless metal sheets, tarpaulin, transparent flexible plastic, fibreglass or rigid PVC sheets. Incubation jars can be made easily out of tarpaulin and the fibreglass jars are extremely durable. These two types of devices are discussed here. The criteria for a good incubation jar are:

- Made out of a neutral material that does not emit anything toxic.
- Oxygen should be supplied continually to it with flow-through water current. This also removes CO_2 and NH_3. Jars where water is not continuously changed or in which oxygen is provided only by aeration are not suitable.
- Overflowing water should not wash out eggs or larvae. Therefore the jar should have a larger filter surface against which the larvae will not be pressed by continuous water current. When the filter surface of the incubator is small or insufficient, large numbers of larvae that swim up will be killed because the water current presses them against the sieve. The movement of water should therefore be gentle in an incubation jar.

Strong, reinforced tarpaulin (the kind used for covering lorries) is widely available at a reasonable price in many countries. Jars cut out of this material can then be strongly welded. As a result, practically any shape or volume, up to about 100 l can be conceived in the event that a professional welder is available. Steps for making and installing such devices are presented in Figure A1-5.

The best possible, widely tested fibreglass incubations jars are presented in Figures A1-6 and A1-7.

Sieves in the incubation jar often become blocked, especially after the hatching of larvae. In order to ensure water flow through the sieve, the blocked sieve should be cleaned only from outside with gentle flushing; if the sieve is cleaned from the inside many larvae may be killed by pressing them against the sieve. In the event there is not enough labour available, especially at night, the use of a porous stone air diffuser operated by an aquarium aerator can prevent blocking of the sieve if the diffuser is placed outside the sieve from where air will backwash the screen continuously.

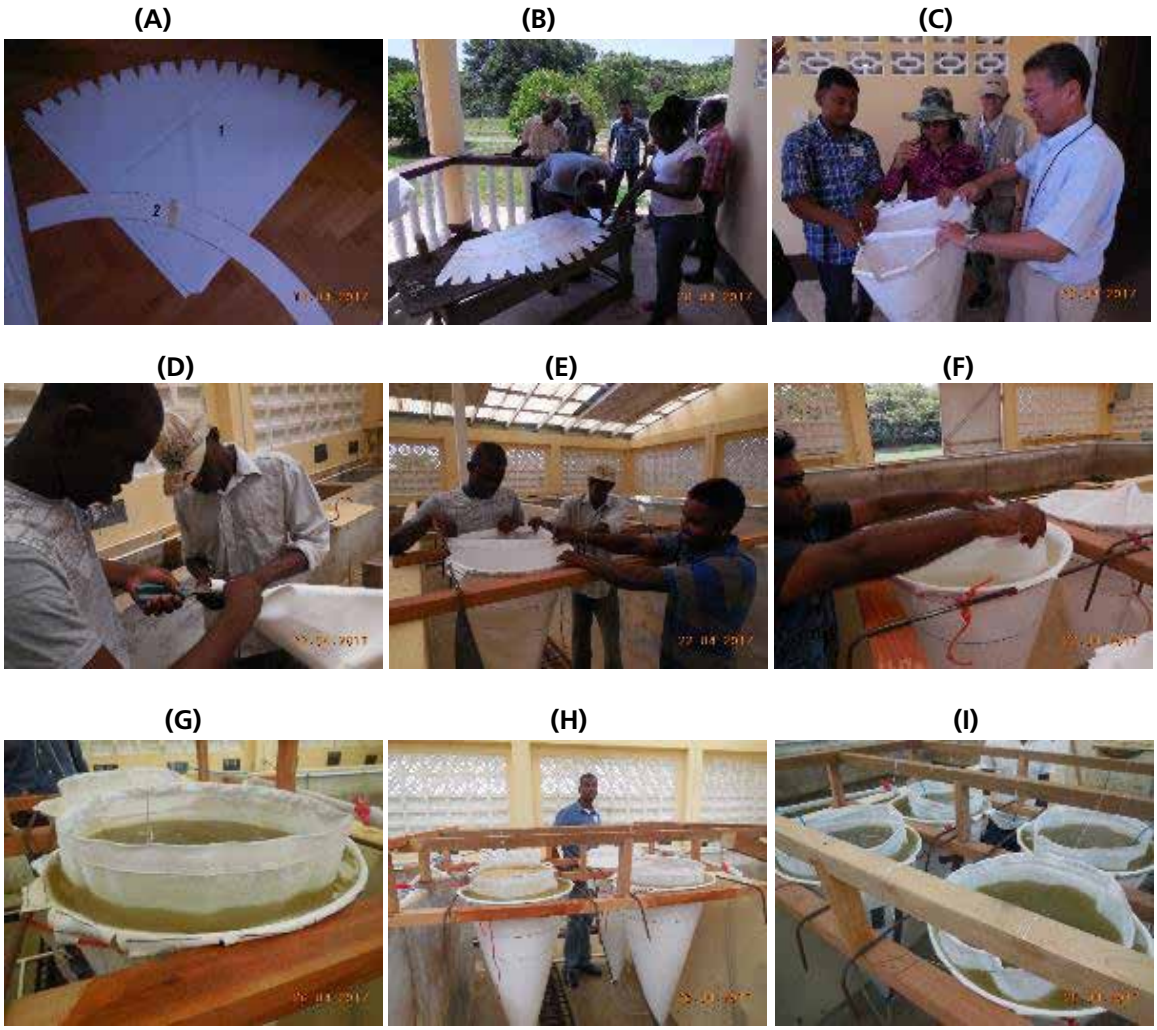
The high pressure and strong current in the incubation jars usually occur in those fish hatcheries where the design of the water supply system has followed the same principles as the water supply of kitchens and bathrooms in households. When water comes from an elevated tank in a narrow pipe the result is a sharp and strong water current which will strip the shells of the eggs. To avoid the current being too strong, it is recommended that water supply pipes to the large diameter jars of (3/4 to 1") be installed, and that the central water supply tank should be placed only about 0.5–1.5 m higher than the water level in the incubators.

4. HATCHERY EQUIPMENT

There are only a few important items of equipment used in the hatchery. These are the different scoop nets to catch and weigh brood fish (see Figure A1-8), a brood fish carrier (Figure A1-9), siphon, and a bucket for concentrating the larvae siphoned from the jar (Figure A1-10).

Infertile eggs, egg shells (after hatching) and larvae should be removed from the incubator jar by siphoning. For this purpose a pipe of approximately half an inch in diameter should be employed. This usually is a flexible pipe fixed to a stiff one, which sinks into the incubator or larvae rearing jar (see Figure A1-10 and A1-11). In order to concentrate the siphoned larvae, either a double bucket with fine mesh sieve or a single bucket/container with a removable sieve should be used. One important aspect of siphoning larvae is that the water current which takes them into the bucket should not press against the wall or the sieve in the bucket: the sieve should always have a counter-support. It is the larger bowl displayed in Figures A1-10 and A1-11.

FIGURE A1-5
Farm made 40 l large tarpaulin incubator



A 40-litre incubator is enough to hold a maximum of 100 000–120 000 fertilized eggs and rear 80 000–100 000 feeding larvae.
(A) Parts of the incubation jar to be cut; (A1) the wall: a section with 105 cm perpendicular sides (up to zig-zag to be folded back) at an 85° angle. (A2) The inside collar to which the sieve is fixed. (B) Welding of the jar. (C) Sieves sewed earlier are welded into the jar. (D) Inlet fittings of the jar are fixed. (E, F and G) The jar with the internal screen. (H and I) Arrangement of a block of four incubators.

Photographs: ©FAO/András Woynárovich.

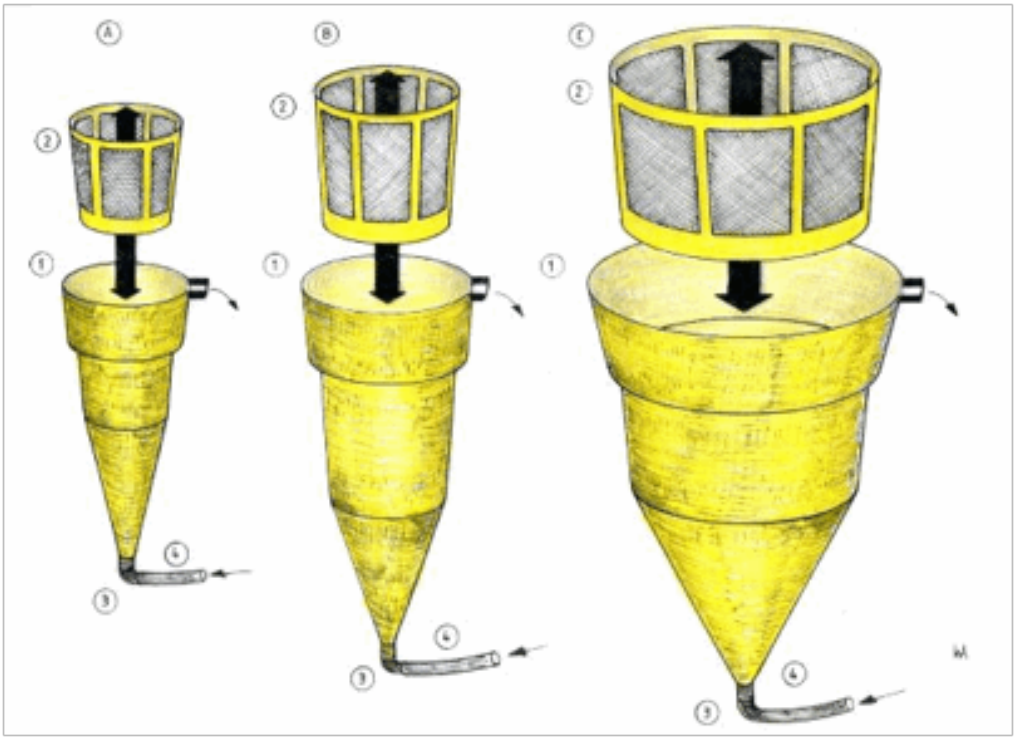
5. MATERIALS USED IN THE HATCHERY

The most important and expensive material used in a fish hatchery is the pituitary gland. Though it can be purchased, it has a high price. Therefore, locally collected hypophyses from any matured fish adequately serve the purpose, and reduce the costs of hatchery operations.

Traditionally glands are taken as demonstrated in Figure A1-12, but this process considerably reduces the sales value of fish: a fish with a hole in its head is considered unappetizing even if the fish is otherwise fresh. A new technique was introduced in 1985, thanks to which the pituitary gland is extracted through the roof of the mouth. This technique is summarized in the figures that follow. Extracting the pituitary through the roof of the mouth is done with a hand tool made out of a strong steel pipe, with a wooden handle (see Figures A1-13 and A1-14).

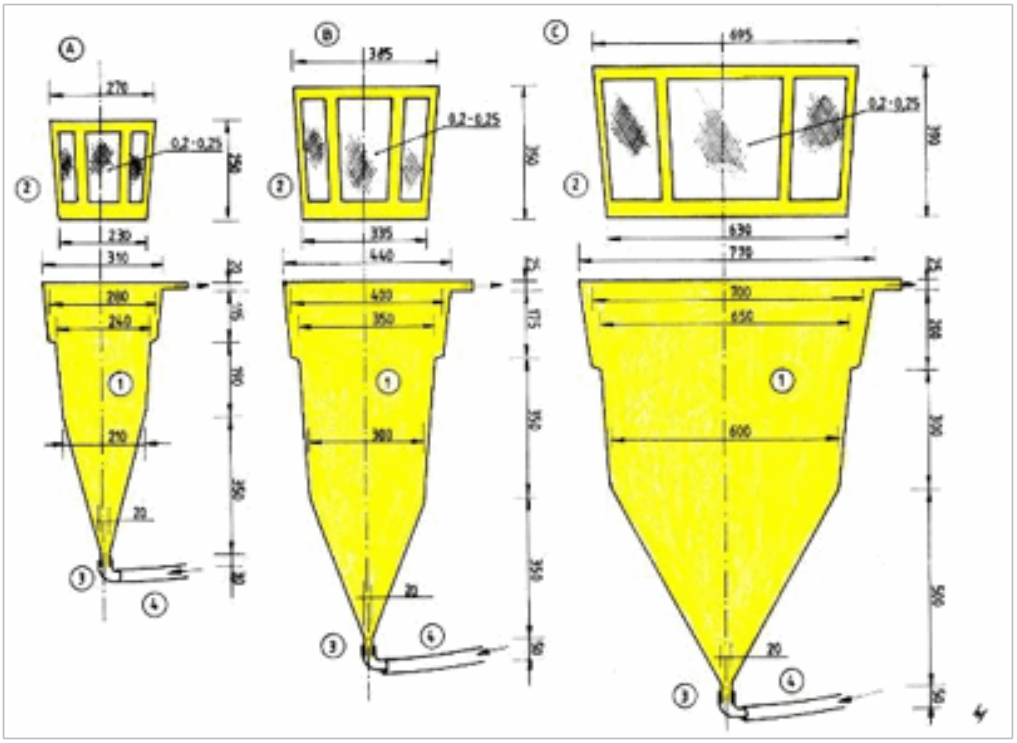
Glands collected this way are preserved as detailed in the glossary at "Acetone-dried carp pituitary glands".

FIGURE A1-6
The shape and sieve of fibreglass incubation jars



(A) 20-litre jar. (B) 60-litre jar. (C) 200-litre volume.

FIGURE A1-7
Dimensions of fiberglass incubator and larvae rearing jars



(A) 20-litre incubator jar. (B) 60-litre jar. (C) 200-litre jar for the rearing of larvae. The mesh size of the sieve should be between 200–250 microns.

FIGURE A1-8
Open-ended scoop net

This type of scoop net is a simple but vital tool, which is indispensable for proper handling of brood fish during selection, hormone treatment and stripping.

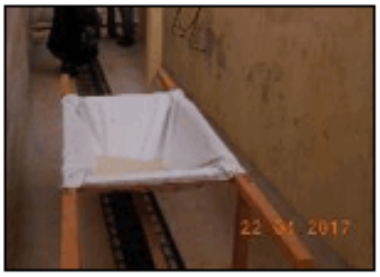
Photographs: ©FAO/András Woynárovich.

