# **RESEARCH ON NEW FISH SPECIES FOR AQUACULTURE IN NORTHERN ISRAEL**

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# **CHAPTER 1**

## A MODEL FARM FOR THE CULTIVATION OF EELS (ANGUILLA ANGUILLA)

# **1.1 Introduction**

The main objective of Northern R&D is to increase the region's profitability and ability to compete on the agricultural market by introducing new crops chosen for development potential and economic and marketing advantages. Eels are one such crop. A model farm for cultivating eels was established at Kibbutz Dan in the upper Galilee in 1997. The model farm is a good example of the correct and modern approach to developing new agricultural branches by combining advanced high-quality commercial opportunities with a research infrastructure that can provide professional and experienced solutions to limitations and problems and, thereby, increase the economic viability of the crop.

This chapter describes the model farm and contains data assembled during three years of work. The material provides a basis for growers who may be interested in cultivating eels.

I would like to thank all those who contributed to this work and the establishment of the branch, in particular my colleagues Yehudah Yehudah, Avshalom Horowitiz, Ofir Degani, and Amiram Efrati.

# 1.2 General information on the eel project

The European eel (*Anguilla anguilla*; Fig. 1.1) is new to Israeli aquaculture and grown exclusively for export. The eel commands high prices in an almost unlimited European market where increasing pollution and limitations on fishing in rivers have created a diminishing market supply. Israel has an advantage in that its climate provides a greater number of days than Europe's during which growth can take place, justifying the high investment involved in developing the industry.

The cel cultivation project in Kibbutz Dan began in 1997. The project involved a large investment and high costs since a number of different techniques are required to raise eels from elver to market size. Facilities include ponds, concrete tanks and intensive cultivation in controlled indoor water systems. Eel cultivation is divided into three stages: primary nursing to 5 g in an indoor recycled water system, secondary nursing to 30 g in 40 m<sup>3</sup> concrete outdoor ponds, and fattening to 200 g for males and 600 g for females in 250 m<sup>3</sup> concrete lined ponds.

The project was carried out by the Dan Fisheries in the hope that it would become a major source of income for the kibbutz and, indeed, the summary of its activities in the first year already indicated a remarkable degree of success.

To ensure that the first years of cultivation would be used to learn as much as possible about the many aspects of this specialty, it was agreed that the farm at Kibbutz Dan would serve as a model for the entire region. As such, the project received experimental support and scientific monitoring from the Galilee Technological Center Research Institute (MIGAL) and Northern R&D.

The first cycle of eel cultivation began in 1997 with glass eels (elvers) imported from England. Altogether 250 kg of glass eels were grown, with a production potential of 100 tons. This stage was successfully completed when the fish were moved to 12 outdoor ponds. Information on this stage was collected and processed by Northern R&D for use by other growers in the region.

#### 1.3 The order Anguilliformes

This large order of teleost fish is divided into 20 families numbering more than 600 species. A few, especially the European and Japanese eels, are reared in aquaculture.

Eels are widely distributed throughout the world. They grow mostly in seas, but a small number grow in fresh water. Their morphology and tubular shape are signs of their primitive evolutionary nature. Eels are bottom feeders. They lack ventral fins and some lack pectoral and tail fins. The odd-numbered fins (dorsal, tail, and anal) are fused into a single long fin running over one third to one half of the body length. The body is long and snakelike. Eels have leathery gill covers and small gill openings; the maw is frontal and large, reaching below the eyes, with many sharp teeth.

The *Anguilla* family contains 18 species worldwide (Table 1.1), some of which reproduce in the same region. All species share an identical two-stage life cycle, the first in the sea and the second in flowing fresh water.

Location of Anguilla species (Tesch, 1977).				
Species	Region	Distribution		
A. megastoma	S.E. to S. Pacific	Solomon and Cook Islands, Fiji, New		
		Caledonia		
A. reinhardti	"	E. coast of Australia		
A. australis australis	"	S.E. Australia		
A. australis schmidti	"	New Zealand, New Caledonia		
A. dieffenbachi	н	New Zealand		
A. b. bicolor Schmidt	Equator and	New Guinea		
	Pacific			
A. celebesensis	11	New Guinea, Philippines		
A. ancestralis	H	Manadu		
A. borneesis	11	Borneo		
A. interioris	н	New Guinea		
A. obscura	"	Fiji, Samoa, Tahiti, Cook Islands		
A. b. bicolor	N. Indian Ocean	Sumatra, Java, Kenya coast		
McClellend				
A. nebulosa nebulosa	11	Sri Lanka, India, Myanmar		
A. mossambica Peter	н	Zanzibar to Cape of Good Hope		
A. nebulosa labiata	11	Kenya coast, Mozambique, Lake Malawi		
A. anguilla	N. Atlantic Ocean	Europe, N. Africa		
A. rostrata	н	N. and S. America		
A. japonica	N.E. Pacific	Japan, China		

# Table 1.1.

# 1.4 The European eel (Anguilla anguilla)

This species is common in Europe and the Mediterranean. In Israel, it is found in all rivers and streams that flow into the Mediterranean Sea. Thanks to their flexible bodies, eels are able to move long distances over moist ground and reach locations remote from their original habitat. They grow to about one meter in length, with a body weight of 1.5 kg. They are brown and have a pale belly. Eels are carnivorous, feeding on animals in riverbeds, in mud, and between stones. They are exclusively benthic, living between rocks or hiding in mud. All eels migrate from fresh to sea water to reproduce and develop through metamorphosis.

# 1.5 Life cycle of the European eel

The course of the European eel's life is especially interesting. It reproduces in the Sargasso Sea near the coast of Florida (Fig. 1.2). Newly-hatched leaf-shaped larvae are carried for two to three years on the Gulf Stream until they reach the coasts of Europe and the Mediterranean. There they metamorphose into the characteristic snakelike shape and enter river estuaries. They live in rivers 5-9 years until adulthood, then return to the sea and migrate back to the Sargasso Sea to reproduce. These migrations are evidently governed by the conditions necessary for reproduction, the search for food, and growth.

# 1.5.1 Reproduction

The European eel presumably reproduces in the Sargasso Sea in the western Atlantic. However, no adult eels have yet been found in this area and the eel reproduction process remains largely unknown. Schmidt was the first to suggest, in 1922, that eggs of the European eel are laid in the Sargasso Sea. In 1967, Bertelsen more precisely defined the area as a result of the Dana Mission in the previous year. The researchers found a great number of larvae in April 1966 in the southwestern part of the area designated by Schmidt, and this was after a voyage in February of the same year failed to find any larvae at all. From this, Bertelsen drew the conclusion that laying begins at the end of March. Hydrographic data from the Sargasso Sea show that laying takes place at a depth of 100-200 m below the surface. In 1967, scientists conducted research on silver eels they had caught, finding that the maximum temperature for reproduction is 25-26°C and the optimal temperature for sexual maturation is 25°C (see comprehensive review by Tesch, 1977).

The exact method of coupling and fertilization is unknown but the accepted hypothesis is that fertilization is external. The ripe eggs and embryo yolk sacs of the larvae contain lipid drops that nourish the larvae during their first days. An adult female lays 5-10 million eggs (0.1 mm) from which larvae hatch. It is currently impossible to commercially reproduce eels.

# 1.5.2 Leaf eels (leptocephali)

Larvae development and migration to the coasts of Europe were described by Schmidt (1922), as follows. Eggs are laid throughout the spring. The larvae are small (5-17 mm) and leaf-shaped (Fig. 1.3a). They float 200-300 m below the surface of the sea where the temperature is 25°C. They grow rapidly for the first two months reaching an average 25 mm by summer, then float to the upper water level. Most remain at a depth of 25-50 m, but sometimes they can be found on the surface. The larvae move eastward with surface water currents towards the European coastline. During their first summer, they can be found in the western Atlantic Ocean. As they reach their second summer, they attain a length of 50-55 mm and mid-Atlantic waters. They reach the Atlantic coasts of Europe only in the third summer.

Not all researchers agree with Schmidt that crossing the Atlantic takes three years. Brongersma (1967), in his work on the loggerhead turtle, opined that these species can cross the ocean with the currents in as little as a year. Other researchers, prior to Schmidt, were of the opinion that some larvae reach the European coast from the American side of the ocean within 13 to 17 months.

The European eel passes through a major portion of its development in the ocean prior to reaching the European coast and migrating inland to fresh water. By the time they reach Europe, the larvae average 75 mm, still in their leaflike shape. They do not migrate under their own power but depend on their shape, which is ideal for passive movement, to float with the current. The direction and speed of movement are governed by ocean currents, especially the northern Gulf Stream.

Once the larvae reach their maximum size, near the coast of Europe, there is a pause in migration. For the first time, they use their own power to remain where the water is 100 m deep. In this area, they undergo metamorphosis into small eels, known as glass eels or elvers, for a period lasting several months until autumn. It is not known how the larvae identify the continental shelf, but it is possible that there is some influence of the nearby fresh water.

#### 1.5.3 Glass eels (elvers)

Migration continues after the larvae metamophose into glass eels. Since they take no nourishment during this avatar, they are small and thin. It is not known how the glass eel migrates through the sea, but it is hypothesized that it makes use of tidal forces and ocean currents to reach the coast rather than actively swim. This comparatively short migration ends when the eel reaches the shore. The glass eel makes a second pause in migration when it reaches a river estuary where it undergoes an additional change - it develops the ability to swim. It practices swimming near the surface of the water, prefering to swim along the length of the coast and showing no tendency to migrate against fresh water currents. The eels remain

at the outlet of the estuary while passing through the physiological changes that adapt them to life in fresh water (Fig. 1.3b). During these changes, they cease to eat, as did larvae undergoing the first metamorphosis. When the changes are complete, they swim upstream and find habitats in rivers and lakes. Penetration into the continent involves swimming against the current and circumventing waterfalls by crawling up the banks. At this stage, they are fully carnivorous, feeding on insects and other small animals. Their food closely resembles that of trout. Some eels remain in brackish or even salt water; the eel can pass from salt to fresh water or the reverse without suffering any harm.

# 1.5.4 Life in fresh water and maturation

Glass eels that reach fresh water spend a number of years feeding and developing. The lifespan of the eel depends on how long it takes it to reach the adult silver eel stage, which is partly governed by sex and size and strongly influenced by the environment.

The female of the species lives longer than the male. Research by Frost (1945) in the Lake Windermere area in England determined that the average age of females was 12-13 years, with a maximum age of 19, while males averaged 9 years, reaching a maximum of 12. The maximum age ever recorded for an eel was 85 years, for a specimen kept in an aquarium in Sweden.

This period of feeding and growing is accompanied by an additional change in which the glass eel matures into an adult with enlarged sex organs. The male is usually smaller than the female. Glass eels develop a green-brown color, towards the end of their growth they become metallic silver-bronze with black shading.

The adult eel ceases to feed once it has grown enough and its body contains sufficient fat reserves to provide it with enough energy to make the return journey against ocean currents to the reproduction site. A number of known factors influence the timing of this journey, including the season, the phase of the moon and the depth of the currents. Experiments with eels marked before their return migration provided information on their speed in the sea. Man and Lupmann (1958) postulated a minimum speed of 13 km per day while Trybon and Schneider (1908) established a average of 36 km per day with a maximum of 50 km. Many reports and signs indicate that this migration takes place in upper water levels. Migration back to the laying ground in the Sargasso Sea can take as long as 9-12 months.

The lifespan of the eel, from larva through glass eel to brown eel to adult silver eel, varies from 15 to 20 years and includes two periods in salt water and one in fresh.

# 1.6 Aquacultured eel species

There are 18 known *Anguilla* species, some of which reproduce in the same area as the European eel. Three are important to aquaculture: the European eel (*Anguilla anguilla*), the American eel (*A. rostrata*) and the Japanese eel (*A. japonica*). These three species share a similar life cycle and resemble each other in shape, size and behavior. The European eel can be found in every river that flows into a sea. It is remarkable that millions of larvae pass through the narrow Straits of Gibraltar every year, especially since this species depends, for part of its life, solely on ocean currents for movement and considering its wide distribution.

The highest concentration of eels in Europe is in the rivers that flow into the Bay of Biscay, especially the Loire and the Gironde. In 1973, the French caught 220 tons of glass eels in this area which they sold in Japan, Europe and other parts of the world. An estimated 800 million glass eels are caught here every year. In Italy, most eels are found in the rivers that flow into the northern Adriatic in the vicinity of Venice. In Ireland, the main concentrations of glass eels are in the Shannon River in southwest Ireland and the Bann River in Northern Ireland, where there are well-organized eel industries. In England, the main eel concentration is in the Severn, with a smaller number in the Parrett: some 50 tons of larvae reach these two rivers together. The number of glass eels caught in Europe every year reaches the billions.

#### 1.7 Eels as an agro-economy

The commercial culture of eels entered the Israeli economy in 1980 after the idea was raised in the Association of Fish Farmers based on two suppositions: (a) eels have a large market in Europe and command good prices, and (b) higher water temperatures give Israeli growers an advantage over European growers.

At the time, a number of farms began to raise eels by various methods: nursery, outdoor ponds, monoculture, polyculture. In 1983, research supported by the Ministry of Industry and Trade began in the Galilee Technological Center (MIGAL) to study the nursery stage of eel culture in specially-built facilities and develop edible eel products. In the course of this research, methods for fattening the fish in controlled water conditions, including water recycling, were developed and a great deal of knowledge accumulated. MIGAL conducted research on the composition of feed (dough) for the nursery and fattening stages. A growth-rate of 1-1.2% per day was achieved at a food conversion ratio of 1.5-2:1. Model farms in Kibbutz Malkiya and Kibbutz Yiftach continued on their own to develop eel culture techniques with recycled water in a closed cycle, adding experience they gained during visits to Denmark. As a result, a nursery planned by MIGAL and the model farm at Malkiya changed from an open to a closed water system in which the oxygen content and temperature were controlled and wastes were removed. Whenever problems of disease or parasites arose, the fish were examined at the Nir David laboratory and a considerable amount of knowledge on health disorders specific to eels was gained.

Since imported feeds for eels are expensive enough to prevent commercial eel culture from being economically viable, it was necessary to develop feeds produced from local sources with a minimum amount of imported components (Table 1.2). A feed was developed which gave results only slightly lower than those obtained with imported feed at a quarter of the cost. The locally produced feed comprised 50% fishmeal, 20% chicken meal, 10% soluble starch, 11% wheat flour, fats 6-8%, and minerals, vitamins and other additives (see review by Degani and Gallagher, 1995). Next, experiments were conducted to produce the feed in pellet form. Research was also conducted on food products, especially smoked eel. The lack of suitable fuel was a problem, but the product was shown at an international exhibition and favorably received.

	Feed				
Ingredient	Α	В	С	D	E
Fishmeal	50	50	50	35	45
Chicken meal	20	20	20	35	20
Soluble starch	10	10	-	10	10
Wheat flour	11	11	21	11	11
Mineral & vitamin concentrate	3	3	3	3	3
Milk powder	-	-	-	-	5
Stabilizer	1	1	1	1	1
Fats added at preparation of dough	5	5	5	5	5
Analysis (before addition of fats)					
Total protein	49.5	49.4	50.6	50.8	48.5
Fats	6.5	6.5	6.4	8.0	7.0
Ash	5.7	5.7	5.5	6.7	5.9

Table 1.2.

#### 1.8 The glass eel market

The main season for catching glass eels in the estuaries of western Europe is November-May (especially January- April). A smaller number is caught at the beginning of summer (June-July). The glass eels are transparent, 7 cm long and 0.3 g. They are tubular, with a diameter of some 2 mm. In northern waters, their arrival is later. Their entrance into the rivers is determined largely by water temperature, speed of the current and rainfall. Most eels penetrate the land when the water temperature of the rivers rises and approximates that of the Atlantic, the moon is full, and rain is falling.

In Spain and South America, some 80% of the annual glass eel catch is consumed in their present physical state or in the first stages of growth. A further 10% are purchased to stock aquaculture ponds and the remaining 10% are stocked into natural water bodies to increase the native eel population.

Catching eels is a delicate procedure, especially those destined for culture or repopulation. They are caught in fine nets, generally 80 cm wide and 35 cm deep, or in plankton nets towed behind motor boats. Glass eels are drawn to light and fishermen exploit this fact to collect glass eel as they migrate to fresh water. Once caught, the eels are placed in tanks supplied with oxygen to keep them in good condition until they are taken to collection points where they are put into floating boxes to await transport to their final destination. They are then either transported "wet" in plastic sacks or oxygenated water tanks, or "dry" in flat polystyrene boxes with ice to keep them moist and at a low temperature of 5-10°C.

The annual European harvest of glass eels is estimated at 1,000 tons: France -500 tons, Portugal -300 tons, Spain -100 tons, England -40 tons, with smaller quantities in Italy, Scotland, Ireland and Holland. The main harvest sites are the estuaries of the Bay of Biscay off the shores of France and Spain, and the Severn River estuary in England.

Because of the high prices that elvers command (\$150-350), the market is wellorganized under the control of "veteran forces". The eels are mostly caught by small local fishermen from the shore or from boats, and transferred to wholesale suppliers. In France, the largest supplier of glass eels, there are some 100 such companies located at over eight river esturaries.

Quality is a major consideration for glass eels destined for culture. Factors taken into consideration are size, season, pigmentation, size variation, concentration of solid matter and methods used to catch, store and transport the elvers. The optimal size is 70-75 mm, 0.22-0.38 g, with 2,600-4,600 fingerlings to the kg. During growth from glass eels to market size (150-400 g), the eels pass through a number of facilities.

#### **1.9 Eel nurseries**

The primary nursery stage of eel culture is defined as the period between capture and attaining a weight of 5 g. Some culture methods keep eels in primary nurseries to 10-20 g, or even more. Two nursery methods are: (a) the closed system, used in Sweden, Denmark, Holland and Germany, where eels are kept in closed heated structures involving a considerable investment and high operating costs; and (b) the open system, used in warm countries such as Japan, Taiwan and Italy, where eels are nursed in outdoor or concrete ponds with geothermal water, where available. This method requires less investment and lower operating costs.

The first container into which the glass eels are put after being caught must be in a closed building with water heated to 23-24°C. Here, the elvers grow to 6-8 cm in 30-60 days. The containers are round with a diameter of 60-80 cm and a jet stream causes the water to swirl in a circle. In the second tank, the eels reach approximately 12 cm in a closed system. The second tank is wider than the first and has a depth of 1 m. Larger eels are hardier and can grow outdoors in concrete or earthen pools with concrete walls to prevent the eels escaping on rainy nights. Containers should be furnished with nets for the eels to rest on; the nets must be well aerated with fresh oxygen-rich water so that, even if the water in the container is of poor quality, the resting area provides shelter where the eels can find water of better quality.

6

Eels must have good quality water during nursing. The water must have an oxygen content of at least 4 ppm, a pH of 7.5, an ammonia content no higher than 5 ppm and a nitrate content no higher than 0.25 ppm. Rearing eels in enclosed conditions produced better results than rearing them in outdoor pools with green water. When the elvers arrive from the airport, they must be acclimated in water at a temperature close to their body temperature, and then it is gradually raised.

Many methods have been tried to cause glass eels to eat in captivity including providing invertebrates, different sorts of meat, artificial food, etc., as feed. This topic is comprehensively reviewed in Degani and Gallagher (1995). Kibbutz Dan adopted the method used in Europe, gradual transition to artificial feed. For the first days, the eels are fed cod roe, then a mixture of roe and dough, and finally they are introduced to pelleted feed.

During the nursery stage, eels are grown at various densities. The optimal density depends on factors such as temperature, tank, oxygen content, etc. A drop in the growth rate occurred at densities above 5 kg/m<sup>3</sup>, according to research carried out at MIGAL (Degani and Gallagher, 1995). Today, eels are grown at an initial density of 7-8 kg/m<sup>3</sup>, provided that high quality water and feed are constant throughout the day.

# 1.10 Fattening

Since eels are undomesticated, they have not been genetically selected and they grow at different rates. The most rapidly growing eels can reach market size in a year and a half whereas slow-growing eels take 4-5 years to reach this size. Stronger fish suppress the growth of weaker ones. When the largest eels are removed from a pond, the next in size begin to grow more rapidly. By the end of the growing period, females reach 300-800 g while males grow no larger than 200 g.

In most areas, eels are grown on artificial food in paste form, however, there is a current trend towards using pellets, even though there is no clear preference towards either. The advantage of pellets is that they allow greater control of feeding times, which has a positive influence on water quality. The disadvantage is that the amount of fat that can be put into pellets is limited.

Research conducted in the mid-1980s studied two factors: (a) temperature adaptation and pond conditions in Israeli eel cultivation; and (b) the use of locally produced feed in these conditions to achieve a commercially feasible growth rate. The results of the research were positive. Eels adapted to cultivation conditions in the upper Galilee when imported Italian feed was used. Some 5% of the eels reached 100 g after 10 months, 30-40% reached an average of 50 g over this period, and the rest remained small. At the same time, a feed in the form of dough developed at MIGAL produced results as good as, or better than, the imported feed. The feed, however, did not become commercially used. Local farmers preferred the pellets to which they were accustomed; pellets allowed them better control of the time and quantity of feeding, thereby aiding maintenance of water quality.

Feeding with pellets can lead to problems, especially during the later fattening period. Optimal fattening in eels is achieved with food containing 15-20% fat, a concentration that cannot be incorporated into pellets. The eel accumulates fat when it approaches market size (over 100 g) and the proportion of fat in its body can reach 30% of its body weight or more. Supplying feed for this fat content in protein form is wasteful. The problem can be solved by feeding a high proportion of carbohydrates, with which the eel apparently copes better than other aquacultured tropical fish.

#### 1.11 Eel nutrition

The eel is carnivorous, hence it grows well on any feed based on animal protein. In the past, it was common to provide fresh foods during nursing and fattening. Such foods are generally made of fish, fresh fish wastes or fishmeal. In addition, small quantities of fresh or frozen weevils were used. Fish are provided in small pieces or hung on lines in the water. For the first two weeks, the glass eels receive thin slices of snails or worms, then they are transferred

to tubifex, with a gradual transition to pieces of fresh fish. In the initial period of growth (the first three months), the daily ration varies between 5-15% of their body weight. Dry foods which were fed after adaptation to an artificial diet in the form of dough or pellets now constitute a sizable portion of the feed in Japan and Europe (Holland, Denmark, German, and especially Italy). The advantage of dry foods is that they are cheaper and easier to store than fresh or frozen. The daily food ration decreases as the growth period progresses (from 5 to 20 months) from 5% to 1.5% of the body weight, depending on the season and water temperature. Even where dry food is used, glass eels receive codfish flakes and dough for a transitional period, before being gradually weaned over two weeks to dry pellets fed in an automatic trough. Pollution of the water by organic material affects the health of the eels, so feeding must be conducted at a fixed place at the edge of the pool. The eels learn to go to this place at feeding time.

The dry food components of eel feed in Japan include fishmeal, liver meal, milk powder, starch and yeast. Food for young eels is slightly different from food in the fattening stage: the protein content is 45-52% in the fattening stage and 2% during the nursery stage, with 2.5-6% fat, 0.1-0.6% cellulose, 15% ash, plus vitamins, minerals and trace elements. Amino acids essential to eels are methionine, tryptophen, trionine, and valine. Before dry food is fed to the eels it is mixed with water and 5-10% fats are added. When it is fed as a dough, it is placed in floating framed nets with 4-10 mm mesh, depending on the size of the eels. Although most literature on eel cultivation recommends feeding in dough form, some farmers in Japan and Europe use 4 mm pellets containing 42.5% protein, 8% fats and 11.5% ash, with 10% fats added at feeding time. Pellet feeding has produced 30% better results than dough, with a 20% advantage to pellets in the food conversion ratio. More material on the nutrition of eels can be found in Degani and Gallagher (1995).

#### 1.12 Sex determination

The sex of fish, unlike mammals, is determined after hatching (or birth) and during growth. There are major differences between fish species in sex determination. In some species sex is determined genetically, in others not, while there are yet other species that change sex during the life cycle. The literature on eels presents a variety of opinions regarding sex determination. While some researchers believe that sex in eels is determined genetically, other disagree, arguing that male and female eels have an identical chromosome structure (Degani and Kushnirov, 1989).

Physiological differences between males and females can be detected in histological gonad sections when the eels reach 15-25 cm. In commercial cultivation, males reach sexual maturity at 150 g, after which their growth rate slows, they become silver, and their commercial value for smoking decreases. At this stage, an experienced farmer can distinguish between males and females. On the other hand, males can be kept until they reach their maximum of 50 cm and 300 g. The female reaches a minimum of 300-350 g before growth slows and can reach a maximum of 5-6 kg. Consequently, it takes fewer females than males to produce 1 kg of fish and they fetch higher prices in the market. Hence, it is important to obtain as high a percentage of females as possible.

The gender determination of eels is influenced by feed quantity and quality, and environmental factors: population density, water temperature and salinity. In extensive cultivation on Italian farms, 25-40% females are obtained, whereas only 10-15% females are obtained in super-intensive closed-circuit facilities in northern Europe where the fish are maintained at high density. European glass eels maintained in Japanese farms at a low density grow into populations with 35% females. Farms that transferred from fresh to salt water achieved a higher percentage of females. As with density, high water temperature raises the percentage of males in the population. In farms where glass eels were raised in a water temperature of 26°C, the majority of the population became males. In farms where the eels were fed cod eggs and higher quality feed, a higher percentage of females was obtained. The gender of eels is determined when they reach 5 g. Some of the population changes sex during growth in response to environmental conditions.

Sex determination can also be influenced by the administration of hormones. Appropriate doses of steroids are an important supplement in sex determination. The use of estradiol has raised the proportion of females to 90%. Feeding ovaries and testicles of fish or other animals raised the percent of females to 40-60%. It is hypothesized that feeding a supplement of estrogens of plant origin to glass eels will increase the proportion of females (Degani and Kushnirov, 1989).

# 1.13 Sorting

Since eels grow at different rates, cannibalism and competition may occur in populations containing eels of different sizes, resulting in heavy economic losses (Degani and Levanon, 1983). Cannibalism and competition can be avoided by frequently grading and separating the eels. The eels must be graded five or six times and re-stocked according to size before they reach 15 cm. Another reason to sort the eels, as with many fish species, is that large fish inhibit the growth of smaller ones. The sorting must be conducted very gently while the eels are fasting. Transfer to a new pond must be carried out carefully, allowing the eels to become accustomed to the change of water. In commercial cultivation, eels are sorted every 6-7 weeks during all stages of growth.

# 1.14 Growth stages

The growth of eels commences at the glass eel stage. Since eels reproduce in distant seas, elvers must be caught in river estuaries. At this stage, they are 6 cm long and weigh 1.5-2 g. A mortality rate of some 20% (mainly eels that did not learn to eat) during the first two months after capture is considered reasonable. It is important at this stage to dispose of weak, sick, and slow-growing eels. The first sorting is conducted five weeks after the elvers have been stocked. The fish are sorted into two groups, separating the slower-growing eels from the rest of the group. A second sorting occurs six weeks later when the eels reach 12 cm. Eels of this size are transferred to larger ponds. At 20-30 cm, the eels are again sorted for size and the large ones are transferred to outdoor secondary nursery pools. Finally, they are transferred to fattening pools where they remain until reaching market size at the end of their second summer. Data from a Japanese farm show that eels reach 28 g (on average) in their second year and 83 g in their third, at 25 and 40 cm, respectively. Mortality during the first year is about 34%, dropping to 6-7% in the second and third years.

There is no certain answer to how long it takes the eel to reach market size (150-400 g). The growth rate depends on the quality of the containers, the skill of the farmer, and the management methods. In experiments conducted at MIGAL, eels that weighed up to 3 g after three months of initial nursing reached 40 g by the end of the first year and market size by the end of the second. Eels that weighed over 3 g at the end of the first three months reached an average of 100 g by the end of the first season and market size by the middle of the second. Eels are expected to reach 60 g by the end of one year of cultivation and 150-200 g by the end of the second (Degani and Gallagher, 1995).

#### 1.15 Growing eels for the table

Eels for consumption are grown in extensive (earthen or concrete pools) or intensive (facilities with a controlled water system) systems. Their growth rate is very slow, they do best in oxygen-rich water at a temperature above 18°C. The optimal temperature for eel growth is 22-25°C, depending on age and size. Eels weighing over 100 g prefer a lower temperature for growth (22°C) than smaller eels. There are additional factors to take into account (Degani and Gallagher, 1988).

# 1.15.1 Extensive cultivation

This system is used mainly in Europe, especially in polyculture with other fish species. Eels are grown as a secondary crop in cyprinid fattening ponds. There are ponds, mostly salt-water

ponds on the shores of Italy, in which eels are the main crop. The ponds are stocked with glass eels during the spring and large eels are harvested in autumn and winter. Production by this method is 30-40 kg/ha, with a maximum of 100 kg/ha.

#### 1.15.2 Intensive cultivation

In some countries, such as Denmark, Germany and Holland, eels have been cultivated for several years under intensive conditions where water temperature, oxygen content and secretions are controlled. The transition to intensive systems has made possible the successful cultivation of eels in high density. This new branch of biotechnology can boast a number of achievements. In some of the installations, intensive cultivation takes place only in the early nursery stages, but other growers have adopted it for the entire cycle. It has been found that when stocking the ponds with young eels (5-35 g) the density should be high -15,000 kg/ha. Under these conditions, production reaches 50-75 ton/ha by the end of the season, with eels averaging of 90 g. Males can reach 150 g and females 500-600 g in intensive cultivation.

In Japan, eels have been grown by the intensive method for 100 years in some two thousand small eel farms which produce over 50,000 tons of eels per year. As in Europe, more and more Japanese eel farms are transferring to dry food in pellet form. Eel farms in Japan can be divided into two types: (a) single-year cultivation farms where glass eels, caught in river estuaries at 6-7 cm and 0.16-0.2 g, are stocked into ponds until autumn, when they reach 15-20 cm and 6-8 g. Single-year cultivation takes place in two types of ponds, the first is 100-300 m<sup>3</sup> where the elver density is high and growth is slow, the second is 0.2 ha where real growth takes place. In both cases, the pond depth is no more than 40 cm; and (b) farms where eels are grown for consumption - here, cultivation takes place in ponds of 0.4-1.0 ha with a depth of 1.0-1.5 m. Cultivation is from April to November and the eels are fed only when the water temperature exceeds 15°C. The eels are fed dry food in the early morning. At the end of the period they are marketed at 100-120 g. The ponds are stocked with about 30 eels/m<sup>2</sup> at an average weight of 15 g. The expected yield is 16 ton/ha.

#### 1.16 Indoor facilities at the Kibbutz Dan model farm

#### 1.16.1 Central building

The eel-growing installation is located in a building that was erected in 1949 as a dairy and converted in the 1970s to rear calves. The building was used for storage since Kibbutz Dan closed its dairy farm. It measures  $43.35 \times 9.50$  m, with an area of  $411.8 \text{ m}^2$ . In 1977, the building was converted into an eel nursery. The conversion included making the windows smaller and closing them with mosquito netting and glass, pouring a new floor and converting the storerooms into an office and a place for two gas burners.

#### 1.16.2 Installations and equipment

The building contains 12 large fiberglass tanks (3 m diameter, 6 m<sup>3</sup>) and 12 small tanks (2 m diameter, 4 m<sup>3</sup>; Figs. 1.4 and 1.5). Each tank is attached to a 75 mm closed water circuit pipe and a 50 mm pipe to the fresh water supply (Fig. 1.6). The fresh water outlet is protected by a stainless steel plate with netting of 0.8-2.0 mm, depending on the size of the fish in the tank. Dirt and dead fish drain out through this plate. To prevent the outlet from becoming blocked, which would raise the water level and allow the fish to escape from the tank, a revolving brush continuously cleans the netting. Each tank is fitted with a float connected to an alarm that warns of a rise in the water level. Each tank contains a ring of pierced 25 mm piping connected to an emergency oxygen system that operates whenever there is a failure in the oxygen supply. The floor of the tank slopes inward toward a hole for removing the fish. The hole is closed by an upright stopper with stainless steel sides. An upright pipe on the outside of the tank is used to control the water level. Fish are removed through this upright for weighing, gender determination or transfer.

The European eel grows best in a water temperature of 23-28°C. Growth is best at 26°C in the early stages and 23°C later on. Since the water of the Dan River reaches the farm at a

temperature of 16°C, it has to be heated as well as enriched with oxygen. This can only be conducted in a closed system (Fig. 1.7).

# 1.16.3 Feeding system

Each tank is fitted with an automatic feeder and a timer to adjust the interval (1-1.5 h) between the 1-2 min feedings. There is a net below the automatic feeder upon which the eels can rest (Fig. 1.8). The food falls onto a plastic plate (Fig. 1.9), so that the feeding rate can be monitored. Feed intervals can be adjusted for all the tanks, according to the biomass of the eels, or for each tank separately. The eels receive feed imported from Italy, composed of 53% total protein, 18% total fat, 0.7% total cellulose, 10% ash, 8.5% water, plus vitamins A, C, D3, E, and trace elements. The eels are carefully watched during feeding to allow them to eat as much as they want, yet prevent waste since imported feed is expensive (about 1/kg) and accumulated excess food spoils and adversely affects water quality.

#### 1.16.4 Biofiltration

The nitrate-rich food creates a surplus of ammonia. Each kilogram of food contains some 90 g nitrate. Twenty percent of the nitrate is retained in the fish body. The remaining 72 g/kg is secreted into the water, 20% of it in solid form; a revolving drum filter removes the solids. The remaining 60 g/kg is secreted in the form of ammonia; a biofilter removes the ammonia.

Water from the tanks drains through 100 mm pipes to a main 300 mm drain (Fig. 1.10) which carries it to the revolving drum filter with a 70-micron sieve (Fig. 1.11). Sprays wash the food remains and other solids from this sieve (Fig. 1.12) to the general drainage system, while the filtered water is pumped by two pumps with a capacity of 150 m<sup>3</sup>/h, each, to a silo adapted for use as a biofilter (Fig. 1.13). A sprinkler, placed at half the height of the tower, spreads the water onto plastic sheets to increase the internal surface, so that each cubic meter of the tower has an internal surface of 250 m<sup>2</sup>. The biofilter system can remove ammonia at a rate of 0.4 g/m<sup>2</sup> biofilter per day. Since the internal surface of the biofilter is 25,000 m<sup>2</sup>, can remove 10 kg ammonia/day. Since 1 kg of food "contributes" 60 g ammonia, the size of the biofilter can treat ammonia from only 166 kg feed per day.

The treated water drains into a reservoir of 30 m<sup>3</sup> at the bottom of the tower. From there it returns to the tanks through 250 mm pipes, being oxygenated on the way, by two pumps with a joint capacity of 300 m<sup>3</sup> per hour.