FIGURE A1-9
Making of brood fish carrier

(A)

(B)

(C)



(D)

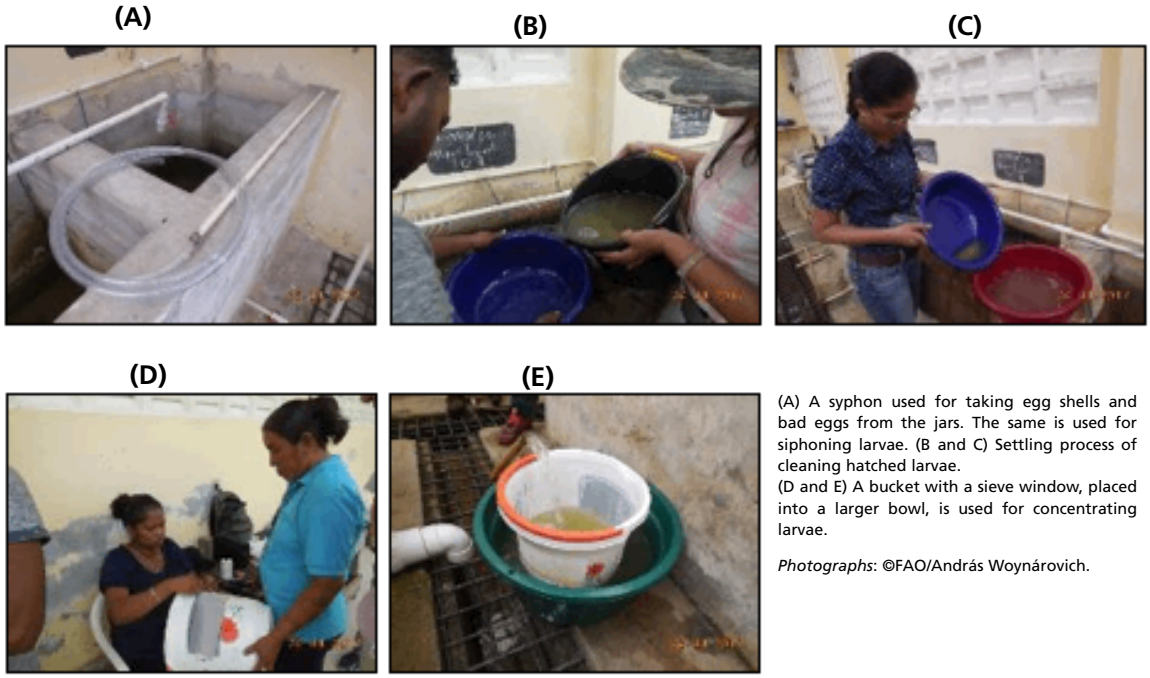
A fish carrier (0.6 x 0.8 m) is important when brood fish are selected at the pond side or carried to and injected in the hatchery. Usually two of these carriers are adequate for the moving and handling of males and females separately.
It is important to make the carriers as deep as possible. Material such as a workshop banner, tarpaulin or strong but flexible plastic sheets can equally be used. The material featured in the images was slightly smaller than required but still served the purpose.
(A and B) Making the carrier. (C and D) Ready to use.

Photographs: ©FAO/András Woynárovich.

The donor fish species does not have to be carp, but can be any species that is available on a large scale. In this case the needed quantity of glands can be collected easily. A guiding rule is that 1 kg of sexually mature donor fish (of common carp, for example) is needed for obtaining as much hypophysis as will induce the ovulation of 0.8–1.2 kg of a female fish. The same quantity of extracted hypophysis is needed for the treatment of about 1.5–2 kg male fish.

According to Kubitza (2004) carp hypophyses can also be replaced with hypophyses collected from native fish species such as curimata (*Prochilodus margrave*), curimbatá (*Curimata macrops*) or jaraqui (*Semaprochilodus* sp.).

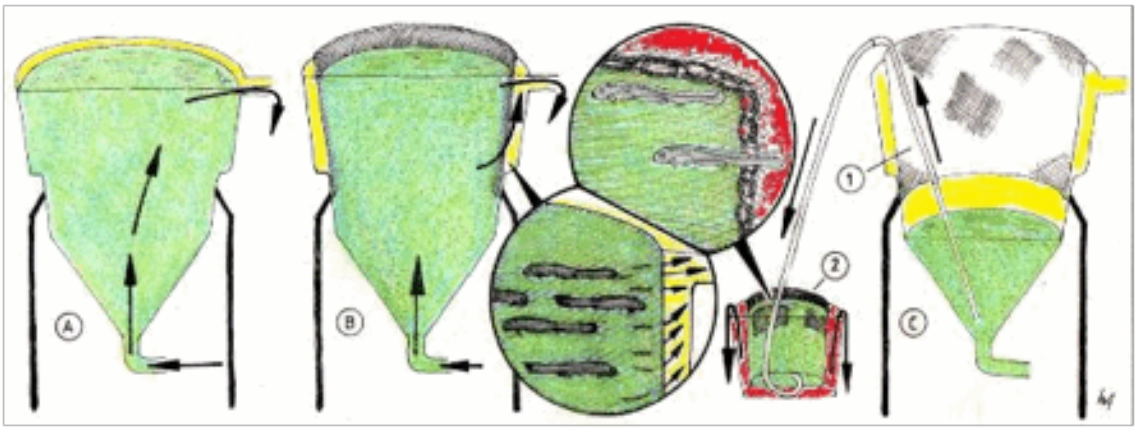
FIGURE A1-10
Equipment for the cleaning of developing larvae



(A) A syphon used for taking egg shells and bad eggs from the jars. The same is used for siphoning larvae. (B and C) Settling process of cleaning hatched larvae.
(D and E) A bucket with a sieve window, placed into a larger bowl, is used for concentrating larvae.

Photographs: ©FAO/András Woynárovich.

FIGURE A1-11
How to siphon larvae from the jar



(A) An incubation jar showing the water entering at the bottom and leaving at the top. (B) An incubator jar fitted with the sieve preventing hatched larvae from escaping. (C) Siphoning the feeding larvae from the jar. For this purpose a stiff pipe of about half an inch is used, which fits into the jar and is equipped with a band on top (C1). Feeding larvae are concentrated in a bucket which has a sieve on its side (C2). Through this water outflows. To avoid larvae being pressed against this sieve bucket is placed into a big one.

FIGURE A1-12
Collection of carp hypophyses through the front of the head


Photographs: ©FAO/András Woynárovich.

FIGURE A1-13
Removing the hypophyses through the roof of the mouth



Common carps after removing their hypophyses through the roof of mouth and the equipment used.
Photographs: ©FAO/András Woynárovich.

FIGURE A1-14
Steps to remove pituitary glands from fish through the roof of the mouth

(A)

(B)

(C)

(D)

The actual diameter of this pipe depends on the size of fish from which the glands are taken. Accordingly, the dimension may vary from 0.5 to 1.5 inches. It is therefore advisable to make a set of pipes.
The key steps to removing the hypophyses from freshly killed adult fish are illustrated above. (A) Cutting the throat of fish, (B and C) removal of the lower section of the brain with the pipe and (D) removal of the pituitary gland with a special "hypophysis spoon". (Woynárovich, 1988).

Photographs: ©FAO/András Woynárovich.

Annex 2
REQUIRED PRODUCTION CONDITIONS FOR THE REARING OF TAMBAQUI IN PONDS AND CAGES

1. INVENTORY OF FISH PONDS: TYPES AND PURPOSES

Fish ponds are typically shallow earthen structures of 1–2 m deep, in which fish culture of various intensities can be practised. One essential criteria for the construction of a fish pond is that it should be built on impermeable soil, thereby avoiding water loss through seepage.

Ponds constructed on flat or very gently sloping sites are called contour ponds, while ponds built on hilly sites are called barrage ponds. Ponds can also be grouped by how they are supplied (filled in) with water and drained:

- non-drainable, dug ponds in which the actual water level is determined by the level of (under)ground water.
- barrage ponds accumulate rain and/or water from a stream, but in the pond will overflow in the event of excess water due to heavy rains. This makes managing the water challenging.
- The water in barrage ponds built with an independent water supply source can be fully managed, in the same way as contour ponds.

It is an advantage if a pond can be fully filled and supplied with water, as well as completely drained whenever needed, although this is not a vital criterion for success. The supply and drainage of water in a pond should be achieved by gravity, if possible, to reduce the electricity costs associated with pumping. A combination of the force of gravity and pumps for water supply and drainage is common practice.

1.1 Nursery ponds

It is important that a nursery pond can be supplied with water continuously during the rearing season, as well as drained completely and left dry until the next shift of advanced fry rearing (see Figure A2-1). Additional criteria for a suitable nursery ponds are:

- The size of a nursery pond may very between a few hundred and a few thousand square metres, but ponds of a few hectares are also often used for producing advanced fry.
- There are examples of shallow ponds no deeper than 0.5–0.6 m on average, which are used for the rearing of advanced fry. However, these ponds may warm up excessively on sunny days. Nevertheless, several years of experience rearing advanced tambaqui fry in such ponds has proven their suitability, especially when stocking figures are calculated by water volume instead of water area. An average depth of about 1 m should be the minimum, especially when constructing a new pond.

FIGURE A2-1
Fully drainable nursery pond prepared for inundation

Though earthen ponds are the best, lined ponds or those with concrete walls may also serve the purpose. However in such ponds the application of manure should be calculated carefully and applied more precisely, by being washed into the water from a sack, as described in Chapter 5.3.

1.2 Rearing and keeping fingerlings, table fish and brood stock in ponds

Considering that fingerlings, table fish and brood fish are much larger and less fragile than advanced fry, it is possible to compromise on some of the ideal pond characteristics. However, the principles of a continuous access to water, as well as the ability to drain and harvest fish properly are still essential.

Brood stock ponds should be small enough to allow easy fishing and handling of brood fish.

2. ESSENTIAL PRODUCTION CONDITIONS IN CAGE CULTURE

2.1 Cage materials

On large commercial cage culture farms set in marine waters, the cages are typically made out of nets. This is also the material used in inland commercial cage culture farms. However, for small-scale and household production of fish in cages, rigid materials such as wooden planks, bamboo, fine steel mesh or steel fencing material covered with plastic are often employed for concrete structures (see Figure A2-2).

FIGURE A2-2
Material used for making cages

Photographs: ©FAO/András Woynárovich.

When selecting the material it is important to ensure that fish will not be able to escape and that predators will not be able to enter.

Easy access to the cages is important; the cages should therefore be fixed to a pontoon, allowing them to float and adjust to the fluctuating water level.

2.2 Location of cages

When selecting a location for cage culture, two important factors must be considered: 1) the depth of water below the cage (at least 2 m or more), and 2) the strength of the current, which should not force fish to swim unnecessarily against it. When rearing younger fish, the speed of the current should be about 1–2 m per minute, while 2–6 m per minute is acceptable for larger fish. If the current is too strong, the front of the cages should be adequately covered to reduce the strength of the current in the cage.

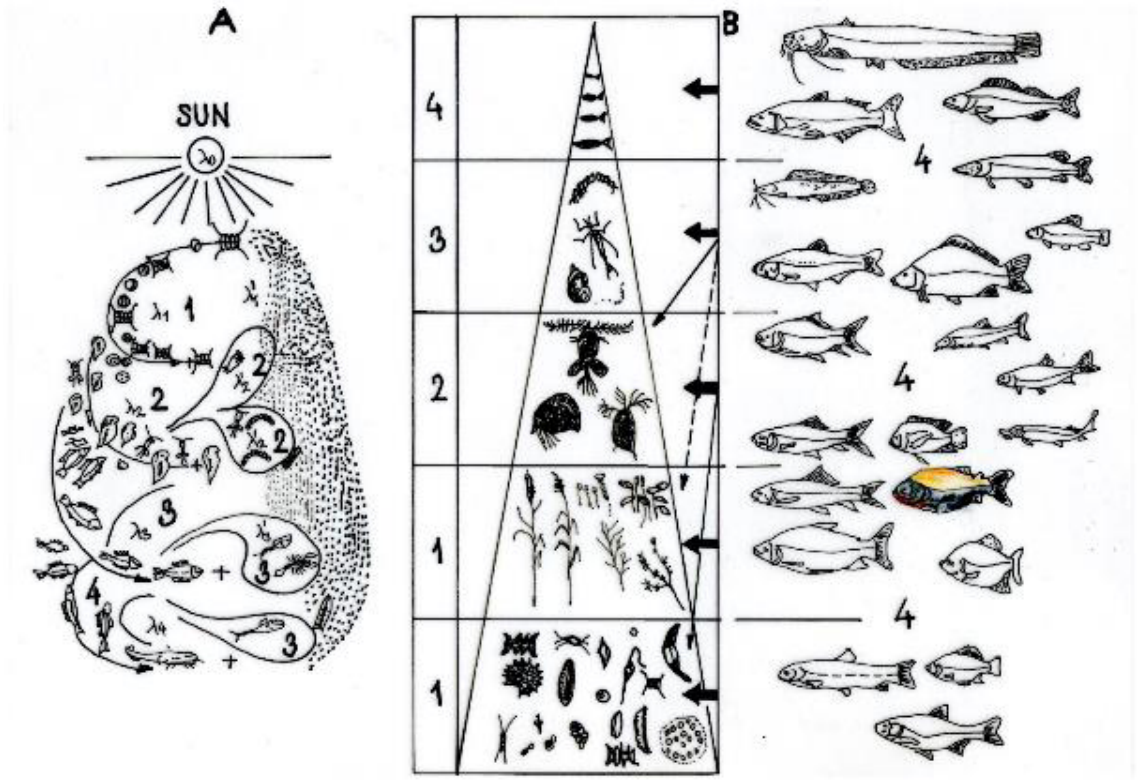
When cages are placed in still water they should either not be loaded with too many fish, or a paddle aerator must be operated to ensure the circulation of fresh, oxygen-rich water into the cages.

Annex 3
FEEDING CONCEPTS AND FEED FORMULATION OF TAMBAQUI

1. THE ROLE OF FEEDING IN DIFFERENT CULTURE SYSTEMS

Figure A3-1 below and Table A3-1 provide good summaries of how natural fish food develops and how the wide range of different fish species and their age groups share the natural fish food resources in different water bodies.

FIGURE A3-1
Fishes and fish food organisms



A: Due to plant nutrients and the energy of the sun, phytoplankton and water weeds (A1 and B1) grow in water. These are a food source for zooplankton (A2 and B2) insects, mollusks (A3 and B3) and fish (A3 and B4), which also eat each other.
B: Fishes grouped by their natural foods: phytoplankton and zooplankton feeders; herbivorous fishes; omnivorous and predator fishes..

Different fish species consume either broadly similar or totally different foods. The type and range of food items consumed changes during their life time. Many species, including tambaqui, have a high level of flexibility in food consumption: this is because fish usually adapt to the availability of food.

Usually the range of actually consumed natural foods depends on the diet and feeding habits of fish. In this regard tambaqui is an outstanding fish.

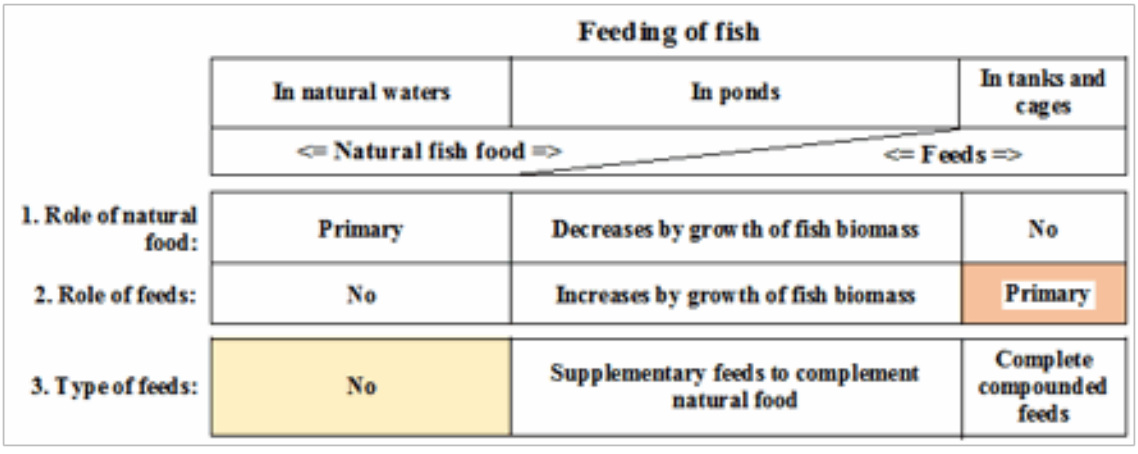
Figure A3-2 presents the role of feeding, which varies considerably according to the culture system and its intensity. These culture systems are:

- culture-based fisheries, or fish ranching
- pond culture
- tank culture and cage culture.

TABLE A3-1
Grouping of fish according to their natural food spectrum

Groups of fishes by their main natural food		Feeding larvae (First 10 days)	Advanced fry (First 4-6 weeks)	Fingerling (up to 100 g)	Grower (up to table fish size)	Adult (brood fish)
Phytoplankton feeders	Main	Zooplankton	Zooplankton	Phytoplankton and anything within the size range of filtrating		
	Occasionally	Phytoplankton	Phytoplankton	Floating detritus after roiling pond bottom		
	Accept feeds	Readily	Readily	May filter feed particles of appropriate size, but it is not suitable source of nutrition for the fish		
Zooplankton feeders	Main	Zooplankton	Zooplankton and anything within the size range of filtrating			
	Occasionally	Phytoplankton	Phytoplankton	Perhaps larger specimens by change		
	Accept feeds	Readily	Readily	May filter feed particles of appropriate size, but it is not suitable source of nutrition for the fish		
Herbivorous fishes	Main	Zooplankton	Zooplankton, larger green algae	Fresh soft/tender green plants		
	Occasionally	Phytoplankton	Phytoplankton and smaller plant particles	Harder water plants including their stems, fish larvae and smaller fish, insects		
	Accept feeds	Readily	Readily	Consume carp feed, but it is not healthy for the fish		
Omnivorous	Main	Zooplankton	Zooplankton, insects	Zooplankton, insects and their larvae, worms, molluscs, tender parts of water weeds and their seeds, zooplankton etc.		
	Occasionally	Phytoplankton	Phytoplankton	Fish larvae, smaller fish dead and alive		
	Accept feeds	Readily				
Predators	Main	Zooplankton	Insects, fish	Fish		
	Occasionally	Phytoplankton	Zooplankton	Insects	Insects, smaller aquatic animals	
	Accept feeds	Readily, after weaning				

FIGURE A3-2
Source of fish nutrients in different culture systems



1.1 Culture-based fisheries – fish ranching

Culture-based fisheries (CBF) is a widely practised means of utilizing natural and manmade water bodies, including large, state-owned water reservoirs or smaller waters used for irrigation or/and as drinking water for livestock.

The basic principle of CBF is to stock a quantity of young fish proportional to the natural fish food production capacity of a given water body. It is possible to estimate the natural fish food productivity of water, and use this to estimate the water body's fish growing capacity. This capacity can be rather considerable – exceeding 100 kg/ha in case of eutrophic water – in situations where fish species with different food spectrums and feeding habits are stocked together.

BOX A3-1
Feeding options for fish in ponds

Feeding of fish can be direct and indirect. Direct feeding is when feed is given to fish. Indirect feeding is when natural fish food production is engineered by increasing the natural productivity of the water in which the fish are reared.

1.2 Feeding fish in ponds

Feeding fish in ponds is usually done both indirectly and directly. The intensity of pond culture depends on how these two feeding techniques are combined. In extensive pond culture the manuring and fertilization of the pond is more dominant.

TABLE A3-2
Chemical composition of the manures of different farmed animals

Values	Dairy cattle	Beef cattle	Ox	Pig	Chicken		Horse
					Layers	Broiler	
Dry matter (DM) as % of fresh manure	12.7	11.6	25.0	9.2	25.2	25.2	20.9
Dry matter (%)	100	100	100	100	100	100	100
Organic material (%)	82.5	85.0	85.0	80.0	70.0	70.0	80.0
Total N (%)	3.9	4.9	4.5	7.5	5.4	6.8	2.9
Total P (%)	0.7	1.6	0.7	2.5	2.1	1.5	0.5
Total K (%)	2.6	3.6	3.2	4.9	2.3	2.1	1.8
BOD* 5 days	16.5	23.0	9.0	33.0	27.0	-	-
COD*	88.0	95.0	11.8	95.0	90.0	-	-

Adopted from: Miner, J.R. and Smith, R.J. 1975

TABLE A3-3
Applicable quantities by type of manure

Type of Manure	Daily		Weekly		Biweekly		Monthly	
	(kg/1 000 m³)							
	From	*To*	*From*	*To*	*From*	*To*	*From*	*To*
Poultry	2.5	3.5	20	25	40	50	80	100
Pig	5	6	35	45	75	90	150	180
Cattle	6	10	50	75	100	150	200	300

1.2.1 Indirect feeding of fish in ponds

Indirect feeding is done with manuring and fertilization if available. The objective of this is to increase the natural fish food productivity of the pond water. Organic materials are important for zooplankton, but also serve as a source of plant nutrients

BOX A3-2
Main characteristics of the pond fish culture system

Pond culture is a fish production system in which fish and their food are grown in the same water body – i.e. in a pond. In addition to increasing natural fish food production, the fish receive supplementary feeds which, together with the natural food, cover all their nutritional demands.

i.e. nitrogen and phosphorus (N and P). All these are supplied with fresh manure, while the essential mineral nutrients (N and P) can also be ensured with the application of fertilizers (see Table A3-2, A3-3 and A3-4). Indirect feeding reduces the overall feed costs in pond culture.

According to Hepher and Pruginin (1981) a maximum of 100–120 kg/ha of dry matter can be utilized by a pond in sunny days. Such intensive manuring may however cause algae bloom and oxygen shortage in the early mornings.

The easiest and most economical way of providing natural food for fish is the application of fresh manure. The frequency of manuring can be daily, weekly, biweekly or monthly. Table A3-3 shows the recommended quantities of different types of manure to be applied in rearing ponds.

TABLE A3-4
Widely used fertilizers

Type of fertilizer	Description
Nitrogenous fertilizers	Liquid ammonia (NH_4OH) or (NH_3 x H_2O) with a nitrogen content of 12–16% Ammonium sulphate ($(NH_4)_2SO_4$) with a nitrogen content of 20–21% Urea ($CO(NH_2)_2$) with a nitrogen content of 44–46% Ammonium chloride 25% Ammonium nitrate 35%
Phosphoric fertilizers	Calcium superphosphate ($Ca(H_2PO_4)_2$) H_2O with 12–18% of P_2O_5 Single superphosphate (SSP) 18-21% P_2O_5 Double superphosphate (DSP) 32% P205 Triple or treble superphosphate (TSP) 43-50% P_2O_5

Adapted from: Agropedia (2018); Lawes (2018); Mengel (2018)

TABLE A3-5
Recommendable quantities of manure and fertilizers for a production period of approximately 3 months

Name	Total quantity *(ton/ha)*	% of Total quantity	
		Start	Later
Manure	3–5	25	75
Carbamide (urea)	0.4–0.5	25	75
Superphosphate	0.3–0.4	25	75

Adapted from: Horváth and Pékh (1984).

The quantities of manure and fertilizers to be applied preferably are presented in Table A3-3 and A3-5. When manuring and fertilizing pond water it is better if the amounts are distributed frequently in smaller portions.

Manuring and fertilization of ponds should not be done without conscious thought. Check the concentration of nitrogen and phosphorus in the pond water and compare this to the figures presented in Table A3-6. In addition, Hepher and Pruginin (1981) recommend the following practices for manuring and fertilization, which make these procedures safer and more economical:

- There is no need to add doses higher than 0.5 mg/l (5 kg/ha) of phosphorus and 1.4 mg/l (14 kg/ha) of nitrogen active ingredients.

TABLE A3-6
Values when checking the concentration of nitrogen and phosphorus

	Desired (mg/l)	Acceptable	Poisonous (mg/l)
Total ammonium	1 below	below 2.5	1.54 at 12 pH, 5.55 at 9 pH, 33.3 at 8 pH, 100 at 7 pH
Nitrite	around 0.1	below 0.3	
Nitrate	around 20	below 40	
Orthophosphate	around 0.3	below 2	

TABLE A3-7
Application of lime during pond preparation and the production season

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

- The proportions of carbon (C), nitrogen (N) and phosphorus (P) in phytoplankton are: 1:0.18:0.024. As a consequence, the requirements for N and P are 0.9 gr/m^2 (9 kg/ha) and 0.12 gr/m^3 (1.2 kg/ha) of active ingredients respectively.
- Phosphorus, in particular, is trapped in insoluble forms in mud. Techniques exist to make it more soluble by aerating (i.e. stirring) the mud cautiously. In smaller muddy ponds a chain pulled on the bottom is one of the options to select.

Lime disinfects as well as improves alkalinity – and hence the productivity of pond water. Applicable doses are presented in Table A3-7. Lime is also useful for reducing/elimination of algal blooms:

Quick lime (200 kg/ha is distributed in stripes over the water surface. Do not use at high pH).

Calcium hypochlorite ($Ca(OCl)_2$) (used in basic waters (pH > 7), when 7–10 kg/ha is distributed in strips over the water surface. To be repeated maximum three times every fourth or fifth day).

Coppersulfate ($CuSO_4$) (used against filamentous algae; annually a total quantity of 8–10 kg/ha can be supplied, divided and distributed evenly over the pond's water surface in three equal portions when needed, with an interval of 3–4 weeks) (Molnár *et al.*, 2019).

Using straw (maximum 5000 kg/ha/season in 90–500 kg/ha doses) is a recently discovered and tested, environmentally friendly technique for eradicating phytoplankton blooms (Tamás *et al.*, 2008; Horváth, 2015; Stiller, 2012).

The results of manuring are measured through the quantity and quality of zooplankton found in the pond water: that is why the sampling of zooplankton is so important, especially when rearing young fish and different generations of tambaqui. When 100 litres of pond water are passed through a 60–70 micron plankton net, an accurate

BOX A3-3
Application of nitrogen fertilizer against blue-green algae

When there is enough accessible inorganic nitrogen for unicellular green algae, these will become dominant, because of their vigour and competitiveness. The applicable quantities of nitrogen fertilizers to reduce blue-green algae vary according to the composition i.e. nitrogen content. The dose should be based on a joint investigation of both phytoplankton and the quantity and quality of nitrogen in the water. (Nagy *et al.*, 2007).

TABLE A3-8
Estimate of the quantity of zooplankton

Settled zooplankton from 100 l pond water (ml)	Quantity of zooplankton in 1 ha pond water (0.8–1.4 m depth) (kg/ha)
0.1	8–14
0.5	40–70
1.0	80–140
5.0	400–700

estimation can be made (see Table A3-8). Such a procedure is not always necessary. If samples are taken in the same way as shown in Figure A3-3, the state of zooplankton life can easily be judged.

1.2.2 Direct feeding of fish in ponds

Natural food grown in fish ponds generally has a high protein content but is poor in energy (see Table A3-10 and A3-11). Therefore, the objective of direct feeding in ponds is to supplement the natural food with the missing nutrients. Traditionally, the most widely used feeds are energy feeds in which the crude protein content varies between 8 and 15 percent. Feeds such as good quality food industry waste and byproducts are also widely used as supplementary feeds. For ease of understanding and application, a detailed summary of, and key figures for, the chemical composition of a range of feeds is presented in Figures A3-4 and A3-5, as well as in Table A3-11). The feed conversion ratio (FCR) in case of fry and fingerlings may be as low as 1. As fish grow this figure will increase and vary between 2 and 3, when energy feeds are supplementing natural food. When feeding simple, compounded supplementary feeds this figure should be around or even below 2 (see Table A3-16).

As the intensity of pond culture increases, single supplementary feeds should be gradually complemented with a simple compounded feed, which has a crude protein content of 20–25 percent. These compounded feeds are made out of several components discussed in Chapter 2 of this annex. The actual proportions of single and simple compounded supplementary feeds are determined by the standing crop of fish in the pond (see Box A3-4).

There are a few practical considerations for feeding fish in ponds:

Preparation of feeds

- Both energy and protein rich feeds should be freshly ground for all age groups of tambaqui;
- grains should be soaked before distribution;
- when preparing compounded balanced feeds, pelleting is practical especially if many different components are used (see Table A3-9).

Way of feeding

- The more frequently fish are fed the better. Dividing the daily portion into smaller rations will therefore give better results in terms of feed conversion rates and growth.
- Though automatic feeders are more convenient than manual feeding (with a shovel or feeding boat), the latter provides a good opportunity to observe the fish and their health and growth.

Determination of the actual daily portions of given feeds

- Fingerlings should be fed at least twice a day at fixed locations. It is good if all the feed given is consumed within 1 hour. This forces fish to search for natural food.
- Growers should also be fed at least twice a day at fixed locations. The given feed should be consumed within 1–2 hours.

Checking results of feeding

- Verification of feed consumption is to be done daily.