#### 1.16.5 Alarm system

Four oxygen sensors and four temperature sensors are distributed among the tanks to read the levels of these parameters. If either falls below the acceptable level, the alarm system triggers a siren and broadcasts a signal to a beeper carried by a worker at all times. The alarm triggers off when the power supply fails, the oxygen in any tank with a sensor falls below 4 ppm, the temperature in any tank with a sensor exceeds 27°C, the float in any tanks rises above a certain point, the heating system fails, or the water level in the pump pit falls.

#### 1.16.6 Size grader

The high density in the tanks (up to 100,000/tank at low weights and 120 kg/m<sup>3</sup> in tanks containing fish larger than 100 g) causes competition for food that harms weaker fish. Consequently, the eels are sorted by size every 6-8 weeks. They are first sorted 8-10 weeks after arrival, not including the removal of weak fish from the tanks at delivery time. When the fish are smaller than 5 g, they are sorted by a static sorter into two size groups (Fig. 1.14 and 1.15). Above 5 g, they are sorted by a grader with revolving drums into three size groups.

To remove the eels from the tank for sorting, the exterior upright pipe is lowered and the eels pour through it into a woven plastic sack. They are manually transferred (for the time being) to the grader. Fish are sorted only after fasting for about 24 h and attention is paid to the oxygen regime in the sorting tank. Currently, we transfer eels from the nursery tanks to the fattening ponds at an average weight of 15 g. Before the eels are stocked into the fattening ponds, they are graded again and the small ones (6-7 g) are returned to the nursery. Transferring the fish at a weight higher than 15 g is liable to create a population of 90%

males, with the economic disadvantage that this implies, unless the contrary is proved in research being conducted on the subject.

# 1.17 Primary nursing at Dan Fisheries

The first 250 kg glass eels from England were received at the end of March 1997 and nursed to 5 g indoors in a closed water circuit. This stage was successfully completed with the transfer of the eels to 12 small concrete secondary outdoor nursing ponds. A second batch of 500 kg of glass eels was received in the middle of March 1998, intended to produce 200 tons grown eels. Many improvements were made to the primary nursing process based on results from the first batch. The glass eels of the third batch arrived in five shipments between January and April of 1999. In this batch, 750 kg of glass eels were brought from England and France. At the time, there were still eels from the 1998 batch at the farm.

#### 1.17.1 Receiving glass eels

Glass eels were purchased from companies specializing in catching and marketing eels in England (U.K. Glass Eels, which has a branch in France) and France (V.T.C.). The temperature of the shipment was generally low  $(1.6-5.2^{\circ}C)$  and in most cases mortality was 0.3-0.5%. The elvers were transported to the farm in refrigerated trucks at a temperature of 10°C. The first commercial batch was preceded by a trial batch of 50,000 elvers that were raised in unsuitable conditions.

#### 1.17.2 Transfer to containers

The glass eels underwent a slow process during which they acclimated from about 10°C to 23°C. The initial temperature of the Dan River water (16°C) was lowered by adding ice to the containers until it reached 10°C. Only then were the elvers transferred from their shipping boxes to the containers at about 30 kg per container (about 100,000 elvers in 4 m<sup>3</sup> water). Adding more water from the Dan River gradually increased the temperature during 48 hours until it stabilized at 16°C. The water heating system then raised the water temperature to a steady 23°C.

# 1.17.3 Veterinary treatment

The day after arrival, the glass eels were immersed for six hours per day for three days in oxytetracycline at a concentration of 30 g active ingredient/ $m^3$  water. The treatment was conducted in a closed system with oxygen-enriched water.

# 1.17.4 Nutrition

The elvers were not fed on the day of their arrival. On following days, they were fed cubes of cod roe which, although their nutritive value is low, their smell strongly awakens the appetite of glass eels and this is the food to which they are accustomed. On the fifth day, generally, they were fed roe mixed with dough of imported floury feed, an equal amount of water and 10% fish oil. The amount of roe was gradually decreased until only dough was fed. From two weeks after their arrival, crumbs were mixed into the dough in increasing proportions until the dough no longer existed. At the end of the third week, the elvers were eating only dry food through the automatic feeder. The daily feeding rate gradually decreased from 5% to 1.0-1.5% of the body weight. If necessary, dough was fed for an additional week and roe was added to the diet of weak elvers, mainly as a food attractant. 1.17.5 *Batch of 1997* 

#### 1.17.6 Batch of 1997

a. Reception of glass eels - This first commercial batch was preceded by a trial batch of 50,000 elvers that were raised in unsuitable conditions.

On March 31, 1997, a shipment of 250 kg elvers arrived from U.K. Glass Eels, England. Twelve kg were transferred to Dr. Appelbaum at Sde Boker College in the Negev. The price of these glass eels was £230/kg. Mortality during shipment was 0.35%. The average weight was 0.275 g (i.e., 865,000 elvers), with the average weight of the dead eels 0.3 g.

The eels arrived at too low a temperature  $(1.6-5.3^{\circ}C)$  instead of at the recommended 10°C as agreed with the suppliers. The reason was that the frozen cod eggs sent with the glass eels were insufficiently insulated from them. The glass eels were transferred to containers half full of water. Ice was added and by the end of the day the elvers had acclimated to the Dan River water of 16°C. Due to a lack of experience, the containers were not hermetically closed and, apparently, a great many eels escaped. On the second day, the temperature of the water was raised to 20°C and, on the third, to the desired 24°C.

b. Nutrition – On the day after their arrival, the eels were fed cod roe, whose smell strongly awakens the appetite of glass eels although their nutritive value is low. The eels received cod roe at a rate of 100 g per container. The food was placed directly on the resting nets to enable the eels to squeeze through and reach the food from underneath. They were fed once on that day. On the second day, they were fed 290 g cod roe per container together with 50 g dough consisting of 50% dry matter and 50% water with 3-10% added fish oil. On the fifth day, they were fed 900 g roe per container with 300 g dough, divided into two feedings. On day 17, feeding with roe was discontinued so that dough became the main food, fed at 350 g per container twice a day.

On day 22, we began to scatter crumbs of Trouvit 2.0 feed into the dough and the water with the aim of transferring the elvers to dry feed as quickly as possible. The automatic feeders were first operated at the beginning of the sixth week. In the eighth week, dough was discontinued and each container received 1.4 kg crumbs. Feeding plates were fitted to each container to allow feedings to be monitored. At no point during this period were we able to reach the recommended level of feeding, 80 kg per biomass of 4 tons (approximately 5%). Generally, the fed amount ranged 50-60 kg per day.

When we operated the biofilter, there was a decrease in appetite, although the quality of the water (at least in the parameters we measured) and the level of parasites did not explain this phenomenon. After treatment with formalin and a change of most of the water, appetite surged and remained high for several days. Ten days after the treatment, a decrease in appetite was again observed. A vicious circle ensued, whereby the automatic feeders spread food, the eels did not eat, and food remnants degraded the water quality, leading to further loss of appetite. Finally, eating began and interrupted the pattern.

When the eels in a container reached an average weight of 10 g, the food was changed and the size of feed was increased. Up to the transfer of the eels to outdoor ponds, the food conversion rate was estimated at 1:1.3.

The automatic feeding regime was 1.5 h operation, 1.5 h pause. During operation, the feeder moved clockwise, feeding for 2 min and stopping for 1 min. To obtain the maximum food conversion and prevent accumulation of uneaten food in the container, feeding should be constantly monitored and adjusted to the appetite of the eels by the timer that controls the rate of food supply.

c. Treatments – Due to higher than expected mortality (Fig. 1.16) in the first week, the elvers were treated with oxytetracycline at a dose of 80 g per container (containers were half filled with water) against mixed bacteria and *Aeromonas* sp. The presence of parasites, namely large *Trichodina*, *Girodactylus* and *Ichtiopterius*, was identified in the early stages. During the six-hour treatment, no fresh water was allowed into the containers and oxygen was pumped in from gas cylinders through dispersal air stones.

Treatment with formalin was applied from day 18 at a dose of 50 ml per container. As with the antibiotic treatment, the containers were half-full and during the six-hour treatment, the flow of fresh water into the containers was stopped and oxygen was supplied from cylinders through dispersal air stones. Feeding was stopped the evening before treatment, a procedure that was used during all medical treatments. A second formalin treatment was applied on day 30 when the containers were full of water, this time at a dosage of 100 ml per container. A third formalin treatment was applied ten days later (day 40) at 150 ml per container.

On July 3, 1997, three months after their arrival, the first "graduates" from Sde Boker College were sent to Dan. The parasite *Pseudodactylogitus* arrived with them and became the only parasite to infect the entire system. All subsequent treatments were intended to keep this parasite under control. They were treated with Mebendazol at 2 g/m<sup>3</sup> water. When this proved ineffective, an experimental treatment with Bromax (0.03 ppm) was applied. In week 15, a further course of treatment was applied, at 150 cm<sup>3</sup> per container. Between September 15 and 25 (weeks 24-25), a formalin treatment was applied at 150 ppm to each container. A second formalin treatment was given immediately after this to effect a drastic reduction in the parasite infection. This time the treatment was given via the system as a whole, as follows:

1. dispersal air stones were placed in the containers, with a manual emergency oxygen supply.

2. pumping was stopped.

3. treatment was applied for two hours.

4. at the end of one hour, only the column pumps operated to supply oxygen-enriched water to the tanks without pumping the water through the biofilter, to prevent the concentrated formalin from destroying the bacteria in the biofilter.

5. at the same time, fresh water was pumped through the heating system, at a rate of roughly 20 m<sup>3</sup>/h for 6 hours, to dilute the formalin.

6. when approximately two thirds of the water had been changed, the biofilter pump was operated again, together with the automatic feeders. In spite of the absence of food since the previous evening, several hours passed before the eels returned to full feeding.

Three days after the completion of this treatment, a third formalin treatment was applied and, until the end of the growth period, treatment with 200 ppm formalin was given every ten days to two weeks, when a drop in appetite was noted.

At the end of November and beginning of December -8 months after arrival - signs of distress were observed in the ponds. The eels were thrashing wildly and trying to escape from the ponds. Dozens succeeded and it was necessary to recruit workers from another farm to help all night to catch and return them. Neither the parasites nor measured water quality parameters provided an explanation.

At the beginning of February, 10 months after arrival, a formalin treatment was given to the eels as a booster, before they were transferred to the nursery ponds.

d. Body weight – When the glass eels arrived at Dan, the average weight of the live fish was 0.275 g and of the dead fish, 0.3 g. The first weighing took place in the fifth week. From each tank, 20 fish were weighed separately to determine the average weight and the total biomass was weighed to determine the number of fish in the tank. In the fifth week, the average weight was 0.47 g. During week 7, the average was 0.53 g; during week 9, it was 0.86 g and during week 11, 0.91 g. At the beginning of the fourth month, the average weight in 10 tanks was 1.06 g (Table 1.3).

To measure total weight, the eels went through the grader, were sorted into two sizes and weighed in net boxes. Thus, the total weight is an accurate figure. A sample of 20 eels from each tank were individually weighed to calculate the average weight in the tank and the number of eels was calculated by dividing the total weight by the average weight. During the entire period of cultivation, we had the feeling that the sample of individually weighed eels was not representative because larger eels avoided the hand net and the smallest were not captured. However, these were the most accurate figures we could obtain for average weight. Further, we are certain that close to 300,000 fish managed to escape, apparently during the first months.

Three weeks later (week 16), the total weight rose to 811 kg with an average of 1.67 g. At the beginning of week 20, the total weight was 1,524 kg with an average of 3.16 g. There were 481,572 eels, with the smallest weighing 0.58 g and the largest 9.45 g. In week 28 (October 13), the total biomass in the farm was 4,190 kg, representing a gain of 3,952 kg, achieved with 3,931 kg food, giving a conversion ratio of 1:3. One week later (October 20, week 29), the eels were transferred to the nursery ponds, as summarized in Table 1.4.

An intermediate summary was conducted on November 17, 1997, and provided the following results:

Total in farm: 5,406 kg, 377,483 fish at an average weight of 14.32 g.

Transferred to ponds: 2,217 kg, 62,098 fish at an average weight of 35.70 g

Remained in tanks: 3,189 kg, 315,385 fish at an average weight of 10.10 gThe highest density was in tank 15, with 575 kg in 5.6 m<sup>3</sup> water, i.e., over 100 kg/m<sup>3</sup>.

Tank 17 contained 17,000 fish at an average weight of 32.5 g.

Table 1.3.						
Average weights in week 13.						
Tank no. Total wt Avg wt No. of						
	(kg)	(g)				
3	61.230	1.20	51,116			
4	67.600	2.98	22,685			
5	61.870	1.03	60,067			
6	63.520	2.69	23,615			
7	60.868	0.62	98,174			
8	62.220	1.27	49,007			
9	62.880	0.64	98,250			
10	65.720	1.72	38,209			
11	53,418	0.47	113,655			
12	55.272	2.03	27,227			
Total	615.708	1.06	582,005			

Table 1.4.							
Weight of eels in week 29.							
Pond no.*	Total wt	No. of eels	Avg wt				
401	544 kg	20,571	26.22 g				
402	571 kg	10,748	53.11 g				
404 470 kg 17,882 26.20 g							
405**	334 kg	6,512	51.30 g				
Total							

\* Ponds 403 & 406 were stocked with fish from the experimental batch. \*\* During week 32 (November 13, 1997) an additional tank of 299 kg was transferred to pond 405, representing 6,203 fish averaging 48.2 g.

On January 14, 1998, another 34,510 fish (1,197 kg at 34.70 g average) were transferred to nursery ponds. The remaining 4,160 kg were transferred on February 19, 1998. The total transferred to nursery ponds was 7,575 kg, a weight increment of 7,337 kg.

At the end of the first year of nursing, the weight distribution was as shown in Fig. 1.17.

e. Technical operation – The first cultivation batch was characterized by continual technical changes, some of which were very costly in every sense of the word.

The farm was operated with up to  $24 \text{ m}^3$  fresh water, from  $16^{\circ}$ C (the ambient temperature of the Dan River) to  $23^{\circ}$ C (the minimum required temperature; the optimal temperature for European eels is  $23-28^{\circ}$ C). As the biomass increased, it became clear that the oxygen supplied by the water and compressed air supplied by two blowers through eight dispersal air stones per tank was insufficient. Between feedings, the oxygen content rarely exceeded 5 ppm and sometimes dropped to 3 ppm. Despite the constant oxygen stress, the mortality rate was not exceptional. The fact that the water was continually changed, instead of circulated in a closed system, prevented problems that appeared when the closed system was operated. Following is a list of the changes that took place during 1997:

June 23 - first sorting by static grader

July 27 - beginning of work on casting floor and building central drain

July 30 - trial operation of biofilter pumps

Aug 8 - addition of oxygen from small cylinders into the water column

Aug 10 - operation of blowers stopped. Oxygen content of water 7-8 ppm

Aug 12 - introduction of liquid oxygen tank

Aug 17 - one of the heaters partially operated to compensate for a 2-3°C drop in water temperature during the night, although it was the peak of the summer

Aug 24 - failure of the pumps by "Ha'ogen Plast" caused the death of some 2000 eels, half weighing 4 g or more

Oct 1 - perforated pipes replaced stones to disperse oxygen

Nov16 - closed circuit water heating system put into operation. From this point on, water was pumped from the tower and heated to  $3^{\circ}$ C instead of  $6^{\circ}$ C.

In general, the biofilter proved itself with respect to reducing the ammonia and nitrate levels in the water and enriching it with oxygen. There was a significant saving in water heating costs. The reason for the loss of appetite every 10-14 days, despite the significant improvement after formalin treatments and changing water, remains unknown. The result was that the eels did not fulfill their growth potential. Nonetheless, compared to an eel farm abroad, both the weight gain and the food conversion ratio were quite satisfactory.

On August 13, 1997, the biomass in the facility, according to a sample weighing, was 1500 kg; by October 21, it had grown to 4200 kg. On these dates, the oxygen tank was refilled, allowing us to estimate the cost of oxygen per weight gain. From August 13 to October 21, the cost of oxygen was NIS 2,155 (not including tax), resulting in NIS 0.80 per kg. The volume of oxygen used was converted to kg by multiplying it by 1.337, resulting in a utilization of 2,800 kg for the 69-day period, i.e., 40 kg/day or 1.66 kg/h. The gain in biomass was 2,700 kg, resulting in about 600 g oxygen/h per ton of biomass gained.

We transferred close to 400,000 eels from the first batch to secondary nursery ponds. If all these were males, production of 50-60 tons of marketable eels would be expected. If the eels included 20% females of 300 g each, production would rise to 70 tons. With 30% females of 350 g, production would be 84 tons. By February 1998, some 160 kg eels at NIS 50/kg had been sold.

f. Preparation for next batch – In preparation for the second batch of elvers, the piping was changed. Instead of two 75 mm pipes, water was supplied through a single 200 mm pipe, with one 75 mm pipe remaining in place for auxiliary use. The system was cleaned thoroughly with pressure pumps used for the trout farm and chlorine for washing and disinfecting (75 liter in the pump pit). The automatic feeders were thoroughly cleaned and checked for repair, the trial netting for the filter was replaced by 0.8 mm netting, all the plastic nets on the posts were replaced. Nipples were soldered onto the drainage system of the large tanks, similar to those that had proved themselves on the small tanks. The resting nets were fitted with nets with a smaller mesh. The operation of five new tanks was postponed, due to lack of need. A week before the planned arrival of the new batch of glass eels the system was ready to receive them, allowing some flexibility in the discussions on their price.

It was hoped that mortality in the next batch of eels would be less than the approximate 50% in the first batch, bearing in mind that most of the loss was due to escaped eels. We hoped to identify the factors that led to the cyclic loss of appetite, and thereby enable the elvers to fulfill their growth potential in the optimal conditions provided. What remained to be tested were the secondary nursery ponds, the fattening ponds, our assumptions regarding the hot water supply and the availability of oxygen. Other questions were whether the high density of the eels during this period of sex determination raised the percentage of males and whether the eels grew faster in large tanks rather than in smaller ones. We planned to stock the next batch of glass eels in small tanks to test this factor.

1.17.6 Batch of 1998

a. Reception of glass eels – There were two shipments of glass eels this year. The first arrived on March 20, 1998. It contained 300 kg eels averaging 0.27 g (i.e., 1,111,000 fish) at a cost of  $\pounds 265/kg$ . The fish arrived at Ben Gurion Airport (Tel Aviv) at a temperature of 3-5°C. They were transferred to the farm and stocked in containers for acclimation. The water in the containers was cooled from the ambient 16°C to 10°C by adding ice; by evening, it rose to the ambient temperature. The following day, the water was heated until, by evening, it reached 23°C. At the same time, an effort was made to interest the fish in a meal of cod roe.

The second shipment arrived on April 5, 1998, and consisted of an additional 200 kg (825,000 elvers averaging 0.24 g) at the astronomical price of £380/kg. Together with the first shipment, the total number of glass eels for 1998 was 1.9 million. The temperature of the shipment at Ben Gurion Airport was 5-6°C. Again the eels were transported to the farm and stocked into containers at 10°C. Water was pumped through the 75 mm piping until evening. When the water in the containers reached the temperature of the Dan River water, it was mixed with the system water. By morning, the water in the entire system reached a uniform temperature and cubes of cod roe, put into the containers during the evening, were totally consumed. The day after the second shipment arrived, the glass eel market in Europe collapsed. The Chinese agents stopped buying, and the price dropped to half. We had thought about buying an additional 100 kg to lower our average cost, but a lack of available funds made us abandon the idea.

b. Nutrition – Elvers received cod roe on the day of arrival. Five to six days afterwards, dough pellets were added to the roe. The dough was given several times a day, with dry matter at 4-5% of the biomass in the tank. The dough pellets were placed on two resting nets per tank, four lumps on each net, to provide access to the food to as many fish as possible. Feeding dough in the recommended quantities resulted in accumulation of huge amounts of sticky food remnants which the brushes on the filter were unable to remove. The water level rose and the water became murky. It is likely that the *Aeromonas* disease that broke out later was linked to the bad hygienic conditions, especially in the large tanks. Dry food was scattered on day 27 with excellent results. The transition from dough to dry food was completed 4 weeks after the second batch of eels arrived, 6 weeks after the first batch arrived, due to a recommendation to shorten this stage as much as possible. In week 14, when the average weight of the eels in the tank passed 3 g, the fine size 2 food was replaced by size 0. According to the producer's recommendation, size 0 is suitable for eels weighing 3-10 g. Since this is the size at which the eels were transferred outdoors, we did not use coarser food. Eels in tanks averaging below 3 g continued to receive size 2.0 food.

The total amount fed to the end of December was 9,214 kg. The weight gain by this date was 7,072 kg, giving an overall food conversion ratio of around 1.22 (Fig. 1.18) compared with 1.3 the previous year. During the entire year, we never fed more than 50 kg/day and the load on the biofilter remained acceptable.

c. Treatments – Bacteriological examination of the glass eels upon arrival revealed the presence of *Aeromonas* bacteria. The eels were treated by immersion in oxytetracycline (2 kg/100 m<sup>3</sup> water) for 24 h.

Signs of *Aeromonas* were found again on April 16 and a second treatment at the same concentration was applied. Mortality rose, first in a single tank and then as a general outbreak. The immersion treatment did not work, so antibiotics were mixed into the dough at a concentration of 80 mg active ingredient per kg biomass. Since the active ingredient was mixed into a powder at 50% concentration, 160 g powder/kg biomass was administered. Since treatment with oxytetracycline did not work, an anti-biogram test was made and we replaced the medication with Nurafloxatine at 30 mg powder per kg biomass. The disease was overcome only after 10 days of feeding antibiotics, plus a treatment with formalin. The disease cost us 400,000 fish and resulted in a loss of about one month of growth, which expressed itself in a lower averáge weight than the previous year.

In July, another *Aeromonas* attack broke out despite the fact that this is a very rare event at this stage. In our opinion, this was the result of accepting the advice of an expert to

return fish weighing under 5 g to the indoor tanks from the outdoor ponds. A formalin treatment was applied and the antibiotic trimerzine was fed at 60 mg/kg for 6 days. This treatment did not work; the mortality rate did not drop. After immersion in oxytetracycline, we fed a course of antibiotics, with nurafloxitine instead of trimerzine, at 30 mg/kg for 10 days. The July outbreak cost us 26,000 large fish averaging over 1 g.

Formalin treatments were applied every two weeks throughout the year: immersion for 30 min at 120-130 ppm, followed by 30 min at 200 ppm. After the treatment, while the biofilter pumps were still switched off, new water was pumped in for 6 h at roughly 25  $m^3/h$ , meaning that most of the water was replaced. Then the biofilter was turned on, together with the heating system. Feeding was stopped the evening before treatment and restarted the evening after, when the water temperature had reached 23°C.

d. Body weight – In general, the 1998 batch did less well than the 1997 batch. The first "graduates" from 1997 were moved outdoors at a maximum weight above 50 g, compared with 34 g in 1998 when most were less than 20 g. Whereas in the first batch we assigned the low survival to the escape of small fish through the nets during the first weeks, in 1998 the nets on all the posts were made of stainless steel and it was possible to identify any escapes from the closed system. The 1998 batch was attacked by *Aeromonas*. The survival rate at the end of December 1998 was 998,700 of the original 1,900,000 (52.6%). In comparison, 440,000 survived of 865,000 in 1997 (51%). The 1997 batch produced almost the same final weight as the 1998 batch, despite the fact that there were almost double the number of glass eels in the 1998 batch. Table 1.5 summarizes the mortality of eels in the two batches.

Month	1997 batch*	%	1998 batch	%
3	Not yet arrived		15,480	0.81
4	17,880	2.06	385,540	20.29
5	10,130	1.17	83,230	4.38
6	6,240	0.72	26,080	1.37
7	4,880	0.56	26,150	1.38
8	3,650	0.42	16,540	0.87
9	1,720	0.19	3,590	0.18

Table 1.5

\* Not including those that escaped

#### 1.17.7 Batch of 1999

a. Reception of glass eels – Following a trip to glass eel suppliers in Europe, a trial was made to vary the source and time of delivery. The first shipment of 200 kg arrived on January 12,1999, from the French branch of U.K. Glass Eels. The average weight was 0.35 g, giving 2850 units per kg, altogether 570,000 fish. Mortality on arrival was less than 1%. The glass eels arrived at temperatures of 2.5-5°C. They received the same acclimation method as in previous years: the water in the containers was cooled by ice to 10°C. They were then stocked in tanks at a density of 7.5-8 kg/m<sup>3</sup> water and fresh water was pumped into the tanks at 16°C. The water did not flow through the closed system to prevent lowering the water temperature.

Unlike in 1998, the nursery tanks were not emptied of the previous year's fish, nor were they thoroughly cleaned and disinfected, because the 1998 fish had not yet been transferred outdoors.

On February 9, 1999, a shipment of 100 kg glass eels arrived from V.T.C. Co. in France. The shipment consisted of 300,000 elvers at 0.33 g average weight. They underwent acclimation as described above. On February 11, a shipment of 150 kg glass eels came from the French branch of U.K. Glass Eels; they averaged 0.36 g, though the supplier said their weight was slightly lower. According to our calculations, the shipment contained roughly 430,000 fish. Another 150 kg (450,000 fish) were received on March 5 from V.T.C., France.

A final shipment of 150 kg (450,000) was received on April 6 from the English branch of U.K. Glass Eels.

Each batch was treated with 3 days' immersion, 6 h per day, in oxytetracycline at 30 g active ingredient/m<sup>3</sup> water starting the day after its arrival. The water flow was interrupted for this treatment and oxygen was pumped into the water. No significant differences were found between the two French companies with regard to speed of adaptation or mortality - 27% - but mortality of the fish from England was 7%.

There were no advantages to spreading out the shipment dates, except that it allowed us to exploit the nursery tanks to the maximum for the bigger outgoing fish. The possibility of a bi-annual growth cycle was suggested, i.e., purchasing two batches close to each other around March-April from the English supplier once every two years, so that at the end of two cycle, no small fish would remain from the previous year and the building could be emptied and thoroughly cleaned and disinfected.

b. Nutrition – No food was given to the elvers on the first day. After the immersion treatment on the second day, a number of cubes of cod roe were fed to each tank. The response of the eels was initially hesitant, but by evening, their interest grew. On the ninth day, dough pellets mixed into cod roe were fed, until dough entirely replaced the roe. Two weeks following arrival, the dough was mixed with crumbs of dry feed. The automatic feeders began operation in the end of the third week with a gradually decreasing proportion of dough in the feed. The transition to dry food was not complete until the end of the first month. The response of the elvers to dough was excellent, but the resulting dirt in the tanks was unacceptable. Greasy deposits formed all over the tank floor and walls. In the future, we will try to reduce the transition to dry food to as soon as possible.

Because of the high price of the elvers, the number that did not learn to eat was disturbing even though it was no higher than considered normal abroad. For the future, we recommend scattering *Artemia* in the water during the first week, together with the cod roe. When the fish reach 4 g, they transfer from size 2.0 to size 0 food, which they will receive until transfer to the next stage. Some of the eels in this batch reached 70 g by that time.

c. Treatments – The eels received a preventative treatment of immersion in oxytetracycline (30 g active ingredient per  $m^3$  water for 6 h) upon arrival. When the parasite concentration rose, a treatment of 200 ppm formalin was given for 2 h, to the biofilters as well. When there were also glass eels in the facility, a loss of appetite was generally observed and the eels were given a separate treatment at a lower dose that was gradually increased until it reached the standard level.

In February, a distressing rise in mortality began because of *Aeromonas* infection. Treatment by immersion in oxytetracycline did not help, so we added flumaquine (10% flumazol) to the food at a dose of 15 mg active ingredient per kg biomass per day for 6 days. The medicated food was distributed manually so that it would reach even the weaker eels that could not reach the feeding troughs. At the beginning of March, a treatment with flubanol (1.25 g active matter per m<sup>3</sup> water) was given against *Pseudodactylogirus*. We repeated the treatment after a week and this solved the problem.

*Ichtyophthirus* parasites were resistant to the Fluvenol, so in April we applied a formalin immersion treatment at the low concentration of 50 ppm. In July, we diagnosed a combination of *Dactylogirus* parasites and *Aeromonas* bacteria. This time immersion in Fluvenol brought disappointing results. We began to supply medication through the feed - Nurafloxatine at 20 g per ton biomass daily. The feed was manually distributed. After 10 days of treatment, mortality sharply dropped. During the event, we lost some 7,000 fish averaging 5 g. In October, another attack by *Pseudodactylogirus* and *Trichodina* took place. Treatment by immersion in formalin, followed a week later with Fluvenol, solved the problem.

d. Summary – The year 1999 differed from the two preceeding years in two ways. (a) The glass eels were not received into a clean and disinfected facility, rather, they were absorbed into a working system that contained fish from 1998. While it cannot be proven that any

problems arose from this fact, instinct suggests that the facilities should be emptied, sludge removed, cleaned and disinfected, if not every year, at least once in two years. (b) The elvers arrived in five shipments. This provided no advantage; in fact, the care of the glass eels was spread over four months when it could have been concentrated into two.

The price of the glass eels was much lower in 1999 than in previous years. In French francs, it was 1360, 1500, 1175, respectively, for the three shipments from France and \$150 for the last shipment from U.K., compared with £380 for the second shipment in 1998. We had no control over the prices. Mortality was much lower amongst the eels from Britain. Therefore, if the price is reasonable, it is preferable to buy from England.

# 1.17.8 Summary of growth in the primary nursery stage

The outstanding phenomenon was the high mortality in the early stages with a significant decline later. Since new stainless steel nets in the Dan Fisheries prevent the escape of eels, it is reasonable to assume that the mortality at the beginning occurred among elvers that did not learn to eat artificial food.

During the nursery year, the eels grew from 0.26 g (avg wt) to 19 g. This was considered an achievement and compared well with the best nurseries in Europe. The eels learned to eat crumbs within their first weeks using cod roe and dough as intermediary feeds. We tried reduce the dough to a minimum because it significantly polluted the tank water. In the future, we intend to expose the elvers to crumbs as early as possible, even during their first days.

The eels ate well during the entire period. The daily amount of food was important to the operation of the biological filter. The greatest amount of food given in a single day (70 kg) was during October, and the biofilter was able to cope with it. Graph 7 shows that the food conversion rate, in general, was good, ranging between 1.1-1.7. There were two cases outside this range, both occurred when the eels were under stress. In June 1999, they suffered from a shortage of oxygen as a result of opening the big ponds, while during the period between the end of November and the middle of December 1999, they suffered a period of stress for reasons which we have not been able to identify.

Most of the eels (87%) did not exceed 30 g while 33% reached only 5-10 g, 29% reached 10-20 g and 25% reached 20-30 g. The rest weighed 30-60 g. The growth extremes are very important since only the last group (13%) would reach market size of 150 g by the end of the following year. The rest would require three years or more.

Table 1.6 compares the results the three primary nursery batches. The food conversion rate was similar for all three (1.2-1.3) while the survival rate was reasonable in 1999, but relatively low in 1997due to escape and 1998 due to bacterial infection.

Comparison of results of three primary nursing cycles.				
	1997	1998	1999	
No. of glass eels received	865,000	1,900,000	2,200,000	
No. of eels at end of calendar year	377,483	980,700	1,575,000	
Total weight at end of year (kg)	5,406	7,572	16,177	
Weight taken out of indoor nursery (kg)	2,217	5,072	8,854	
Survival in indoor nursery	51%	52.6%	70%	
Net growth in indoor nursery	5,156	7,072	15,427	
Food conversion rate in indoor nursery	1.3	1.22	1.36	

 Table 1.6.

 Comparison of results of three primary nursing cycles.

#### **1.18 Outdoor installations**

The location of secondary nursing and fattening ponds for eels must meet several conditions. A regular water supply in sufficient quantity is essential. The water must be free of chemicals and have a neutral pH (acidic water is not suitable for eels). The location must receive plenty of sunlight to encourage oxygen-producing phytoplankton and be exposed to wind that

oxygenates the upper level of the water. The outdoor installations in the eel farm contain two kinds of ponds: small ponds for secondary nursing and larger ones for fattening. Secondary nursing begins when the eels reach 5 g and continues until they reach 20 g. At the Dan Fisheries, this stage is conducted in concrete ponds, into which eels are transferred from the indoor tanks. The final stage of growth is in the fattening ponds, where they remain until marketing. The walls of the fattening ponds are lined with concrete and a plastic covering that also forms the floor: this is to prevent the eels from escaping or burying themselves in mud at harvest. All the outdoor ponds were covered with netting to prevent bird attacks.

# 1.18.1 Secondary nursing ponds

Twelve nursery ponds of 40 m<sup>3</sup> (Fig. 1.19) are used for the elvers that are transferred from the indoor tanks at a size of 7-40 g. Two similar ponds are used most of the time to store large fish that were ready for marketing. The water supply consists of (a) fresh cold water from the Dan River pumped at 5-10 m<sup>3</sup>/h and (b) in summer, recycled water pumped from a 2 ha earthen pond at 5-10 m<sup>3</sup>/h. The stocking density is 5-35 kg/m<sup>3</sup>. The eels are graded every 6-8 weeks. Dough is fed on floating nets (Fig. 1.20), once a day in winter, twice a day in summer.

#### 1.18.2 Fattening ponds

Fifteen 250 m<sup>3</sup> ponds (Fig. 1.21) are used for fattening eels weighing 10-40 g until they reached market size. The water supply consists of (a) cold water from the Dan River following use in trout ponds at a pumping rate of 20-30 m<sup>3</sup>/h per pond through two pipes at opposite sides of the pond and (b) in summer, recycled water from 2 ha earthen ponds pumped at 10-20 m<sup>3</sup>/h. The eels are stocked at 4-16 kg/m<sup>3</sup>, assuming the density rises to 30-40 kg/m<sup>3</sup>. The eels are graded approximately every three weeks, depending on their size. Males are separated from females when the fish reach 100-160 g on a special sorting table. The eels are fed dough on floating nets, once a day in winter, twice a day in summer. The dough is prepared in a mixer stored in a nearby 5 x 15 m shed that is also used to store feed and oil and to sort and weigh the fish.

#### 1.18.3 Oxygen and temperature control

The ponds are supplied with auxiliary oxygen by mushroom oxygenators connected to a liquid oxygen tank. Every pond has "Oxigard" oxygen electrodes connected to a control box to monitor oxygen and temperature. The oxygen level is maintained at 6-10 mg/l and the water temperature at 22-25°C for as much of the year as possible, by means of manual control.

### 1.18.4 Removing and sorting

For the removal of eels, piping with a diameter of 250 mm connects all the ponds to a central removal pit in which there is a perforated basket with doors. By removing an upright pipe, the eels can be removed from the pond into the basket in batches of 400 kg. A forklift transfers the fish from the basket to an intermediate tank placed above the sorter. The doors of the tank are opened and the fish spill onto the sorter at the required rate. Fish exit the sorter into a perforated tank, are weighed and counted for calculation of the average weight. Then they are transferred to ponds for additional growth.