BOX A3-4
Production intensity and the standing crop in fish ponds

The standing crop or standing biomass is the total weight of fish found in the pond at a given time.

Usually, single energy feeds (CP below 15 percent) are used until a polyculture, in which a fed omnivorous fish such as common carp, tilapia or tambaqui are the main (dominant) species, reaches about 700 kg/ha of standing biomass.

Above 700 kg/ha, as the biomass increases, a certain portion (25, 50 and later 75 percent) of single energy feeds are gradually replaced with simple compounded feed (see Table A3-14).

When standing biomass is over 1 800 kg/ha only simple compounded feed should be fed. (Hepher and Pruginin, 1981).

- Calculation of the FCR should be done either weekly or be-weekly. In case of testing new feed formulas a weekly checking/calculation of the FCR is recommended.
- During sampling, in the event the fish stock in a pond consists of different size groups, the sample should be divided into "small", "medium" and "large" fish when measured.

Quality and storing of feeds

- Only good quality feeds should be given to fish. Broken feed is not a problem, but it should not be spoiled, infected or contaminated with pollutants. Dry, well ventilated storage space is indispensable.
- The entrance of insects, rats, birds etc. into the storage room should be avoided.

1.3 Feeding fish in tanks and cages

Characteristics summarized in Box A3-5 distinguish cage and tank culture from pond culture. Usually there is no considerable natural food production when fish are reared in a tank or cage. Though passing through water may carry some natural food, the density of fish within a tank or cage is relatively high. Natural food contributions to tank and cage culture are normally not counted as a result. This means that all nutritional requirements for the healthy life and a good growth of the fish must be secured through the diet provided.

BOX A3-5

Main characteristics of tank and cage fish culture systems

Both culture systems are based on a continuous exchange of water in the rearing space (i.e. tank or cage) and on the use of compounded, fully balanced feed or a wide range different feeds, which together ensure a biologically complete balanced diet.

1.3.1 Commercial fish feeds

There are many different commercial fish feeds. These are relatively expensive, but are also more effective. Their usual FCR varies between 1.2 and 1.3, but can be as low as 1:1 or even lower. When using commercial feeds, farmers should follow the feeding charts recommended by feed producers.

The commercial feeds facilitate intensive, environmentally friendly fish production. As they are well digested less faeces are produced, meaning they pollute less.

TABLE A3-9

Correlation between the size of fish and the particles and pellets fed to them

Age group		Size of fish during rearing from – to (g)	Size of feed particles to be ground when fed directly (mm)	Size of the pellet of balanced compounded feeds (mm)
Fry		1	0.2–2	-
Fingerling		1–100	2–3	2–3
Grower	1st option	100–500	3–5	3–10
	2nd option	500–2 500	3–5	10–15
Brood fish		above 4 kg	10–20	15–20

1.3.2 Farm-made compounded fish feeds

Farm-made, compounded fish feeds are widely used. Typically these feeds are either simple compounded supplementary feeds or (in the event that all the required ingredients are readily available, including mineral and vitamin premixes) biologically fully balanced fish feeds. The FCR of such feeds is higher than for commercial feeds, but should be below 2 at least.

BOX A3-6
Exchange calculations between weight and percent of feed components

To calculate the components' contents from "as-fed" to DM percentage:

- "As-fed" CP (CL, NFE, CF, etc.) content is multiplied by (100 divided by the percent of DM). The resultant figure is always higher than the original one.
- "As-fed" CP (CL, NFE, CF, etc.) content is multiplied by (1 000 divided by the weight of DM given in grams). The resultant figure is always higher than the original one.

Calculating components' contents from DM% to "as-fed":

- DM% CP (CL, NFE, CF, etc.) content is multiplied by (DM divided by 100). The resultant figure is always lower than the original one.

Adapted from: New (1987).

2. ON-FARM PREPARATION OF COMPOUNDED FISH FEEDS

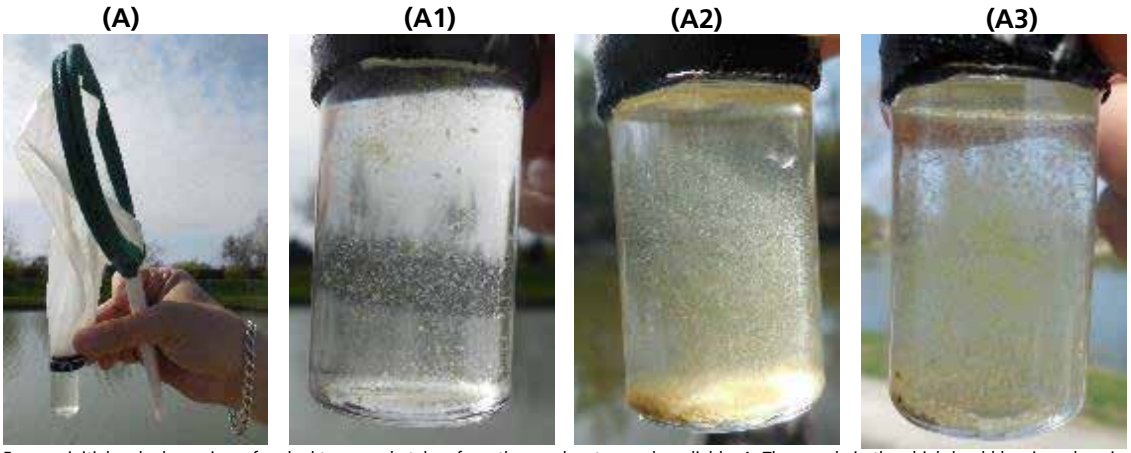
2.1 Potential ingredients

Practically anything which is consumed by fish and which contributes to the healthy life and growth of fish is a potential ingredient of compounded feeds. To facilitate understanding and the preparation of fish feeds the ingredients are grouped into classes as summarized in Table A3-10.

Knowing the chemical composition of the ingredients is indispensable when fish feed is formulated. For this reason, the key analytical information for specific foods and feeds is summarized (New, 1987) in Figures A3-4 and A3-5 and explained below:

- **Moisture** (water) is the diluent of the nutrients in foods and feeds. Moist content provides essential information about the actual quality and consistency of fish foods and feeds.
- **Dry matter** of the food or feeds is the part containing all ingredients, except for water. The sum of moister and dry matter is always 100 percent or 1 000 g in 1 kg of food or feed.
- **Proteins** are large, complex organic compounds. These play an essential role in the structure, propagation and growth of plants and animals. Proteins are mostly composed of amino acids (around 20). Their range (number of amino acids) and their actual quantities depend on their origin. Some of the amino acids can be synthesized by fish itself but others, the essential amino acids, cannot. These should be supplied with natural food or feeds.
- **Lipids** – Lipid or oil content, fat or fat content are used as synonyms. The words oil, fat and wax also indicate their actual consistency and increasing melting point of these materials. Dietary lipids are energy sources as well as fatty acids, some of which are essential to the survival and growth of the fish.

FIGURE A3-3
Judgement of zooplankton by naked-eye



Even an initial, naked-eye view of a plankton sample taken from the pond water can be reliable. A: The sample in the phial should be viewed against sunlight. (A1) shows a modest quantity of mixed zooplankton, while (A2 and A3) suggest that plankton crustaceans are dominant.
Photographs: ©FAO/András Woynárovich.

FIGURE A3-4
Structure of chemical composition of feeds

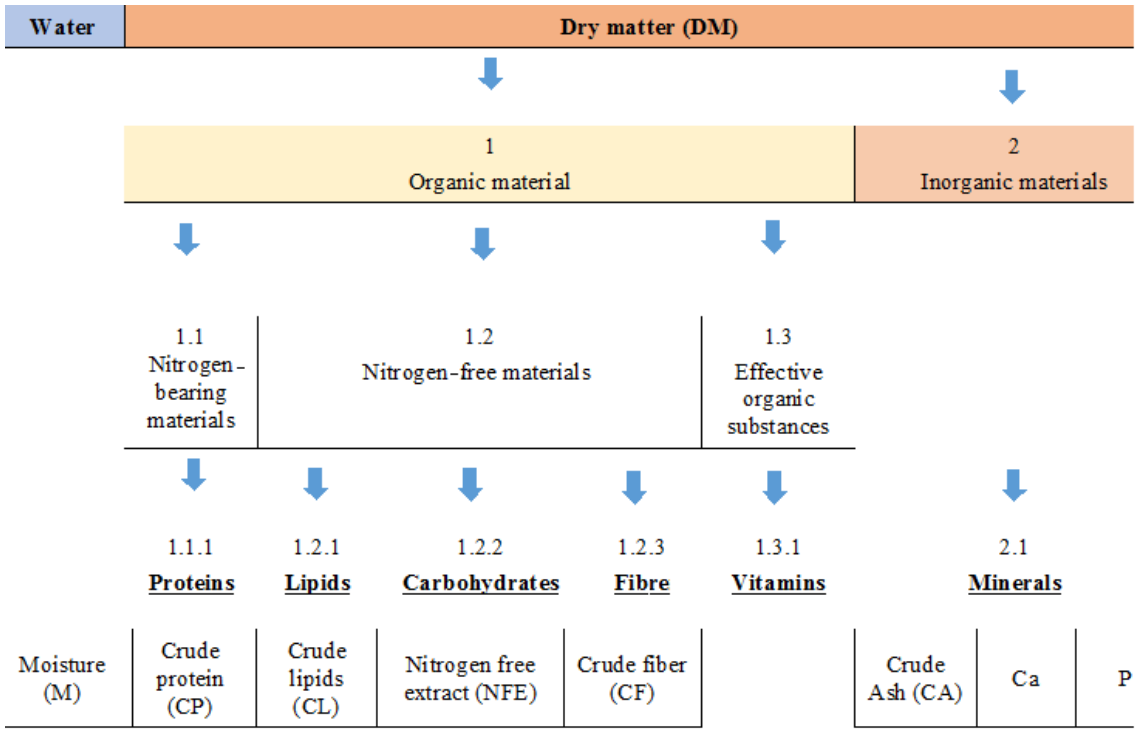


FIGURE A3-5
Energy of feeds

	Protein (kJ/gr)	Lipids (kJ/gr)	Carbohydrates (kJ/gr)	Fiber (kJ/gr)
GE:	23.01	38.00	17.15	20.05
DE:	AP: 17.78 PP: 15.90	33.47	Legume: 8.37 Non-leg.: 12.55	
	AP: 88.5% PP: 79.0%	88.0%	Legume: 48.8% Non-leg.: 73.2%	

(**GE** : Gross calculated energy)
(**AP**: animal protein)
(**PP**: plant protein)
(**DE**: Digestible energy in fish)

Within the different feed classes listed in Table A3-10, feeds have a similar composition. This can be observed in Table A3-11. The same or similar figures shown in this table can be found in specialized textbooks for the feeding of farmed animals. Highlighted cells show the main characteristics of the different groups. Following Göhl (1975), the key for the columns of this table (and others of its kind) is as follows:

- DM (dry matter) – all analyses are given on DM basis; this figure also helps to calculate the real quantities of chemical composition "as fed basis".
- CP (crude protein) – based on nitrogen analysis, hence not the entire figure but only its smaller or larger part indicates the true protein content. The actual value depends on the type of feeds.
- CL (crude lipid) – earlier EE (Ether extract or crude fat), which includes oils, fats, fatty acids, resins, chlorophyll, etc.
- NFE (nitrogen-free extract) – this group includes sugars, starch and cellulose (i.e. carbohydrates).
- CF (crude fibre).

- Ash indicates the quantity of minerals.
- DE (digestible energy) expressed in MJ (=1000 kJ) or kJ.

Analytical information on foods and feeds appear in two forms in the feed composition tables. These are either presented as a percentage or a weight of the components (see Figure A3-6).

The presentation of the components' composition in weight (called "as fed" or "as fed basis") facilitates an easy and precise composition of balanced diets.

FIGURE A3-6
Information as it is usually found in composition tables

DM (%)	CP (%)	CL (%)	NFE (%)	CF (%)	CA (%)	Ca (%)	P (%)	BE (MJ/kg)
DM (gr/kg)	CP (gr/kg)	CL (gr/kg)	NFE (gr/kg)	CF (gr/kg)	CA (gr/kg)	Ca (gr/kg)	P (gr/kg)	BE (MJ/kg)

In these two cases the sum of all chemical ingredients found in the dry material plus water contents should be either 100% (above) or 1000 g (below), which is referred to as, "as fed".

2.2 Formulation of farm-made compounded feeds for tambaqui

Tambaqui is usually the main fish to be fed in a pond polyculture. Moreover, as a fish it is suitable for intensive production in tanks and cages, provided for the required quality of feeds can be ensured during the entire production cycle. In order to build capacities to formulate and produce such suitable feeds this chapter, how to formulate and produce the different farm-made compounded feeds for tambaqui was elaborated.

Similarly to other omnivorous fish species the relative protein intake of tambaqui reduces by size. Developing fry require about 42 percent protein in its diet, which gradually reduces to 20 percent. Inversely, adult fish need more energy, about 23 kJ/g of food/feed consumed, while this value is only around 18–20 kJ/g in the case of fry and fingerlings.

BOX A3-7
Use of commercial poultry and pig feeds in tambaqui culture

In many cases, widely available commercial feeds for different farmed animals can also be tested and used in the intensive pond culture of tambaqui. Feeds with guaranteed quality produced for poultry or pig and sold in bulk can be adjusted to the nutritional needs of tambaqui, and after regrinding to be (re)pelleted and used.

2.2.1 Principles of fish feed formulation

It is important to prepare a nutritionally complete diet with the lowest possible cost of ingredient in the culture of fish (New, 1987).

Feed formulations require the following information to be provided:

- **Nutritional requirements of a species' targeted age group** – this depends on the amount of information available, which in the case of tambaqui is often contradictory.
- **Nutrient content of feed ingredients** – Can be obtained easily from textbooks on feeding farmed animals, including fish. For some initial guidance, see Table A3-11.
- **Availability of feed ingredients** – it is important that the different ingredients for a compounded feed can be sourced at a local market.
- **Availability of the nutrient to the animal** – This is the most critical information regarding the digestibility/availability of nutrients within the feed ingredient.
- **Minimum–maximum restrictions on levels** – These can be set on the basis of relevant literature, combined with personal experience.
- **Cost of feed ingredients** – can be obtained easily through market surveys.