#### 1.18.5 *Water quality*

Quality assurance of the water in the ponds and in pool 609 (the water source) is carried out at the Dan Fisheries laboratory every 2-4 weeks.  $N-NH_4^+$ , pH, nitrates,  $N-NO_2$  and temperature are measured.  $N-NH_4$  ranged 0.1-1.0 ppm with one exceptional case of 2.0 ppm.  $N-NO_2$  ranged 0.01-0.6 ppm, pH was 7.5±0.4 and temperature ranged 12-25°C.

### 1.18.6 Diseases and treatments

The eels are frequently examined for parasites, with preference given to ponds in which the appetite of the eels was relatively poor. In cases of mortality or suspicious symptoms, tests were conducted for the presence of bacterial pathogens.

The following external parasites have been encountered: large and small *Trichodina*, *Ichtyophthirus*, *Chilodonella* and *Pseudodactylogirus*. *Angvilicola carsus microcystis* has also been found. Most of the external parasites are successfully treated by formalin at a concentration of 200 ppm for 3 hours. Only in the case of *Dactylogirus* was this treatment ineffective, in which case Mebendazol or Fluvenol was used.

The only bacterial disease that was diagnosed was *Aeromonas hydrophilia*, which was treated by adding Quinabic to the food at 30 mg/kg fish for 10 days. The symptoms were redness around the head, belly or pectoral fins, and damage to the gill tissue.

## 1.19 Secondary nursing and fattening

While results from the primary nursing were successful when compared to data from eel farms in Europe, secondary nursing and fattening had comparatively slow growth rates, high food conversion ratios and unsolved health problems. As a consequence, a trip to Italy was organized to find ways to in the fattening stage, with emphasis on changing the grading regime. Frequently removing the eels from the pond to grade them was seen as the source of the difficulties in the fattening stage, so the sorting regime was changed. It was important to provide hiding places for the eels in the ponds. Some growers put corrugated asbestos or plastic sheets into the pond for this purpose. Experience showed that it was possible to reduce size variation by reducing aggressive confrontations between eels. Food consumption rose, resulting in higher rates of growth, so it was advised to increase the density in the ponds. Since the food was fed on the surface of the pond, it was important to protect it from direct sunlight: a 10 m<sup>2</sup> roof above the feeding point proved to be a good solution.

# 1.19.1 Operation of the systems

Towards the end of summer 1998, apart from some occasional problems, all systems worked well including the control system; the water system; the gradual transfer to fattening ponds; the system for draining and removing eels, including cleaning the piping after the eels pass through it; the supply center, including daily mixing of dough; and the sorting process, including transfer of the eels to the holding pond, through the blade sorter, weighing them and returning them to the appropriate ponds according to size.

#### 1.19.2 Sorting and grading

Eels were graded during secondary nursing every 6-8 weeks and during the fattening period every 3-4 weeks, as needed (Figs. 1.22 and 1.23). Males were sorted from females on a sorting table when the eels reached 100-160 g. Using the mechanical drum sorter from the primary nursery proved unsuccessful, causing disease and mortality in the secondary stage. This problem was solved when the new blade sorter arrived. The eels in the secondary and fattening ponds were graded a number of times with good results in both size and subsequent health. After sorting males from females a number of times, Dan Fisheries concluded that 90% of the fish were males, so this was not worth the work involved in sorting.

#### 1.19.3 Nutrition

Food in the secondary and fattening stages was fed in the form of dough, mechanically mixed from a powder mixure, water, and fish oil at a ratio of 1:1:0.05. The powder mix was imported from Italy and the fish oil was bought from a feed plant at Tsemach, Israel. Later, the oil was oil imported from Italy to reduce growth extremes. When there was no powder from Italy, the dough was prepared from a powdered mixture made by Tsemach which was tested by MIGAL and gave good results.

The feed was prepared daily prior to distribution in a mixer located at the supply center (Fig. 1.24), and distributed twice a day on floating nets. During the cold months, the eels were fed only once a day. Food remains were collected and removed. The food conversion ratio during fattening was high in 1998 (3.75) and growth rates were relatively slow. In 1999, the eels in fattening consumed 78,825 kg (dry weight) of dough, producing 18,609 kg live weight, at a conversion ratio of 4.23.

# 1.19.4 Health

The only serious disease in the outdoor ponds took place after grading in the drum sorter. Once the drum grader was replaced by the blade sorter and the eels were treated with formalin and oxytetracycline, they suffered no further health problems. Once an *Ichtyophthirus* attack was suspected, but after treatment with formalin treatment the phenomenon disappeared. Whenever 2-3 dead eels were found afloat on the water, samples of fish were sent to the laboratory for examination, but they were usually free of disease.

During the winter, when temperatures dropped to 13-15°C, the nursery ponds were treated with formalin at 200 ppm for two hours, every two weeks, as a preventive measure. The fattening ponds were not treated.

#### 1.19.5 Water temperature

Water temperatures remained above 20°C until the end of October and usually ranged 22-24°C, which was suitable for secondary nursing. These results pointed to the suitability of the temperature in the open system growing ponds. This important parameter showed that the basic hypothesis – that conditions at Dan Fisheries were suitable for growing eels – was correct.

#### 1.19.6 The control system

The control system of the growing ponds worked well. The oxygenation system was not operated at least half the time, resulting in considerable electricity savings. During the summer, two oxygenators were put into the fattening pools, a period during which the control system was particularly important.

# 1.19.7 Results from eels in the growing ponds

There was a wide range of initial weights (7-137 g). The growth rates during 3-4 months were generally low (0.01-0.25%) and, while in some ponds the food conversion ratios were acceptable (1-2), in others the ratio was very high (up to 376), testifying to a problem in cultivation. No two ponds produced the same results, even when the initial weights were similar. Mortality also ranged widely from pond to pond (1-53%) although mortality figures included escapees.

# 1.20 Nutrition of eels at Kibbutz Dan

The food fed in Dan Fisheries was the same as that used for growing eels in Europe. In the primary nursing stage, the eels were fed cod roe and dry food crumbs from two companies: Coppens and Hendrix. The crumbs were fed in three sizes, according to the size of the fish. All the food was imported. The cod roe were sent with the glass eels and the crumbs were imported mainly from Italy and Spain. During the secondary nursing and fattening stages, feed was a powdered mix imported from Italy and a similar feed produced by Tsemach Feeds in Israel, from which dough was prepared daily.

During the primary nursing stage, elvers are still very small. They have an undeveloped digestive system and require expensive food. However, the required quantity is relatively low and the cost does not represent a significant proportion of the total costs. In later stages, the digestive system is fully developed and eels can accustom themselves to normal foods. Large quantities of food are required, however, and food conversion ratios ranged 3.0-5.0. The price of imported food (including the cost of mixing the powder) is so high - \$1200-1300/ton - that there seems to be no chance of achieving economical eel cultivation if this food is used for fattening. In the mid-1980s, a number of powder mixes for preparing dough were developed in Israel for fattening fish. These mixes were based on raw materials available in Israel. All contain similar levels of protein and lipids.

Table 1.7 shows the formulation of the feed mixed at Tsemach. The initial dough mixtures were coarse in texture and crumbled easily in water. To prevent this, the wheat was replaced by wheat flour and the gluten was ground into grains smaller than 1 mm. The new

mixture was stable in water and readily eaten by the eels. Guar was added to bind the mixture and glycine was added as an attractant.

Table 1.8 summarizes the nutritive value of the feed as analyzed in the MIGAL laboratory. The Tsemach Feeds mixture contains more protein, less lipids, and the same energy value as the feed imported from Italy. Experiments conducted at MIGAL by Northern R&D showed that consumption and growth results were the same for both feeds. Table 1.9 shows nutritive factors of the crumbs fed during primary nursing. Initially, size 2.0 crumbs were fed through the automatic feeder. When the eels reached 3 g they were given size 0 crumbs from Trouvit. In tanks containing eels averaging above 10 g, AA10 feed was used. When they exceeded 20 g, they were fed Coppens feed.

Table 1.7.							
Formula of powder mix fed to eels (	Tsemach 1999).						

Ingredient	%
Wheat flour	27.9
Fish oil	2.16
Fishmeal	62.78
Gluten	5.0
Guar	1.0
Stay-C	0.06
Vitamins & minerals	0.5
Glycine	0.05

Table 1.8.				
Chemical analysis of fattening mix.				
Ingredient	Tsemach	Italy		
	(%)	(%)		
Protein	53.4	50.7		
Lipids	7.8	8.2		
Ash	9.1	9.2		
Moisture	9.8	8.4		
Carbohydrates	18.9	22.3		
Cellulose	1.0	1.2		
Energy (kcal/kg)	4534	4530		

Three kinds of crumbs fed to eels during primary nursing.

Factor	А	В	С
Name of institute	RaananBarjak	Trouvit	Trouvit
Size	1.5 (extruded)	AA10	3.0, 2.0, 0
Company name	Coppens	Hendrix	Hendrix
Country of origin	Spain	Italy	Italy
Protein (%)	48	50	53
Water (%)	9	8.5	8.5
Crude cellulose	1.1	1.0	0.7
Lipids (%)	22	22	18
Ash (%)	7.7	8	10
Vitamin A (units/l)	30000	18000	19000
Vitamin D (units/l)	3000	2000	2000
Vitamin E (mg)	100	2000	2000
Vitamin C (mg)	200	100	4000

### 1.21 Control of water quality

Maintenance of water quality during all stages of cultivation, especially primary nursing, was a key factor. The most important water quality parameters were oxygen concentration, pH, nitrates, ammonia, temperature, and organic matter content. The water quality changed constantly depending on the amount of food fed, the amount of oxygen that the eels used, and the amount of water that was replaced on a daily basis. Efforts were made to keep the water clear and oxygen-rich (at least 4 ppm), the pH as close to 7.5 as possible, and the nitrate and ammonia levels low (1 ppm and 0.25 ppm, respectively). High water quality ensured that the eels remained healthy and maintained a fast rate of growth. During primary nursing, water quality was determined primarily by its rate of turnover. An efficient biofilter and steady flow of oxygen before the water was returned to the tanks ensured good quality water. The source of health problems and slow growth was stagnant water.

The most important factor was oxygen content. Eels cannot breathe in water containing less than 1 ppm oxygen and rise to the surface to breathe if the oxygen content falls below this figure. In the primary nursing tanks, the oxygen content was maintained by controls that injected dissolved oxygen into water returning from the biofilter. In addition, each tank was fitted with perforated pipes that injected oxygen into the water in times of emergency.

The secondary nursing ponds received oxygen through mushroom-shaped oxygenators connected to a tank of liquid oxygen. The fattening pools were fitted with normal wing oxygenators and, in 2000, were connected to liquid oxygen as well. Every pond had Oxiguard oxygen electrodes connected to the oxygen and temperature controls. In these ponds, the oxygen level was maintained at 6-10 ppm.

The optimal temperature for growing eels at the Dan Fisheries was 23°C. In indoor facilities, a heating system ensured that the water was kept at the optimal temperature. In outdoor ponds, the temperature was manually controlled by mixing cold and heated water to maintain the temperature with a range of 22-25°C for as long as possible throughout the year. Water quality was examined at the Dan Fisheries laboratory on a bi-weekly basis.

Table 1.10 shows some of the water quality records from the growing ponds. On the whole, ammonia and nitrate levels were low, with ammonia levels never above 2 ppm and nitrate always below 0.5 ppm. The pH was kept neutral, 6.1-7.6. Apart from a number of particularly cold days, the temperature was kept in the desired range of 22-25°C.

# 1.22 Parasites and diseases

Eels are subject to a variety of parasites and disease factors such as the fungi *Saprolegnia parasitica*, the bacteria *Aeromonas liquafacines* and *Chondrococcus columnaris*, and the parasites *Lernaea cyprinacea* and *Ichtyophthirus multifiis*. Additional parasites were found in the Dan Fisheries eels, namely *chaldon*, large and small *Trichodenia*, *Unvillicola carsus*, and *pseudodactylogirus*. A loss of appetite was the first symptom of health problems. Tests of water quality and the presence of parasites were conducted as soon as a loss of appetite was observed. If parasites were found, treatment was given to the affected tank or throughout the system, depending on the test results. Formalin treatment was given by putting 200 ppm formalin into the pumping pit while arresting the operation of the biofilter. Following approximately two hours of treatment, the biofilter pump was restarted. Monitoring the nitrate and ammonia concentrations showed that the biofilter was not affected by pumping water with formalin through it. This treatment was effective against external parasites, however, attacks by *Pseudodactylogirus* required the use of Fluvenol. *Aeromonas* was the only bacterial disease diagnosed at the Dan Fisheries; it was successfully treated with Kwinavik at 30 mg in the food per kg fish for 10 days.

# 1.23 Harvesting, packing and transporting

The greater part of the market in eels consists of live fish (80%). Frozen eels constitutes an additional 15% of the market, while smoked eels comprise only 5%. The eels are caught by

draining the growing ponds through a catching basket, after which they are sorted by size. The eels undergo 5 days of fasting before harvest to empty their digestive tracts. This results in a 3% loss in body weight. The eels are then transferred to cold, clean water to wash them. Eels can be sent to market in four ways:

a. live, in double plastic bags with ice and plenty of oxygen, for shipments up to 30 h; b. live, in boxes with a little ice, for shipments up to 5 h;

c. live, in tanks with an air supply and 1 ton water/1 ton fish, to keep their bodies moist;

d. frozen, cleaned or not, at a temperature of -20°C. They are quickly cleaned by washing in cold water and scraping off the mucous or immersing them in very salty water.

Water quality in eel fattening ponds in 2000.					
Date	Pond	Nitrates (ppm)	Ammonia (ppm)	pН	Temperature (°C)
Jan 9	В	0.38	0.36	6.53	23
Juli J	A	0.34	0.85	6.59	23
	426	0.14	0.33	7.57	13.1
	609	0.14	0.79	7.47	12.7
	341	0.12	0.42	7.41	13.5
	434	0.11	0.37	7.39	13.5
	407	0.04	0.20	7.33	14.3
Feb 7	407	0.12	0.42	6.74	14
100 /	426	0.12	0.35	6.85	14
	431	0.13	0.37	7	14
	434	0.09	0.32	7.1	14
	609	0.16	0.20	7.1	14
Feb 29	409	0.03	0.22	6.92	15
	431	0.06	0.25	7	15
	435	0.04	0.25	7.04	15
	В	0.56	2.64	6.46	24
	Ā	0.53	3.20	6.2	24
	609	0.10	0.40	6.67	13
Mar 5	В	0.47	1.97	6.3	23
	Ā	0.47	1.97	6.33	23
Apr 9	В	0.33	1.96	6.2	23
	А	0.29	1.96	6.1	23
	402	0.15	0.64	6.54	16
	422	0.20	0.65	6.8	16
	432	0.23	0.79	6.9	16
May 1	407	0.24	1.03	6.79	22
•	409	0.14	1.30	6.75	22
	421	0.12	1.39	6.91	22
	424	0.17	1.32	6.97	22
	425	0.13	1.40	7.08	22
	429	0.15	1.58	7.14	22
	431	0.16	1.45	7.17	22
	609	0.17	0.95	7.2	22
	В	0.23	1.90	6.54	23
	A	0.16	0.87	6.23	23

Table 1.10. Water quality in eel fattening ponds in 2000.

Note: A = Indoors, before filter; B = indoors, after filter.

1.23.1 Before harvest

Five days before the harvest, feeding is stopped. Three days prior, the cels are transferred to clean, cold water. Two days before, the growing ponds are washed. The day before harvest,

ice bags are prepared, each contains approximately 100 ml water, at a rate of one bag for every 10 kg fish. Eels at Dan Fisheries should be harvested 20 hours before flight-time, determined by the following timetable: Takeoff – H hour Arrival at airport – H minus 8 h Leave the farm – H minus 11 h Begin packing – H minus 13 h

Begin cooling fish – H minus 19 h. Cooling to 6°C should proceed as gradually as possible, so that from the initial temperature of 15°C, the eels will be cooled 1.5°C per hour, requiring six hours, by adding ice to the container. Harvesting the pond – H minus 20 h

# 1.23.2 Shipping preparations

Essential equipment includes cartons, staples, a carton machine, an oxygenator, hand nets, cut-up plastic produce boxes, strapping for pallets, stick-on labels, pierced buckets, small ice bags, disposable cups, a good work team of at least five people, a large tractor with a stainless steel tank, and a forklift. Make sure there are enough containers and that the packing house is available for use.

### 1.23.3 Harvest and transfer to packing station

Remove the fish from the holding tank and weigh them (take extra). The first fish to reach the basket (Fig. 25) should be stocked into a different pond, because they may have been in the pipe a long time. The eels should be weighed together with the basket, washed and then poured into the container. Pay attention to oxygen, cold water, etc.

Transfer the eels to the packing house in the stainless steel tank, with the blower and oxygenator working, by tractor. Make sure that someone takes care of the pond when you leave it – water, oxygenator, piping, catchment pit, etc. At the packing house, connect the electric blower, measure the oxygen and temperature, and begin cooling the eels, taking care not to cool them too rapidly.

### 1.23.4 At the packing station

Prepare the shipping cartons. The lids should be larger than the base. Keep the work area dry. Stick labels only onto the lids. Proceed as follows.

a. One pair of workers unloads the water containing the eels into containers, stocking an equal load in each, not more than 100 kg.

b. A second pair of workers weighs the eels to an accuracy of  $\pm 10$  kg, using the hand nets and pierced buckets.

c. The third pair, after pouring the fish into the plastic frame, lifts it carefully and closes the carton with the lid. One of this pair takes care of the water; half a cup of cold water and an ice bag in each frame. The second person stacks the cartons.

d. The cartons are fastened with black strapping.

e. Ensure that the driver has all necessary information and paper work

f. Leave the packing area clean and locked, with the lights switched off.

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# CHAPTER 2

# AYU (PLECOGLOSSUS ALTIVELIS) CULTIVATION IN JAPAN AND ISRAEL

# 2.1 Biology and life cycle of the ayu

The ayu (*Plecoglossus altivelis*, Fig. 2.1) is a member of the Plecoglossidae family of the Salmoniformes order (Nelson 1994). Relatively small compared to other salmonids, this fish is common to shore areas of streams and lakes in Japan (Fig. 2.2), Korea, Taiwan, and China. It has also been transferred to various areas of the USA.

The ayu has a traditional fin structure of 10-12 bristles along the back fin, 9-17 bristles along the rear end fins, and 5-6 bristles on the chest fins. The spinal column consists of 59-64 vertebrae up to 12 inches long. The fish is territorial.

The ayu spawns in flowing freshwater streams of the mountainous regions where the fish reach sexual maturity. In nature, the spawning process of the ayu concludes with egg laying, insemination, and the demise of the parents. The eggs are laid in high quality water during the autumn, by one-year-old fish. The 0.7-1.2 mm eggs (Fig. 2.3) are stuck onto bedrock and hatch after 10-17 days. The fish like clean, oxygen-rich (5 ppm) water and are sensitive to nitrates and ammonia. In captivity, a fertile female ayu can lay 30-40 thousand eggs.

The young fish grow to one inch and head for the sea where they spend the winter. By the end of the winter, they have reached three inches and return upstream where they remain until sexual maturity. The fish have a well-developed set of teeth and feed on moss growing on rocks along the stream banks during the spawning period.

# 2.2 Methods of cultivation and marketing in Japan

#### 2.2.1 Ayu breeding at Yabe River Cooperative

The author visited Japan during the first two weeks of October 1998 to learn about ayu breeding and larviculture, and to arrange shipment of inseminated ayu eggs to an experimental station at the Kibbutz Dan Fish Nursery. The author made several visits to the Yabe River Cooperative on Kyushu Island in southern Japan. This fishery is privately owned by a number of wholesale fish growers who cultivate fingerlings for stocking in streams and other farms, and fully-grown fish for market. The farm is managed by Mr. Hisami Koga and two full-time assistants. During breeding and egg-laying, they are reinforced by an additional 3-4 employees. The information in this chapter was gathered during these visits.

### 2.2.2 Breeding preparedness examination

Ayu reach sexual maturity at the age of one year, at which time the average weight is 150-230 g. The average Gonado-Somatic Index (GSI) is used to anticipate the breeding date for the majority of fish in a specific group. To determine the GSI, a random sampling of 30 fish is taken at the beginning of August, September, and October. The body weight (BW) and gonad weight (GW) of each fish is measured and the GSI is computed according to the equation GSI =  $GW/BW \times 100$ . When plotted on a graph (Fig. 2.4), the GSI values produce a straight line. The date that this line reaches 23 is considered the most suitable date for breeding.

On the day of our visit, there were approximately 3000 fish (avg wt 180 g) in one pool that were to be tested for breeding readiness. The pool was 100 m<sup>3</sup>, circular, and had drainage and harvest systems in the center. The fish were 'first generation', i.e., they had been captured in the stream in March 1998 at a weight of 2-5 g. Feeding was stopped two days prior to the examination. On the morning of the examination, the fish were lowered into nets in the harvest pits; the pits were aerated by a closed circulating system.

The fish were transferred from the harvest pit by hand net to tanks filled with water and an anesthetic. By manually pressing on the stomach area, the fish were sorted into separate tanks, filled with clean water and no anesthetic, according to the following groups: (a) mature females, (b) mature males, (c) immature fish, and (d) overly mature fish (fish from which eggs were expressed but that had nearly empty stomachs or eggs of bad color).

The mature fish were then placed into  $2 \text{ m}^3$  tanks with circulating water systems. The immature fish were returned to the breeding pools and overly mature fish were destroyed. The mature fish were used immediately for breeding. After the insemination process (section 2.2.3), the selection process was repeated and another group of mature fish were bred.

# 2.2.3 Breeding

A worktable was prepared with a scale to weigh the eggs, large metal and small plastic bowls, towels, and two wings of a night heron (egret).

The staff began with the females. Some 30 females were taken from the tanks and placed in a tank with an anesthetic. Each female was removed from the water, towel dried and placed on the worktable. Teams of two workers took the dried female and squeezed her eggs into the small bowls (Fig. 2.5). As each bowl filled, it was covered with a thin plastic wrap to prevent dehydration. The egg-producing females were collected into a plastic container and counted. Females that did not produce eggs or produced eggs of unsuitable quality were placed in a separate container. Eggs were collected from 60-80 females and each group of eggs weighed 1.4-1.8 kg.

The males were removed from the tanks and dried in the same manner as the females. Semen was squeezed from the males onto the eggs in the small bowls (Fig. 2.6). A male to female ratio of at least 3:10 is required however the actual ratio was higher and a ratio of 1:1 is desirable.

The mixture of eggs and semen was transferred to a large metal bowl, weighed, and thoroughly mixed with a heron feather. The mixture was spread with a feather onto plastic incubation beds (Fig. 2.7). The beds were stacked, a clean bed was placed on the top and the stack was placed into a tank of water (Fig. 2.8). The beds were vigorously shaken for about a minute to activate the semen and cause insemination. Then the stack of beds was taken to an incubation tank filled with cold (16-17°C) aerated fresh water. The beds were separated from one another and hung on metal wire hooks connected to poles leaning against the sides of the tanks.

Selection and breeding continued until the fish supply was exhausted. Seven workers labored four hours to remove the 3000 fish from the circular pond. Seven hundred eighty-four were suitable for breeding (317 females and 467 males). Each kg of eggs contained about 2 million eggs and a total of 6.6 kg eggs was collected, i.e., some 13.2 million eggs. Therefore, each female provided an average 20 g (40,000) eggs. While the expected insemination was 50-60%, only some 30% of the eggs were inseminated.

Two days later another selection was conducted in the same pool. Of 1600 fish, only 40 females were fertile. Therefore, 40 fertile males were selected and the rest were returned to the pool. The processes of extraction and insemination were conducted as above, but more quickly and meticulously. Seven hundred and ninety grams (1,580,000 eggs) of eggs were harvested and designated for shipment to the Dan Fisheries in Israel. The percent insemination in this batch was approximately 60% or 940,000 eggs.

### 2.2.4 Care of the eggs

On the day following the insemination process, the beds with the eggs were transferred to tanks filled with water and green malachite  $Cu_2$  (CO<sub>3</sub>)(OH)<sub>2</sub> at a density of 0.26 ppm for 30 min with aeration. This treatment was repeated every two days. The rate of insemination was determined with a binocular (Fig. 2.9) by counting the number of live and dead eggs in a section of a bed (dead eggs are white).

After 4-5 days of incubation, the staff removed dead eggs by spraying water with a moderate flow through the incubation beds and washing the dead eggs into an empty tank (Fig. 2.10). The live eggs remained connected to the beds.

# 2.2.5 Marketing at Yabe River Cooperative

The fish were sorted into two sizes -(a) 40 g and greater, and (b) below 40 g - for marketing. The fish were sorted with a wooden crate with slats (Fig. 2.11). Smaller fish fell through the crate while larger fish were caught by the crate. The fish were gathered into tubs filled with ice water and sprinkled with coarse cooking salt (NaCl). The fish were packed by hand into plastic bags at 0.5 kg for per bag for the larger fish and 1 kg per bag for the smaller. Each bag had one layer of fish. The bags were packed into styrofoam boxes and deep frozen. The price was 1,300 Yen (¥) per kg for large fish and 1,000 ¥ for small fish.

### 2.2.6 An ayu fish farm at Kochi

This is a privately owned farm and possibly the most modern of its kind in Japan. The farm is owned by a partnership of several investors and contains 30 pools of 100 m<sup>3</sup> for raising ayu and 3 pools of 50 m<sup>3</sup> for cultivating rotifers (*Aschelminthes*). Approximately 28 million ayu are raised annually from the breeding stage until marketing, with the majority destined for stream repopulation and about 30 tons for marketing fresh, frozen or processed.

Most of the farm is covered by a semi-transparent roof. The farm is equipped with centralized water heating and air circulation systems, computerized control of temperature, oxygen distribution, and water pumping, a 4-m<sup>3</sup>/day freshwater well, and a 1.2 m<sup>3</sup>/day saline well. As a result of the extremely limited water resources, all water is recycled. Each pool has a biological filter and independent recycling by pump.

Two pools contain 10,000 breed fish, each. The other pools were mostly empty and ready to receive young fish during the author's visit. The breed fish are kept at 22°C until a week before egg extraction, when the temperature is lowered to 16°C. Breeding is similar to the method described above while incubation is conducted in a similar bedding system in four specially designed containers (Fig. 2.12) that allow for the simultaneous incubation of 4 million eggs with a survival rate of 60-70%.

#### 2.2.7 Incubation and larviculture

During incubation, which lasts 9-10 days, the pools are sterilized with green malachite  $Cu_2$  ( $CO_3$ )(OH)<sub>2</sub> at 3 mg/l for 30 min every two days. As hatching approaches, the beds are transferred to breeding pools so that the eggs can hatch in fresh water. For the first 10 days in the breeding pool, the water remains unchanged and salt is gradually introduced until the water reaches 50-70% that of sea water. Beginning 3 days after hatching, the fingerlings are fed rotifers (plankton) three times a day at a maintenance concentration of 3-8 rotifers/ml for at least one month. The density of the ayu larvae after hatching is programmed to be 20,000/m<sup>3</sup> water or 2,000,000 per pool. Starting on day 5, the young fish are fed dry food in addition to the rotifers. A number of foodstuffs from different manufacturers are used, depending on the price and quality. Initially the fish are fed by hand, later they receive food from automatic feeders. The utilization of the dry food is 1:1 until 1 g and 0.9:1 afterwards. The saltwater acclimatization stage continues 60-90 days until the fish reached 0.3 g. The survival rate at this stage is approximately 40%. The freshwater comes from a well at 20°C.

Towards the end of the saltwater stage, the fish are often afflicted with vibriosis, however afterwards there usually are no problems. The general survival rate reaches only 30%.

Fish are sorted by size when they reach approximately 0.5 g and once a month. At first, extraction is done with hand nets; later, pumps and circular sorters are used.

#### 2.2.8 Rotifer cultivation

Rotifers are cultivated in consecutive batches in three 50 m<sup>3</sup> pools. Twenty percent of the water is removed and replaced with clean fresh water daily. The density of the plankton is approximately 800/ml and the daily production rate per pool is approximately 8 billion. The salinity of the water is 70% seawater and the temperature is 28°C. The rotifers are fed moist yeast and algae of the freshwater *Chlorophyta* variety. An effective device sorts the rotifers by size for feeding small and large ayu.

# 2.2.9 Processing plant

The Kochi farm processing plant has machinery for cleaning the fish and removing the intestines through an opening in the back of the fish. The cleaned fish are placed on racks in an incubator for 24 hours to dry and then packed in a decorative vacuum packaging for sale. The processed product is called "ayu no ichiyabosh" in Japanese, which means "ayu without intestines".

# 2.2.10 Algae

A number of pools outside the enclosed area are coated with algae. When asked the reason for this, it was explained that fish grown in these pools felt better as they enjoyed eating the algae from the walls and floors of the pool. Their color improved, resulting in a higher market price. In the case of breeding fish, the algae contributed to the improvement of egg quality. In other farms that raise both trout and ayu, it is customary to rotate the population in the pools so that the ayu eat the algae, and thereby clean the pool for the trout.

# 2.3 Methods of Cultivation at Kibbutz Dan

### 2.3.1 Breeding

As in nature, the lifespan of the ayu is one year, culminating with reproduction and death. Therefore, a school of breeding fish is selected from each cycle, according to size and other desirable characteristics such as resistance to disease. At Dan, a group of 600 females and 300 males were prepared for breeding. They were kept in 20 m<sup>3</sup> concrete pools in very low density, with good water circulation and an unlimited amount of food.

To estimate the date that breeding would begin, 30 fish were randomly sampled on four dates beginning July 1, 1999. The BW and GW of each fish were recorded and the GSI was determined, as above (section 2.2.2). Since gonadal development is linear in ayu and females are ready to breed when GSI = 23%, it was predicted that the fish would be ready by the end of December 1999 (Fig. 2.13).

On September 28, 1999, weekly selections began. In the first selection, males were separated from females according to exterior appearance. Adult males are thinner and darker than females (Fig. 2.14). If the male was sufficiently mature, white semen was ejaculated by lightly pressing the genital area. The males were separated from the females by placing a plastic fence in the pool. Forty females were transferred to the processing station for egg extraction by milking. An attempt to inseminate and incubate the eggs failed because the eggs were immature (they were not white enough and were extracted with the ovular membrane, i.e., the females had not completed ovulation) and the males produced too little semen.

On October 17, 1999, the first successful extraction took place. The females designated for breeding were transferred to the processing plant and anesthetized in a solution of Benzocain (50 ppm). At the same time, a number of males were also anesthetized. The anesthetized females were towel dried to absorb and light green eggs were removed into a bowl by squeezing the stomachs of the females. There were approximately 2000 eggs/g. Although semen from one male is required to inseminate the eggs from one female, the semen from two males was squeezed onto the eggs in case one of the males was impotent. The semen and eggs were mixed gently but thoroughly without adding any water, similar to the technique at Yabe River Cooperative in Japan (section 2.2.3).

## 2.3.2 Insemination and incubation

A specially designed incubator (Fig. 2.15) was built so that the beds with the eggs would be vertically and totally submerged in the water and so that there would be a constant spray of fresh water from above. The incubator contained two cells (400 liters each), each with its own water supply and drainage systems so that it was possible to simultaneously incubate eggs from different cycles. The eggs were incubated 10 days at the ambient temperature of the Dan River, 15°C. During this period, the eggs were treated to prevent the development of the *Sperolania* fungi that grows on dead and live animals. The treatment included a bath of "Yaraqron" [green malachite  $Cu_2$  (CO<sub>3</sub>)(OH)<sub>2</sub>] at a concentration of 0.3 ppm for 30 minutes, once every two days.

After 48 hours of incubation, it was possible to examine the percent fertilization by binocular and separate dead from live eggs. On day 10, the beds were transferred from the incubator to larvae tanks  $(2 \times 2 \times 0.5 \text{ m})$ . They were vertically submerged in the water with gentle aeration and water exchange. The eggs began to hatch on day 11 and continued for three days. The hatched larvae were tiny and transparent. It was difficult to see them with the naked eye and a good magnifying glass was required.

At the end of hatching, the beds were removed and salt was added to the water. A water recycling system maintained the salinity at 4-6 ppt and the temperature at 17.5-19°C.

The six hatching cycles of 1999 produced 868 g of eggs (Table 2.1), of which 524 g were fertile, producing 1,048,000 larvae. In addition, a shipment of 300,000 eggs was sent from Japan. Aside from the need to select females once or even twice a week, there were no significant difficulties or problems involved with the extraction, fertilization and incubation stages described in this section.

#### 2.3.3 Larviculture

During the primary nursery stage, newly hatched fish are kept in salt water for three months, during which time they are fed live and artificial dried foods. At the end of the primary nursery stage, they are expected to reach 0.4-0.6 g.

The primary nursery stage is conducted in six  $2\text{-m}^3$  fiberglass tanks (2 x 2 x 0.5 m; Fig. 2.16). A central drainage channel collects the water from the tanks and channels it to a solid waste separation facility. From there, water is pumped to a biological filter (two  $3\text{-m}^3$  containers) that removes nitrogen. Ammonia and nitrate are removed by a substrate of plastic particles (macaroni) kept in constant motion by massive air circulation that also enriches the oxygen level of the water. Finally, the water is sanitized by a UV lamp and pumped back into the tanks. The total volume of the system is 21 m<sup>3</sup>.

For the first three days after hatching, larvae are nourished by their vitellus membranes. However, since the hatching of a batch of eggs is a gradual three-day process, when the beds were removed from the incubator on the tenth day, some of the young fish had already begun searching for food. Therefore, approximately 150 million live rotifers were provided per day from the beginning of the primary nursery stage. The fish were fed three times a day. The water flow was stopped during the feedings and the food was spread along the walls of the tank. To guarantee availability of food, a density of 10-20 rotifers per ml was required at the end of the two-hour feeding, when the water flow was restored. The rotifers were enriched by an alga of the *Nanochlorophycsis* order and other supplements such as Algamac. Frozen algae were obtained from the Eilat Center of Oceanic Research.

The young fish reached 15 mm on approximately day 20 and began to receive *Artemia* with dry food (Nihon Nosan Kogyo K.K., Japan; Table 2.2) instead of rotifers. The transfer from rotifers to *Artemia* was gradual, with fish receiving both for a number of days. *Artemia* was provided for two weeks, after which the fish received only artificial feeds. In contrast to food for other Salmonids, the food composition for ayu is low in lipids and the protein, while significant in percentage, originates mostly from vegetarian protein sources. During the primary nursery period, the nutrient efficiency of the dry nutrients were 1:1, with nutrients from the live feeds not taken into consideration.

During the 3-month primary nursing in salt water, the young ayu were expected to reach 0.4-0.6 g (Table 2.3). This occurred late-January or early-February 2000 and at this stage it was able to wean them from salt and transfer them to fresh water. At this stage, the optimal temperature was 19-20°C. The primary nursery stage ended with the transfer to fresh water.

## 2.3.4 Cultivation of rotifers

One to three week ayu larvae are too small to digest dry food or *Artemia*. Therefore, they are usually fed rotifers. Some rotifers of the *Brachionus* species grow in fresh water and some in salt. Two sizes of *Brachionus plicatilis* are cultivated at the Galilee Technology Center (MIGAL) and Dan Fisheries, small (up to  $100 \mu$ ) and large (90-250  $\mu$ ). The ayu larvae at Dan Fisheries were fed the smaller size of the saltwater species.

Table 2.1. Ayu breeding cycles at Dan Fisheries.						
Date	Females	Eggs (g)	Fertilization (%)	Fertilized eggs (g)		
	(no.)					
Oct 17	1	28	70.2	19.66		
Oct 22	1	12	68	8.16		
Oct 29	8	150	62.2	93.30		
Nov 5	5	126	59.1	74.47		
Nov 9	14	262	70.1	183.66		
Nov 29	15	290	50	145.00		
	44	868	Average 63.3	524.25		
Total			_			

Table 2.2.
Feeds for ayu.

Code	Size of	feed	Size of f	ish	Feed con	mponents	s (%)			
no.	particle				_					
	(mm)		(g)	(cm)	Protein	Lipids	Phosphorus	Calcium	Ash	Fiber
0	0.07		< 0.01	<1.5	54	5	1.6	2.2	14	2
1	0.1		< 0.07	<2.5	54	5	1.6	2.2	14	2
2	0.2		< 0.5	<4.0	54	5	1.6	2.2	14	2
3	0.35		<1.8	<5.5	54	5	1.6	2.2	14	2
PC 1	0.5		<2.5	<6.0	49	5	1.5	2.4	17	2.5
PC 2	0.8		<10	<9	49	5	1.5	2.4	17	2.5
PC 3	1.2		<20	<12	49	5	1.5	2.4	17	2.5
PC 4	1.7		<40	<16	45	4	1.2	1.5	15	3
PC 5	2.4		>40	>16	45	4	1.2	1.5	15	3

Table 2.3. Growth rate of young ayu during the primary nursery period. Time from Length Weight hatching (mm) (g) (days) 11 10 --15 13 --20 15 --38 25 0.02 52 30 0.1 66 34 0.12 73 39 0.2 77 46 0.5

In Japan, ayu larvae are fed *Brachionus* during their first 30-40 days and the density of rotifers is 5-30/ml. Larvae of 3.5 mm eat 100 rotifers of 100-200  $\mu$ , each. Therefore, to produce enough rotifers for 100,000 ayu larvae, cultivation in 4-5 m<sup>3</sup> water is required.

56

0.6

90

Rotifers can be cultivated in a system consisting of five to seven tanks, where one is totally harvested each day, or in a continuous culture, where all tanks are partially harvested each day and a portion of the rotifers remain in each of the tanks for continued reproduction.

At MIGAL, rotifers are maintained in two  $1-m^3$  tanks while at Dan Fisheries they are raised in six  $1-m^3$  tanks (Fig. 2.17), five of which are stocked while the sixth is being cleaned.

The 1-m<sup>3</sup> rotifer tanks at Dan contain a heating element, a thermostat and an aeration system in the center of the tank. The tanks are filled with filtered and sterilized water. Salinity is maintained at 30-40 ppt and water temperature at 28°C. The tanks are constantly aerated. Initially, the tanks are densely populated with 100-400 rotifers per ml. Fresh salt water is added daily at a rate of 20-30% of the tank volume. On day 5-7, when the density reaches 100-600 rotifers/ml, they are harvested by filtering them through a 45  $\mu$  net and washed in fresh water for 10 min to clean away foreign protozoa. They are served to the fish in large quantities throughout the day. A concentration of 10-20 rotifers per ml is maintained to make sure that food is always accessible to the fish.

Rotifers feed through a filtering method and therefore require very small food particles. Their primary foodstuff is phytoplankton and yeast. Phytoplankton can include *Chlorella*, *Nannochloropsis* and *Isochrysis*, etc., while common baking yeast (*Saccharomyces cerevisiae*) is inexpensive and always available. The daily portion per million rotifers is 1-3 g yeast, served 2-4 times daily, or 0.5-1g yeast plus 30-80 l *Chlorella* (10-15 million cells per ml) per day. The rotifers received a diet of moist yeast enriched with "Algamac" and *Nanochlorphysis* plankton. After a period of experimentation regarding feed quantities, enrichment materials and feeding frequency, the system produced a good and stable daily output of 400-600 million rotifers.

# 2.3.5 Water quality in the closed water system

A closed recycled water system was used to control salinity and temperature in the primary nursery tanks. However, as time passes, the water quality in a closed system may diminish, primarily due to increasing levels of ammonia (NH<sub>4</sub>-N), nitrates (NO<sub>2</sub>-N) and sulfur. To maintain water quality, a mechanical filter for the removal of solid wastes and a biological filter for the treatment of nitrogen and sulfur were installed. Water quality was constantly monitored and controlled. Salinity was measured with a refractometer (an optic salinity measurement tool). The required salinity range for young ayu is 4-8 ppt. A salt composition similar to that of seawater is most desirable. A minimum of fresh water was exchanged (average 5% per day) at the same time that salt compounds were added to maintain the required salt composition (Table 2.4). The ammonia level did not exceed 0.2 mg/l and the nitrate level was no higher than 0.5 mg/l. Accumulation of sulfur was prevented by weekly disposal of residues from the filters.

	raute	. 2.7.	
Salt composition of	the ayu nurs	sery tanks at the Dan Fisheries	5.
	Salt	g/m <sup>3</sup>	
	NaCl	3,096	
	MgSO <sub>4</sub>	784	
	MgCl	596	

Table 24

11401	2,070
$MgSO_4$	784
$MgCl_2$	596
CaCl <sub>2</sub>	176
KC1	84
NaHCO <sub>3</sub>	24

During incubation and up to a week after hatching, the water temperature was  $16^{\circ}$ C. The temperature was gradually increased to  $19^{\circ}$ C using electric water heaters in the tanks. The water was enriched with oxygen by the closed circulation system that operated through the biological filter and directly to the tanks. Aeration in the filter was strong enough to create movement in the plastic particles while aeration in the tanks was gentle so as not to harm the fish. The minimum oxygen level is 5 mg/l. However a higher level, close to saturation at 8.0-8.5 mg/l (at 19°C) is more desirable.

# 2.3.6 Advanced nursery and fattening pools

The advanced nursery stage begins with the transfer of the fish to large pools and can happen when the fish reach anywhere in the range of 5- 20 g. In Dan Fisheries, the advanced nursery stage was conducted in large rectangular 20 m<sup>3</sup> concrete pools ( $10 \times 2 \times 1$  m; Fig. 2.18) with a closed water circulating system at 17-19°C. Pools were separately filled with water but shared a common drainage system. A submerged pump with a maximum capacity of 20 m<sup>3</sup>/h was placed in the drainage hole of each pool and the water was pumped through a 75 mm polyethylene pipe to the water treatment plant. Water treatment consisted of two 1-m<sup>3</sup> tanks connected by a 110 mm sewage pipe. The first tank served as a separator of solid wastes and was filled with plastic (macaroni) particles; water entered from the bottom of the tank. The second tank served as a biological filter and was filled with plastic macaroni in constant movement, caused by strong circulation; water entered from the solid waste separator. The recycled water returned to the two advanced nursery pools from the biological filter.

Because of the gross weight stocked in the pool (90 kg) and the daily feeding level (3.6 kg), substantial aeration was required. The aeration caused strong turbulence that disturbed the fish. Therefore, on March 6, 2001, the recycled water was stopped, although the water quality was good, and fresh water of 15°C was introduced to provide aeration. At this stage, the average fish was 1.43 g. The water was cleaner in the open system, the condition of the fish improved and they grew nicely in spite of the lower water temperature.

Fish were sorted by size and restocked in other pools periodically while monitoring growth, nutrition efficiency, mortality and general health. Nutrition efficiency was approximately 1:1.6 and the rate of expected growth was 3-4% per day.

On May 24, 2001, when the largest fish reached 20 g, 17,000 fish were transferred to a round 80 m<sup>3</sup> fattening pool (Fig. 2.19). The rest of the fish remained in the rectangular pools for continued growth.

The floors of the fattening pools slope towards the center where the water exits to an external drainage ditch and tank where it is possible to control the water level and collect dead fish. The pool is covered with a shadow net (75% protection) to reduce reflection from the sun and protect the fish from birds. Fattening pools were given foodstuffs imported from Japan that contained fatteners at 3% of the fish mass of the pool per day (Table 2.5).

The results of the fattening pools during 2001 were good (Fig. 2.20). By the beginning of August, it was already possible to harvest fish for market and on September 21, 2001, the fattening pool was emptied (Table 2.6). The total amount of food given during the fattening period was 1,880 kg.

Daily 1000 faile	m, acc	Stung it	water t	emperati	ine anu i	isii weig	m (78 01	bouy w	eigin).
Water temperature				Fis	h weigh	t (g)			
(°C)	<10	10-20	30-20	40-30	50-40	60-50	70-60	80-70	+80
12	3.9	3.6	3.0	2.3	2.0	1.7	1.5	1.3	1.2
13	4.1	3.8	3.2	2.5	2.1	1.8	1.6	1.4	1.3
14	4.4	4.1	3.4	2.7	2.2	1.9	1.7	1.5	1.4
15	4.7	4.4	3.7	2.9	2.4	2.1	1.8	1.6	1.4
16	5.1	4.7	3.9	3.1	2.5	2.3	2.0	1.7	1.5
17	5.4	5.1	4.2	3.3	2.7	2.4	2.1	1.8	1.6
18	5.9	5.5	4.5	3.6	2.9	2.6	2.3	2.0	1.7
19	6.3	5.9	4.9	3.8	3.1	2.8	2.4	2.2	1.8
20	6.9	6.3	5.3	4.1	3.4	3.0	2.6	2.3	2.0
21	7.4	6.8	5.7	4.4	3.6	3.2	2.8	2.5	2.2
22	8.0	7.3	6.1	4.8	3.9	3.5	3.1	2.7	2.3

Table 2.5.

In the second fattening pool, populated with smaller fish, the results were less satisfactory due to morbidity that caused a reduction in their appetite and subsequent mortality (section 2.4.2b). The males reached sexual maturity in September 2001 and ceased to grow. Their skin darkened and they lost weight to such an extent that it was impossible to

market them. During the first year, approximately 34% of the males reached maturity prior to marketing, significantly reducing the amount of marketable fish. In 2001, lighting was installed in the fattening pools and, as of August 1, the pools were illuminated automatically from 18:00 to midnight. The extended daylight hours delayed the sexual maturity such that a relatively small percentage of the fish (12%) were disqualified for marketing.

T11 20

Size	Weight (g)	Amount (kg)	No. fish
Giant	105-up	270	1,900
Large	80-105	850	9,400
Medium	60-80	308	4,400
Small	<60	24	500
Total		1,452	16,200
Stocked on May 24, 2001		340	17,000
Depreciation			800 (4.7%

# 2.3.7 Marketing of ayu cultivated at the Dan Fisheries

During the first year of operation all of the first quality fish and a smaller amount of the second quality were marketed at a total weight of 650 kg. The prices per kg for first and second quality fish were \$12 and \$10, respectively. The majority (500 kg) was marketed in Holland where contact was made with a local distributor who supplies fish to Japanese restaurants. The rest was shipped to England and France, primarily to customers who were willing to try the fish. After a lengthy search for potential customers, including an Internet search, emails, and faxes to tens of customers and personal contacts in Japan, two customers requested samples of the fish. Following is a description of the activities in relation to these customers.

a. Yamaguchi Shokai Co. – This company imports various products from Israel, primarily phosphates. They import fish from Israel and were involved in an initiative to capture and/or cultivate tuna off the Mediterranean coast near Israel. Their representative visited Dan Fisheries and was impressed with the farm. Discussions about potential business opportunities were held. Following his return to Japan, it was decided that a 10 kg experimental shipment would be sent, followed by a semi-commercial shipment of 100 kg fresh and 30 kg frozen ayu. The price for the fresh ayu was \$12.50 per kg; the frozen fish were sent as free samples. The fish arrived in relatively good condition.

The semi-commercial shipment was sent to Japan on September 4, 2001, and arrived in the evening of the following day. Two and a half days before departure, feeding was stopped. The fish were removed from the pool by net at 06:30 and sorted. The fish were held in water until 11:00 and then placed in containers with ice water. The fish to be frozen were packed in nylon bags, individually frozen and packed in styrofoam boxes containing 10 kg fish plus 5 kg dry ice. The fresh fish were packed in layers, separated by nylon sheets, in styrofoam boxes, 10 kg fish plus 5 kg regular ice.

b. Chuo Gyorui Co. Ltd. – Following a number of email communications including pictures (the Japanese had difficulty believing that ayu were grown in Israel), a shipment of 10 kg fresh fish was air-shipped in ice packed styrofoam boxes. Because of problems with the connecting flight in Frankfort, it took 48 hours for the shipment to reach Narita International Airport in Tokyo and another 24 hours until the fish arrived at their destination at the Tsukiji Fish Market. The delay in the journey impaired the freshness of the fish.

The customer's response to the taste was satisfactory. He noted that the percentage of males and females was 50:50. Regarding a commercial shipment, they were only willing to consider purchasing on consignment and at an estimated price of \$5.40 CIF Japan.

- c. Customer remarks concerning the ayu market in Japan
- 1. Fresh ayu is sold only during spring and summer (May-August) at \$12-14 per kg.
- 2. Males and females must be sorted as females bring a better price.
- 3. Fish must be individually wrapped in polyethylene packaging.
- 4. Two customers were willing to purchase the ayu on a consignment basis.
- 5. Frozen fish must be placed in polyethylene packaging prior to freezing.
- 6. The ideal packaging is 5-10 kg.
- 7. The market for frozen ayu is very small and it is difficult to sell the goods.

d. Conclusions regarding of marketing in Japan

1. Since fresh ayu are generally consumed during May-July when the price is satisfactorily high (\$12-14 per kg) while consumption is significantly less and prices much lower during the rest of the year, and since culture conditions at Dan Fisheries do not allow fish to reach market size before September-October, there is a problem with marketing at a fair price.

2. While frozen ayu are sold throughout the year, the price is extremely low (\$2-3 CIF/Japan. The customers were willing to purchase frozen ayu, but only on consignment, without even guaranteeing the estimated price.

3. Conclusion - there is no economic justification to continuing the project under these conditions.

# 2.4 Maladies and treatments

#### 2.4.1 In Japan

The harvest of ayu from nature significantly decreased during 1996-1999. At the same time, a variety of diseases on ayu farms caused death or bodily injury to the fish, disqualifying them for marketing. The most common or unique maladies are discussed below.

a. *Vibriosis* – this disease afflicts fish worldwide and is caused by the *Vibro anquillarum* bacterium. The disease harms the ayu population of Lake Biwa, a landlocked lake near Kyoto, and ayu populations that migrate to the sea. Recently, the disease is on the decrease in Japan.

b. *Glugea plecogloss* – this is a tiny parasite related to *Microsporiasis* and is particular to ayu. The parasite procreates in internal tissues of the fish body such as gonads, liver, and the digestive track. The parasite is transferred directly between fish without an intermediary. Today no treatment exists for this malady, however, mortality caused by this parasite is not high as long as proper sanitary conditions are maintained.

c. *Lantan Disease* – a malady that expresses itself in wounds in the area of the rear fin and occurs in cases of crowdedness, fatty foods, too shallow water, and stress. A single cause is not known. Treatment entails improving living conditions and removing the source of stress.

d. *Pseudomonas* – a systematic bacterial disease caused by the pathogenic bacterium *Plecoglosisida pseudomonas putidea*. Symptoms usually include bloody ascites in the stomach, escoptalmia, interrites, anemia, spots on the kidneys and liver as well as forced wounds. The disease is very common in Japan, primarily in aquaculture where it causes significant damage. It is very difficult to treat the disease with antibiotics as the Japanese strain of bacteria is resistant to almost all forms of medication.

e. RTFS – a disease also common in Israel among young trout and caused by *Flavobacterium* psychrophilus. This illness has caused heavy damage to ayu in Japan, in both nature and aquaculture. The following antibiotics are used for treatment when introduced through fish nutrition: Oyxolinic, Acid, Flurpheyncol, Sulfusozil, or Amoxicillin. An alternative treatment is to heat the water to a temperature of 25°C for 15-30 min, but the antibiotic treatment is preferable as heat treatments may encourage outbreaks of *Pseudomonas*.

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f. *Streptococcus* – there have been reports of isolated events of *Streptococcus* in ayu, however, it is not known as a serious ayu-afflicting disease in Japan.

### 2.4.2 In Israel

None of the above diseases is prevalent in Israel. On the other hand, the Dan Fisheries encountered other difficulties related to ayu health and mortality.

a. *Primary nursery stage* – Survival was the most serious problem in this stage. During the first four reproduction cycles in 2000, including amongst the eggs shipped from Japan, the mortality rate was extremely high 7-10 days after hatching. The overwhelming majority of the larvae starved to death, in spite of the fact that there was a sufficient supply of rotifers and dry feed was also provided. In some cycles, almost all the larvae died from starvation. The high mortality was caused by mycobacterium bacteria. Following treatment with baths of oxytetracycline type antibiotics, at a dose of 15 g powder (50% active material) per 1 m<sup>3</sup> water for two hours, the mortality was arrested and the condition of the young fish improved.

At about three weeks after hatching, a creeping mortality began in all the cycles due to a bacterial disease that affected all the tanks. On plates of blood samples, a gram-negative bacterium grew. In tests conducted at the Veterinary Institute, the bacteria was diagnosed as *Aeromonas hydrophila*. Attempts to treat the disease with antibiotics together with dry foods and *Artemia* produced only partial success. Medications such as Oxytetracycline and Tseflosporin were ineffective. Tseforel caused a temporary reduction in mortality, however after a few days the mortality renewed. Unfortunately, the larger fish from each tank usually did not survive, including amongst larvae that hatched from eggs shipped from Japan.

A. hydrophila is not a primary pathogen in fish. It is a secondary pathogen that afflicts fish when they are weak or sick due to a virus or other bacterium or some factor in the cultivation of the species is awry and the fish are under stress. In the case of the young ayu, the conclusion was reached that the fish suffered from stress due to some sort of nutritional or other deficiency.

During 2001, the massive mortality of larvae ended, largely due to the fact that algae were added to the diet of the rotifers. The addition of algae, rich in fatty acids such as EPA and DHL, to the diet significantly improved the physiological situation of the young ayu and greatly reduced the mortality rate and need for antibiotic treatment.

b. Advanced nursery stage – A new disease, previously unidentified among ayu, appeared during this stage. This disease had occurred in schools of trout at the Sion Fisheries and was known to develop in poor quality water. The disease appeared in ayu of 25 g and more. The primary symptom is an oval shaped lesion on the side of the fish that begins as a white skin stain and develops into a deep flat wound until all the skin in the region is destroyed and the muscle tissue is exposed (Fig. 2.21). A gram-negative bacterium on blood samples, with white-beige colonies that changed to a green-gray, grew without any hemoliza. The bacteria were identified as *Aeromonas salmonicida*. However, there was no confidence that this was the primary, rather than a secondary, pathogen. Experimental medications included Linko-Spectin and a vaccine serum prepared from the bacterium that was thought to be a possible pathogen, however both had limited influence. There was some decrease in mortality.

In addition to mortality, the disease slowed the growth rate of the fish and a large portion could not be marketed because of the ugly wound on the side of the body.

Disease among ayu in Japan was one of the reasons for attempting to cultivate this fish in Israel; it was hoped that the majority of maladies would be avoided. However, the ayu is extremely sensitive and disease became one of the important factors in deciding to end the project in Israel.

## CHAPTER 3

# CULTIVATING HYBRID STURGEON (ACIPENSER GUELDENSTAEDTII X A. BESTER) IN NORTHERN ISRAEL

# 3.1 Biology of the sturgeon

The sturgeon (Fig. 3.1) is most common in the northern hemisphere, i.e., in Europe, Asia, and North America. It can survive in both fresh and salt water, however, it spawns exclusively in freshwater streams. For the first year after hatching, the young fish live in fresh water, during the second they can be found in coastal regions, and only from the third year onwards do they migrate and populate the deeper ocean waters until maturity. Once in the sea, their primary food is mollusks. During the annual spring migration, the fish head upstream into large rivers to spawn. Sturgeons live together as heterosexual couples. Females lay millions of 2 mm roe each year.

The sturgeon family (*Acipenseridae*) is the primary member of the *Chondrostei* suborder and is similar in many aspects to the shark. The sturgeon body is mostly scaleless with only a small portion covered by five rows of shield-like fins along the length of the fish. One row runs along the back, while the others are paired on each side. The mouth, similar to that of the shark, is inferior, located beneath an elongated pointed snout used to dig into the ground. The teeth are molar-like.

The family includes four genera. The most significant are the *Huso* and the *Acipenser*. *Huso* includes two species, *H. huso* (beluga) and *H. caricus*. *Acipenser* includes 20 species: <u>A. baerii</u> (Siberian sturgeon), <u>A. brevirostrum</u> (shortnose sturgeon), <u>A. dabryanus</u> (Yangtze sturgeon), <u>A. fulvescens</u> (lake sturgeon), <u>A. gueldenstaedtii</u> (Russian sturgeon), <u>A. kikuchii</u>, <u>A. medirostris</u> (green sturgeon), <u>A. multiscutatus</u> (Japanese sturgeon), <u>A. naccarii</u> (Adriatic sturgeon), <u>A. nudiventris</u> (ship sturgeon, spiny sturgeon, thorn sturgeon), <u>A. ornatus</u>, <u>A. oxyrinchus</u> (Atlantic sturgeon, esturgeon noir, Gulf sturgeon), <u>A. persicus</u>, <u>A. ruthenus</u> (sterlet), <u>A. schrenckii</u>, <u>A. sinensis</u> (Chinese sturgeon, Sagami sturgeon), <u>A. stellatus</u> (sevruga, star sturgeon, stellate sturgeon), *A. sturio* Linnaeus, (European sturgeon, French sturgeon), <u>A. toliapicus</u>, <u>A. transmontanus</u> (white sturgeon). All are economically significant due to their heavy weight, red meat, and large delicious roe known as caviar.

The sturgeon has a long life, according to some researchers, up to 100 years. Sturgeon grow slowly, with the growth rate depending on environmental conditions. Some sturgeon species reach mammoth sizes. The maximum known weight is 2,000 kg. In Asia and North America, white sturgeon weighed in at 816 kg. Most other species weigh less, 10-90 kg.

The sturgeon is omnivorous. Food is located by smell and touch. The sturgeon searches for food with its sensitive rostrum snout, prowling and eating benthic organisms that live in the substratum of deep aquatic ecosystems such as crabs, worms, mollusks, snails, and various zooplanktons.

#### 3.2 Production of caviar

Several species have been domesticated for caviar production. Other species are fished in nature for their roe and not farmed. Because of environmental pollution and unregulated fishing, these species are being threatened by extinction.

One of the problems of caviar production is the fact that roe are consumed when they are in a stage prior to divergence and the female sturgeon must be killed to remove them. This reduces the sturgeon population, especially the fingerling population.

One species used to produce caviar is the beluga (*H. huso*) that lives in the Caspian and Black Seas. At maturity, this fish may reach 9 m and weigh hundreds or even thousands of kilograms. A 1,400 kg beluga may produce 300 kg of caviar. Another species used for caviar is the Russian sturgeon (*A. gueldenstaedtii*), a 2 m fish that at 20-80 kg may produce 3-12 kg of caviar. A third species is the fringe barbel or ship sturgeon (*A. nuvudentxis*) that is caught

while swimming upstream in rivers to spawn. Mature fish weigh several hundred kilograms and are capable of producing a mere 2-8 kg of caviar.

### 3.3 Freshwater species used for meat production

The sterlet (*A. ruthenus*) is common in the Danube River from Germany to Hungary and in the Caspian and Black Seas. This fish, which can reach 1 m and 12 kg, has a sharp elongated snout and is one of the most common and most fished varieties in the sturgeon family.

The French sturgeon (*A. sturio*) is nearly extinct and primarily found in the Garonne River in southern France or in captivity. This 2 m sturgeon inhabits the Mediterranean Sea and Atlantic Ocean. During the spring, it migrates throughout the rivers of western Europe to spawn.

The Siberian sturgeon (*A. baerii baerii*) has been acclimated to conditions in France and attempts are being made to transform it into a commercial fish.

The Adriatic sturgeon (A. naccarii) has been acclimated to aquaculture in Italy.

The white sturgeon (*A. transmontanus*) grows in the Pacific Ocean and spawns in the upstream rivers of California, USA. This species has been acclimated to aquaculture in California.

# **3.4 Reproduction**

Compared to most studied fish species, the sturgeon matures late. The first spawning occurs anywhere between 5 and 25 years of age. Most of the sturgeon that reach maturity at a relatively early age (4-8 years) can be found in streams and are comparatively small. Among the larger species (beluga, Atlantic, and Siberian sturgeon), sexual maturity occurs at 20-25 years of age. In many species, the fish form heterosexual couples at ages 2-9 years.

Researchers found that sturgeon swim upstream to spawn and that the gonadosomatic index (ratio between the weight of the sexual organs and the body weight) increases 20-30% during vitellogenesis (the formation and accumulation of vitellus in the ovum).

Each species has a different season for spawning. In Russia, spawning occurs from the end of winter until the end of spring. Spawning takes place a number of times during the life cycle. Some species spawn deep in the stream, others on rocks. Roe development is similar to that of amphibians. The optimal temperature for embryo development in the roe is 10-20°C although the range of temperature tolerance varies widely among species. Fingerlings hatch after 5-10 days of incubation, they are 8-12 mm and survive on their vitellus for a number of days. After approximately 8-14 days (depending on the water temperature), the fingerlings begin to eat. Metamorphosis occurs after 20-30 days.

#### 3.5 The sturgeon in aquaculture

The cultivation of sturgeon begins with artificial insemination using the hypophysis of adult sturgeon or carp, or LH-RH. Results show that it is possible to cause divergence of the ovum by injection. The dose of hypophysis from carp is 4-5 mg/kg, given in two injections at an interval of 15-40 hours between the first smaller injection and the second larger dose depending on gender, size, and body temperature.

The semen is milked from the males and may be stored at freezing temperatures up to 24 hours. A female beluga weighing 150-200 kg can produce 500,000-800,000 ovum. The resulting larvae may be cultivated in tanks at a density of 20,000-30,000 larvae per 2 m x 30 cm circular tank. It is important to maintain cleanliness by, for example, circulating the water. A circulating water flow directs extra food and other wastes to the center of the tank, facilitating their disposal.

When the larvae are capable of eating, they are fed a variety of zooplankton such as *Artemia salina*, white worms (*Enchytraeus albidus*), and water fleas (*Daphnia*). During the first 7 days, larvae are fed every 3-4 hours throughout the 24-hour day. After 2-3 weeks, it is possible to gradually introduce artificial foodstuffs such as trout larvae feed. After 2-4 weeks,

the fish can be transferred to a larger growth pond. After a week in the larger pond, it is possible to stop providing live food.

Relatively little is known about the growth of sturgeon fingerlings. Depending on conditions, 5-20% of the inseminated roe survive the first year. At this rate, 100,000-1,000,000 roe are needed to produce 100 tons of sturgeon. With a healthy stock and quality maintenance, mortality can be reduced and survival increased.

The growth rate of sturgeon is relatively fast with fish reaching 500 g within one year and 3 kg after 1.5-2 years. The growth rate of the hybrid *H. huso x A. ruthenus* is good, with Russian fish farms producing 3,000-4,000 tons. A 50-80% survival rate is common. Artificial feed is composed of fishmeal, vitamins, and minerals, while the mainstay of the hybrid diet, has not been sufficiently studied. Today, feed formulas used for other predatory fish are used for sturgeon.

Fish should be regularly sorted. In a constant water temperature of  $16^{\circ}$ C year-round such as at Dan, the fish should be sorted when the fingerlings reach 1 g, again at 10 g, and again at approximately 400 g. Publications and observations indicate that it is possible to store up to 7,000 larvae in a 3 m<sup>2</sup> tank until they reach 1 g. Then the population should be reduced to 1,500 fingerlings until 40 g. The recommended density for cultivating sturgeon is 7-20 kg/m<sup>2</sup>, resulting in an average of 150 tons per hectare. Larger fish can be kept at a greater density of 50-100 kg/m<sup>3</sup>.

Before beginning to cultivate sturgeon in Israel, the above subjects needed to be carefully examined and adapted to Israeli conditions, especially conditions prevalent at the Dan Fisheries. In Europe, sturgeon are cultivated in water with an oxygen level of 4-6 ppm and a temperature of 12-21°C, conditions very similar to those at Dan. However, the European cultivation season is limited to 5-6 months while conditions at Dan permit year-round cultivation, a tremendous advantage for Israeli growers. Sturgeon are fed a trout or cel diet. Israeli fish farmers plan to use a trout diet until an optimal food formula suitable to the commonly accepted nutritional coefficient of 0.8-1.5 has been developed.

# 3.6 R&D in Israel

The objective of the R&D was to determine the feasibility of raising sturgeon at the Dan Fisheries in northern Israel. The production plan of the R&D, described in this chapter, was based on published studies and knowledge accumulated during visits and observations at sturgeon farms in Russia, Hungary, and the USA (California), adapted to growth conditions in Israel which are similar to those under which trout are cultivated.

It was recommended that the hybrid *Acipenser gueldenstaedtii* x *A. bester* be grown. In any case, because of problems in introducing new fish to Israeli aquaculture, permission was received to raise only this hybrid. Roe was imported by techniques similar to those in trout culture. The experimental program is shown in Table 3.1 and results are reported in sections 3.6.1 and 3.6.2.

### 3.6.1 Incubation and nursery stages

Batches of 70,000 inseminated eggs were sent from Russia in ice-packed trays. The eggs averaged 0.056 g. The eggs were incubated in the same kind of incubation jars that are used in trout cultivation. Density was 10,000 eggs per container. The water temperature was  $16^{\circ}$ C (Dan River water temperature). The eggs had been kept at this temperature 5 days prior to arrival in Israel. Eggs hatched 3-5 days after arrival in Israel. The 20,000 hatched larvae were transferred to six large 2 m<sup>3</sup> (4 m<sup>2</sup>) fiberglass tanks. Approximately 14 days after the beginning of incubation (8 days after hatching), the larvae released a black plug from the digestive system, signifying that they finished feeding on the embryonic sac and were prepared to accept foodstuffs. They stopped crowding the tank floor and began searching for food. At this stage, mortality was 14-18%. The larvae were given a dry trout starter (feed no. 0, EWOS Ltd.) and *Artemia* nauplii. Salomon Trout Feed Grade 1 was given on day 22 and Salomon Trout Feed Grade 2 on day 44 after the beginning of incubation.

were transferred to rectangular 20 m concrete pools at a density of 4,000 fish per pool.
Survival during this stage is shown in Fig. 3.2. Growth is shown in Table 3.2.

Table 3.1.

Feed Grade 3 was begun on day 58 and Salomon Trout Feed Grade 4 on day 72 when the fish

Table 3.1. Production program for sturgeon according to growth cycle (A, B, C, D).						
End of year no.	No. eggs	No. fish of 400 g (tons)	No. fish of 3 kg (tons)	No. fish of 8 kg (tons)	To market (tons)	No. of pools*
First roe import	80,000 A	· · · · · · · · · · · · · · · · · · ·				6 nursery
1	80,000 B	16,000 A (6.4)				6 nursery 1 concrete
2	80,000 C	16,000 B (6.4)	10,000 A (30)			6 nursery 1 concrete 3 plastic
3	80,000 D	16,000 C (6.4)	10,000 B (30)	7,500 A (60)	60	6 nursery 1 concrete 6 plastic
4	80,000 E	16,000 D (6.4)	10,000 C (30)	7,500 B (60)	60	6 nursery 1 concrete 6 plastic

\*Nursery tanks are 4 m<sup>2</sup>, concrete pools 100 m<sup>2</sup>, plastic pools 625 m<sup>2</sup>

	Table 3.2.	
•	Fish growth and production in 4 m <sup>2</sup> ta	anks.
Date	Avg wt, $n = 60$	$kg/m^2$
	(g)	
Sept. 1, 1992	0.01	0.035
Oct. 1, 1992	0.15	0.5
Nov. 1, 1992	5.00	1.19
Dec. 1, 1992	17.81	4.2
Jan. 1, 1993	42.42	10.0
Feb. 1, 1993	107.16	25.0
Mar. 1, 1993	180.36	43.0
Apr. 1, 1993	266.58	63.0

## 3.6.2 Fattening stage

During the growth period, significant differences in growth rate develop between fish. Therefore, after a few months, the sturgeon must be sorted according to size. Large sturgeon grow faster than small sturgeon. It is unclear whether the variance in growth rate is a genetic or competitive trait. It seems that both factors influence growth. During the first 3 months in the nursery, some fish reached 140 g. Compared to other pondfish, this is especially rapid growth. Larger fish were moved to 600 m<sup>2</sup> pools and, after 18 months (in March), the production rate was 37 kg per  $m^2$  while the average weight exceeded 1 kg.

On June 1, 1994, the fish were sorted and separated according to size. This activity clearly influenced the average fish weight.

During the third year of cultivation, large fish weighing 1 kg achieved a weight approaching 5 kg and became suitable for marketing (Fig. 3.3). The average fish weight increased some 400% in 8 months. Rapid growth is characteristic of sturgeon and justifies cultivating it for meat products.

A summary of breeding, population and production statistics is presented in Table 3.3, which shows that, in Israel's conditions, the fish reach 300-400 g in the first year. To raise a large multi-portion fish of 4-8 kg, 3 years are needed. The decision as to the proper size and weight to cultivate was made according to market demand and product development. The general survival rate from egg stage to 3.2 kg was 14%.