TABLE A3-10
Classes of fish feeds by composition

Classes	Observations
Natural fish food	
Phytoplankton	
Water weeds	Tambaqui consumes their seeds and edible parts
Zooplankton	Tambaqui extensively feeds on this throughout its life
Insects and their larvae	Tambaqui extensively feeds on this throughout its life
Worms, molluscs, water snails	Tambaqui extensively feeds on this throughout its life
Fish	Smaller and dead specimens are grabbed and eaten
Green plants, forage and roughage	
Fresh	Might be used as ingredient of compounded feed
Dry	Might be used as ingredient of compounded feed
Roots, tubers, fruits, vegetables	
Roots and tubers	Traditional type of feeds in pond fish culture
Fruits	Traditional type of feeds in pond fish culture
Vegetables	Traditional type of feeds in pond fish culture
Byproducts	
Mill byproducts	Widely used in all forms: grain, meal or flour
Brewery byproducts	Widely used
Oil pressing	Widely used
Miscellaneous byproducts	Depends on item
Energy feeds	
Grains	Widely used in all forms: grain, meal or flour
Protein feeds	
Plant origin	Widely used in compounded fish feeds
Animal origin	Widely used in compounded fish feeds
Lipid feeds	
Plant origin	Widely used in compounded fish feeds
Animal origin	Widely used in compounded fish feeds
Supplements	
Minerals	
Food lime, bone meal, etc.	Widely used in compounded fish feeds
Premixes	Widely used in compounded fish feeds
Vitamins	
Natural substances	Fresh plants ground and mixed into the feed
Premixes	Widely used in compounded fish feeds
Binders	Widely used in compounded fish feeds
Additives	Antibiotics, flavour, hormones, antioxidants, drugs
Concentrates	These products contain all important ingredients, except for bulky energy rich grains, which are added to it at the farms.

Adapted from: New (1987).

TABLE A3-11
Fish foods and feeds by class and their composition on a "DM basis"

Classes of fish feeds	Average of					
	Water (%)	*CP as % of DM*	*C. Lipid as % of DM*	*C. Fiber as % of DM*	*NFE as % of DM*	*DE (MJ/kg)*
01 Natural fish foods	76.7	50.0	12.8	0.2	0.8	13.0
1.1 Phytoplankton	83.1	12.1	3.4	0.0	0.0	3.2
Algae - Bacillariophyceae (diatoms)	85.0	30.7	9.9	0.0	0.0	8.1
Algae - Chlorophyta (green algae)	83.2	17.6	3.7	0.0	0.0	4.0
Algae - Phaeophyta (brown algae)	85.9	0.0	0.0	0.0	0.0	0.0
Algae - Rhodophyta (red algae)	78.3	0.0	0.0	0.0	0.0	0.0
Algae – (Chlorella + Scenedesmus)[1]	-	42.8	7.2	-	27.4	15.0
Algae – (in ponds Phytoplagellates) [1]	88.0	33.3	10.8	3.5	16.7	-
1.2 Water weeds	84.2	14.6	4.5	0.0	0.0	3.8
Aquatic macro-vegetation	84.2	14.6	4.5	0.0	0.0	3.8
1.3 Zooplankton	89.6	57.7	22.0	0.0	0.0	16.9
Zooplankton[1]	88.0	61.5	1.0		64.8	
Crustacea – Cladocera	90.2	56.5	19.3	0.0	0.0	16.4
Crustacea – Cladocera[1]	94.0	52.6	10.7		15.2	21.8
Crustacea – Copepoda	89.7	52.3	26.4	0.0	0.0	18.0
Rotifers	88.8	64.3	20.3	0.0	0.0	18.1
Rotifers[1]	90	40	15.0		40.0	
1.4 Insects	78.1	53.0	7.8	0.7	2.2	12.3
Artemia	89.0	61.6	19.5	0.0	0.0	17.4
Artemia – Adult	65.0	56.4	11.8	2.9	12.1	15.4
Artemia – nauplii	80.0	50.2	18.9	5.0	14.8	17.0
Crustacea – Malacostraca	75.4	49.9	20.3	0.0	0.0	15.6
Crustacea – Ostracoda	65.0	41.5	0.0	0.0	0.0	7.4
Insects	76.8	55.9	18.6	0.0	0.0	16.1
Insects - Chironomids (larvae)	80.9	59.0	4.9	0.0	0.0	12.1
Aquatic insects (chironomids larvae)[1]	84.0	58.3	10.0			24.2
Insects – Diptera	84.0	55.3	0.0	0.0	0.0	9.8
Insects - Ephemeridae (mayflies)	82.4	50.2	0.0	0.0	0.0	8.9
Insects - Hemiptera (water bugs)	74.0	68.8	0.0	0.0	0.0	12.2
Insects - Odonata (dragon flies)	78.9	51.9	0.0	0.0	0.0	9.2
Insects - Trichoptera (caddisflies)	85.2	34.7	0.0	0.0	0.0	6.2
1.5 Worms and mollusks	78.8	49.9	8.9	0.0	0.0	11.8
Leeches	76.0	61.0	0.0	0.0	0.0	10.8
Mollusks	67.8	39.5	7.8	0.0	0.0	9.6
Oligochaetes	92.7	49.3	19.0	0.0	0.0	15.0
Planorbid snails						
1.6 Fish	74.0	69.8	24.1	0.0	0.0	20.3
Common carp cultured fatty	63.9	36.8	60.9	0.0	0.0	26.7
Common carp cultured not fatty	73.4	63.5	32.7	0.0	0.0	22.1
Common carp wild	78.9	74.4	21.8	0.0	0.0	20.4
Eel	58.2	29.2	65.8	0.0	0.0	26.9
European catfish	76.8	80.2	15.1	0.0	0.0	19.2
Pike perch	79.0	91.4	2.4	0.0	0.0	17.0
Trout	77.5	85.8	9.3	0.0	0.0	18.3
Sturgeon	79.0	75.2	6.2	0.0	0.0	15.4
02 Green plants, forage	33.2	19.4	3.0	24.9	43.0	8.1
2.1 Fresh	80.7	22.0	3.3	21.6	42.5	8.1
Clover (flowering)	80.3	18.8	3.6	22.3	45.7	8.0
Clover (young)	83.3	21.6	4.2	19.2	44.9	8.6
Lucerne (flowering)	75.5	20.0	2.4	28.2	38.4	7.2
Lucerne (young)	83.7	27.6	3.1	16.6	41.1	8.8
2.2 Dry	9.4	18.2	2.8	26.5	43.2	8.0

Classes of fish feeds	Average of					
	Water (%)	*CP as % of DM*	*C. Lipid as % of DM*	*C. Fiber as % of DM*	*NFE as % of DM*	*DE (MJ/kg)*
Lucerne flour (2nd)	8.6	20.9	2.7	26.0	40.2	7.6
Lucerne flour (3rd)	6.5	19.0	2.6	30.4	38.6	7.1
Lucerne hay (dry)	13.8	18.6	2.3	31.6	37.8	6.9
Maize - whole plant flour	8.1	7.1	3.8	20.2	64.7	10.5
Pasture hay (dry)	12.1	11.5	2.7	34.0	44.0	8.2
03 Roots, tubers, fruits, vegetable	52.2	7.9	0.6	10.3	75.5	10.9
3.1 Roots and tuber	52.2	7.9	0.6	10.3	75.5	10.9
Fodder beet	88.9	10.8	0.9	8.1	70.3	10.8
Potato	76.4	8.5	0.4	3.0	83.5	12.0
Potato pulp (dry)	10.0	5.0	0.6	13.6	77.3	10.7
Sugar beet slides (dry)	9.2	10.2	0.8	21.7	61.8	9.6
Manioc (cassava) – fresh sweet peeled[2]	71.5	1.7	0.7	1.7	92.0	
Manioc (cassava) – bitter[2]	68.1	2.7	0.5	3.1	91.0	
Manioc – fresh leaves wet season[2]	85.5	22.8	6.2	22.8	40.6	
Cassava – pomace (pulp)[2]	16.5	2.2	0.6	26.9	66.9	
Cassava – seed oil meal[2]	10.9	2.5	0.3	5.5	89.1	
Sugar beet	76.7	5.2	0.4	5.2	84.5	11.6
Sugarcane tops, fresh mature[2]	74.4	6.3	2.2	35.0	50.3	
Sugarcane tops only leaves[2]	69.5	5.6	1.7	36.3	47.0	
Sugarcane bagasse[2]	9.7	1.9	1.0	45.0	44.1	
Sugarcane - molasses[2]	23.1 - 43.0	1.2 - 4.7	0.0	0.0	87.2–95.9	
3.2 Fruits	47.0	11.8	14.2	12.7	54.9	
Guava[2]	64.9	11.7	8.7	16.1	55.8	
Mango – fresh leaves[2]	56.0 - 58.4	8.1 - 9.5	2.7 - 4.8	22.6–28	50.0 - 51.2	
Mango – fruit pulp mature[2]	82.7	5.6	0.5	2.3	89.4	
Banana leaves[2]	5.9	9.9	11.8	24.0	45.5	
Banana fruit ripe[2]	69	5.4	0.9	2.2	88.2	
Banana peelings ripe[2]	85.9	7.9	11.6	7.7	59.4	
Rubber-tree seeds (Hevea spruceana)[1]	39.0	15.9				
Rubber-tree seeds (Hevea brasiliensis)[1]	4.1	19.2	44.2	9.3	24.4	26.1
Munguba seeds (Pseudobombax)[1]	14.3	21.3	31.8	15.9	25.7	
Mesquite meal[2]	12.9	8.3		22.8		14.62
Pupunha palm						
3.3 Vegetables						
Cabbage[2]		20.0	3.5	10.3	38.9	
Pumpkins[2]	92.4	14.5	2.6	13.2	61.8	
04 Byproducts	12.4	24.1	5.3	8.6	56.5	12.7
4.1 Mill byproducts	11.6	16.2	5.5	9.9	62.8	12.3
Barley – polished	10.8	13.9	2.2	1.3	80.5	13.1
Barley bran	12.1	13.2	3.5	13.0	64.5	11.4
Barley fodder flour	12.7	13.4	3.3	7.3	72.2	12.3
Oat bran	11.0	9.1	3.5	23.7	57.8	9.8
Oat fodder flour	12.0	13.2	6.0	12.0	63.6	12.1
Pea fodder flour	11.3	25.1	2.3	8.0	60.8	12.4
Rice bran	11.4	13.0	14.5	10.8	50.7	13.2
Rice fodder flour	11.2	14.8	15.5	9.3	49.5	13.7
Rye bran	11.3	16.2	3.4	12.3	62.5	11.5
Rye fodder flour	13.1	15.8	2.6	3.3	74.5	12.7
Wheat bran	11.4	17.2	4.9	10.9	62.3	12.1
Wheat bran	12.0	16.5	4.4	11.8	60.9	11.7
Wheat fodder flour	12.2	17.7	4.4	6.0	67.7	12.8
Wheat germ	9.3	27.8	6.9	8.8	52.0	13.2
4.2 Brewery byproducts	15.1	31.1	5.1	11.6	47.6	12.6
Apple marc dry	7.2	2.8	18.8	70.6	5.4	7.3

Classes of fish feeds	Average of					
	Water (%)	*CP as % of DM*	*C. Lipid as % of DM*	*C. Fiber as % of DM*	*NFE as % of DM*	*DE (MJ/kg)*
Beer marc (fresh)	76.1	27.2	8.8	16.7	42.7	12.6
DDGS – corn	11.5	27.5	13.0	6.3	48.4	14.7
Maize starch	8.4	0.7	0.1	0.2	98.8	12.5
Maize gluten (CGF)	8.4	71.4	1.5	1.6	23.4	14.8
Malt germ	6.6	28.5	1.1	15.1	48.5	11.0
Potato starch	12.0	0.5	0.1	0.0	99.0	12.5
Yeast	8.0	48.9	1.1	0.0	41.5	13.3
Yeast – beer	10.0	53.9	1.3	0.0	36.4	13.6
4.3 Miscellanea	9.3	29.8	5.2	0.1	58.0	14.0
Casein	10.5	89.7	1.2	0.0	4.9	15.3
Milk powder - f/full milk	5.5	26.8	28.4	0.0	38.4	18.9
Milk powder - f/skimmed milk	7.5	36.8	0.9	0.0	54.8	13.7
Molasses	22.0	10.8	0.0	0.0	79.6	11.7
Sugar	4.2	2.0	0.1	0.8	93.6	12.1
Whey powder	5.8	13.0	0.8	0.0	76.8	12.2
Copra meal[2]	10.5	22.7	7.7	10.5	48.2	21.1
Babassu oilcake[2] (*Orbignya speciosa*)		24,9	6.8	15.0	47.4	
Coroza palm oilcake[2] (*O. cohune*)	9.2	21.8	7.9	24.0	41.6	
Soap stock		35.0				
05 Energy feeds	11.1	11.8	3.0	5.3	77.2	12.5
5.1 Grain	11.1	11.8	3.0	5.3	77.2	12.5
Barley	12.0	12.4	2.1	5.2	77.5	12.4
Maize	9.7	9.7	4.1	2.5	82.1	13.2
Millet	10.4	11.8	4.4	8.0	72.7	12.4
Oat	11.4	11.7	4.6	12.7	67.5	11.9
Rice (grain, meal)	11.0	10.2	2.8	9.5	71.8	11.6
Rye	11.8	10.5	1.7	2.9	82.7	12.6
Sorghum	12.0	11.8	3.5	3.7	78.7	12.9
Triticale	11.9	11.7	1.2	3.0	82.0	12.6
Wheat flour	10.3	13.9	1.9	2.9	79.2	12.8
Wheat grain	10.3	13.9	1.9	2.9	79.2	12.8
06 Protein feeds	8.8	49.1	7.6	7.8	22.6	11.9
6.1 Plant origin	9.8	39.1	7.2	11.2	35.8	11.8
Cotton meal extracted	10.0	42.9	1.7	15.6	33.6	11.6
Horse bean	11.3	29.5	1.2	8.3	57.0	12.3
Linseed (full fat)	9.6	24.3	37.6	6.7	26.5	19.6
Linseed meal extracted	9.5	37.9	3.0	9.8	42.2	12.3
Lupine (sweet)	12.0	43.5	5.2	16.5	29.9	12.4
Maize germ meal extracted	6.0	25.7	2.1	9.1	56.8	11.9
Pea	11.2	24.8	1.4	6.5	63.6	9.7
Peanut meal extracted (1st)	9.8	56.2	1.1	8.6	27.7	11.6
Rape cake	9.2	33.5	13.5	11.5	34.7	12.7
Rape meal extracted (00)	8.4	37.8	2.5	12.9	39.1	10.1
Rapeseed (full fat)	10.0	21.7	48.0	6.8	18.0	20.8
Rapeseed meal extracted	10.0	39.6	2.9	12.8	35.5	10.2
Soya (full fat)	10.2	37.5	20.7	7.7	28.3	15.2
Soya meal extracted	10.0	52.3	1.9	5.5	33.1	11.7
Soya meal extracted (1st)	10.6	53.9	1.8	6.4	30.3	11.7
Soya meal extracted (2nd)	11.1	51.9	1.8	7.1	32.1	11.5
Soya meal extracted (3rd)	10.7	49.3	2.1	7.6	33.8	11.4
Sunflower meal extracted	10.4	41.2	1.7	20.1	29.2	10.8
Sunflower meal extracted (1st)	9.2	43.1	1.9	14.9	31.5	11.4