Table 3.3

	140	ne 5.5.	
Fish gro	wth and production	on in feedin	
	Date	Avg wt	kg/m <sup>2</sup>
		(g)	
	May 1, 1993	279	8
	Jun. 1, 1993	344	10
	Jul. 1, 1993	428	13
	Aug. 1, 1993	637	19
	Sep. 1, 1993	754	23
	Oct. 1, 1993	874	26
	Nov. 1, 1993	1,015	30
	Dec. 1, 1993	1,158	35
	Jan. 1, 1994	1, 328	40
	Feb. 1, 1994	1, 524	46
	Mar. 1, 1994	1,725	52
	Apr. 1, 1994	1,977	59
	May 1, 1994*	2,258	68
	Jun. 1, 1994	3, 263	26
	Jul. 1, 1994	3, 717	30
	Aug. 1, 1994	4,237	34
	Sep. 1, 1994	4, 644	37

\*First harvest

# 3.7 Research on gonadal development of sturgeon

The goal of this research was to examine the development of the sexual organs of hybrid sturgeon (*A. geuldenstardtii* Brandt x *A. Bester*) during their growth at the Dan Fisheries. Sexual organs from three fish of varying sizes (1g, 50 g, 150 g) were extracted (Fig. 3.4) and histologically prepared for examination. All were females of approximately 13 months. There was a clear correlation between the size of the fish and its ovaries, as expected. Ninety-eight percent of the gonad was fatty and only a small part had begun to develop at this early stage (Fig. 3.5). From the exterior, the ovary of the largest fish resembled a male gonad due to the large amount of fat cells from which it was constructed.

Only 28% of the gonad had differentiated into gender cells in all three fish. The cells were still undergoing oogenesis. Accordingly, we concluded the gonads were young and immature. However, we could not determine whether the gonads were under-developed because they came from hybrids or because they came from young fish.

Tissue portions in the oogenesis stage with oocytes can be found in sterile female sturgeon hybrids (Bortzev A.A., 1962, *The Lack of Possibilities for Reproduction in Hybrids (Asioter x sterlet)*, Soviet Acad. Sci., 144:6, in Russian). However, these oocytes are unable to mature and are destroyed by diffusion of the chromatin, infiltration of phagocytes, and destruction of the protoplasm. There is almost no difference in the overall histological picture between gonads of sterile hybrid fish at age 4 and those at age 8 years. This is contrary to the situation in *A. Bester*, where there is a constant and gradual development of sex cells in the

gonads. Further research on gonadal development in the sturgeon cultivated at the Dan Fisheries is necessary.

# 3.8 Chemical treatment trials

On November 12, 1994, sturgeon were brought from the Dan Fisheries to the MIGAL Laboratories and stocked into 1-m<sup>3</sup> tanks (Fig. 3.6). Fourteen tanks were stocked with 25 fish, each. The rate of water exchange was 90 l/h.

During the first two weeks, temperature ranged 17.5-20.5°C and oxygen 7.1-9.7 ppt. On December 12, muddy erosion entered the water system. A few days later, the fish began to change color (whiten) and became lethargic. Mortality began immediately, 31 fish died by January 11.

In a number of fish there were indications of disease, e.g., rust and spirulina in the gills. They were treated with formalin 25 ppm plus green malachite  $Cu_2(CO_3)(OH)$  at 15 ppm for six hours. Bacterial cultures taken from most of the dying fish were found to be *Aeromonas hydrofila/caviae* and *Flavobacterium*. Both bacteria are sensitive to Nitrofurazone, therefore they were treated with 3 g Nitrofurazone per kg food for six days (Jan. 9-15, 1995).

Following recovery, they were tested for resistance to Formalin at 25 ppm and Bromax at 0.25 ppm. For each type of medication, the fish were divided according to tank+trials and control. In each tank, there were at least 10 fish per m<sup>3</sup> water. Water quality was measured for levels of oxygen, temperature, ammonia, pH, and nitrates. During treatment, all unusual swimming and breathing behavior, as well as changes in color and dizziness, were recorded.

There were indications that sensual perception, especially smell, was damaged or disrupted in the medicated fish. Since the sturgeon's sight is not well-developed, proper functioning of other sensual receptors is required to locate food. Therefore, the times for allocating feed were changed for medicated fish so as to assure free and unobstructed approach to feed.

a. Formalin treatment @ 25 ppm for 8 h in 2 tanks containing 10 fish/tank plus one control tank. Oxygen content was 14.2 ppm, temperature  $17^{\circ}$ C, ammonia 0 ppm and nitrates 0 ppm. The treatment was administered from 09:00 until 15:00 with water quality parameters remaining constant. The next day, at the 07:30 feeding, all fish approached food with no trouble, change in color, or manner of swimming.

b. Formalin treatment @ 25 ppm for 6 h. Identical to experiment (a), except that feeding resumed 5 hours after a total water exchange. Here, too, the fish approached food with no problem.

c. Bromax treatment @ 0.25 ppm for 8 h in 2 tanks with 10 fish/tank plus one control tank. Oxygen content was 14.0 ppm, temperature  $17^{\circ}$ C, ammonia and nitrates 0 ppm. Bromax was diluted 1:100 and given in doses of 15 cc per 600 m<sup>3</sup>. The fish were treated from 09:00 until 15:00 and fed the following day at 07:30. All fish approached food with no difficulty, change in color, or manner of swimming.

d. Bromax treatment @ 0.25 ppm for 6 h. Identical to experiment (c), except that feeding followed 5 hours after a total water exchange. The fish approached food with no problem. All food was consumed during the night.

This initial research showed that the Dan water quality was suitable for sturgeon cultivation and that the concentrations of the two medications used were more or less suitable for treating various illnesses and parasites that might afflict the sturgeon (Table 3.4).

Table 3.4. Sturgeon mortality following experiments a-d.						
28.1.95	29.1.95	30.1.95				
Tank treated w/Formalin a	1	0				
Tank treated w/F ormalin b	1	0				
Tank treated w/Bromax c	0	1				
Tank treated w/Bromax b	1	2				
Control tank	2	0				

Additional research was required during the second year to improve methods of disease prevention and treatment. The following medications were examined: Formalin at 25 ppm, bromax at 0.25 ppm, Yaraqron [green malachite  $Cu_2(CO_3)(OH)_2$ ] at 0.26 ppm, copper sulfate ( $Cu_2SO_4$ ) at 1-2 ppm (according to the hardness of the water), grain salts at 0.1%, nitrofurazone at 5 g/m<sup>3</sup>, and Oxytetracycline at 10 g/m<sup>3</sup>. On April 4, 1995, the fish were divided into groups of 25 fish weighing 40-400 g. Each tank had 700 l water with a controlled flow of 1.5 l/min. Fish of all sizes were placed in each tank. Prior to initiation of the experiments, the fish were examined for external parasites and all were healthy.

e. Formalin treatment @ 25 ppm. On May 17, 1995, 17.5 cc Formalin was introduced to one 700-1 tank for 6 hours. A second tank was untreated. The water valves were closed. The water temperature was 19.5°C, pH 7.35, oxygen 8.6 ppm, ammonia and nitrates were 0 ppm. No mortality or change in color or behavior was recorded. Food was served 4 hours after opening the water valve and no cases of disorientation or lack of appetite was observed, all the food was eaten.

f. Bromax treatment @ 0.25 ppm. On May 17, 1995, 17.0 cc Bromax was introduced to one 700-1 tank for 6 hours. A second tank was untreated. The water valves were closed. Water temperature was 19.5°C, pH 7.35, oxygen 8.6 ppm, ammonia and nitrates were 0 ppm. No mortality or change in color or behavior was recorded. Food was served 4 hours after opening the water valve and no cases of disorientation or lack of appetite was observed, all the food was eaten.

g. Green malachite  $Cu_2(CO_3)(OH)_2$ ] @ 0.26 ppm. On May 21, 1995, liquid Yaraqron was diluted at a level of 1:100 and injected into one 800-1 tank at 208 cc/800 l water for 6 hours. A second tank was untreated. The water valves of both tanks were closed. Water temperature was 19.0°C, pH 7.35, oxygen 8.9 ppm, ammonia and nitrates 0 ppm. No mortality or change in color or behavior was recorded. Food was served 4 hours after opening the water valve and no cases of disorientation or lack of appetite was observed, all the food was eaten

h. Copper sulfate (Cu<sub>2</sub>SO<sub>4</sub>) @ 2 ppm. On May 21, 1995, 1.6 g/800 l water (calculated according to the hardness of the water, 1.90 ppm) was introduced into two tanks for 6 hours. A third tank was untreated. The water valves of both tanks were closed. Water temperature was 19.0°C, pH 7.35, oxygen 8.9 ppm, ammonia and nitrates 0 ppm. During the 6-h treatment, two fish from each of the treated tanks displayed "slap-happy" behavior: rapid breathing, recumbent behavior expressed by remaining on the tank bottom. Movement towards food was slow. Feeding occurred after reopening the water valve 4 hours after treatment was stopped. Mortalities were recorded in the days following treatment (Table 3.5).

Fish mortality following copper sulfate treatment.							
Date/Hour	Date/Hour	Date/Hour	Total dead	Weight of dead fish			
21.5/19:30	22.5/07:30	22.5/15:00		(g)			
1 dying	1 dead 3 dying	3 dead	4	402.5, 80, 105, 87.5			
2 dying	2 dead	3 dead	5	245.2, 223.1, 95, 130.2, 85			
	Date/Hour 21.5/19:30 1 dying	Date/Hour         Date/Hour           21.5/19:30         22.5/07:30           1 dying         1 dead           3 dying	Date/Hour         Date/Hour         Date/Hour           21.5/19:30         22.5/07:30         22.5/15:00           1 dying         1 dead         3 dead           3 dying         3 dying         3 dead	Date/HourDate/HourDate/HourTotal dead21.5/19:3022.5/07:3022.5/15:0011 dying1 dead3 dead43 dying333			

Table 3.5.

i. Grain salts (NaCl) @ 0.1%. On May 23, 1995, two tanks were treated with 800 g grain salts per 800-l water for 6 hours. A third tank was untreated. Water valves of all tanks were closed. Water temperature was 18.5°C, pH 7.5, oxygen 8.9 ppm, ammonia and nitrates 0 ppm. No mortality or change in color or behavior was recorded. Food was served 4 hours after opening the valves and no cases of disorientation or lack of appetite were observed, all food was eaten.

j. Nitrofurazone @ 5g/m<sup>3</sup>. On June 7, 1995, two tanks were treated at 4 g/800 l water for 6 hours. A third tank was untreated. The water valves were closed. Water temperature was 20°C, pH 7.4, oxygen 8.9 ppm, ammonia and nitrates 0 ppm. No mortality or change in color or behavior was recorded. Food was served 4 hours after opening the valves and no cases of disorientation or lack of appetite were observed, all food was eaten.

k. Oxytetracycline @ 10 g/m<sup>3</sup>. On June 7, 1995, two tanks were treated with 16 g at 50% medication per 800 l water for 6 hours. A third tank was untreated. The water valves were closed. Water temperature was 20°C, pH 7.3, oxygen 8.9 ppm, ammonia and nitrates 0 ppm. No mortality or change in color or behavior was recorded. Food was served 4 hours after opening the valves and no cases of disorientation or lack of appetite were observed, all food was eaten.

On June 12, 1995, it was decided to conduct another trial of Formalin and Bromax, both of which put out strong odors and are thought to disrupt sensory perception of foods. As part of the experiment, it was decided that the fish would be fed immediately following the 6-hour treatment and that the water valve would be opened prior to introducing clean non-medicated water.

l. Formalin. Two tanks were treated at 17.5 cc per 700-l tank for 6 hours. A third tank was untreated. The water valves were closed. Water temperature was 20.5°C, pH 7.5, oxygen 7.5-8.5 ppm, ammonia and nitrates 0 ppm. No mortality or change in color or behavior was recorded. Food was served immediately after opening the valves and no cases of disorientation or lack of appetite were observed, all food was eaten.

m. Bromax. Two tanks were treated at 0.25 ppm for 6 hours. A third tank was untreated. The water valves were closed. Water temperature was 20.5°C, pH 7.5, oxygen 7.5-8.5 ppm, ammonia and nitrates 0 ppm. No mortality or change in color or behavior was recorded. Food was served immediately after opening the valves and no cases of disorientation or lack of appetite were observed, all food was eaten.

Conclusions – In the copper sulfate treatment, 25% of the fish exhibited signs of distress or mortality. No signs of distress or difficulty in finding food were recorded during other treatments, even immediately after treatment or after treatment with odor-emitting Formalin or Bromax.

### 3.9 Adapting feed for sturgeon cultivation

Feeding experiments were conducted with products prevalent on the market at the time. Since, according to relevant literature, sturgeon requires a high protein diet, trout, carp, and tilapia feed and combinations of the three were chosen for the trial. Trout feed contains approximately 40-45% protein, while carp feed contains 30-35%. Tilapia feed has a lower protein component, around 25%.

Preliminary results showed that the growth rate with trout feed, at a feeding rate of 2% of the body weight, was best (Fig. 3.7). The results suggest that to obtain the maximum growth rate, sturgeon must be fed a diet similar to that of carnivorous fish, containing a high percentage of both protein and fishmeal. In the preliminary experiment, the feeding rate was only 2% of the body weight. It is possible that this was less than required and reduced the growth rate.

Further experimentation was required to determine the optimal protein percentage and source (fishmeal, soymeal, or meat meal). Another important parameter was the optimal feed ration in relation to body weight. During the second experiment, three diets for sturgeon were examined: low protein (35%), trout feed (40%), and protein rich (45%).

The experimental model was based on 3 x 3 treatments with three trials of each treatment. Each treatment included 50 sturgeon with an average weight of 307-318 g. The food was served in pellet form (4 mm) from a hanging feeder at a daily rate of 4% of the fish body weight.

During the 46-day experiment, food consumption and growth rate were measured. The fish were individually weighed four times throughout the experiment: on July 19, 1995 (the start of the experiment), August 2, August 18, and September 3 (the end of the experiment).

The chemical composition of the experimental diets is presented in Table 3.6. The diets were prepared at the laboratory at MIGAL and transformed into feed pellets at the Tzemech Feed Facility. Although the diets were originally planned to contain protein concentrations of 35%, 40%, and 45%, chemical analyses conducted at MIGAL showed the concentrations to be 3-5% higher. During planning and design, efforts were made to standardize the nutritional factors in all three diets and, except for ash, there were almost identical percentages of fats and energy in the diets. Also, the ratio between calcium and phosphorous were similar in all three.

Table 3.6.

Chemical analysi	s of the experim	ental diets (% of	f dry material).
Component	35% Protein	40% Protein	45% Protein
Moisture	11.06	10.14	9.63
Dry material	88.94	89.86	90.37
Fats	13.09	13.52	12.95
Ash	7.42	8.27	10.03
Protein	38.8	43.42	51.37
Calcium	1.59	1.78	2.26
Phosphorous	1.08	1.22	1.47
Coarse cellulose	1.54	1.97	1.91
Energy (K/g)	5132	5172	5130

Table 3.7 summarizes the food consumption throughout the experiment. Although the amounts in the table are the amounts served (approximately 4% of the fish body weight), the researchers observed that the fish ate all the food that fell from the feeder into the water. The amount of feed was based on the previous weighing and remained constant until the next weighing of the fish. On the day that weight was measured, feed was not served. Table 3.8 shows the growth and the ratio between growth and body weight. Fish body weights are shown in Table 3.9.

Results showed no significant influence of the 5% difference between protein levels on the growth rate. All the fish in the 46-day experiment grew approximately 180 g. Although the fish that received the 45% protein diet grew faster, the difference was statistically insignificant.

A third experiment dealt with the digestibility of fishmeal, poultry meal, and soybean meal as a protein source in sturgeon feed. The sturgeon in the experiment weighed 500-700 g. Two groups of 9 fish each were maintained in a controlled environment in 600-1 plastic holding tanks. The temperature was 19-22°C and the air supply maintained an oxygen level of at least 5 ppm. Only healthy fish that ate immediately following the serving of the food participated in the experiment.

The experimental diets were composed of ingredients used to produce fish feed in Israel. In the first stage, a basic diet suitable for carnivorous fish such as salmon and trout (40% protein, 13% fats, 1.5% guar, 0.75% chromic oxide) was given to the sturgeon. After enough feces for chemical analysis was collected, half of the basic diet was changed. The

experimental diets were composed of 50% of the basic diet and 50% of either soybean meal, fishmeal, or poultry meal. All the experimental feeds were prepared at the MIGAL Laboratories and marked with chromium oxide ( $Cr_2O_3$ ). The daily diet was 4-5% of the body weight of the fish. Five days preceding commencement of the experiment, the fish were adapted to the diet. Feed was given at 12:00 and feces were collected at 09:00 the following day. The feces were maintained at -20°C.

The feed composition and feces specimens were examined at MIGAL. The feces were collected on petri dishes by stripping the stomach and collecting them from the water with a fine net immediately after excretion. Total protein, ash, lipid, course cellulose fibers, and energy concentrations were measured. Carbohydrates were calculated as the surplus between all the ingredients and the organic materials. Components of the diets are shown in Table 3.10 and of the experimental ingredients in Table 3.11. Components of the feces are shown in Table 3.12.

The protein and energy digestibility coefficients and the ratio between the amount of feed digested and the amount consumed were higher with soybean and fishmeal than with poultry meal but generally lower than in other carnivorous fish (Tables 3.13 and 3.14).

Group	Treatment	Jul 19-Aug 1	Aug 1-17	Aug 18-Sep 3
	(% Protein)			
1	35	0.6	0.716	0.782
2	35	0.6	0.714	0.814
3	35	0.6	0.724	0.808
Total		1.8	2.154	2.404
4	40	0.6	0.708	0.803
5	40	0.6	0.74	0.794
6	40	0.6	0.784	0.892
Total		1.8	2.232	2.489
7	45	0.6	0.704	0.75
8 •	45	0.6	0.72	0.816
9	45	0.6	0.756	0.864
Total		1.8	2.18	2.43

Table 3.7.

Table 3.8. Fish growth initial average weight 307-318 g

			in, initial aver				
Group	Treatment	Jul 19-	Aug 1	Aug 1-	-17	Aug 18	3-Sep 3
		g/day	% Growth	g/day	% Growth	g/day	% Growth
1	35	3.8	1.22	2.2	0.61	4.81	1.23
2	35	3.7	1.19	2.33	0.93	2.69	0.66
3	35	4.2	1.38	2.8	0.77	5.56	1.38
Average		3.9	1.26	2.78	0.77	4.35	1.09
4	40	3.77	1.24	3.73	1.05	4.25	1.04
5	40	5.00	1.64	1.27	0.34	3.63	0.93
6	40	6.46	2.1	3.73	0.96	4.38	0.98
Average		5.08	1.66	2.91	0.78	4.09	0.98
7	45	3.46	1.13	1.53	0.43	2.69	0.72
8	45	4.15	1.36	2.67	0.74	6.88	1.72
9	45	4.62	1.45	3.6	0.95	6.88	1.6
Average		4.08	1.31	2.6	0.70	5.48	1.35

Average fish body weight (g).									
Group	Treatment	July	19	Augu	ust 2	Augus	t 18	Sep 3	
		g	S.D.	g	S.D.	g	S.D.	g	S.D.
1	35	309	27	358	50	391	60	468	91
2	35	309	28	357	51	407	72	450	88
3	35	307	26	362	43	404	56	493	86
Average		308	27	359	48	400.7	63	470.3	88
4	40	305	29	354	43	410	52	478	70
5	40	305	34	370	54	389	62	447	74
6	40	308	32	392	57	446	63	516	81
Average		306	35	372	51	415	59	480.3	75
7	45	307	31	352	40	375	60	418	76
8	45	306	32	360	43	400	51	510	64
9	45	318	39	378	46	432	66	542	97
Average		310	34	363	44	402	59	490	79

Table 3.9.

	Diet				
Component	Soybean meal	Fishmeal	Poultry meal	Basic	
Total protein	47.38	42.52	41.75	`44.7	
Total lipid	8.30	11.82	16.91	13.2	
Course cellulose	4.20	1.59	1.50	2.8	
Calculated carbohydrates	30.88	29.81	26.98	28.57	
Ash	8.9	13.72	12.43	10.3	
Chromic oxide	0.34	0.44	0.43	0.43	
Total energy (cal/g dry matter)	4900	4794	5390	5111	

Table 3.10.Composition of experimental diets (% dry matter).

Composit	Table 3.1 tion of protein sou		ter).
	]	Protein source	
Component	Soybean meal	Poultry meal	Fishmeal
Protein	49.8	59.4	71.95
Lipid	1.33	10.28	7.41
Course cellulose	6.4	1.12	0.45
Energy (cal/g)	4623	4638	4786
Ash	3.56	22.64	18.51

	Table 3.12.	
Chemical composition (	6 dry matter) and energy of fecal specimens.	

		D	viet	
Component	Soybean meal	Fishmeal	Poultry meal	Basic
Total protein	23.40	19.50	25.30	45.07
Total lipid	2.80	3.83	5.80	3.87
Course cellulose	16.10	4.10	4.20	8.70
Calculated carbohydrates	41.33	38.38	26.17	18.13
Ash	15.40	33.10	37.7	21.50
Chromic oxide	0.97	1.09	0.877	2.73
Total energy (cal/g dry matter)	1665	2822	3005	3894

Tał	ble	3.	13	i.

Digestib	ility and energy co	befficients (%	() in experimenta	al diets.
Component	Soybean meal	Fishmeal	Poultry meal	Basic diet
Total protein	82.70	81.49	70.29	84.12
Total energy	88.10	76.24	72.66	88.00

Table 3.14.	
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Digestibility and energy coefficients (%) of protein sources.									
Component	Soybean meal	Fishmeal	Poultry meal	Basic diet					
Total protein	91.86	81.01	64.65	84.12					
Total energy	88.21	64.44	52.34	88.00					
Digested energy (cal)	4078	3084	2428	4498					

# 3.10 Sturgeon processing

3.10.1 Products

The sturgeon can be sold in a number of ways (Figs. 3.8, 3.9).

a. *Head*. The head is especially fatty and is used in soups. It can be used whole or halved, as is or following extraction of the gills. There is a popular Russian soup known as *ukha* that is served with or without vegetables and spices. The soup has many varieties and may be prepared from sections or the whole fish (for the wealthy).

b. *Tail*. The tail includes the area from the dorsal/anal fins to the caudal fin lobe. Its primary use is in soup. Tail fins are discarded in the production process.

c. *Steaks*. Steaks (50-300 g) may be prepared from all parts of the body, however they are usually taken from between the tail and pectoral fins.

d. *Fillets.* The boneless fillet is prepared with the skin as the skin helps maintain the shape of the cut during cooking. Fillets may be prepared as quarter, half, or third cuts. Sturgeon fillet, like other fish fillets, are used to prepare delicacies such as smoked and salted sturgeon.

e. *Underbelly*. Similar to other fish, the underbelly is considered one of the tastier sections as it has a high percentage of fat. It may be fried or smoked and is very popular.

f. Whole fish. Whole fish are prepared without the spine, abdomen, and liver.

g. *Fresh meat.* Following removal of the underbelly, pectoral fin, and spinal cartilage, fresh cuts include the belly, the rear end and pectoral fin, the head, the fat, and the liver.

h. Shashlik. Pieces of meat for preparation on a skewer.

i. *Salted or smoked*. Made from fillets, all smoked fish are first salted for several hours to 10 days. Smoked and salted fish may be processed by hot or cold smoke or a combination of both and have a long shelf life.

Tables 3.15-3.18 show the weights and percentages of sturgeon sections. There is an apparent advantage to large fish in amount of marketable flesh. The examinations were made on 11 fish, a sample not large enough to draw long-range conclusions, but the data can be used as general indicators. The deviations are within individual percentage points because the weights of the fish were in a relatively small range of 4-6 kg. These findings can be used to calculate costs and prices for fish within this range. The same kind of examination should be made for larger fish.

For steaks, only one 6-kg fish was examined and the following figures are based on this single sample. After removing the head, anal fin, and rear section, the body was divided into 12 steaks of 20 mm. In one day (8 h), one person can process 14 fish of 6 kg, producing 31 kg steaks (37% of the whole fish weight), 7 kg rear sections (8.6%), two small quarters of 6 kg each (14%), 12 kg head (14%), and 7 kg pectoral fin (8%). The number of hours required to produce 1 kg of steaks is 0.052 h, rear sections 0.012 h, two small quarters 0.020 h, head 0.020 h and pectoral fin 0.012 h.

For shashlik, only three 6-kg fish were examined and the following figures are based on this small sample. After removing the head, anal fin, and rear section, the meat was removed from the skin and cut into shashlik. In one day (8 h), one person can process 13 fish of 6 kg, producing 29 kg shashlik (37% of the whole fish weight), 7 kg rear sections (8.6%), 11 kg head (14%), 6 kg pectoral fin (8%), and 9 kg underbelly (12%). The number of hours required to produce 1 kg of shashlik is 0.06 h, rear sections 0.014 h, head 0.023 h, pectoral fin 0.012 h, and underbelly 0.019 h.

At 13 NIS per hour for labor costs, Table 3.19 shows the number of hours and labor cost for each sturgeon section.

## 3.10.2 Processing facility

The fish processing facility includes:

a. cleaning room  $(86 \times 40 \text{ m})$  that contains an electric saw, a freezer (-20°C), work benches, a meat grinder, vacuum packaging equipment, and a sausage press.

							Fish no.					
	1	2	3	4	5	6	7	8	9	10	11	Comments
Full length (cm)	94	86	87.5	95	94	93.5	97	99	97.5	98	95	
Whole fish wt <sup>1</sup>	6020	4050	3920	5120	4840	5480	6600	6225	5925	4905	5855	
Internal organs	390	255	145	160	225	300	5995	5605	5450	4270	5435	Discarded
Liver and fat	175	185	30	90	50	120	245	335	100	60	190	Sold
Clean fish wt <sup>2</sup>	5455	3610	3745	4870	4565	5060	1085	820	765	765	720	
Head	800	615	815	920	820	845	360	285	375	75 <sup>4</sup>	230	Sold
Anal fin	90	55	70	95	105	120	105	85	85	90	105	Discarded
Body wt <sup>3</sup>	4565	2940	2860	3855	3640	4095	4805	4700	4600	3415	4610	Sold
Body sections												
Rear	535	370	345	435	435	470	545	545	520	475	560	Sold
Pectoral fin	505	345	360	435	395	415	530	485		420	600	Sold
Underbelly	695	440	365	530	510	590	805	760	760	543	565	Sold
Fresh meat & skin	2295	1370	1385	1860	1675	2240	2770	2710	3795	1830		Sold
Skin		210	215								335	Discarded
Meat thickness (mm)	35	27	28	37	37	37	40	38	40			
Spine and cartilage	435	270	295	375	400		425	430	240	387 <sup>5</sup>	300	Discarded
Shashlik (meat only)		1165	1240								2045	Sold
Small quarters										675		
Steaks (unit)										12		20 mm
Labor and weighing (min/fish)							9	6	6	14	12	
Processed product wt												
Salted cuts	2090			1560	1694	1609	1795	1995				Sold
Cold smoked cuts												Sold
Smoked sturgeon												Sold

Table 3.15. Weights of sturgeon sections (g).

<sup>1</sup>Whole fish weight = weight when removed from water <sup>2</sup>Clean fish weight = weight after removal of fat, liver and abdomen <sup>3</sup>Body weight = weight after removal of head, fat, liver, abdomen and anal fin <sup>4</sup>Cartilage <sup>5</sup>In internal parts

		Boo	ly wt	Spinal co	ord & cartilage		portion		rters		oral fin	Unde	erbelly	Fres	n cuts <sup>2</sup>
Fish	Whole	g	% of	g	% of whole	g	% of	g	% of	g	% of	g	% of	g	% of
no.	fish wt	e	whole	U	fish wt	C	whole	U	whole	U	whole	U	whole	0	whole
	(g)		fish wt				fish wt		fish wt		fish wt		fish wt		fish wt
1	6600	4700	71.2	425	6.4	545	8.3	3730	56.5	530	8.0	805	12.2	2940	45
2	6225	4615	74.1	430	6.9	545	8.8	3640	58.5	485	7.8	760	12.2	2940	47
3	6020	4475	74.3	435	7.2	535	8.9	3505	58.2	505	8.4	695	11.5	2840	47
4	5925	4515	76.2	240	4.1	520	8.8	3755	63.4	520	8.8	706	11.9	3049	51
5	5855	4505	76.9	300	5.1	560	9.6	3645	62.3	560	9.6	565	9.6	3080	53
6	5480	4095	74.7	325	5.9	470	8.6	3300	60.2	414	7.6	590	10.8	2766	50
7	5120	3705	72.4	375	7.3	435	8.5	2895	56.5	435	8.5	530	10.4	2365	46
8	4905	3605	73.5	387	7.9	475	9.7	2743	55.9	420	8.6	543	11.1	2255	46
9	4840	3450	71.3	400	8.3	435	9.0	2615	54.0	395	8.2	510	10.5	2145	44
10	4050	2885	71.2	270	6.7	307	9.1	2245	55.4	345	8.5	440	10.9	1830	45
11	3920	2730	69.6	295	7.5	345	8.8	2090	53.3	360	9.2	365	9.3	1710	44
Avg	5358	3935	73.2	353	6.7	476	8.9	3106	57.7	452	8.5	592	10.9	238	47
	largest fisl	h													
1	6600	4700	71.2	425	6.4	545	8.3	3730	56.6	530	8.0	805	12.2	2940	45
2	6225	4615	74.1	430	6.9	545	8.8	3640	58.5	485	7.8	760	12.2	2940	47
3	6020	4475	74.3	435	7.2	535	8.9	3505	58.2	505	8.4	695	11.5	2840	47
Avg	6282	4597	73.2	430	6.9	542	8.6	3625	57.7	507	8.1	753	12.0	2907	46
Three	smallest fi	sh													
9	4840	3450	71.3	400	8.3	435	9.0	2615	54.0	395	8.2	510	10.5	2145	44
10	4050	2885	71.2	270	6.7	370	9.1	2245	55.4	345	8.5	440	10.9	1830	45
11	3920	2730	69.6	295	7.5	345	8.8	2090	53.3	360	9.2	365	9.3	1710	44
Avg	4270	3022	70.7	322	7.5	383	9.0	2317	54.3	367	8.6	438	10.2	1895	44
Salted	l fresh cuts	(dry)													
	6600													1795	27
	6225													1995	32
	6020													2090	35
	5480													1609	29
	5120								•					1560	30
	4840													1694	35
Avg	5714													1791	31

Table 3.16. Weights and percentage of portions of sturgeon.

<sup>1</sup>Quarter cuts = Body weight less meat and skin + pectoral fin <sup>2</sup>Fresh cuts = Body weight less meat and skin + rear portion

			Weigh	its and per-	centage of	`shashlik a	and steak.			
		Body	wt	Less:					Shashlik wt*	
Fish no.	Whole fish wt (g)	g	% of whole fish wt	Spinal cord & cartilage	Rear portions	Pectoral fin	Underbelly	Skin	g	% of whole fish wt
5	5855	4505	0.77	300	560	560	565	335	2185	0.37
10	4050	2885	0.71	270	370	345	440	210	1250	0.31
11	3920	2730	0.70	295	345	360	365	215	1150	0.29
Avg	4608	3373	0.73	288	425	422	457	253	1528	0.33
-									Steak	wt*
8	4905	3605	0.73						1830	0.37

Ta	ble 3.17.
ights and nercen	tage of shashlik and steak

\*Data on shashlik were taken from the largest and 2 smallest fish. Data on steak were taken from only one fish. The two small quarters that remained were unsuitable as steaks.

	Sturgeon sections (% of	whole fish weight).	
	Avg of 3 largest fish	Avg of 3 smallest fish	Deviation
Internal organs	6	5	1
Fat and liver	4	3	1
Spine & cartilage	6.9	7.5	0.7
Anal fin	1	2	1
Skin		5.5	
Fresh cuts	46	44	2
Head	14	18	4
Pectoral fin	8	9	1
Quarters	58	54	4
Shashlik	37	29	8
Steak		37	
Underbelly	12	10	2
Rear portions	8.6	9	0.4
Net fish	90	92	2
Fresh cuts	46	45	1
Salted cuts	31.33	31.33	0.0

Table 3.18.

Table 3.19.

					Table (	3.19.				
Num	ber of h	ours and la	abor cost	(New I	sraeli Sł	nekels) for	processin	g 1 kg stu	rgeon produc	ets.
Cut for:	Clean fish	Quarters	Fresh cuts	Head	Steaks	Shashlik	Pectoral fin	Rear sections	Underbelly	Fat & liver
Clean	0.27									0.001
fish										
Quarters		0.054		0.013				0.008		0.004
Fresh cuts			0.029	0.009			0.005		0.008	0.002
Steaks		0.020		0.020	0.052		0.012	0.012		
Shashlik				0.023		0.060	0.012	0.014	0.019	
Avg (h)	0.27	0.037	0.029*	0.016	0.052	0.060	0.010	0.011	0.014	0.002
Cost (NIS)	0.351	0.481	0.377*	0.211	0.676	0.780	0.126	0.147	0.176	0.030

\* The additional cost for salting fresh cuts is 0.544 h or 7.449 NIS.

b. drying room and warehouse  $(510 \times 120 \text{ m})$  that contains a UV ceiling lamp for sanitization, two work benches, and hooks on rails for hanging products. The room is darkened, has constantly controlled air  $(15^{\circ}C)$ , and minimal humidity. Depending on the type of product, a one-week salting period is followed by drying for 48 h or more.

c. cold smoking room (43 x 25 m) that is totally sealed, has a thermometer, and an opening to allow the introduction of smoke. The room is connected to the smoking oven in the central shed by a 7-m pipe (15 cm diameter). The length of the pipe was determined to allow proper cooling of the smoke prior to reaching the smoking room. The combustion material is wood or sawdust from various sources, according to the desired taste. Cold smoking lasts a number of hours. Within minutes after starting the oven, the room is filled with smoke and the temperature in the room is between 15-20°C.

d. two large cold storage rooms, one maintained at a temperature of  $4^{\circ}$ C for cold storage and processing (salting and soaking), the second for deep-freezing (-20°C).

e. central shed, containing two ovens for cold and hot smoking and combustion materials such as wood and sawdust.

The facility must maintain a high standard of sanitation to prevent contamination. To guarantee successful production, all tools are reinforced plastic or stainless steel. The facility includes the following equipment and tools:

- a. electric saw for cutting the sturgeon into sections
- b. four stainless steel work tables for processing the fish
- c. a small freezer and a large storage freezer
- d. a large meat grinder
- e. vacuum packing unit for packaging
- f. knives of various sizes and shapes
- g. brinometer and thermometer to measure salinity and temperature

h. combustion materials - sawdust, woodchips, logs of pear, apple, cherry, pine, etc., to produce smoke

i. salt. All salts derived from the sea are suitable. Salt must be completely dry during weighing.

j. potassium nitrate that is added in small amounts to the salt to prevent the development of bacteria and to provide color to the final product

k. sugar, either brown or white, added to soften the fish meat during the salination process

1. herbs and spices, added separately or together with the salt for flavor, such as cloves, rosemary, juniper, etc.