Classes of fish feeds	Average of					
	Water (%)	*CP as % of DM*	*C. Lipid as % of DM*	*C. Fiber as % of DM*	*NFE as % of DM*	*DE (MJ/kg)*
Sunflower meal extracted (3rd)	7.7	36.4	1.8	22.8	31.3	10.3
6.2 Animal origin	7.4	64.9	8.2	2.5	1.7	11.9
Blood meal	4.4	95.9	0.3	0.0	0.3	17.2
Carp meal	7.5	81.1	10.3	0.0	0.0	17.8
Feather meal	10.0	46.2	9.3	0.0	1.5	11.5
Feather meal – hydrolyzed	8.7	86.4	6.6	0.0	4.1	18.0
Fishmeal	7.7	72.0	8.0	0.0	1.6	15.6
Fishmeal (60%)	8.7	67.6	11.5	0.0	1.9	16.0
Fishmeal (65%)	8.6	70.2	10.3	0.0	1.4	16.1
Fishmeal (70%)	6.7	76.5	3.8	0.0	3.3	15.3
Mixed animal protein meal (54%)	6.4	58.7	18.7	0.0	2.7	16.9
Mixed animal protein meal (58%)	5.5	62.4	15.6	0.0	4.0	16.7
Mixed animal protein meal (62%)	6.7	66.9	12.6	0.0	3.4	16.5
Sea crustacean meal	7.5	33.5	1.8	11.9	0.0	6.6
Shrimp meal	7.5	45.4	3.2	11.9	0.0	9.1
07 Lipid feeds	0.9	0.0	99.2	0.0	0.0	32.7
7.1 Plant origin	0.8	0.0	99.1	0.0	0.0	32.7
Coconut grease	0.5	0.0	98.8	0.0	0.0	32.6
Palm oil	0.8	0.0	98.8	0.0	0.0	32.6
Sunflower oil	1.1	0.0	99.8	0.0	0.0	32.9
7.2 Animal origin	1.0	0.0	99.3	0.0	0.0	32.8
Fish oil	1.0	0.0	99.4	0.0	0.0	32.8
Pig fat	1.1	0.0	99.2	0.0	0.0	32.7
Poultry fat	0.8	0.0	99.3	0.0	0.0	32.8
Tallow	1.0	0.0	99.3	0.0	0.0	32.8

Sources: Kakuk and Schmidt (1988), Schmidt (2015).
Feeds marked as [1] and [2] originate from Araujo-Lima and Goulding (1997) and Göhl (1975).

The feed formulation can be done thanks to specialized commercial computer programmes using linear programming software. Without such a computer programme a step-by-step method must be followed. Calculations can be made on Excel worksheets as well.

2.2.2 Feed formulation sheets

In the event that a computer is not available, the feed formulation can be done on spreadsheet with the help of linear programming. One of the sheets (forms) is presented in Figure A3-7. However, this paper-based form is generally replaced by professional computer programmes that are widely available online. A simple Microsoft Excel spreadsheet as presented in Figure A3-8, may also be able to calculate both simple and complex compounded, farm-made feeds.

When calculating farm-made fish feed ingredients, the person square method is used to blend energy- and protein-rich feeds into a mixture containing the desired protein content. The Microsoft Excel adaptation of the improved person square method is presented in Figure A3-9. With this the proportion of the different ingredients of the main feed groups can be calculated with an improved person square method (Woynárovich and Péteri, 2016).

The presented aspects and forms, together with the figures in Table A3-12, facilitate the compilation of feed receipts for the different age groups of tambaqui reared in ponds and cages.

During the selection and use of compounded feed ingredients no stale, rotten, poisoned or rancid lipids should be used.

Similarly to other fish species the protein and energy intake of tambaqui changes by age and size, together with the change in the range of natural food consumed. Finding

TABLE A3-12
Most frequently used ingredients and their limitations in compounded commercial feeds of tambaqui, as per the principal age groups

Ingredients		Age groups to be fed		
Name	**Restrictions**	**Fry**	**Table fish**	**Brood fish**
Protein in commercial pellet feed[2]	About:	40%	18%	
Fat in commercial pellet feed[2]	About:	3-10 %		
Carbohydrates in commercial pellet feed[2]	About:	20-50 %		
Fibre in commercial pellet feed[2]	About:			7-20 %
Ash in commercial pellet feed[2]	About:			7-13 %
Energy in commercial pellet feed[2]	About:			11-14 kcal/kg
Soya meal[1]	More or as much as:	10%	10%	10%
Cotton cake meal[1]	Less or as much as:	5%	15%	0%
Peanut cake[1]	Less or as much as:	15%	15%	15%
Sorghum[1]	Less or as much as:	5%	30%	30%
Powdered milk serum[1]	Less or as much as:	5%	5%	5%
Dry solution of distillate or fermentation[1]	Less or as much as:	15%	15%	15%
Clover meal (17% CP)[1]	Less or as much as:	0%	10%	10%
Molasses[1]	Less or as much as:	0%	5%	5%
Corn gluten[1]	Less or as much as:	12%	12%	12%
Fish meal[1]	More or as much as:	10%	5%	5%
Blood meal (80% CP)[1]	Less or as much as:	7.5%	5%	5%
Meat and bone meal (49% CP)[1]	Less or as much as:	5%	5%	5%
Poultry byproduct meal (55% CP)[1]	Less or as much as:	10%	10%	10%
Milk sour (without lactose)[1]	Less or as much as:	6%	6%	6%
Skimmed milk[1]	Less or as much as:	10%	10%	10%
Citrus pulp[1]	Less or as much as:	0%	8%	8%
Yellow maize[1]	More or as much as:	5%	10%	10%
Soluble fish (condensed)[1]	Less or as much as:	6%	6%	6%
Broken rice meal[1]	Less or as much as:	10%	10%	10%
Rice bran[1]	Less or as much as:	20%	20%	20%
Tankage[1]	Less or as much as:	5%	5%	5%
Yeast[1]	Less or as much as:	10%	10%	10%
Wheat[1]	Less or as much as:	10%	20%	20%
Animal fat[1]	Less or as much as:	3%	3%	3%

Adapted from: (1) CEPTA (1986); (2) Araujo-Lima and Goulding (1997).

and mixing the suitable ingredients of farm-made compounded fish feeds for the different age groups of tambaqui can be conducted by taking into account how tambaqui's natural food changes over its lifetime. Table A3-12 and Table A3-13 provide guidance.

Only in highly intensive pond culture or cage/tank culture, may vitamin and/or mineral deficiencies endanger the proper health and growth of the reared fish. In cage culture in remote regions, feeding with fresh fruits generally satisfies the vitamin and mineral demand of the fish.

2.2.3 Steps of diet formulation

1. Selection of needed/available ingredients
2. Filling in the worksheet step by step:
   - 2.1 Protein-rich main components
   - 2.2 Energy-rich main components.

The MS Excel sheets presented in Figures A3-8 and A3-9 will help to model ideas and choose between different choices of ingredients.

FIGURE A3-7
Traditional paper-based worksheet for fish feed formulation

Ingredient			Nutrient contribution on "as-fed" basis (%)								Price of feed (USD)
Name	Price (USD/kg)	%	H_2O	CP	CL	CF	NFE	C. Ash	Ca	P	BE	
Total											

Source: New (1987).

FIGURE A3-8
Traditional paper-based worksheet for fish feed formulation adapted to Microsoft Excel

Entry sheet											
Ingredient		**Nutriant value "as-fed" basis (%)**								DE	Price
Name	%	CP	CL	CF	NFE	C. Ash	Ca	P		(MJ/kg)	(USD/kg)
Maize	25.0	**9.7**	**4.1**	**2.5**	**82.1**					**13.2**	0.136
Soya meal	25.0	**52.3**	**1.9**	**5.5**	**33.1**					**11.7**	0.358
Fish meal	25.0	**67.6**	**11.5**	**0.0**	**1.9**					**16.0**	1.296
Meat meal	25.0	**66.9**	**12.6**	**0.0**	**3.4**					**16.5**	0.475
Total	**100.0**										
Missing	**0.0**										
Result sheet											
Ingredient		**Nutriant value "as-fed" basis (%)**								DE	Price
Name	kg	CP	CL	CF	NFE	C. Ash	Ca	P		(MJ/kg)	(USD/kg)
Total	**1.00**	**49.1**	**7.5**	**2.0**	**30.1**	**0.0**	**0.0**	**0.0**		**14.4**	**0.566**
Maize	0.25	2.4	1.0	0.6	20.5	0.0	0.0	0.0		3.3	0
Soya meal	0.25	13.1	0.5	1.4	8.3	0.0	0.0	0.0		2.9	0
Fish meal	0.25	16.9	2.9	0.0	0.5	0.0	0.0	0.0		4.0	0
Meat meal	0.25	16.7	3.2	0.0	0.9	0.0	0.0	0.0		4.1	0
0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0
0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0
0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0
0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0
0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0
0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0

Source: Woynárovich and Peteri (2016).

FIGURE A3-9
Improved person square method on xls worksheet to calculate proportions of more than two components of feeds to obtain the desired CP contents of feed mix

	Entry					Calculated % of ingredients in the mixture	Contribution from the ingredient to CP content (%)
	Name	CP%	% in the group	Planned CP content (%)	→	↓	↓
		↓		↓			
	CP of the **1st group** of ingradients with more CP than wanted:	49.2			11.7	28.7	14.1
				20.0			
	CP of the **2nd group** of ingradients with less CP than wanted:	8.3			29.2	71.3	5.9
1st group:	Soya meal	47.1	90.0			25.8	12.7
Ingradients with more CP than wanted:	Fishmeal	67.6	10.0			2.9	1.4
						0.0	0.0
						0.0	0.0
						0.0	0.0
		Total (%):	*100.0*		*Total (%):*	*28.7*	*14.1*
2nd group:	Maize	7.9	85.0			60.6	5.0
Ingradients with less CP than wanted:	Wheat	10.3	15.0			10.7	0.9
						0.0	0.0
						0.0	0.0
						0.0	0.0
						0.0	0.0
						0.0	0.0
						0.0	0.0
		Total (%):	*100.0*		*Total (%):*	*71.3*	*5.9*
					Grand total (%):	*100.0*	*20.0*

Source: Woynárovich and Peteri (2016).

TABLE A3-13
Composition of vitamin and mineral premixes for tambaqui

Vitamins – Quantity per kg of premix			
Vitamin A	600 000 UI	Colin chloride	55.0 g
Vitamin D3 (Cholecalciferol)	100 000 UI	Vitamin B12	2.4 mg
Vitamin E (Alfa Tocoferol)	6 000 UI	Inositol	10.0 g
Vitamin K3 (Menadione)	1.2 g	Antioxidant BHT	5.0 g
Ascorbic acid	50.0 g	Minerals – Quantity per kg of premix	
Vitamin B1 (Thiamine)	2.4 g	Iron	5.0 g
Vitamin B2 (Riboflavin)	2.4 g	Copper	0.3 g
Pantothenic acid	6.0 g	Maegan	2.0 g
Niacin	12.0 g	Zinc	3.0 g
Vitamin B2 (Pyridoxine)	2.4 g	Iodine	10.0 mg
Vitamin H (Biotin)	24.0 mg	Cobalt	1.0 mg
Folic acid	600.0 mg	Selene	10.0 mg

Source: CEPTA (1986).

2.3 Preparation of farm-made compounded feeds

There are a wide range of capacities and brands of machines used to produce fish feeds on the farm. These include a hammer mill with different screens, mixer and a pelletizer.

When deciding on the actual capacities of these machines, it is worth noting that pelleted feed should not be produced in large volumes and stored for long periods.

When making pellets and deciding on the binder to use, the actual eagerness of tambaqui for feed should also be calculated. This is important because the production of pellets with a water stability of longer than 10–15 minutes may be more expensive than feeding fish more frequently; the fishes' eagerness to feed is also better maintained with regular feedings.

TABLE A3-14
Development of feed formulation for warmwater omnivorous fishes

Ingredients	1. A simple 18% CP feed for use in ponds[1]			2. A simple 25% CP feed for use in ponds[1]			3. A complex 32% CP feed for use in cages[2]		
	Min.	*Fixed*	*Max.*	*Min.*	*Fixed*	*Max.*	*Min.*	*Fixed*	*Max.*
Fishmeal (%)	5		10		15				10
Soybean meal (%)	5		10	15		25	30		40
Cotton cake meal									10
Maize							20		
Wheat	80		90	60		70	25		
Fat							6		
Soapstone oil				3		4			
Vitamin premix (standard complete)								0.1	
Mineral premix (standard complete)								0.1	

Adapted from: (1) Hepher and Pruginin (1981); (2) www.soyaqua.org

3. FEEDING PRACTICES AND CALCULATION OF FEEDING EFFICIENCY

As the intensity of production increases, the single largest production cost in the culture of tambaqui is the feed. A regular follow-up on feeding and a calculation of how efficient the feed practices therefore remain among the fish farmer's most important tasks.

Tambaqui feeds aggressively both in ponds and cages/tanks, especially when hungry and when the water temperature is high. Similarly to other animals, when fishes are fed regularly around the same time they will gather and wait to receive the feed. This is also a good occasion to observe them.

The actual daily quantity of feed given to the fish is calculated in proportion to the total standing biomass and expressed as a percentage of the estimated total weight of the fish fed in the pond. This may vary between the culture system used and the age/size of fish reared (see Table A3-16).