# 3.10.3 Salted and smoked products

a. Salting and soaking – Salting is part of the process to manufacture salted cuts. Processing in dry salt or a salt solution causes significant changes in muscular composition: the salt withdraws the water from the cells of the muscle and the water is replaced by salt. Salting can be done with dry salt or a salt solution and with or without additives such as sugars and spices. Sugar helps soften and color the meat. It also serves as a substrate for bacteria that decompose the sugar into organic acids that flavor the smoked sturgeon. The amount of added salt must be as small as possible yet sufficient to achieve the desired flavor, according to subjective judgment and tastes. The salting process causes a 17% weight loss as a result of dehydration. The loss in chicken reaches 20% and in beef 25%.

There are two approaches to salting fish: use of a high concentration of salt (80%) for a short time, and gentler salting over a longer period. The salt solution requires boiling water and adding a suitable amount of salt. Other additives (e.g., spices, sugars, etc.) must be boiled with a small amount of water and only then added to the solution. This process must be conducted at 4°C, a temperature at which the bacteria that cause decomposition are inactive. Salting solutions are re-useable as long as the solution is clean and clear, has no sour odor or blood residue, and there is no doubt whatsoever regarding contamination. Successfully salted

fresh cuts smell good and look fresh. Liquids should be allowed to drip out of the fish prior to smoking.

b. *Smoking* – Smoking as a means of preserving fish has a long history. However, few smoking methods and recipes from the past have survived. As the need for preserving food in this fashion decreased, methods were lost and forgotten. Smoking probably began in ancient times; man hung pieces of meat close to the ceiling to distance them from animals and discovered that those pieces that were affected by smoke were better preserved. Perhaps it was discovered that meat near the sea was better preserved than meat from other locations, because it was washed in sea rather than river water. It is indeed these two facts that inspired many recipes that combine smoking and salting.

Similar to salting, the smoking of fish reduces the amount of water in the fish meat. Chemical changes in the meat provide it with a resistance to bacteria and lengthens its shelf life. The special flavor of smoked sturgeon (fresh cuts) comes from the type of wood or sawdust used and the method of smoking, however, it is primarily influenced by the flavor of the soaking solution. The composition of the soaking solution is the fisherman's most closely guarded secret, specific to each product. The flameless burning of sawdust, woodchips, or logs releases smoke at a low temperature. The ensuing smoke is pressed upon the surface of the fish meat and penetrates it, bringing out the characteristic flavor and color. There are two methods of smoking; hot and cold.

Cold smoking is done over a period of time at  $15-20^{\circ}$ C. The cold smoking room is hermetically sealed. The oven that supplies the smoke is usually some 10 meters from the room. The smoke travels from the oven to the room through a pipe, cooling along the way. The products intended for smoking are hung in the room, the thermostat is set, and the door is sealed. Once the oven is activated, the room immediately fills with smoke and, depending on the product, this continues for several hours. Cold smoking is a relatively long process that cannot be hurried or shortened.

Hot smoking occurs at a hot temperature for a short period of time, primarily to fish that have already been cold smoked. The charcoal is ignited and brought to glowing embers. The smoke comes from branches placed on the embers. The branches (10-20 cm, 3-4.5 cm diameter) may be from apple, pine, pear, plum, or berry trees and should be totally dry. However, just prior to use, the branches are soaked in water so they are wet on the outside. They are then placed over the burning charcoals to produce the desired smoke. A metal plate with 5-6 cm holes is placed over the smoking branches and a rack with the fish above the plate. The smoking lasts approximately 2 h with the fish being rotated every 20 min.

A step-by-step description of the smoking process:

- remove the fish from the pond
- weigh the fish
- slaughter the fish, remove internal organs, weigh again
- prepare a soaking solution at 4°C
- place the sturgeon in the solution for the desired time
- remove the fish from the solution and hang to dry
- light the smoking oven
- hang the fish in the smoking room for the time desired

- remove the fish from the smoking room and place in a refrigerator for cooling. The final color and flavor will develop within 24 hours of the conclusion of the smoking process.

- vacuum pack and freeze

## **CHAPTER 4**

# CULTIVATION OF GUPPIES (*POECILA RETICULATE*) IN AN ENCLOSED WATER SYSTEM

# 4.1 Introduction

Commercial cultivation of tropical ornamental fish began approximately 70 years ago. The branch rapidly developed following World War II with the improvement in air transportation. Commercial cultivation is primarily concentrated in southeast Asia, particularly Singapore, and the USA, particularly Florida. The principal customers of ornamental fish are amateur fish enthusiasts whose knowledge of fish maintenance is limited. A key demand comes from community aquariums in which a variety of ornamental species are held together. During the past few years, there has been a continually increasing demand for ornamental fish throughout Europe and the USA. Millions of mollies, swordtails, flatheads, and guppies (Fig. 4.1) are sold to tropical fish enthusiasts annually. Of all of the live bearing (ovoviviparous) aquarium fish, they are the most popular. There are hundreds of live breeder farms in Florida, Singapore, Hong Kong, and Thailand.

In its natural state, the wild guppy (*Poecila reticulate*) is found in Trinidad, Barbados, Venezuela, Guyana, and various regions of Brazil, and is characterized by a multitude of shapes. There is limited documentation regarding the domestication of the wild guppy and selections to separate guppies with unique colors and tail shapes from the natural population. The swordtail, speartail, pintail, and fantail guppies, with their unique colors and tail shapes, were the first mutants separated and domesticated from the wild population in a process that began in the early 1930s. In 1941, an albino guppy was developed from a domesticated populace. In 1937, a number of wild schools of guppies were brought to Singapore in an effort to biologically exterminate mosquito eggs. The fish were placed in rivers and channels and adapted well to the new environment. The nurturing of guppies accelerated greatly in Singapore during the 1950s when a number of ornamental fish enthusiasts began to foster unique varieties of guppies with distinctive colors and fin shapes. The work was conducted in an intensive process of collecting mutants in which body and fin colors appeared together with a wide variety of body shapes and sizes. A number of breeding schools of pure linage were developed from these mutants.

Hahnel was the first to develop guppies with enlarged tails by using selected males. The guppies he developed were relatively large compared to those collected from natural habitats. This can be credited to Hahnel's growth conditions, methods of nutrition and nurturing. His guppies are known today as veiltail and have been further developed by other growers in a variety of colors and tail shapes.

In Florida, for example, Strenke and his wife worked to produce a guppy with improved coloring while maintaining Hahnel's tail shape. Further north, along the east coast, Alger developed a wide tail guppy. The creation of the American Guppy Association (AGA) and later the International Fancy Guppy Association (IFGA) significantly contributed to the development and distribution of guppies, particularly species with a delta tail (a tailfin with a spread of at least 60°).

In Europe, on the other hand, growers developed a guppy with a large body and a tail smaller than that developed in the USA, primarily because of differences in nutrition methods.

Common to all species of this order is the simplicity of care and procreation that create an especially large demand worldwide. The operation of a tropical ornamental fish farm combines a wide range of subjects such as filtration, water exchange, system cleanliness, nutrition, population density, treatments, sanitation, sorting, etc. All these issues are interdependent and impossible to separate. Partial implementation cannot result in success; if just one link in the chain of procedures is absent or inadequate, the whole cultivation process can fail. Every procedure conducted at the farm should be documented. Accumulated data help growers amass information and learn from past errors and allow professional advisors to diagnose the source of problems. At present there is no competition among Israeli growers. However, there is competition between Israeli growers and growers from countries such as Singapore, Sri Lanka, Hong Kong, Malaysia, Thailand, the Czech Republic, Poland, etc. By producing a variety of species in the right quantities, Israel will be able to successfully compete on the market. Coordination between Israeli farms will help create a more efficient and effective marketing program.

# 4.2 Biology of the guppy

The Poeciliidae family is common from the northern USA to the estuaries of the Rio de la Plata in northern Argentina. The origins of this family are in South America and the Caribbean islands of Barbados and Trinidad. The most extensive and diverse taxonomy of family members is found in Central America and Mexico. Family members inhabit a wide variety of habitats such as springs, lakes, creeks, seashores, and salty mangrove swamps. Species exist and reproduce in a wide range of temperature, in springs as hot as 44°C, indicating the high resistance and adaptability of the family to a variety of environmental conditions.

The shape of the guppy (Fig. 4.2) is determined in large part by the spinal column composed of many vertebrae. The head is comprised of a skull and jaws made of bone. The ribs are connected to the vertebrae and protect the internal organs. A bony structure known as the operculum covers each of the two gills. The fins protrude from the body in a number of locations. The fins are supported by two types of fin rays, one hard and undivided and one soft and branched. Shoulder blades and the pelvic bone assist in supporting the fins. The dorsal fin is often the source of the fish's beauty. It may stand erect, lie recumbent, or spread out beyond the tail fin. The two triangular fins on either side are the pectoral fins while the tail is the caudal fin. The fins on the underside of two fin rays that are so closely placed that they give the impression of being a single ray. The guppy moves almost exclusively by an 8-shaped tail movement. The other fins are important for directional swimming: upward, downward, and sideways. Melanophores, microscopic colored dots in the skin, create the guppy's colors. The number and organization of colored areas provide the large variety among species.

a. *Digestive system* – Guppies have teeth in both the jaws and the roof of the mouth. They have a tongue, behind which is the pharynx, located between the two gills. The gills permit water to enter the gill chambers. The digestion process begins in the pharynx and continues as food passes through the gullet or esophagus to the stomach. Food then passes from the stomach into and through the intestine, a winding canal in which digestion continues. The remaining residue is excreted through the rectum, forward of the anal fin. The liver produces bile, an important liquid for digestion, and a spleen that aids in cleansing the blood. The rapid digestion is evidence of the large quantity of food that guppies eat.

b. *Circulatory system* – The guppy's heart (Fig. 4.3) is composed of two chambers: the atrium and ventricle. The blood is pumped by contraction of the ventricle to the gills where there it is enriched with oxygen and releases carbon dioxide. The oxygen-enriched blood is then carried by the dorsal artery and auxiliary blood vessels throughout the body in an ever-expanding arterial network. Oxygen-poor blood, loaded with  $CO_2$ , returns to the heart through a system of veins. The lymphatic system assists in collecting the blood from the capillaries and bringing it to the heart where it is returned to the gills. The gills, because of their exposed location, tend to be more sensitive to disease. Each gill is covered by a bone arch that holds a gill rake (a comb-like structure) and a gill filament, a capillary structure that is washed by incoming water from the mouth that exits through the operculum.

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c. *Air saccule* – The guppy maintains its equilibrium and location in the water by using the air bladder (saccule) within its body. There are many blood vessels in the walls of the bladder that help the bladder maintain gasses by regulating the oxygen supply. Hence, the saccule acts as an air reservoir as well as the organ that controls the body volume. Experiments have shown that the air saccule contains a higher percentage of  $CO_2$  than the regular atmosphere. When the guppy needs oxygen, it rises to the water surface by inflating the air saccule, and exposes its body (except for the tail).

d. *Nervous system* – The brain of the guppy with the neural spinal cord passing through the vertebrae is the center of the nervous system. The guppy smells through neural centers in smell sensory organs unrelated to the respiratory system. It hears by way of two empty cavities at the sides of the head containing otoliths that translate sound vibrations and aid in maintaining equilibrium. Guppies have a sense of taste that allows them to taste their foods.

e. *Secretion system* – The kidneys filter the blood, secreting by-products that were transferred to the bladder by the ureters. Both the kidneys and bladder are small. The bladder is emptied through the urogenital duct located behind the rectum.

# 4.3 Life cycle of the guppy

From the age of 80-90 days, the guppy begins the process of sexual maturity. The male usually enters this stage earlier than the female. From the age of 3 months, the fertile female is capable of giving birth every 35-40 days to approximately 20-100 fingerlings per spawning. After achieving sexual maturity the female continues to grow slowly until the age of 2 years and continues to be fertile until an average of 2.5-3 years.

a. *Spawning* – The birth of live creatures occurs in many vertebrate classes. In fish, this method of breeding is known as spawning and appeared quite early in the course of evolution. Although there is an impression that this manner of breeding is successful, the majority of fish species procreate by laying eggs. Among the 800 species of cartilage fish, there are only 420 ovoviparous species that reproduce by live births. Amongst the 20,000 species of Grammicolepidids, only about 510 spawn their offspring.

Ovoviparous females store fertilized eggs in their bodies until the eggs are completely developed and fingerlings hatch. All stages of egg development, including incubation and nutrition of the young larvae, occur *in vivo* in the female body. Germinal epithelium, minute cells that meiotically divide, are deposited in the ovary. Following a number of meiotic divisions and development including the appearance of the vitellus, these cells become eggs. The developing offspring are cultivated within the egg and do not receive nutrition from the mother's body through a placenta. They receive oxygen and discharge  $CO_2$  through minute capillaries that line the follicles in which they develop. The exchange of gases is conducted between these capillaries and minute veins in the mother's reproductive system. The developing offspring hatch and pass through a narrow opening that is common to the sexual organs and urinary tract and is known as the urogenital pore. Usually, spawning fingerlings initially move downwards, then rest for a period before resuming activity.

b. *The male guppy* – The male guppy, similar to other ovoviviparous fish, has a gonopodium. At birth, the anal fin of the male is similar to that of the female, however, as the male matures the fin gradually becomes a reproductive gonopodium that, in a relaxed state, is narrower and longer than the anal fin of the female (Fig. 4.4). As the male grows, the pelvic fin moves closer to the front of the body than it is in the female (Fig. 4.5). When the growth of the male is complete, the nine fin rays crowd together. At the edge of the third and longest ray, there is a covering with a hook that faces the rear of the fish. There is no conduit in the gonopodium. Rather, by drawing the gonopodium and two pelvic fins forward into a duct-like shape, sperm are transferred from the nearby urogenital pore into the female. The spermatophores implanted into the female are transferred to the oviduct.

Sperm is produced in the male fish in the germ plasm in the testicle, also known as the spermary. A short conduit connects the testicles to the urogenital pore. Spermatogenesis in fish differs somewhat from that in mammals. The testicles are filled with tissue composed of primordial sex cells that undergo infinite meiosis to create identical cells. Although it was once thought that they wear out due to exhaustion, a series of transplantation experiments in mammals showed that these cells are worn out by aging. Cells that are created from the primordial sex cells are known as spermocytes and, following the growth of a tail, spermatozoa or sperm. Sperm cells are stored together in cavities known as spermatocysts from which they are transferred packed in capsules known as spermatophores through the *vas deferens* or sperm duct to an area close to the urogenital pore known as the emmectory.

This process of sperm transfer from the male to the female occurs so quickly that the human eye is incapable of discerning it.

c. *The female guppy* – The female produces eggs or ova. The sperm is received through a pipe known as the oviduct, although this actually is a misnomer, as the female does not lay eggs and therefore has no need for a tool of transport. In the oviduct, the sperm releases itself from the membrane enveloping it, apparently with the help of a secretion from the female's body that weakens the membrane. The sperm are then absorbed into the folds of the organ where they wait until the creation of ova. Sperm cells are maintained within the female for up to eight months during which the sperm may be used for 3-4 spawning cycles.

The creation of ova is similar to that of sperm, by the division of primordial cells. The number of ova created is a decisive factor regarding the number offspring spawned. There are thousands of spermatozoa for each egg and the number of sperm must be quite large, as one insemination is enough for a number of spawns. Even if the female is inseminated by a male and gives birth to his offspring, another male may still inseminate the female. The fresher sperm will inseminate, it seems, only the following batch of ova.

The female can store 75 fingerlings together with their embryonic membranes in her stomach. The vagina grows and darkens during the gestation period that usually lasts 2-8 weeks, depending on the species, season and water temperature. Under optimal conditions, gestation lasts 22-24 days and offspring are created every 27-30 days. The 5-6 days separating each gestation and spawning is the time required for oogenesis. Insemination in guppies is very interesting. The sperm enters the ovary and waits for the maturation of the first available batch of eggs: A second batch will not mature until the last of the first batch of fingerlings has left the mother's body. Following spawning, new ova quickly develop and are inseminated by the waiting sperm. Each female spawns 10-40 live fingerlings.

d. *Sexual maturity* – In captivity, if spawning occurs in a tank populated with other fish, the fingerlings will be eaten immediately upon release from the mother's body. To obtain a high survival rate, the larvae must be defended from birth. A multitude of hiding spots in the birth tank and immediate transfer to separate nursery tanks according to size increases the chance of survival.

Mating between fingerlings has been observed by age 30-35 days and is visible by the larger body of the female and a distinguishable gravid spot (Fig. 4.6), unseen in males. Males are distinguished by their developing gonopodium. Unique colors or black spots develop to a recognizable point by day 40, however, in fast-developing species, tail colors may be noticed as early as day 28. In this context, there are significant differences between species. Some species mature as early as two weeks before others. It seems that the characteristic pigments of black, red, and yellow depend on hormones produced in the testicles. Female hormones depress the colors that are seen on males.

Females that are exposed to male hormones develop male characteristics. Maturing females that receive sperm from adult males can carry it until they fully mature. Virgin female guppies have a recurring period of 4-6 days in which they are willing to accept a male. To indicate this period, they place their bodies on a diagonal angle in the water. For the most part, even though a male might pursue her, this phenomenon occurs in temperatures no less than 27°C. Evidence such as this and others indicate that the guppy is more fertile in warm

tropical waters. Evidence also exists according to which a male, a day after being placed into the tank, produces a substance that causes the female to enter into the diagonal position even in water colder the  $27^{\circ}$ C. Some growers reported better results with shy females when a higher ratio of males to females was used. This may be the result of a higher concentration of the stimulating substance.

# 4.4 Selection of phenotypes (external attributes) and genotypes (genetic structure)

Stringent selection and nurturing are required to create a parental school. It is insufficient to choose parents only according to desired qualities such as body structure, anal and dorsal fin sizes, and colors. Such selection was tried on fish farms in northern Israel, resulting in a high percentage of fingerlings that did not resemble the parents and significant financial losses to the growers.

Selections are made when a grower observes a fish with unique qualities of color, shape, etc. If the quality is attractive, it is separated from the rest of the fish and used to create a new lineage. Presumably, such exceptional fish would not survive in nature; an unusual appearance attracts predators and an exquisite fin might limit movement or impede reproduction, locating of food, or escape from predators. Almost all lineages of ovoviviparous fish in pet shops are mutants or hybrids created by amateurs. Usually a mutant with desirable qualities is repeatedly inbred with females of the same brood and their descendents until a clean lineage with the desired quality is obtained. Another technique, more commonly used by cultivators, is to inbreed the mutant male with his female parent and then with one of their descendents that has the desired quality. By using this method, it is possible to obtain clean lines of guppies such as blues, reds, greens, blacks, etc., within a few generations. Professional insemination facilities operate in a similar fashion.

Commercial insemination schools usually include 150-250 females and 30 males in a production unit of 1m<sup>3</sup>. In contrast, insemination schools that produce parental schools usually contain two females and one male. Young fish should not be chosen as parents; the selection must be made when the fish are at least 4-5 months old. In principle, only one quality is nurtured in each breeding. For example, if the objective is to obtain guppies with a larger body, parents with the largest bodies will be bred. If the objective is to obtain a certain color, then the most colorful males of that color will be used. Selection of males for the desired trait is relatively uncomplicated.

Females, however, must be carefully selected over a period of time. Many growers failed to realize this; they selected unsuitable females and obtained fingerlings of reduced value. In general, the body of the female must be compact, attractive, and not elongated. The anal fin should be adequately colored, with a wide spread, whole and symmetric in shape. The majority of selected females have a delta tailfin (a tailfin with a spread of at least  $60^{\circ}$ ) although females with shark tails often spawn fingerlings with excellent delta fins. Attractive females with exquisite colors do not always spawn attractive males. On the other hand, physically wretched males have produced some of the most attractive and high-quality females.

Males are usually placed in the mating tanks 24 h before the females so that they can establish their territory. If the females are placed in the tanks before the males, they tend to become aggressive and attack the males. The fish that is placed first into the tank tends to attack those that follow.

Different species should not be stocked in the same tank; even fish of the same gender. Different genetic lines should not be stocked together, primarily because of variations in susceptibility to disease, growth rate, etc.

The methods for maintaining or improving a particular trait of a certain strain vary from grower to grower and do not depend on the lineage. The foundations of all reproduction programs are identical and based on one of three commonly accepted reproduction methods: inbreeding, line breeding, out-crossing. In each case it is possible to hold the parents in 10 or 20-1 tanks equipped with a filter and some sort of shelter that provides protection for the

fingerlings from their hungry parents. The majority of growers maintain their species through line breeding.

a. *Inbreeding* – In this method, the grower attempts to purify his guppy lineage, in other words make it genetically homozygotic, so that all offspring will be similar to their parents. Inbreeding is usually between siblings, offspring and parents (backcrossing), or offspring and aunts/uncles. The best coupling depends on the genetic characteristics and quality of the lineage. Since inbreeding is intended to maintain distinctive characteristics, care must be taken throughout the process not to develop undesired characteristics. Special consideration must be given to the selection of the parents. They should be suitable in terms of either color or shape and size. Parents must be without any defect in the desired characteristic. When breeding to achieve a large body, the parents should not be genetically close (e.g., offspring and uncle) since a close genetic kinship causes a loss in weight. A number of couples of varying kinships and a variety of breeding methods should be used. Especially in pure-lines, weight or color loss might occur between close kin. In nature, the most resistant and fertile of the species become reproducers. While resistance and fertility are desirable traits, they usually do not appear together with more commonly desired traits of shape, size and color.

To assure that the chosen method of reproduction does not produce undesirable results, the process must be carefully documented and changes be implemented as required. <u>Under no circumstances should inbreeding be the exclusive method of maintaining a selected lineage</u>. If the line deviates, incorrect selection of parents may be indicated. In such a case, backcrossing may help to restore the lost genetic quality.

b. *Line breeding* – In this method, two or more sets of related parents (e.g., one or two males with two or more females) are kept together in the same pool. The offspring of each set of parents (from the same group) are held separately to protect the lineage's genealogy, be able to trace the offspring, and control any undesirable crossbreeding with unsuitable parents. Occasionally, crossbreeding is required between related sets to maintain the general quality of the species. When procuring a threesome for a new lineage it is best that the females come from two separate lines and that the male come from a display line or a line that provides the best examples of the most desired qualities (in this way it will be possible to know to what to aspire). All data such as the name and origin of the species, the ages of the fish, and the date of breeding should be documented. Each female and her genealogical lineage should be marked, for example "Line A" or "Line B". It is to be expected that 6-10 aquariums per line will be of a specific color.

Attention should be paid to the quality of each group of offspring and only two females of each should be held if space is limited. Each tank holding a group of offspring should be marked with its familial lineage (e.g., F1, F2, etc.), day of spawning, reproduction cycle number, and method of feeding. Line maintenance includes frequent feedings of rich nutrition (section 4.7), weekly water exchange, distancing undesirable fish, and transferring growing fish to larger aquariums. The majority of females should be separated from the rest of the school to allow the male fish room to grow. To protect against disaster, disease, and technical problems, a number of females may be left together with the males from the same line to save the line if the male dies. A well-balanced program of line breeding will include a variety of combinations of inbreeding, semi-cross line breeding (crossing fish from the same region or farm), and, often, outcrossing (fish from other farms or countries).

The principles of inbreeding may be applied to preserve a particular characteristic. It is important to preserve at least two lines from identical and related species while cultivating different characteristics so that there is diversity between them. Fish lineage may be cultivated according to size, shape, color, fertility, courage, behavior, changing colors, etc. The most difficult quality to preserve is not size, as many growers tend to believe, but shape and color of the body and fins. The loss of a specific shape in a particular lineage will hinder advancement in a cultivation program for generations to come.

Maintenance of two similar yet separate lines ensures genetic purity (if one line losses the homozygosis of a desired trait, it may still be intact in the second) and allows crossbreeding between two lines to combine related genetic characteristics and obtain a larger hybrid for exhibition that will potentially serve as a repository of parents. Since it is difficult to preserve in any one fish all the desirable traits while removing all the undesirable, this is the safest method known for preserving or implementing methodical and planned improvements. When crossbreeding two related lines, it is possible to obtain a repository of parents that may serve to improve each of the reproductive lines, especially in those that have been inbred over a long period.

The preservation of separate lines is likely to improve many aspects in colored species, e.g., symmetry, background colors to dual-colored tailfins, density in semi-black body colors, fertility, size and shape of the dorsal or anal fin, and body shape. In red-tailed species such as half-black yellow, half-black-AOC and half-black pastels, the red color can be removed by careful selection of parents or gradual purification of the lineage in gradually increasing intervals.

c. *Outcrossing* – Application of this method requires detailed instruction by an experienced grower or field instructor. Prior to crossing, the line should be inbred for a number of generations according to species. Luke Roebuck related how he started with a black species that was unfit for exhibition since it had several defects that required repair. Roebuck decided to develop a new species by outcrossing. The species was carefully line bred for 5-8 generations before outcrossing. Several good species have been stably preserved many years through line breeding, without any need for outcrossing. Growers should obtain all documentation regarding fish purchased for reproduction and ask the seller what is required to maintain the acquired characteristics of the fish.

The outcrossing method serves as a starting point for a desired species to create a larger hybrid suitable for exhibition or for introducing new genetic characteristics into an existing lineage. There is a 10-20% chance of obtaining the preferred result under optimal conditions. Twin species should be used if results are desired in early F1 or F2 generations. The grower should define the objective of the breeding before selecting the species. It is recommended not to deviate greatly from the original genetic qualities of the species selected for improvement. If the desired results are obtained after the initial breeding, they should immediately be based during the next 3-6 months. Early signs of desirable characteristics should be ascertained (e.g., growth and activity rates, color, size). Suitable parents should then be selected and backcrossed with the species selected for improvement. A repeat breeding should be conducted on both species since it will not yet be known which will provide better results. If both repeat breedings are successful, it is possible to begin developing a new lineage of the original species. During experimentation, two or more original generations (not crossbred) should be preserved in case of malfunction. Some of the fish in the species should be transferred to an associate grower or farmer willing to assist in their maintenance. With time, the species will undergo small changes. However, if the grower stringently follows the rules of reproduction, the species will maintain their trait and serve as a source of improvements of the original species.

## 4.5 Cultivation of ornamental fish in Israel

Producers of ornamental fish in Israel have several advantages over producers in other areas of the world. The proximity to Europe means fish are shipped a shorter distance in less time than, say, from Singapore, 10-15 h as opposed to 35-40 h, at a cost of US\$1.5-2 per kg instead of US\$4-5. The shorter shipping time allows denser packing of fish. Again compared to Singapore, 2-3 times as many fish can be packed in the same size container, if the fish are shipped to Europe from Israel. Israel has excellent scientific support systems and technology, including recycling systems that allow better control of fish health.

On the other hand, in contrast to Singapore, a limited number of species are currently raised in Israel. Israel is new in the world ornamental fish market (5 years as opposed to 40 years of fish growing in Singapore) and has a limited clientele and limited experience. A solid marketing system has yet to be established and there is lack of organized growers and sharing

of knowledge. Since there is no continuity of supply, supply from Israel is unreliable. Labor costs are high.

There are three fish farms in the Galilee that cultivate guppies - the Yitzhak Farm (1998) at Kanaf, the Hefetz Farm (1999) at Korazim, and the Center of Ornamental Fish at Kibbutz Kfar Szold. Each farm cultivates 5-7 guppy lines, some of which are unique to the farm on which they are raised. Most of the parental schools on the fish farms were purchased from other farms and did not undergo a selection process or minimal nurturing.

Today, purification of parental schools is being implemented in two ways: (a) on fish farms, by removing unsuitable parents, selecting parents at a more advanced age (5-6 months), and eliminating weak parental schools, and (b) at MIGAL, by nurturing two guppy lineages since June 2000, a clear blade and a black/yellow.

At MIGAL, the nurturing is conducted in the following stages: (a) receiving mature black/yellow guppies, (b) selecting suitable males and females, (c) inseminating 10 females with a single male, (d) separating the males from the females after 10 days, (e) placing the females in spawning cells on day 25 (presumably, the females were not virgin and at least some were impregnated by males before beginning the program), (f) cultivating spawned fingerlings in separate nursery tanks, (g) selecting fingerlings according to color for the next parental generation, and (h) comparing the distribution of color in the fingerling and parental populations.

Maintaining pure lineage of guppies is a challenge. This is most difficult in the albino line where there is a problem of sterility. To prevent repeated inbreeding, albino males are bred with gray females. Maintaining purity in the red guppy is also difficult as miniature gray or black spots on the fin are enough to significantly harm the purity of the lineage in the future.

In all cases, density should be no greater than one fish per liter, at least until the water quality and daily routine are stabilized. The age range of fish held in one tank should be no greater than 7-10 days, less is recommended. After approximately 5-6 weeks of cultivation, fish should be sorted according to size and large fish separated from smaller. To adequately populate two subsequent tanks, sorting should be conducted from two tanks of fish of a similar size. Fish should be sorted after a fast of 24 hours in the tank in which they were raised, provided the water quality is suitable.

#### 4.6 Description of cultivation systems

#### 4.6.1 Greenhouse structure

Fish farms may be established in a variety of structures, however greenhouse/tunnel structures (Fig. 4.7) are relatively low cost and can absorb heat and natural light during winter.

According to economic calculations of MIGAL researchers, Yochai Yechieli and Yehuda Yehudah, the recommended size for a family farm is 200 m<sup>3</sup> of water volume on an area of at least 500 m<sup>2</sup>. If the farm is established in a plastic tunnel, the braces should be 3" in diameter, the opening 10 m wide and 4 m high. Because of the sensitive nature of fish cultivation, the tunnel should be constructed of a reliable material, such as reinforced plastic sheeting, that is durable even in inclement weather. The concrete floor should have channels along the sides of the tanks to allow water exchange and drainage (Fig. 4.7C,D). Sanitation must be taken into consideration when designing a floor of this type, as a high level of sanitation is required. Within the facility, 30-50 m<sup>2</sup> should be allocated to routine daily activities, containing two aquariums, one for cultivating fingerlings to create a school of breeders using genetic programs and virgin females, and one for quality control prior to marketing.

A separate 30 m<sup>2</sup> structure will serve as packinghouse, warehouse, office and field laboratory, and contain a microscope and laboratory equipment for examining fish, a computer and software for management and control, a freezer for foodstuffs, a telephone and fax, pH and oxygen meters, an analytic scale to measure medications and food additives, water quality kits, medications, cleaning materials, office equipment, and professional literature.

The greenhouse/tunnel facility must be shaded by a 90% shade net to prevent development of algae in the water. In some farms, greenhouse containers are covered to prevent overheating. In winter, the structure should be shaded from the interior to allow solar radiation to penetrate the greenhouse/tunnel (Fig. 4.7E), effectively heating and dispersing the heat throughout the tunnel. During the summer, the net also helps reduce radiation. In addition, two large fans (Fig. 4.7F) placed at the ends of the greenhouse/tunnel expel hot air and help maintain a lower inside temperature. The tunnel or greenhouse walls can be raised for greater aeration.

#### 4.6.2 Tanks and water quality

Fish should be maintained at a temperature range of 23-29°C, in semi-hard water with a pH of 6.8-7.4 and no poisonous residues (ammonium nitrate and salts). In addition, there should be sufficient oxygen in the water (maximum solubility in the optimal range of temperatures is 6.5-9 mg/liter) and lighting for 12-14 hours. Sunlight during a number of hours in the day stimulates the production of Vitamin D. The most important water quality factors are hardness and pH.

a. *Type and size of tank* – There are a variety of options for suitable cultivation tanks including plastic Dolav or fiberglass tanks (Fig. 4.8A) and portable plastic tanks hung on metal frames (Fig. 4.8C). The recommended volume for a cultivation tank is 0.5-4 m<sup>3</sup>, while sorting tanks for shipments should be larger. The breeding baskets (80 cm radius x 40 cm depth) contain no more than 200 females and 30 males. Several baskets can be placed in a single tank, but not too close to one another (Fig. 4.8B). The size of the tanks should be limited. Tanks of a large volume are not recommended because of difficulties in controlling the system and harvesting the fish. Also, ornamental fish must be stocked in similar size and gender groups, requiring many small tanks. Tanks that are larger than 5 m<sup>3</sup> can be partitioned into smaller compartments.

b. Chemical quality - Aquarium water contains organic waste products originating from fish secretions, food residues, and vegetable and animal decay. When these materials decompose, they form a nitrite  $(HNO_3)$  that is poisonous to fish. The small streams in which fish live have a constant exchange of clean water so there is no build-up of poisonous remains such as ammonia, nitrites, and salts (NaCl). In tanks, filters containing bacteria are used to control the nitrites. The bacteria turn the nitrite into a less poisonous nitrate compound by a process that requires oxygen. In tanks with effective biological filters, water should be continually exchanged at a daily rate of 5-10% of water volume. When the water is turbid or density is higher than usual, the water should be exchanged at a greater frequency. Without proper water replacement, fish mortality can increase and fertility stops. The most important element in limiting nitrites is preventing over-population in the tanks. High levels of nitrites are often found in new tanks. To prevent nitrite poisoning, new tanks should operate with a filter for two weeks prior to stocking. Nitrite distress is can be diagnosed in fish if they are observed to be gasping. In this case, a third of the pool water must be replaced immediately with water at the same temperature as the remaining water or one sixth if the new water is colder than the remaining water. To aid in converting nitrites, oxygen emitting aquatic plants may be placed in the aquariums.

Water quality is controlled through a series of filters placed above each tank. Since most of the sanitation problems in fish stem from inferior water quality, these filters are of great importance. The filters are constructed from a tank or bucket hung on boards or hooks above the tanks (Fig. 4.8E). The filter has sealed sides and a perforated bottom and is packed with bacteria. The filter is connected to a 8 mm pipe sunk in the water through which air is forced, creating an elevator of air on which water is carried to the top of the pipe and filtered through the bacteria material back into the tank. It is highly recommended to prepare the bacteria-enriched porous substrate in advance by allocating a tank to the preparation of the biological substrate. Bacteriological colonies can be created in the absence of fish by adding ammonia or a proteinic material that decomposes into ammonia to the water in the specially

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allocated tank. In this manner, when a biofilter needs replacing or the production capacity is expanded, a biological filter is already available. To reduce biological stress, tanks with new filters should be initially stocked at a low density that will be gradually increased, or with small fingerlings whose growth will improve the activity of the biological filter. In addition to sanitizing the pool and filter, the substrate should be replaced following each growth cycle.

Ammonia (NH<sub>3</sub>), nitrate (NO<sub>3</sub>), nitrites (HNO<sub>3</sub>), and oxygen (O) levels should be randomly sampled weekly. In tanks with new filters, water quality should be tested at least twice a week until levels stabilize. The rate of water exchange can be raised to improve water quality. Twice weekly measurements are also recommended for tanks given medical treatments that can impair the effectiveness of the biological filter or suspected of inferior water quality. Careful attention should be paid to changes in the appearance or behavior of the fish.

c. *Temperature* – The water temperature should be maintained at 24-26°C. Minimum and maximum temperatures should be recorded daily. Dzikowski et al. reported that temperature variances ranging 20-32°C influence intervals between spawnings and not the number of spawned fish. The optimum spawning rate occurred at 25-27°C while at 32°C there was increased mortality of females and offspring, degeneration of ovaries, and reduction in the number of fish spawned.

To maintain a regular and stable water temperature throughout the winter, the water in the tanks should be heated (Fig. 4.9) through a series of natural gas or diesel-operated heat exchangers composed of at least two heater ovens equipped with gas or diesel burners that work at an operating strength of 120,000 kcal (approximately 1 kcal per 1.5 l water). The system should have at least two heaters so that the second will serve as a backup for increased heating or in the case the first breaks down. The system includes a chimney, expansion tank, and centrifuge pump that leads the water through central metal plumbing to a series of 16 mm polybutylene pipes that serve as heat exchangers. All dry piping (piping that contains no water) must be insulated to prevent heat loss from the system. The system should also include a series of mechanical and electronic valves, one electronic thermostat for each 20 m<sup>3</sup> of water, metal or 2' polybutylene main plumbing, and 32-40 mm polybutylene secondary plumbing along the length of the pools. Thermostats and electric valves at the opening of each tank control the water flow according to sensory devices that discern changes in the water temperature.

d. pH – The pH level is an expression of the acidic or basic condition of the water. A pH of 7 indicates that the water is neutral. Less than 7 indicates acidic, while water with a pH greater than 7 is basic. Since the pH scale is logarithmic, water with a pH level of 6 is ten times richer in acidic particles than water with a neutral pH level of 7. Guppies are very sensitive to extreme variations in pH, hence the importance of maintaining a stable pH of 6-8. The simplest method of measuring pH is with a liquid meter. Litmus sticks are available, but it is recommended to use the electronic pH meter as it allows for simple and frequent measurements. The pH can be adjusted by using purchased kits. The pH should be measured whenever water properties change.

e. *Water hardness* – Water hardness is measured in dH and categorized as: very soft  $0-4^{\circ}$ dH, soft  $5-8^{\circ}$ dH, semi-hard  $9-12^{\circ}$ dH, hard  $13-20^{\circ}$ dH, very hard  $20^{\circ}$ dH. In nature, almost all live breeders live in semi-hard or hard water. As a result, many species do not thrive in soft water and their development is hindered, especially if the water also has a low pH. Both total (permanent) and temporary (calcium bicarbonate) hardness should be measured weekly. Many commercial kits for measuring permanent and temporary water hardness are available. Permanent hardness should be maintained at a constant 7°dH in both cultivation and breeding tanks.

4.6.3 Water storage and supply

Clean water is pumped into the greenhouse tanks at a controlled rate from the facility's storage tank (Fig. 4.10) through a sunken pump to each cultivation tank. Fresh water flows to each tank through a pipe system that runs the length of the greenhouse or tunnel. The recommended daily rate of water exchange in the tanks is 5-10%, regulated by valves or a drip system. Each tank should have an upright pipe to measure the water level and drain overflow into the drainage channels. The position of the upright pipe can be adjusted so that the pipe can be used to clean the tank and collect fish. An activated charcoal filter (80-100 l activated charcoal per 200 m<sup>3</sup>) should be installed on the main water line to draw away materials such as chlorine and heavy metals that are harmful to fish. From the filter, the water should be stored in a closed water storage tower that holds 5-10% of the total farm volume for a 24-h period prior to its reentry into the tanks. This is because tap water often contains a high concentration of harmful liquefied gases that can harm the health of the fish.

#### 4.6.4 Aeration

Two 3.5 hp blowers (Fig. 4.11) placed outside the structure infuse fresh air for the purpose of increasing the water's oxygen level and whirl the water in filters placed in the tanks. The blowers are connected to 63 mm primary piping for air conduction and 50 mm secondary piping running the length of the pools for temporary replacement or backup in case the primary piping fails. The blowers should be housed in a closed structure outside the greenhouse or tunnel. Dispersion stones can be used to aerate the water in the tanks. One stone is sufficient for a small tank of 0.5 m<sup>3</sup>. In larger pools, aeration should mix the water in a homogenous fashion.

#### 4.6.5 Lighting

During the summer, the average length of daylight is approximately 14 h. With the shortening of daylight hours in autumn and winter, the activity level of the fish, i.e., consumption and growth, decreases. Therefore, to maintain activity at the summer level, lighting for 12-14 h per day should be provided throughout the year using neon lights controlled by timers. A pair of 40 W neon bulbs should be placed in two rows along the length of the tunnel at intervals of 5 m (Fig. 4.12).

## 4.6.6 Additional equipment

Electrical failures endanger ornamental fish farms. Growers should take every precaution to prevent electrical failures by installing an automatically operated generator for backup electrical power (Fig. 4.13). A simple inexpensive electronic alarm system can be installed to provide an alert by phone or pager in case of operating failures of the heating or aeration system. Small hand nets for sorting, fishing nets, thermometers, buckets, plastic containers and feeding plates, etc., are needed. At MIGAL, the quality control system is an 80-100 l aquarium on a stainless steel stand with a closed water system that includes a central filter and heating system.

#### 4.6.7 Experimental breeding area

In the MIGAL laboratory, experiments and selection for breeding are conducted in aquaria with no special devices or plants. Each line consists of at least six aquaria. Aquaria are placed on three shelves along each wall (Fig. 4.14). The top row is placed at eye level and the bottom at knee level to facilitate work. The rows are spaced so that it is possible to comfortably insert a hand net into the water. The fresh water and air supply systems are placed above the upper row. Air is supplied continually for oxygenation of the water through air stones and to activate the air elevator that raises the water in the biological filter. Up to 15% of the water in the aquarium is replaced daily. The center of the room contains tables and chairs for selecting and sorting.

Aquaria of 30 l were found most suitable for breeding guppies and cultivating fingerlings. Larger fish can be held in 60-l aquaria until maturity, however, larger aquaria are more difficult to move and clean. All aquaria are glass to enable viewing the fish for quality control. Five-liter buckets are used as holding tanks for newly spawned fingerlings until

transfer to an aquarium, for females before spawning, and for final sorting of fish for breeding. If there are not enough aquaria, these buckets (with the addition of a small filter) can be used to hold fish up to two weeks, except for pregnant females that cannot be held in small conditions for so long a period.

## 4.7 Nutrition

The nutrition of fish of the Poeciliidae family is very varied and includes carnivores, omnivores, and herbivores. Most omnivores prefer mosquitoes and other small water creatures. In a number of species, parents devour their offspring, especially in captivity.

During the first two weeks after birth, guppy fingerlings should be fed *Artemia* three times a day with a 3-hour gap between feedings. Very young shrimp of other salt-water species may also be fed.

Adult guppies should be fed as extensive a variety of foods as possible. This can include any combination of dry artificial feeds and live feeds used in standard ornamental fish nutrition. They should be fed at least once a day with food prepared by the grower containing vitamin and food additives. Frequent small feedings throughout the day translate into rapid growth and high food efficiency. Excess food should be prevented; if excess foods accumulate they should be removed immediately and the food level reduced. The recommended level of feeding dry foods is around 5% of the fish biomass. This provides an abundant level of nourishment; any more is simply not required. Natural or artificial color pigments must be added to the food. Sometimes guppies refuse to eat a suitable food. Such behavior usually lasts only a day or two, after which the guppies eat what is provided.

Feeding of parent stock should receive special attention. Breeders should receive feed of the highest quality and the exact composition that best meets the needs of the fish. Floating foods are easier for the fish to consume.

#### 4.7.1 Dry foodstuffs

The simplest way to provide the required nutrition is by using dry foodstuffs. Various products sold today include all the vitamins and minerals necessary for healthy fish. However, some minerals and vitamins decompose with time so it is important to purchase no more than two months' supply at a time.

a. Food chips – arc sold in a wide range of shapes, sizes, and packaging and differ in composition. The most common variety contains both animal and vegetable ingredients and is suitable for many types of fish regardless of the fish's preference in nature. Most ovoviviparous fish remain healthy for a long period on this food as long as their diet is regularly enriched with raw animal foods. There are vegetable-based food chips that are unsuitable for guppies. Some food chips contain carotene, a color compound that enhances red shades in fish. A similar effect can be obtained by adding ground paprika to the food once a week. These foods are recommended for fish with a reddish shade. The size of the chips to be used depends on the size of the fish. Standard packaging contains large and small chips. Since the guppy is a small fish, it should be fed the smaller chips.

b. *Tablets* – are sold in two forms, those that sink to the tank bottom for fish that usually feed on the benthos or those that stick to the aquarium walls and gradually dissolve. The latter is usually considered a good food additive for guppies.

c. *Pellets* – are composed of dry food shaped like short strings (imagine miniature spaghetti) compressed together in the shape of a pellet. When the pellets are ground they create an excellent floating food source. However, they must be used carefully as they tend to pollute the water.

d. *Granular foods* - are an excellent source of nutrition, however not all fish eat them immediately. Care must be taken not to over-feed as this type of food tends to spoil rather than dissolve quickly.

#### 4.7.2 Live foods

Live foods are part of the natural diet of most ovoviviparous fish, especially the guppy. Guppies should receive live food at least once a week, preferably more often. Live foods may be fresh, frozen, dried, or freeze-dried, purchased, cultivated, or caught in the wild.

a. *Capturing live organisms from freshwater ponds* – In nature, fish are forced to work hard to obtain their food. The search and capture of food is excellent exercise and prevents the fish from becoming overweight (surplus lipids in the liver are common among aquarium fish). Live foods are healthy as they contain important vitamins and minerals as well as all the basic nutrients. Live foodstuffs can be found in clean freshwater bodies that contain no fish but may contain a rich variety of miniature creatures, some of which are shown in Fig. 4.15.

Care must be taken when placing collected food sources into cultivation tanks since the water containing the food organisms may also contain pathogens or parasites (e.g., fish lice, leeches). Even water from nature that contains no fish can contain polyps of hydra or planaria that attack young fish. To prevent this, place the collected foodstuff in a 20-l tank especially designated for this purpose and aerate it with a strong stream of forced air for 30 min. After 30 min of aeration, the hydra, planaria, and leeches will stick to the walls of the tank, allowing for the collection of the clean animals intended for food.

There are a few animals that live outside the water that are suitable as food for guppies and can be collected by the grower. Among them, the fruit fly (*Drosophilidae*) has the advantage that it cannot transfer disease to fish. Live food should be thoroughly filtered immediately following capture. Large creatures such as dragonflies (*Anisoptera*) and larvae of predaceous diving beetles (*Dytiscidae*) must be removed as they pose a danger to aquarium fish.

b. *Mosquito larvae* – There are three types of mosquito larvae, differentiated by their color. Black larvae can be found between the spring and fall in small water bodies, even puddles. These larvae are excellent sources of nutrition for fingerlings and adults, however care must be taken not to overfeed, as larvae not consumed within a few minutes will complete their evolution into pupa (that are rarely eaten) and, a few hours later, into full-grown *Culex*. Freezing the larvae for a short time prior to using them as feed prevents such development. Black larvae can be cultivated by placing a 50-l tank containing water and a small amount of juice from ground nettles in a shaded outdoor area. Within a short period, larvae are expected to appear which should be gathered before they begin pupation.

White larvae are more transparent than black and are created by fly-like *Corethra* in large swarms on hot summer days. They may be collected from still clean water where they appear in large amounts primarily during winter. They are a premium source of nutrition for large fish but are dangerous (possibly lethal) for fingerlings. White larvae may be stored in a refrigerator wrapped in damp newspaper.

Red larvae originate in non-biting mosquitoes known as *Chironomos*. Guppies are particularly fond of red larvae however these creatures primarily appear on the muddy bottoms of waterways or pools that are polluted by organic matter and even heavy metals. Therefore they should be given to the fish no more than once a week and handled with care, not only because of the danger they pose to fish but also due to serious allergic reactions in humans.

c. *Small crustaceans* – The most common variety of miniature crustaceans is the *Daphnia*. They appear in clean and dirty bodies of still water (although not in significant numbers) and serve as an excellent food for fish. Although not outstandingly nutritious, daphnia contain a number of basic minerals important for maintaining health in fish. Some types of daphnia are common in garden pools. Other crustaceans that are suitable as fish food are *Cyclops* and

*Bosmina*. Unfortunately, *Cyclops* is a rapidly growing predator that can injure fingerlings and *Bosmina*, which is an ideal food, is relatively rare. *Artemia salina* eggs can be purchased and hatched in the farm laboratory (Fig. 4.16).

d. *Worms* – All live breeders hungrily feed on sludge worms (*Tubifex tubifex*) that are rich in vital nutrients, especially for breed fish and in small portions for fingerlings. Unfortunately, as their common name implies, these worms appear exclusively on muddy bottoms of water bodies that contain high concentrations of organic materials, other composites, and heavy metals. Sludge worms are commonly purchased in pet supply stores. Whether purchased or gathered from the mud, the worms must be flushed with clean water for a number of days prior to being fed to fish. Worms can be placed in a tank under a slowly dripping faucet, or covered in a tank in which the water is repeatedly replaced. White dead worms and muddy surface dirt should be removed prior to feeding. Feeding rings, developed especially for sludge worms, can be purchased. They should be used when the fish are not usually fed from the tank's surface. Small amounts of sludge worms may be stored for a few days in a refrigerator, on a shallow dish, covered in water that is replaced twice daily.

e. *Flies* – Some live breeders live close to the water surface and capture flying insects such as flies (*Dipterans*). The addition of flies to the fish diet improves longevity and disease resistance. Small fruit flies (*Drosophilidae*) are easily captured. A glass jar with fruit (banana is particularly suitable) can be placed outside during the summer. After a few hours, there will be enough flies in the jar and it should be closed.

#### 4.7.3 Food additives

Dzikowski et al. examined whether the addition of L-carnitine as a food additive improves the number of offspring. They added 1,100 mg/kg L-carnitine to the fish food and found that this additive advanced the first spawning and increased the number of offspring from 88 to 200. However, these results did not occur during the second and third spawnings.

#### 4.8 Disease and parasite control

Diseases can cause serious damage. They can cause mortality, damage quality lineage, reduce quality (even of fish that recover), waste time spent in selective breeding, and require costly investment in medications and treatments. A number of diseases and parasites may afflict fish. External parasites such as cotton wool disease, velvet, lice, and leeches attack fish fins and skin. Guppies may be affected by internal parasites such as hexamifa, worms such as roundworms or tapeworms, or nematodes such as callmanus. Bacteria such as eye-fungus, finrot, and columnaris attack fins, skin, and eyes and affect internal tissues and the circulatory system. Fish are susceptible to contagious diseases such as tuberculosis and edema as well as genetic diseases. Diseases caused by bacteria are more difficult to prevent and identify than those caused by parasites. Genetic diseases are liable to occur when unsuitable parents are chosen for breeding. Fish with genetic diseases should be discarded immediately without attempting to treat them.

The most effective method of coping with disease is prevention. One of the important aspects of disease prevention in guppies is stress control. A fish in distress is extremely susceptible to disease. Therefore, guppies should be provided suitable conditions, comparable to those in their natural environment. Growers must maintain proper hygiene and sanitation by, for example, sanitizing nets before using them in a second tank or allocating specific nets to each tank. Ponds and their surroundings should be kept clean, tools should be sterilized, ponds should be sanitized at the conclusion of each growth cycle, food should be properly stored, dead fish and plants should be removed daily. The grower should keep track of the state of health of the fish through monthly examinations conducted by certified laboratories. He should know which opportunistic parasites are present on the farm and be familiar with disease symptoms.

There should always be medications on hand such as formaldehyde (CH<sub>2</sub>O), salt (NaCl), and anti-stress compounds. Formaldehyde together with salt has been reported to be effective in preventing external parasites but unsuccessful in treatment following their appearance. Antibiotics should be used only when there is clear indication of a bacterial infection that can be treated with a specific antibiotic. Antibiotics should not be used for prevention since bacteria may develop a resistance to the medication.

Some external and abdominal parasites can be identified by microscope on the site. In the case of the appearance of a disease, an expert should be consulted. Affected fish should be treated with drugs only after diagnosis by a certified laboratory that specializes in fish diseases. Affected tanks should be isolated and no fish, water, or equipment (including workers) should be transferred from affected tanks to healthy tanks without being sanitized. Water in affected tanks should be frequently changed. When pharmacotherapy is ineffective, an increase in water temperature and examination of the salt content may help. Afflicted tanks should be treated only after concluding the routine daily responsibilities in the rest of the farm. The entire farm should be carefully observed to detect the spread of the infection to other tanks

To prevent disease, no extraneous fish be introduced into the system or transferred from another farm, even if the grower has been assured that isolation procedures were carried out. Growers should obtain a copy of the rules and regulations regarding quarantine procedures from their nearest field extension service; these are strict regulations and difficult to follow. Even fish that return to their original farm should be considered suspect and quarantined outside the farm. When receiving a new parental school, all required activities should be conducted in coordination with all involved parties (the seller, the purchaser, field guides, laboratory and research center, etc.). Ponds should always be thoroughly sanitized with chlorine (Cl) negated with anti-chlorine and initially stocked with a small number of fish so that their behavior and state of health can be observed before completing the stocking.

During the selection and shipping process, low concentrations of antibiotics should be introduced into the water to prevent bacteriological infection during this critical and stressful period.

## 4.9 Fish harvest and shipment

Fish should be starved for 24 hours before selection for shipment. Every attempt should be made to avoid injuring the fish. To minimize stress to the fish caused by being removed from the water, the selection process should be carried out manually and as quickly as possible.

Tanks with baskets to hold the chosen fish should be prepared in advance. Water in the tanks must be of high quality; a dispersal stone in each basket or circulating water will provide biofiltration. The tanks should receive fresh water before each batch of fish. Shipping causes prolonged and difficult distress to the fish, therefore, conditions should be as good as possible prior to shipment. Fish should be sorted by uniformity and size and random measurements should be taken with a ruler from each group. Food should be denied the fish 2-3 days prior to marketing. However, if the fish will be held for longer than this before shipment, they should be fed very small amounts of food for maintenance.

At least two days before shipment, the fish should be counted, organized in groups, and packaged in water-filled plastic bags. The bags should be placed in an aquarium to observe their behavior and conduct a more stringent quality control examination. The grower should be very critical and objective regarding the quality of his product. If the product is to be marketed in Europe, it will be competing with fish from many sources and Israeli fish are usually priced higher than those of other countries. Therefore, fish intended for export should be carefully selected and all unsuitable fish rejected. The better the environment in which the fish were grown, the fewer unsuitable fish will be produced.

During each transfer of fish from one tank to another, they should undergo acclimatization to the new water into which they are being transferred. During all sorting or transfer activities, good aeration must be maintained, especially if the fish are to remain in the container more than 15 min.

Some time before or after sorting, the fish should be immersed in an antibiotic solution of formaldehyde or Bromax to prevent bacteriological infection. A variety of parasites can be present in any cultivation system or farm. In general, if a farm is well maintained, the physical state of the fish will be good and parasites will be unable to attack the fish. However, during marketing the fish are in an extremely stressful condition and should be well protected from parasites. Farms achieving export quality will receive specific instructions for selecting and preparing fish for shipment.

## 4.10 International trade of ornamental fish

The estimated value of the international ornamental fish market and associated equipment is US\$4 billion per year. The European wholesale ornamental fish market is US\$250-300 million per year. Annual export from Singapore is US\$40 million. Eighty percent of the fish are raised on fish farms.

The market in the USA includes 5-10 importers, 500 wholesalers and 1000 retailers. Annual production is 60 million fish while another 125 million are imported. In Florida, approximately 300 fish farms annually market nearly US\$22 million of ornamental fish. There are approximately 15 million fish enthusiasts caring for 95 million ornamental fish in the USA.

Large wholesalers in Europe are listed in Table 4.1, sources of tropical ornamental fish are listed in Table 4.2 and the most commonly requested varieties are listed in Table 4.3.

Table 4.1. Large ornamental fish wholesalers in Europe

Large offamental rish wholesafers in Europe.			
Company	Country	Comments	
Schmittkentz	Germany	Largest store in the Frankfort area	
Glazer	Germany	Largest European wholesaler	
Ottlik	Germany		
Fetura	Germany		
Wolfeld	Germany, Israel		
Ryba	Germany		
Carmar	Italy		
Maura <sup>.</sup>	Italy		
Cof	Italy		
Niederhomer	Austria		
Holland Cichlids	Holland		
Petra Aqua	Czech Republic		
Exomarc	France		

Table 4.2.	
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Sources of tropical ornamental fish.			
	Cultivation	Wild Catches	
Southeast Asia	Singapore Hong Kong	Thailand Australia	
	Malaysia Others		
USA	Florida		
Other areas	Israel Eastern Europe South Africa Others	South America, primarily the Amazons Africa	

Table 4.3.				
Popular tropical ornamental fish.				

Family	Species
Characidae	Tetra, neon, cardinal
Poeciliidae	Mollies, swordtails, flatheads, guppies (live breeders)
Anabantidae	Gourami: blue, pearl, miniatures, kissing fish
Cichlidae	Scalare, discus, ramirez
Cyprinidae	Sumatras, rosybarbs

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#### **CHAPTER 5**

## CULTIVATION OF THE HECKEL DISCUS FISH (SYMPHYSODON DISCUS)

## **5.1 Introduction**

This chapter reports on R&D to establish a system for cultivating Heckel discus fish (*Symphysodon discus*) for export. The R&D was conducted at the Shoreshim Ornamental Fish Farm which took primary responsibility for developing cultivation techniques and at the MIGAL Research Center that concentrated on breeding.

Approximately one year is required to raise a school of breeders therefore our initial proposal was for a two-year R&D program. The objective was to create production, logistic, and marketing procedures for discus, as had been done for angelfish (*Pterophyllum scalare*) and gourami (*Trichogaster trichopterus*) at Shoreshim. The goals of the second year were: (a) to develop controlled production, logistic and marketing processes (Shoreshim), (b) to develop a breeding school (Shoreshim), (c) to improve the breeding process by isolating eggs from parents immediately following oviparity, (d) to develop a food resource for larvae during their initial growth stages (MIGAL), (e) to improve the food formula for fingerlings (MIGAL), (f) to develop import/export capabilities by obtaining maximum spawning (Shoreshim), and (f) to improve diagnosis, prevention, and treatment of disease.

Researchers successfully prepared adults for breeding, circumvented the natural breeding pattern whereby parents care for their eggs, and raised fingerlings to approximately mature size. Most of the major diseases that afflict discus were successfully treated. Further, a new method for cultivating a live nutritional source, *Artemia*, was examined at both centers. Without development of this new system, based on the system used at the Eli production facility at Kfar Blum, and the ability to control other live nutritional resources, it is impossible to breed discus.

Together with this success, it was clear that discus is difficult to cultivate and requires significant experience and skill. The researchers were unsuccessful in raising discus without parental involvement but concluded that the ability to do so would improve the growth rate. They also concluded that although food for the discus was developed that can grow in Shoreshim's hard water, further nutritional improvement is required.

## 5.2 Broodstock and oviparity

Three shipments of discus were purchased abroad through an agent. The first shipment of sixty 3-4 cm discus was transferred directly from Ben Gurion International Airport in Tel Aviv to MIGAL in August 1992. Unfortunately, the fish were afflicted with rust fungus and within two days all died. The event emphasized the need for veterinary follow-up to diagnose, treat, and prevent disease. The second shipment included eight blue discus and eight red discus of 6-8 cm in October 1992. Four couples resulted from this shipment: three blue and one red. The third shipment (October 1993) consisted of twenty fish of 7-12 cm. They were distributed into four tanks and observed to identify couples. This task requires constant observation of the behavior of the fish. The process is long, complicated and may take several months. The water throughout the system was hard water that passed through a charcoal activated filter. The water in the hatching tanks was soft water. Ammonia, nitrite, chlorine, pH, etc. were examined every two weeks and suitable in all cases.

Six to eight approximately one-year-old discus were placed in a 160-l aquarium. Among discus, the first male and female that reach sexual maturity establish themselves as a couple and begin nesting. As soon as a couple was observed (Fig. 5.1), it was removed from the group and placed in a separate aquarium that served as a breeding center. A plastic pipe was placed in each breeding aquarium in which the female was able to deposit her eggs. Usually after the couple showed signs of mating they cleaned the nesting area, their body colors changed, and their sexual organs became exposed. The process continued about an hour and most of the eggs were laid in the plastic pipe (Fig. 5.2). Frequent observations were

required to study the characteristics of egg-laying and prevent the parents from destroying the eggs.

Until February 6, 1993, the researchers separated the eggs from the parents and transferred them to a hatching aquarium with soft water and 1 ppm blue methylene. In only one case did the eggs hatch and approximately 50 fingerlings survived until 3 weeks old, receiving nourishment from egg protein. The fingerlings died because of contaminated water. Following February 6, 1993, the eggs were left in the aquarium with the parents. In four oviparous cycles, the fingerlings grew to 2 cm and were removed from the parents' tank at 3-4 weeks. Two other cycles hatched and survived a few days, only to be eaten by the parents. The male of couple no. 4 tended to eat his female's eggs immediately upon being produced. When there were no hatchlings in the tank with the parents, eggs were produced every 4-10 days.

From time to time the parents changed the location for laying eggs from the open pipe to a piece of plastic on the aquarium floor, a screen, the filter, or one of the aquarium walls. Couple no. 1 frequently moved their live fingerlings from one location to another.

The body colors of the female were clearer prior to oviparity and therefore it was possible to predict when she was ready to lay eggs. Oviparity was recorded only when eggs were observed.

Presently there are 18 adult "foreign-grown" red-turquoise discus (*S. discus*) and approximately 40 "home-grown" kolbet-blue discus (*S. aequifasciatus*) and initial attempts are being made to unite them as couples.

## 5.3 Cultivation of eggs and fingerlings

Two treatments for raising eggs and fingerlings were examined during the study. The first was to leave them with the parents for hatching and raising ('natural cultivation'). This stage required four months. The second was to remove the eggs immediately after oviparity for development in separate aquaria ('artificial cultivation'). This stage is relatively shorter due to the quiet season (section 5.4) of the female discus.

#### 5.3.1 Natural cultivation

Immediately following oviparity, the water in the aquarium was replaced with soft water until after the eggs hatched. In most cases, the couples initially took good care of their offspring. During the first few hours they continually stimulated them to supply them with oxygen and prevent the development of fungus. The primary disadvantage of leaving the eggs with the parents is that after 20 hours the parents begin to eat the eggs. The tendency of discus and other cichlids to eat their eggs is not related to their appetite. Only in the case of the first couple did five eggs remain uneaten in the first stage of the research. In the second stage, other couples laid eggs. The numbers of eggs laid and hatched are shown in Table 5.1.

Table 5.1.				
Number of	Number of discus eggs laid and hatched.			
No. of eggs	No. hatched	% hatched		
100	60	60		
30	20	66		
100	30	30		
50	31	62		
50	25	50		
102	35	34		
199	61	31		

The eggs hatched after two days of incubation. Three days later the fingerlings began to swim freely in the aquarium. Until this point, their sole source of nutrition was the yolk. Once they began swimming, they began to eat from the sides of their parents' bodies. The feeding from each parent's body lasts 2-3 minutes and continues as long as there is light. Therefore, the room was darkened during the hours that the parents were used to darkness. At this stage the grower's role is limited to observation. If a quarrel develops between the parents, the grower should remove the weaker fish from the aquarium. In most cases, fingerlings eat from their parents until 4-6 days, when they begin to receive rations of young *Artemia*.

In the literature, it is recommended that fingerlings be separated from their parents at 7 days to stimulate the next oviparity. Fingerlings from later groups were kept two weeks and no difficulties relating to the separation from the parents occurred at any time.

The fingerlings were transferred to 90 or 140-l aquaria with circulating water and filtration in conditions identical to those of their parents. The fingerlings continued to receive *Artemia* until the age of 3 weeks, when they began to receive additional foodstuffs such as bloodworms and ground meat (section 5.5). When the discus reached 8 months and 7-8 cm, eight fish were placed in each tank in preparation for coupling.

#### 5.3.2 Artificial cultivation

Seven attempts were made to remove fish eggs from their parents' aquarium for artificial cultivation. In each case, the eggs were removed immediately after laying and transferred to a small 15 l aquarium with soft water (Fig. 5.3). Blue methylene was added to the water as a sanitizing agent at a concentration of 1 ppm to prevent the growth of fungus and bacteria. Water temperature in the hatching tank was 26-28°C and a dispersal stone providing gentle aeration was placed 10 cm from the eggs. For the first 3 days after hatching, the yolk nourished the young fish. Until this stage they showed no signs of distress.

On day 4, the fingerlings began to rise from the aquarium floor in search of food. The first food given them was chicken yolk. At first, the researchers used the yolk of a hard boiled egg; later they switched to powdered yolk commonly used in bakeries. Water was added to produce a paste that was spread on the water surface of the aquarium.

As recommended in the literature, water was replaced every 2-3 hours throughout the day. At night, the fingerlings were transferred to aquariums with clean water in a dark room until morning. In addition to this method, the researchers tried a method used earlier by some growers - instead of replacing the water every two hours, the fish were transferred to a glass bowl every 4-5 hours. Nevertheless, they died after 2-3 days. It seems that the fish were harmed by the multiple transfers. Later, the researchers prepared plastic bowls with circulating water (Fig. 5.4, 5.5), but this system was utilized only once since the females stopped laying eggs, making further examination impossible.

The difficulty was to discover the ideal conditions for cultivating the fingerlings until the stage in which they are able to eat young *Artemia*, i.e., a week after hatching. Although 80-90% of the eggs hatched, the fingerlings survived, at best, only a few days. The researchers felt that they would have obtained better results from the artificial cultivation if they had had a greater number of fertile couples.

#### 5.4 Cessation of oviparity

Oviparity in discus occurs once every 7-10 days, provided that eggs from the previous batch are removed from the aquarium immediately after being laid. The oviparous cycle can continue 3-4 months, following which the female enters a quiet season of approximately 4 months. The female uses this period for development of tissue and energy. Therefore, she must be supplied superior foodstuffs during the quiet period. Meanwhile, the male still shows significant interest in oviparity and may become violent and aggressive as a reaction to the female's inability to be oviparous and he may harm the unsuspecting female.

The female in couple 1 entered her quiet season after 20 oviparity cycles. During her quiet period, the male harassed her and caused her to hide. After the researchers saw that she was unable to reach food, the female was transferred to another aquarium (there are those who recommend constructing a partition within the aquarium to separate the couple during the quiet season). A similar cessation of oviparity occurred among the other couples.

#### 5.5 Feed

One of the factors that determines good health, growth rate, and maturation of the discus is nutrition. Due to their heightened need for organic compounds, discus require a constant supply of protein. Because of their amino acid composition, animal proteins have a higher nutritional value than vegetable proteins. The discus can utilize carbohydrates, however there is little information regarding their level of digestibility. Very little fiber is digested and fiber is known to be one of the most prevalent sources of pollution in the aquarium.

Animal foods are of significant importance to the health, development and fertility of discus. In addition the chasing of live food improves the health, alertness, and overall activity of the discus.

Until May 27, 1993, all the discus cultivated at MIGAL were fed frozen bloodworms, *Artemia*, mosquito larvae, and *Daphnia*. Adult discus received two meals per day (at 8:00 and 13:00). Egg-laying discus received three meals per day (8:00, 10:00, 13:00). Parents caring for eggs or fingerlings received an additional meal at 18:00. Fingerlings that were separated from their parents received four meals per day (8:00,10:00, 13:00). The menu included *Artemia* (young and adult), mosquito larvae (live or frozen), daphnia (water fleas), bloodworms, and ground meat (composed of chicken hearts, livers, cooked oatmeal, and wheat bran).

On May 27, a new menu composed of ground meat (hereinafter: the MIGAL paddy, Fig. 5.6) was introduced. The MIGAL paddy was prepared according to recommendations taken from relevant literature and included turkey hearts (9%), chicken livers (13%), a vegetable mixture (77%), and vitamins and minerals (1%). The MIGAL paddy was initially given at 10-90% of the animal food ration so that the fish would become accustomed to its color and taste. After a number of days, the ratio was revised to 25-75%. Once it was ascertained that the paddy was properly digested, it was served separately from other animal foods but together with live foods.

In a series of growth experiments, the researchers examined the influence of six different paddies on the growth rate of fingerlings. According to relevant literature, discus eats various foods. The objective of this experiment was to examine some of the foods that are readily available in Israel, such as frozen meat paddies, superior quality flax, live foods (*Artemia*, bloodworms, daphnia, mosquito larvae), meat (turkey hearts, livers, and spleens, beef hearts and kidneys, crabs), aminal foods (fishmeal, powdered eggs, milk) and vegetable foods (oatmeal, wheat germ, spinach, lettuce, peas, mixed vegetables). The compositions of paddies prepared at MIGAL are given in Table 5.2.

Composition of experimental meat paddies.			
Turkey based	Beef based	Fishmeal based	%
Turkey hearts	Beef hearts	Fishmeal	77
Turkey livers Beef liver		Chicken liver	13
Mixed vegetable*	Mixed vegetable*	Mixed vegetable*	9
Concentrated vitamins	Concentrated vitamins	Concentrated vitamins	1

Table 5.2.

\*Mixed vegetable includes oatmeal, wheat germ, spinach.

Feed was served twice a day, at 08:00 and 14:00, and in excess amounts so that all fish would be able to reach food. Leftover food was collected approximately one hour after serving. Six tanks were fed six different menus (Fig. 5.7), as in Table 5.3. The fish were blue discus from two sequential cycles of eggs hatched by couple no. 1 at the beginning of the experiment and cultivated at MIGAL. The fish were 4 months old and 4-6 cm. Since all fish were from the same genetic origin, the source of the fish was not a source of variance in growth. Any growth variance derived from the food source.

The water quality in which the fish were cultivated was suitable for discus: pH ranged 7.4-8.1 and temperature 25.9-29°C (although it is possible to cultivate discus at temperatures

above  $30^{\circ}$ C), NH<sub>4</sub> was 0, NO<sub>2</sub> did not exceed 0.05 ppm, Cl was 3-10 ppm, and total bacteria was 101.1-103.4 per ml in all groups.

The turkey and fishmeal diets (groups 2 and 6) resulted in the best growth (Figs. 5.8 and 5.9). The addition of bloodworms did not significantly improve growth and is therefore considered unnecessary as a food additive. The fishmeal diet was the most cost effective and therefore considered the optimum food source among those examined. The discus grew rapidly from 5-10 g to 20-30 g. Growth was slower beyond this size. Overall, the growth rate of the discus was slow and attempts will be made in the future to improve it.

The discus in these experiments were relatively large (Fig. 5.10). Because of the initial rapid growth rate, it is recommended that the fish be cultivated to a smaller size in the future.