TABLE A3-15

A selection of published compounded feed recipes for the different age groups of tambaqui culture in ponds and cages/tanks

Feeds and ingredients	Ingredient (%)	Estimated chemical composition (%)						DE (MJ/kg)
		Water	Crude Protein	Crude Lipid	Crude Fibre	NFE	Crude Ash	
Experimental feed in pond – A01	100.0	9.8	22.5	3.4	3.4	67.4	0.0	12.8
Maize	70.0	6.8	6.8	2.9	1.8	57.5	0.0	9.2
Soybean meal	30.0	3.0	15.7	0.6	1.7	9.9	0.0	3.5
Experimental feed in pond – A02	100.0	10.6	24.8	3.3	3.7	51.0	0.0	12.5
Maize	50.0	4.9	4.9	2.1	1.3	41.1	0.0	6.6
Soybean meal	30.0	3.0	15.7	0.6	1.7	9.9	0.0	3.5
Chicken broiler feed	20.0	2.8	4.3	0.7	0.8	0.0	0.0	2.4
Experimental feed in cage/tank – B01	100.0	7.7	27.8	2.0	4.6	51.2	6.7	16.2
Composition of ingredients: Soybean meal (49.9% CP) 15.0 %, Maize gluten (69.0% CP) 8.0 %, Meat and bone meal (62.7% CP) 8.0 %, Wheat flour (17.8% CP) 20.0 %, Maize (9.1% CP) 40.2 %, Wheat bran (9.3% CP) 7.0 %, Vitamin-mineral premix 1.0 %, Methionine 0.2 %, Lysine 0.6 %								
Experimental feed in cage/tank – B02	100.0	8.7	32.0	2.2	4.4	45.8	6.9	16.3
Composition of ingredients: Soybean meal (49.9% CP) 19.0 %, Maize gluten (69.0% CP) 13.0 %, Meat and bone meal (62.7% CP) 10.0 %, Wheat flour (17.8% CP) 15.0 %, Maize (9.1% CP) 34.2 %, Wheat bran (9.3% CP) 7.0 %, Vitamin-mineral premix 1.0 %, Methionine 0.2 %, Lysine 0.6 %								
Experimental feed in cage/tank – B03	100.0	7.9	36.6	2.5	4.2	40.8	8.0	16.6
Composition of ingredients: Soybean meal (49.9% CP) 23.0 %, Maize gluten (69.0% CP) 18.0 %, Meat and bone meal (62.7% CP) 12.0 %, Wheat flour (17.8% CP) 10.0 %, Maize (9.1% CP) 28.2 %, Wheat bran (9.3% CP) 7.0 %, Vitamin-mineral premix 1.0 %, Methionine 0.2 %, Lysine 0.6 %								
Experimental feed in cage/tank – B04	100.0	7.0	39.7	1.9	4.1	39.5	7.9	16.9
Composition of ingredients: Soybean meal (49.9% CP) 27.0 %, Maize gluten (69.0% CP) 23.0 %, Meat and bone meal (62.7% CP) 14.0 %, Wheat flour (17.8% CP) 5.2 %, Maize (9.1% CP) 22.0 %, Wheat bran (9.3% CP) 7.0 %, Vitamin-mineral premix 1.0 %, Methionine 0.2 %, Lysine 0.6 %								
Experimental feed in cage/tank – C01	100.0		26.1		8.5			13.5
Composition of ingredients: Wheat flour 11.0 %, Maize flour 33.6 %, Coconut meal 0.0 %, Soya meal 26.2 %, Fish meal 15.0 %, Cellulose 6.7 %, Lysine 0.8 %, BHT (antioxidant) 0.0 %, DL – Methionine 0.3 %, Soya oil 2.8 %, Phosphate bicarbonate 0.6 %, Limestone 1.2 %, Salt (NaCl) 0.5 %, Premix 1.0 %, Alginate 0.5 %								
Experimental feed in cage/tank – C02	100.0		26.1		8.5			13.5
Composition of ingredients: Wheat flour 11.0 %, Maize flour 30.2 %, Coconut meal 13.7 %, Soya meal 19.4 %, Fish meal 15.0 %, Cellulose 5.0 %, Lysine 0.8 %, BHT (antioxidant) 0.0 %, DL – Methionine 0.3 %, Soya oil 1.6 %, Phosphate bicarbonate 0.0 %, Limestone 1.1 %, Salt (NaCl) 0.5 %, Premix 1.0 %, Alginate 0.5 %								
Experimental feed in cage/tank – C03	100.0		26.1		8.5			13.5
Composition of ingredients: Wheat flour 11.0 %, Maize flour 25.4 %, Coconut meal 27.5 %, Soya meal 12.8 %, Fish meal 15.0 %, Cellulose 3.3 %, Lysine 0.8 %, BHT (antioxidant) 0.0 %, DL – Methionine 0.3 %, Soya oil 0.8 %, Phosphate bicarbonate 0.0 %, Limestone 1.2 %, Salt (NaCl) 0.5 %, Premix 1.0 %, Alginate 0.5 %								
Feed of tambaqui in tanks – C03	100.0		26.1		8.5			13.7
Composition of ingredients: Wheat flour 11.0 %, Maize flour 14.6 %, Coconut meal 55.0 %, Soya meal 0.0 %, Fish meal 15.0 %, Cellulose 0.0 %, Lysine 1.0 %, BHT (antioxidant) 0.0 %, DL – Methionine 0.3 %, Soya oil 0.0 %, Phosphate bicarbonate 0.0 %, Limestone 1.2 %, Salt (NaCl) 0.5 %, Premix 1.0 %, Alginate 0.5 %								

Sources: Hancz, 1993 (feeds A1, A2); Oishi *et al.,* 2010 (feeds B1, B2); Lemos *et al.,* 2011 (feeds C1, C2, C3, C4).

TABLE A3-16
Rate of feeding and expectable FCR as per reared age groups, quality of feeds and culture system practised

Description	Fingerling production				Grower production				Table fish production			
	in pond			in cage	in pond			in cage	in pond			in cage
	Ext.	*S-int.*	*Int.*		*Ext.*	*S-int.*	*Int.*		*Ext.*	*S-int.*	*Int.*	
Daily quantity of feeds given and the number of portions to be given per day												
Proportion of SB (%)	1.5–3	1.5–3	1.5–3	4–6	2.5–5	2.5–5	2.5–5	4–6	2.5–5	2.5–5	2.5–5	4–6
Portions (No./day)	2–3	2–3	2–3	3–4				3–4				3–4
Expectable FCR of different feeds												
Supplementary (8–12% CP)	1–1.5	1.5–2	2–2.5	-	1.5–2	2–2.5	2.5–3	-	1.5–2	2–2.5	2.5–3	-
Simple feed (18–20 % CP)	1	1–1.5	1.5–2	-	1–1.5	1.5–2	2–2.5	-	1–1.5	1.5–2	2–2.5	-
Simple feed (25–28 % CP)	-	1	1–1.5	-	1	1–1.5	1.5–2	-	1	1–1.5	1.5–2	-
Complex feed (32 % CP)	-	-	-	2–2.5	-	-	-	2.5–3	-	-	-	2.5–3
Complex feed (36 % CP)	-	-	-	1.5–2	-	-	-	2–2.5	-	-	-	2–2.5
Commercial feeds	-	-	-	1–1.5	-	-	-	1–1.5	-	-	-	1–1.5

Annex 4
HANDLING AND TRANSPORTATION OF DIFFERENT TAMBAQUI AGE GROUPS

1. HANDLING THE DIFFERENT TAMBAQUI AGE GROUPS

Knowing the principles of how to handle fish eggs and the different age groups is very important: if it is not done properly they may be hurt, and in extreme cases killed. This annex therefore explains important practical aspects of how to handle the developing eggs and larvae (feeding larvae). Fry, fingerlings, pre-adult and adult fish are discussed.

A general rule is that any age group of fish should be released into the water gently: eggs, larvae, fry, fingerlings, table fish and brood fish can be harmed if they splash onto the surface of the water. Improper handling can cause fatal wounds or temperature shock in eggs and fish.

1.1 Handling of eggs and larvae

Fish eggs, and particularly those of tambaqui, are very sensitive. They should therefore be kept and transported in clean plastic bowls with smooth walls, regardless of whether the eggs are in a stage prior to, during or following fertilization. As soon as the eggs are fertilized they should be moved in water and poured very gently into the incubator. Once the eggs have started to swell they should not be moved any longer until they hatch – even a gentle transfer in water may cause considerable damage and high mortality.

Hatched and developing larvae, as well as feeding larvae, are not as sensitive during handling, providing they remain in water while they are transferred from one tank or basin to another or to the pond. A basic rule is that they must have no contact with air and they should not be lifted out of water, as it is lethal for them.

1.2 Fishing and handling of advanced fry and fingerlings

To catch these age groups of young fish in ponds, the nets presented in Figure A4-3 should be used.

The handling of fry requires special attention. At this age the fish are still very small and vulnerable; they should not be lifted out of water, or only for a short time, if it is indispensable to move them from the net to the place where they are going to be reared, kept or transported.

When moving fry plastic sieves (presented in Figure A4-1) have to be used even if only one or a few fry are moved. Taking the fry in one's hands is also to be avoided as they may suffer from internal injuries by the grabbing fingers, which may lead to an immediate or imminent death.

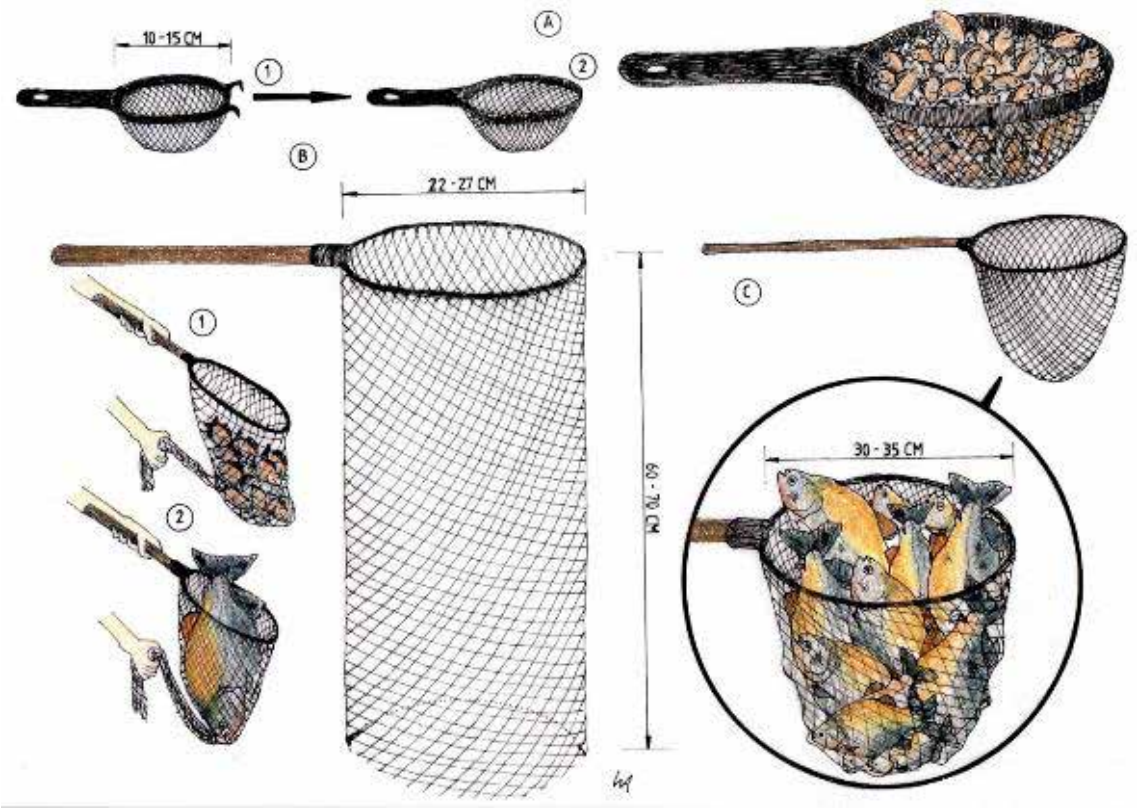
The same plastic kitchen sieve recommended earlier should also be used for counting advanced fry, as it is very important to know how many fry were produced, and how many were sold or stocked for rearing fingerlings out of this number. The technique employed to count advanced fry or fingerlings using a plastic sieve is as demonstrated in Figure A4-4.

Proper and quick handling of fry and fingerlings should be done as shown in Figure A4-2:

- a framed net that fits well into the conditioning tank is used
- a net-box is placed into the conditioning tank.

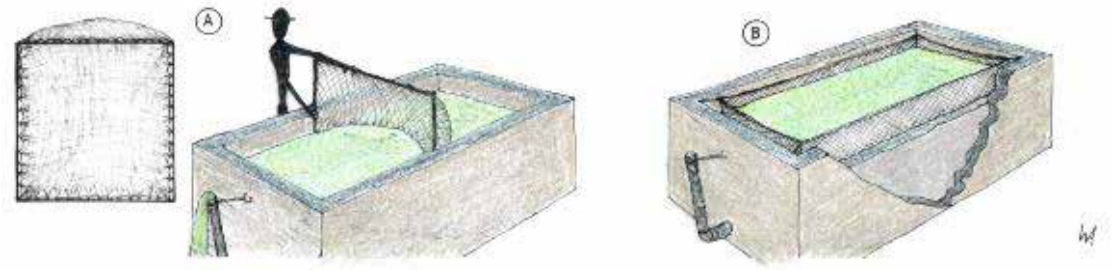
The handling of fingerlings involves the same principles as handling fry. The use of a plastic sieve is still recommended for moving them. If fingerlings are bigger (5 to 10 g) they may be moved from one place to another in batches with an open-ended scoop (see Figure A4-1).

FIGURE A4-1
Equipment of handling the different age groups of fish



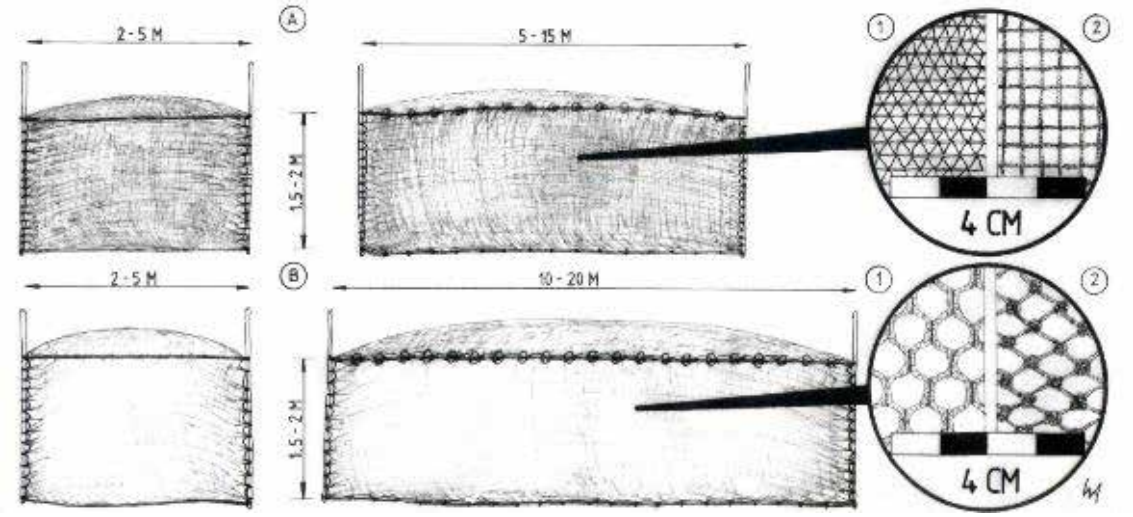
(A) Plastic sieve used for handling and counting fry – an ordinary plastic kitchen sieve commonly available in shops (A1) and altered for counting fry (A2). (B) Open-ended scoop net for handling fingerlings (B1) and brood fish (B2). (C) Scoop net for handling table fish.

FIGURE A4-2
Equipment for the fishing and handling of advanced fry and fingerlings



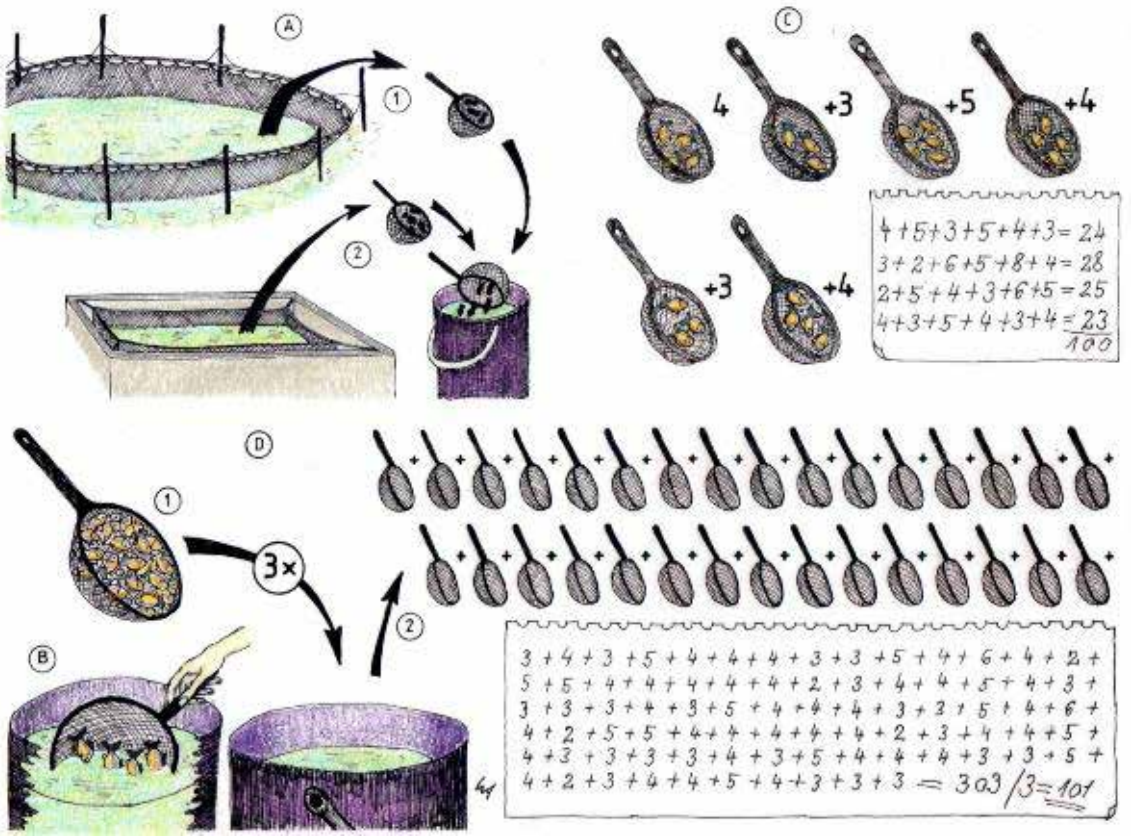
(A) Framed net for taking fry and fingerlings from a tank. (B) Holding net for keeping and handling fry and fingerlings before transportation.

FIGURE A4-3
Nets used for catching advanced fry and fingerling



(A) Seine for catching fry (A1 and A2). (B) Nets for fingerlings. Enlargements show the suitable (B1) and unsuitable (B2) types of mesh structure, because netting materials with knots are not suitable.

FIGURE A4-4
Counting of advanced fry



(C and D) Counting fry is done in small batches by volume. Three sieves full of fry should be taken (D1) and counted (D2). This quantity divided by three will give the average number per one sieve.

1.3 Handling of brood fish

The handling of brood fish also requires special care. This age group is normally moved either by hand or with a open-ended scoop net. Though this age group is not so sensitive to being out of water, it is still preferable not to keep them out of water unnecessarily or for a long period of time. When brood fish are taken for stripping they should be wiped with a soft towel gently so as not to damage the scales of the fish. A frequent mistake is rubbing brood fish dry before stripping. This unfortunately damages their scales and can easily lead to infections.

2. TRANSPORT OF LIVE FISH

2.1 Essentials of fish transport within and between fish farms

Transporting live fish costs money and when unsuccessful the damage is great. On the other hand it is in a farmer's interest to move as many fish as possible in a transporting container. The success of live fish transportation is influenced by many different factors, some of which contradict one another, but all of which should be taken into consideration. The most important factors are:

- duration of transport;
- physical and chemical conditions of the transporting water, particularly the temperature and oxygen content of water are both very important;
- accumulation of metabolic wastes in transporting water, depending on how empty or full the digestive tract of transported fish is;
- size, number, species, health and physical condition of transported fish, in addition to how the fish were handled before transportation;
- uniformity of size of packed fry and fingerlings – i.e. whether there are very big and very small specimens in the same batch.

The above aspects remain valid regardless of whether fish are transported only a short distance within the same fish farm, or for a longer distance to another farm or water body.

2.2 Containers for the transportation of live fish

Depending on the size of the fish, a bucket, plastic bag or a plastic or fiberglass tank can be used as a container for short-distance transportation, when fish are shifted from one pond into another, or from a pond to the fish hatchery, or vice-versa.

While feeding larvae may be transported using any of the transporting containers listed providing they do not leak or bruise the fragile fish,[1] brood fish should never be transported in a bucket or similar device, in which there is not enough space for them. Using oxygen or compressed air for short-distance transportation is not vital, but it is always very useful. A tarpaulin tank moved with a garden tractor is ideal for moving fish within a farm (see Figure A4-5).

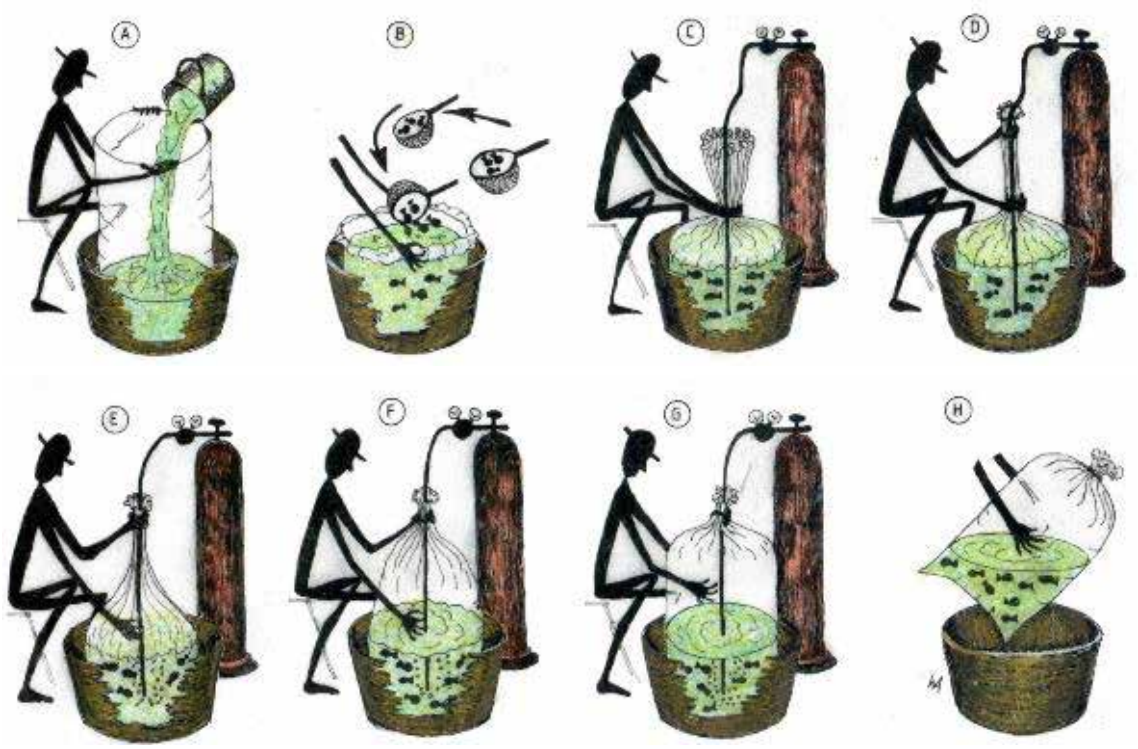
FIGURE A4-5
Motorized transport of fish within a farm

(A) A tarpaulin tank on a lorry and (B) a garden tractor with a tarpaulin water tank.

[1] If a larger, several hundred litre container is used to transport feeding larvae, it is important to siphon them with a suitably long and wide pipe when they are removed from it.

The most frequently employed containers for long-distance fish transportation are the strong plastic bags and stiff plastic or fibreglass tanks of different shapes and sizes. If plastic bags are used, pure oxygen is injected into the bag: this provides the proper oxygen supply for the transported fish (see Figure A4-6). In the case of tanks, either pure oxygen or compressed air is diffused continuously into the water during the entire period of transportation (see Figure A4-7).

FIGURE A4-6
Packing of fish-seed into plastic bag step by step



(A) One third of the plastic bag is filled with water. (B) The fish are put into the bag. (C and D) The air is squeezed out of the bag. (E and F) The bag is filled up with oxygen. (G) The bag is closed. (H) The bag is ready for transport.

If the transportation time is very long, there is a chance that transported fish will produce a lot of ammonia and faeces, which will pollute the transporting water, and eventually poison the fish transported. Another danger of accumulating faeces is that bacteria will develop on them, which also consumes a lot of oxygen. Fish with a full digestive tract also consume more oxygen. It is therefore important to condition (prepare) the fish six to twelve hours before loading or packing. Fry and fingerlings need less time to empty their digestive tract, but if the fish are bigger then the preparation time should be extended accordingly. The conditioning should be done in a tank with continuous water exchange. As good conditioning is the key to successful long-distance transport, specimens that are too big, too weak and too small should also be removed from the batch. This will reduce mortality during transport and increase the satisfaction of the customer receiving the fish.

2.3 Correlation between the number and size of tambaqui to be transported

The most frequently transported sizes of live tambaqui are advanced fry and fingerlings of 2–6 cm (TL). When fry and fingerlings of tambaqui are hungry and overcrowded in a small space they become aggressive and dead specimens are consumed by others.

FIGURE A4-7
Loading of fish-seed transportation tank step by step

(A) When unused the tank should be kept dry. To avoid accumulation of rain in it, hence should be kept upside down. (B) The tank should be rinsed before use. (C) It should be filled up to the half of its volume. (D) The aerator should be checked. (E) Fish are put into the tank. (F) Water is filled to the top of transporting tank. (G) Starting aeration of water. (H) Tank is closed.

Wounded fry are also attacked and smaller, weaker fish suffer from the stress of being threatened by bigger and stronger specimens. With this in mind, tambaqui fry and fingerlings should be conditioned for about five to six hours before loading.

The conditioning tank should be covered, creating total darkness in the tank; and it is also advisable to keep the tank or plastic bags dark and sheltered from sun radiation during transportation, in order to limit mortality. Wounded specimens are unlikely to survive the journey, and so the fishing, transportation and all handling prior to loading or packing should be performed swiftly with extreme care.

Transportable quantities of various sizes of fry and fingerlings are indicated in Tables A4-1, A4-2 and A4-3.

TABLE A4-1
Transportation of advanced fry (2 to 2.5 cm. TL) in plastic bag for longer than 24 hours

Duration (hours)	In 10 l water (pc.)	In 15 l water (pc.)
24	2.000	3.000
48	1.300	2.000

* Water to oxygen proportion: 1:1 or 1:2. Water temperature 27 to 28 °C.

TABLE A4-2
Transportation of advanced fry and fingerlings of tambaqui in a plastic bag for no longer than 6 to 7 hours

Average total length of fish (cm)	Average weight (g)	In 10 l water (pc.)	In 15 l water (pc.)
6	2.9	700	1.000
5	1.8	1.000	1.500
4	1.0	1.200	1.800
3	0.6	1.700	2.500
2	0.3	2.700	4.000

* Water to oxygen proportion: 1:1 or 1:2. Water temperature 27 to 28 °C

TABLE A4-3
Transportation of advanced fry and fingerlings in tanks, with continuous dispersion of oxygen into the transportation water, for no longer than 6 to 7 hours

Average total length of fish (cm)	Average weight (g)	In 100 l water (pc.)
6	2.9	2.000–3.000
4	1.0	4.000–5.000
2	0.3	7.500–10.000

* Water temperature 27 to 28 °C.

As the actual quantity of transported fish depends on the health and physical condition of fish to be transported, it is advisable to carry out a test pack beforehand.

Following a short introduction to the species and its closest commercially viable related species, namely pirapatinga (*Piaractus brachypomus*) and pacu (*Piaractus mesopotamicus*), this field guide provides practical information on the culture and reproduction of tambaqui (*Colossoma macropomum*).

As a field guide it aims to support the understanding and dissemination of applicable technologies for the culture and reproduction of tambaqui, i.e. what should be done – as well as when and how it should be done – in order to achieve success in the artificial propagation as well as the fingerling and table fish production stages.

The concise technical descriptions in this guide are accompanied by self-explanatory illustrations and a reader-friendly glossary of technical terms, which is important for tambaqui aquaculture farmers.

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