	Table 5.3. Diets in feed experiment	•
Group	Feeding time 08:00	14:00
1	Turkey heart paddies (MIGAL)	Bloodworms
2	Turkey heart paddies (MIGAL)	Turkey heart paddies (MIGAL)
3	Beef heart paddies	Bloodworms
4	Beef heart paddies	Beef heart paddies
5	Bloodworms	Bloodworms
6	Fishmeal paddies	Bloodworms

#### 5.6 Overall health

Discus, like other tropical fish, are susceptible to diseases caused by external parasites such as rust, trichodina, and xostia (*Ichthyobodo necatrix*). While they usually are no more susceptible than other fish, they are more prone to stress that can cause bacterial infection and *Hexamita*. In some cases, it is easy to locate and immediately correct the cause of stress, such as an increase in the level of ammonia or nitrites or a change in the pH or temperature. In other cases, such as a lower oxygen concentration or presence of chemical poisons, the cause of stress is more difficult to correct. After one year of growth at Shoreshim and MIGAL, important experience was obtained regarding numerous health related issues.

a. *Protozoa* – Similar to angelfish (*Pterophyllum scalare*), the majority of discus are almost constantly and permanently low-level carriers of the abdominal parasite *Hexamita*, *spironucleus*, and *symphysodonis*. When the number of parasites increases, it is possible to diagnose stress (primarily by a darkening of color) or disease (by rapid breathing, refusal to eat, etc.). This condition is largely caused by drastic changes in water quality that are not always controllable and occurred twice during our experiments when a large amount of floating solids was present in the water. To combat the outbreak of *Hexamita*, it is recommended to add 2.5 g of the drug Flagyl to 1 kg feed (paste) and feed it to the fish for 5 days. If the fish refuse to eat, dissolve the drug in water and stop the water flow.

b. *Intestinal parasites* – The discus were occasionally infected by intestinal parasites such as *Nematoda, Capilaria spp., Nonogenetic,* and *Trematoda xyurid* and gill parasites such as *Dactyloguros.* As with *Hexamita,* affected fish experienced changes in colors and behavior, primarily a lack of movement. Fish were successfully treated with Flubenol 5%. A solution of 200 mg Flubenol diluted in 5 cc acetone was added to the water for 6 hours for 5 consecutive days. When parasites are suspected and the grower does not want to sacrifice a fish for internal examination, they can be treated with Flubenol, followed by Flagyl in the food (see above).

In one case, a fertile female discus refused to eat for a long time, leading to a change in color and significant weight loss. She was successfully treated in isolation by oral administration of 500 mg Flagyl/10 ml water. This resulted in a treatment of 1 ml drug per aquarium over four days.

c. *Bacteria* – Throughout the feed study, some fish were infected by *Myxobacteria* on the ends of their fins and tails. These infections were treated with Imequil 10% at 6 cc per 100 l water for 6 hours on two consecutive days.

d. *Head wounds* – Head wounds were discovered occasionally and treated by external application of Imequil and addition of Flagyl to the food. According to the literature, this type of wound indicates a metabolic problem caused by intestinal parasites.

e. *Chicodonella* – The first shipment of fish imported to MIGAL were carriers of many parasites in the gills, particular of the *Chicodonella* species. They were treated with 25 ppm Formalin for 6 h. However, since the infection rate was so high, few fish survived.

## **CHAPTER 6**

# BREEDING ANGELFISH (PTEROPHYLLUM SCALARE, CICH81LIDAE)

## 6.1 Biology of the angelfish

Angelfish or scalars belong to the order Perciformes, suborder Percoidei, family Cichlidae, genera *Perophyllum*, species *scalare*. The Cichlidae are an important family in aquaculture. There is, however, great difficulty in identifying the different species due to overlapping of morphological characteristics and crossbreeding between species. Also, morphological characteristics are affected by environmental factors.

The scientific name of the freshwater angelfish, *Pterophyllum scalare* Bergmann (1986), describes the fish. *Pterophyllum* derives from the Greek word for "winged leaf" and the Latin *scalare* means "like a flight of stairs" or "ladder", referring to the dorsal fin. Angelfish are thin (laterally compressed), look like a standing disc with long fins extending from the top and bottom of the fish. It has two 'feelers' in front of the anal or bottom fin (Fig. 6.1). The tail is vertically oriented and may be shovel-shaped to long and narrow, depending on the variety. Scalar is found naturally in the Amazon River and some of its tributaries, such as the Tapajoz.

The color of the scalar in its natural habitat is silver but many strains have been developed. Zebra scalars are similar to silver, but have more vertical stripes that continue through the tail. Black lace, veiltail marble, golden, blushing and pearl scale are more colorful. Degani et al. (1997a) studied the DNA variation in black marble, gold marble, and silver angelfish using DNA fingerprint patterns and random amplified polymerase DNA (RAPD). Band sharing within the strains (0.60-0.80) was higher than between the strains (0.43-0.48) and, according to both methods, there were different DNA patterns between strains. The resemblance between the patterns of black marble and golden marble was greater than their resemblance to silver angelfish. According to their study, the calculated band sharing of DNA fingerprint patterns, and RAPD are useful parameters not only to measure variations between strains, but also to establish the genetic relationship between them.

In nature, scalar is carnivorous and its diet consists of various invertebrates. In aquaculture, the diet contains a high percentage of protein (above 45%) and many kinds of live food, e.g., tubifex, water fleas, mosquito larvae, *Artemia*, daphnia, bloodworms (midge fly larvae), and brine shrimp.

Oogenesis and hormonal control of reproduction in *scalare* are described in Degani et al. (1997b). The average cycle of the multi-spawning synchronic angelfish lasts 11 days, during which the oocytes pass through vitellogenesis and mature before spawning. Six major oocytes have been described. The patterns of steroid changes during oogenesis were traced by high pressure liquid chromatography and radioimmunoassay. The levels of the main steroids,  $17\beta$ -estradiol (E<sub>2</sub>) and testosterone (T), rose during vitellogenesis, while  $17\alpha$ ,  $20\beta$ , and dihydroxy-4-pregnen-3-one were very low and did not change markedly. E<sub>2</sub> and T in the plasma increased significantly after injection of carp pituitary extract, which also led to a higher percentage of mature oocytes than in the control group.

#### 6.2 Fecundity and larvae survival in five angelfish strains

*P. scalare* of the third generation, bred in the MIGAL laboratories, were used for this study. Pairs were isolated as soon as they began to display sexual behavior and defend their small territory. This occurred at the age of approximately 8 months. Five laboratory strains were selected for study: black marble, golden marble, gray striped, black coal, and silver diamond. In the black marble, a high proportion of the body is black while other parts are white, with very little yellow. The golden marble has a black and white pattern, with a golden dorsal fin. The gray striped is gray-green, with two lines across the body. Black coal angelfish are entirely black. Silver diamond has a silver body and brown stripes in the dorsal fin.

Fish from each strain were placed into containers  $(1 \times 1 \times 1m)$  divided by nets into 6 partitions of 0.33 x 0.5 x 1 m (Fig. 6.2). The containers were maintained at 30°C, with a light regimen of 12:12 light:dark hours. The fish were fed twice a day with live food (*Artemia salina*) and dry food. A piece of plastic, 2 x 5 x 20 cm, was hung in each partition to serve as a spawning site. Detailed measurements of pH, ammonia, NO2, NO3, O2, conductivity, and hardness appear in Degani et al. (1988).

The fish spawned in the partitioned container and the eggs were transferred to aquaria for hatching (Fig. 6.3). The eggs were kept in distilled water at pH 6.8-7 and 28°C that contained malachite blue (C16H18CIN3H) at 0.001g/l to prevent the development of fungi and bacteria. The larvae hatched three days after spawning and began to swim about six days later. Swimming larvae were fed *Artemia* larvae three times a day (08:00, 11:00, 15:00) for 5 days and then were transferred to a bigger aquarium of 90 l where they were fed *Artemia* larvae and dry food (53% protein, 7% fat, 9% ash, 2% cellulose). They were maintained in the large aquarium 30 days and then transferred to containers of 1 x 1 x 1 m (28°C, pH 7) with filters. Five percent of the water was replaced every day. The number of eggs hatched and the survival of larvae after hatching, adapting to *Artemia*, and adapting to dry food were monitored for each couple.

The interval between spawnings was 7.8 days for the black marble strain, 8.3 for gray striped, 8.8 for golden marble, 8.95 for diamond silver, and 9.1 for black coal. The mean number of eggs per spawning ranged from 181 for black coal to 258 for black marble (Fig. 6.4). The percentage of hatched eggs was lowest in black coal (85%) and highest in golden marble, black marble, and diamond silver (94-95%) while survival after adaptation to *Artemia* and dry food was highest in black marble (87% and 77%, respectively) and lowest in black coal (71% and 59%, respectively).

This work describes the reproduction potential and survival of angelfish larvae in artificial conditions. As far as we know, there is no information on the number of eggs per spawning or percent hatched in natural habitats published in scientific journals. The angelfish grown in aquaculture are strains of new shapes and colors developed by farmers (Degani et al., 1997a) and may vary not only in color but also in other biological parameters. Many parameters affect spawning and larva survival rates, the most important being temperature (Degani, 1989), density, diet, genetic origin, and water quality. The results of this study show the variation between laboratory strains grown in aquaculture where the only variable was genetic origin. Small differences were found in the interval between spawnings (16% between the highest and lowest intervals) and number of eggs hatched (12%), while greater differences was found between number of eggs per spawning (42.5%) and survival after adaptation to *Artemia* (23%) and dry food (31%). These, apparently, genetic variations need more detailed study.

## 6.3 Growth and nutrition

Little information is available on the growth and nutrition of angelfish. Temperature, oxygen, water quality, and diet are very important for larvae growth and development. Degani (1993) studied the growth and body composition of juvenile *P. scalare* at different densities and diets. Results showed that increased density significantly lowers the growth rate, and that the addition of live food (*Artemia*) to the diet significantly raises the growth rate.

#### 6.4 Oogenesis and steroid profiles during the reproduction cycle of female angelfish

The angelfish can serve as a model for the synchronous spawning class of fish. The female angelfish lays her eggs, which stick on a suitable substrate, and the male fertilizes them immediately. Angelfish display lengthy pre-spawning and spawning behavior. To the best of our knowledge, no information has been published on the oogenesis and steroid profile of multi-spawning synchronous fish. Therefore, the purpose of this study was to examine the oocyte at various stages of oogenesis and the biosynthesis of steroids during the gonadal cycle in female angelfish, thereby providing a model for synchronous multi-spawning fish.

#### 6.4.1 Oogenesis of P. scalare

The oogenesis of *P. scalare* is shown in Fig. 6.5. There are six stages, discussed below. The terminology used is modified from Degani and Boker (1992a). Stages 1-3 are described as the first growth phase and Stages 4-6 as the second growth phase.

a. Stage 1 - Chromatin nucleolus stage: The oocyte is a small cell, containing a single, centrally located nucleus that occupies at least 50% of the diameter. The nucleus contains a varying number of identically-sized nucleoli. The cytoplasm is very basophilic.

b. Stage 2 - Perinucleolus stage: The cell is round, with a round centrally-located nucleus, now less than 50% of the diameter. The nucleoli are the same size or larger than in stage 1 and located at the periphery of the nucleus. The cytoplasm is less stained, but still strongly basophilic. There are a varying number of round empty vacuoles at the periphery of the cell.

c. *Stage 3 – Lipid vesicle stage*: The nucleus is irregularly shaped and has large round nucleoli in the entrances of the cariotheca. There are three zones of cytoplasm: (a) a perinuclear cytoplasm formed by a densely granulated matrix, (b) a zone of empty vesicles (probably lipid droplets) which are grouped in several areas, (c) a cortical zone formed by a finely granulated matrix at the periphery of which there are small vesicles containing basophilic material. These vesicles are probably the cortical vesicles described in many teleosts. At this stage it is possible to clearly distinguish between three varieties of membrane in the oocyte: (a) an inner *zona radiata*, a basophilic acellular layer, (b) a *zona granuloso* of elliptical cells, (c) an external thecal layer of sparse elongated cells.

d. *Stage 4 – Granular stage*: Primary - The diameter of the oocyte increases greatly. The central nucleus is convoluted and contains a small number of large nucleoli. The principal feature of this stage is the appearance of cytoplasmic inclusions. The first appear in the perinuclear cytoplasm and are basophilic inclusions of irregular shape. Small, strongly acidophilic globules later appear in the same area. Generally known as yolk protein globules, they are initially found around the nucleus. Also during this stage, the lipid droplets grow larger and the cortical vesicles become organized into a monolayer at the periphery of the cell. The oocyte envelope continues to develop. The *zona radiata* now comprises two distinct regions.

Secondary – The nucleus remains unchanged. The basophilic inclusions and yolk globules increase in size and number and still occupy the perinuclear area together with the lipid droplets. A line of large lipid droplets limits the inclusions. At the periphery of the cell, the cortical vesicles are still seen as an organized monolayer. It appears that some modification of the pH takes place at this stage: the yolk globules, which were strongly basophilic, now have both basophilic and acidophilic components. Gradually, the protein globules occupy the whole area of the oocyte, interspersed with a number of lipid droplets. The *zona radiata* remains basophilic, the *zona granulosa* is formed of tall, palisade-like cells, and the thecal layer still consists of elongated cells.

e. *Stage 5 – Maturation*: During this stage, the oocytes resume meiosis which had been arrested at the diploid stage of the first meiotic phase. The beginning of maturation is indicated by the migration of the nucleus to the periphery of the oocyte and its dissolution (nuclear breakdown). In the cytoplasm, the yolk granules occupy the whole area with some acquiring a polygonal shape. The globules display different staining affiliates and different textures. Some contain acidophilic material included in a basophilic halo; some are formed of homogeneous material; others consist of a densely granulated matrix. These differences probably indicate different stages of development. The *zona radiata* is now highly acidophilic. The *zona granulata* consists of several layers in which the cells contain a highly basophilic cytoplasm and the nucleus is dislocated to one pole. The cells of the thecal layer are still elongated.

f. Stage 6 – Post-maturation: Completely mature oocytes contain a peripheric animal pole (shaped like a half moon) in which the embryo is to develop. The rest of the oocyte is filled with nutritive material: yolk globules and lipid droplets. The cortical vesicles can no longer be identified with regular stain such as Mallory. The follicular envelope is formed by the zona radiata, once again basophilic; the zona granulata, a single layer of round cells; and a stretched thecal laye.

#### 6.4.2 Steroid changes during oogenesis

In fish, two forms of gonadotropin (GtH) have been isolated: follicular stimulated hormone (FSH) and lutin hormone (LH). During vitellogenesis, GtH causes the secretion of 17βestradiol (E<sub>2</sub>) from ovarian follicles, leading to the synthesis of vitellogenin in the liver and its accumulation in oocytes. GtH also effects maturation in oocytes by causing the ovarian follicles to secrete maturation-inducing steroids (MIS). Some studies identified MIS as 17 $\alpha$ , 20 $\beta$ , 21, trihydroxy-4-pregnen-3-one (for example, in *Micropogonias undulates*) while 17 $\alpha$ , 20 $\beta$ , dihydroxy-4-pregnen-3-one (17,20-P) was detected in the plasma of *Oncorhynchus nerka*. Later studies showed that 17,20-P induces oocyte maturation *in vitro* in a number of fish species.

Further studies of the biosynthesis of steroids during oogenesis determined that other steroids are also involved in the process. Sangalang and Freeman (1988) identified 17 $\alpha$ -hydroxyprogesterone (17-P) as a precursor to 17,20-P in *Salmo salar*. Lambert and van der Hurk (1982) showed that a homogenate of post-vitellogenic ovary of *Clarias gariepinus* produced mainly dihydroepiandrosterone and testosterone (T), while a homogenate of post-ovulatory ovary produced 17-P and 17,20-P. Schoonen et al. (1987) found that T was the main product before maturation of the oocytes, together with a number of reduced steroids, including 5 $\beta$ -pregnane-3 $\alpha$ ,17 $\beta$ , 20 $\alpha$ -triol (5-P3,17,20-T). Degani (unpublished data) studied the biosynthesis of steroids during vitellogenesis and maturation in *Trichogaster trichopterus* (typical of multi-spawning asynchronous fish), and found that E<sub>2</sub> is synthesized during vitellogenesis and that the levels of 17,20-P and 5-P3,17,20-T rise during maturation.

The current study found that  $E_2$  and T in the plasma of female angelfish during oogenesis, as measured by RIA (Fig. 6.6), were low after spawning, then rose steeply between spawnings when vitellogenesis occurred. The levels decreased during maturation, ovulation, and spawning. Injection with cGtH significantly raised the levels of  $E_2$  and T in the plasma and also increased vitellogenesis. Injections with cGtH also effected maturation in females that were not previously in a reproductive state.

The pattern of steroid changes in *P. scalare* is different from that found in other classes of fish. Some fish, such as *Salmo gairdneri* (Zohar et al., 1982) and *Cyprinus carpio* (Levavi-Zermonsky and Yaron, 1986), are annual synchronic spawners while *Carassius aureus* (Kobayashi et al., 1988) is a multi-spawner and *T. trichopterus* (Degani, 1990) is a multi-asynchronous spawner. In all these classes of fish,  $E_2$  controlled vitellogenesis and 17,20-P functioned as MIS. Exceptionally, in the Atlantic croaker, *M. undulatus*, the principal MIS was 17 $\beta$ ,21-trihydroxy-4-pregnen-3-one (Patino and Thomas, 1990a,b). In *P. scalare*, however, while  $E_2$  controlled vitellogenesis, 17,20-P was very low or not detected at all.

#### 6.4.3 Conclusions

There are two models of oogenesis in fish. The first applies to seasonal spawners such as *S. gairdneri* and *C. carpio*, one-time spawners such as *Anguilla anguilla*, and species of the genus *Oncorhynchus*. The second model applies to male-dependent species in which the entire process of oogenesis is simultaneous in reproductive females. This study found that the second model applies to *P. scalare*. This class of fish is multi-spawning and makes use of a nest or substrate, or is a mouth-breeder.

The relationship between the morphological changes in oocytes and the steroid changes in *P. scalare* is described here for the first time. Thus, *P. scalare* can serve as a model for the Cichlidae class of multi-spawning synchronic fish. The model resembles that of the asynchronic *T. trichopterus* (Degani and Boker, 1992a,b) in which maturation in nature occurs only in the presence of the territorial male but can be induced by the injection of GtH. It is therefore reasonable to hypothesize that, in male-dependent fish, the presence of the male leads to the secretion of GtH in the female.

In samples of ovaries of females before and after spawning, the level of 17,20-P was very low. Other peaks were found in the steroid profile, however, which were not identified. Additional study of steroids and steroidogenesis are therefore needed in this species, work that is currently in progress in our laboratories.

#### 6.5 Sale of fish

The fish were sold when they reached the mature size of 3 cm (Fig. 6.7), after three months of growth and at age six months.

## **6.6 References**

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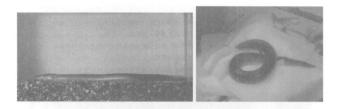


Fig. 1.1. European eel (Anguilla anguilla).



Fig. 1.2. Migration of European (Anguilla anguilla Fischelsohn, 1984) eels.

Α				
	Contraction of the second	ALTA IN INCOMENTAL		
2 months	5-8 n	nonths	10-18 months	1-3 years (elver)
				6-15 years (adult)
	в	<u>e</u>		
		(		

Fig. 1.3. A - shape of eel in different growth stages. B - development of pigment in glass eels (from bottom to top).



Fig. 1.4. The eel nursery (Kibbutz Dan Frame).

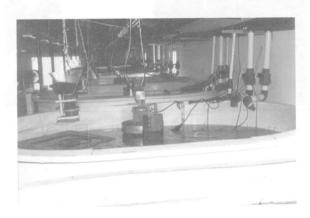


Fig. 1.5. Eel nursery tank (Kibbutz Dan Frame).

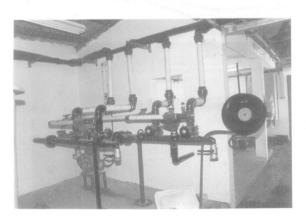


Fig. 1.6. Water transfer system in the indoor facilities (Kibbutz Dan Frame).



Fig. 1.7. Water heating system in the indoor facilities (Kibbutz Dan Frame).

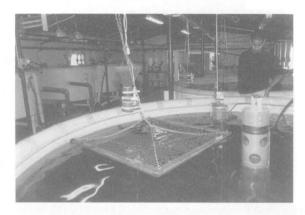


Fig. 1.8. Floating surface for eating and resting (Kibbutz Dan Frame).



Fig. 1.9. Automatic feeding system and plastic plate onto which feed falls (Kibbutz Dan Frame).



Fig. 1.10. Nursery tanks and the water drainage system (Kibbutz Dan Frame).



Fig. 1.11. Revolving drum for separating out solids (Kibbutz Dan Frame).



Fig. 1.12. Installation with brush to remove solids from the water (Kibbutz Dan Frame).



Fig. 1.13. Biofilter tower (Kibbutz Dan Frame).



Fig. 1.14. Indoor size grader (Kibbutz Dan Frame).



Fig. 1.15. Size grading (Kibbutz Dan Frame).



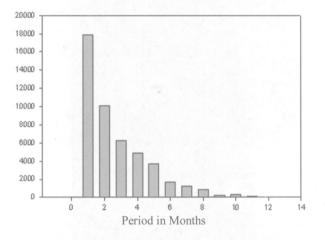


Fig. 1.16. Mortality during first 12 months of growing (1997 batch).

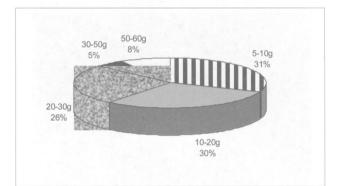


Fig. 1.17. Distribution of eel sizes at the end of the first year of nursing (1997 batch).

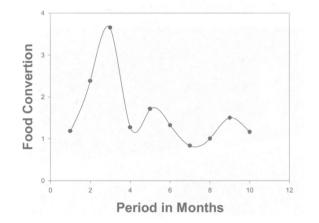


Fig. 1.18. Food conversion rate in second year (1998 batch).



Fig. 1.19. Outdoor elver ponds (40 m<sup>3</sup> each).

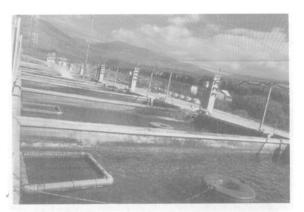


Fig. 1.20. Feeder in outdoor elver pond.



Fig. 1.21. Fattening the eels in concrete ponds (250  $\mbox{m}^3\mbox{)}.$ 



Fig. 1.22. Eel grader during nursing and fattening stages.

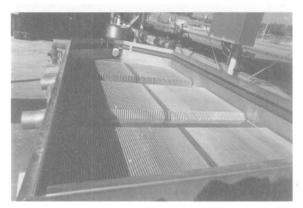


Fig. 1.23. Eel grader during nursing and fattening stages (close-up).



Fig. 1.24. Supply center for preparation of dough.



Fig. 1.25. Basket for harvesting eels from the holding pit.

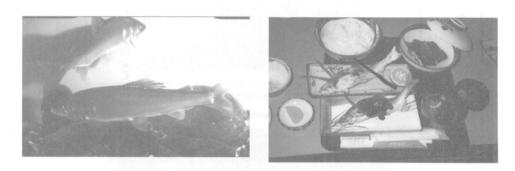


Fig. 2.1. The ayu fish (Plecoglossus altivelis).



Fig. 2.2. The natural spawning area of the ayu in Japan.





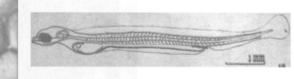


Fig. 2.3. Eggs and larvae of the ayu.

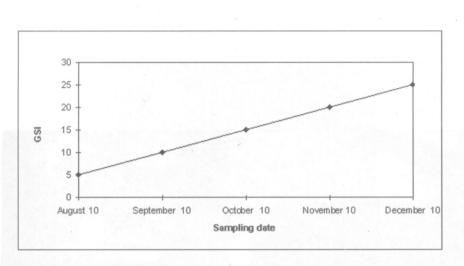


Fig. 2.4. Gonado-Somatic Index (GSI) at various stages of growth in the ayu.



Fig. 2.5. Expressing eggs from female ayu (Photo - Avshalom Hurvitz)

Fig. 2.6. Expressing semen from male ayu (Photo - Avshalom Hurvitz).



Fig. 2.7. Spreading the eggs and semen onto an incubation bed (Photo - Avshalom Hurvitz).



Fig. 2.8. Inseminating the eggs in water (Photo - Avshalom Hurvitz).



Fig. 2.9. Determining the rate of insemination (Photo - Avshalom Hurvitz).



Fig. 2.10. Removal of dead eggs (Photo - Avshalom Hurvitz).



Fig. 2.11. Sorting ayu by size in Japan (Photo - Avshalom Hurvitz).



Fig. 2.12. An egg incubation unit (Photo - Avshalom Hurvitz).

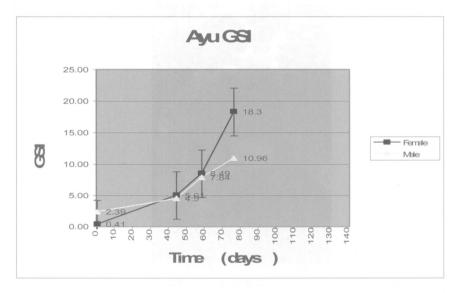
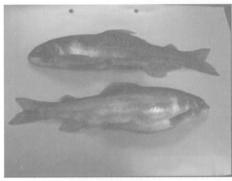


Fig. 2.13. The GSI in male and female ayu at Kibbutz Dan (Photo - Avshalom Hurvitz).



and Fig. 2.15. The incubator and be

Fig. 2.14. A mature male (above) and female (below; Photos - Avshalom Hurvitz).

Fig. 2.15. The incubator and beds at the Dan Fisheries (Photo - Avshalom Hurvitz).





Fig. 2.16. Primary nursery tanks at Kibbutz Dan (Photo - Avshalom Hurvitz)

Fig. 2.17. The rotifer cultivation system at Kibbutz Dan.



Fig. 2.18. Advanced 20  $\mathrm{m}^3$  nursery pools at the Kibbutz Dan Fisheries (Photo - Avshalom Hurvitz).



Fig. 2.19. Fattening pools at Kibbutz Dan Fisheries (Photo - Avshalom Hurvitz).

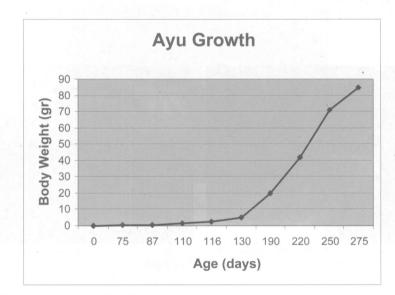


Fig. 2.20. Growth of ayu at Dan Fisheries.

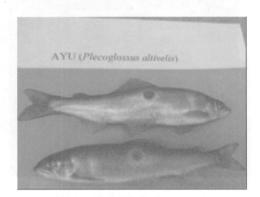
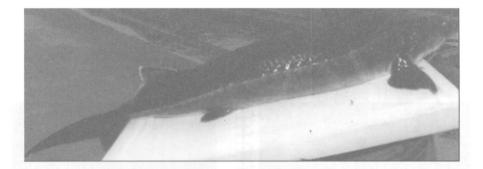
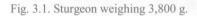
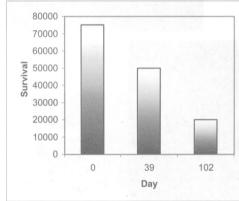


Fig. 2.21. Ayu with a lesion on the side of the body.

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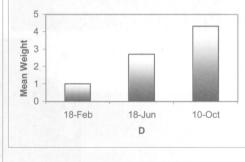


Fig. 3.2. Survival of sturgeon during the incubation and nursery stages.

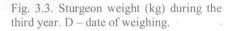




Fig. 3.4. A female 1,110 g sturgeon before (a) and after (b) incision to remove gonad.

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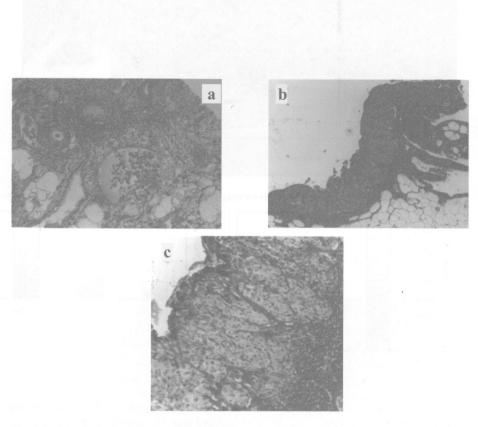


Fig. 3.5. Gonad of a female sturgeon (a) and fat cells (a,b) in the ovary.

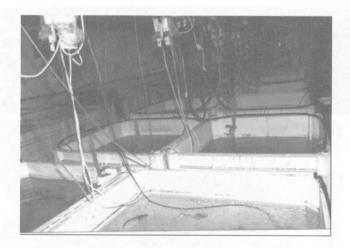


Fig. 3.6. Experimental fish tanks at MIGAL.

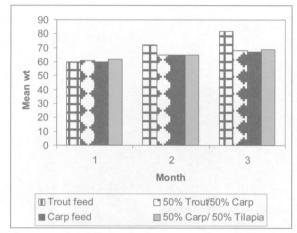


Fig. 3.7. Growth of sturgeon fed different feeds.



Fig. 3.8. Sturgeon products following sectioning.



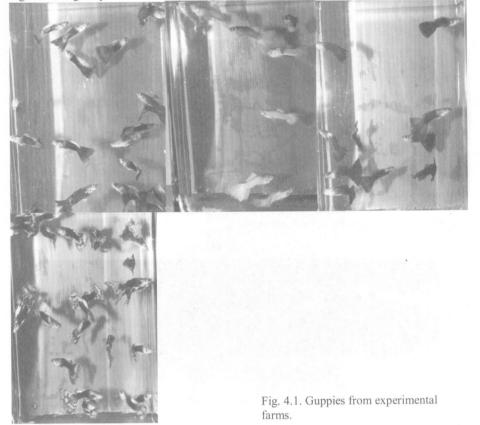


Fig. 3.9. Sturgeon products after vacuum packaging.

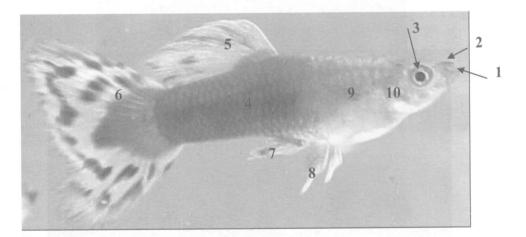


Fig. 4.2. The guppy: 1 – mouth, 2 – nostril, 3 – eye, 4 – lateral line, 5 – dorsal fin, 6 – caudal fin, 7 – gonopodium, 8 – pelvic fin, 9 – pectoral fin, 10 – operculum.

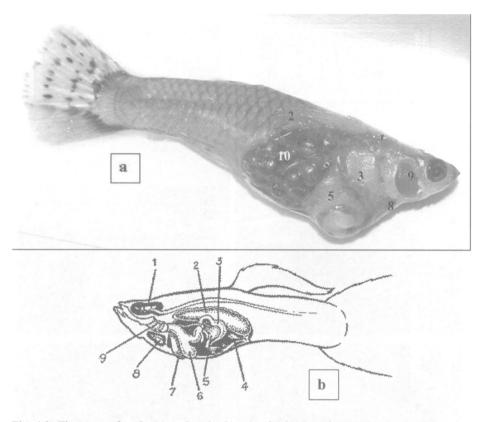


Fig. 4.3. The guppy female (a) and male (b): 1 - brain, 2 - air saccule, 3 - intestine, 4 - spermary, 5 - body lipids, 6 - stomach, 7 - liver, 8 - heart, 9 - gills, 10 - ovary oocytes with eyes stage. Kidneys (not shown in the diagram) are located above the air saccule and wrapped in a membrane.



Fig. 4.4. The pelvic and anal fins in the female (left) and male (right) guppy.

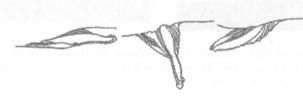


Fig. 4.5. The gonopodium (right and middle) and the organ it creates with the pelvic fin for insemination (left).

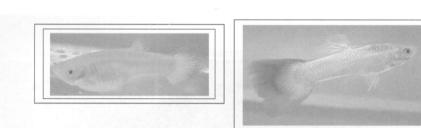


Fig. 4.6. A female (left) and male (right) of the Flamingo species.

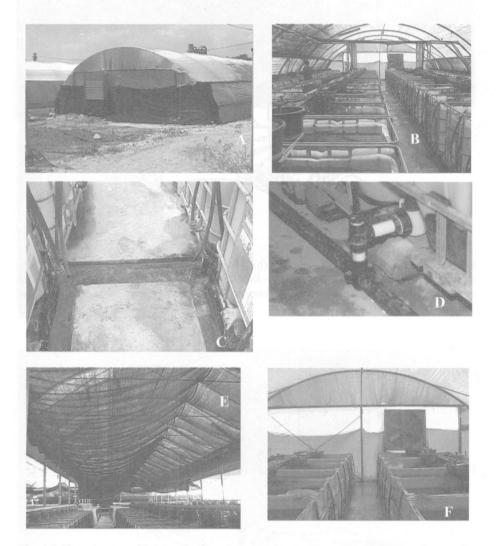


Fig. 4.7. Greenhouse cultivation facility: A – greenhouse; B – tanks inside greenhouse; C – greenhouse floor with drainage canal; D – pipe from tank to drainage canal: E – nets for shade during hot days; F – ventilator (arrow).

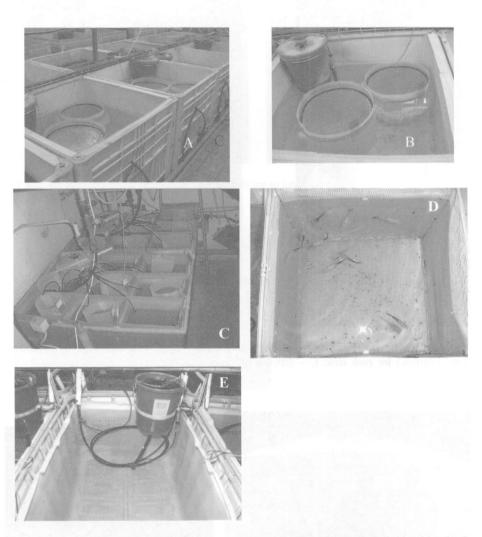


Fig. 4.8. Cultivation facilities at MIGAL: A,B – plastic Dolav tanks; C,D – portable plastic tanks hung on a metal frame; E - biological filter for removing ammonia, nitrites an nitrates from the water.

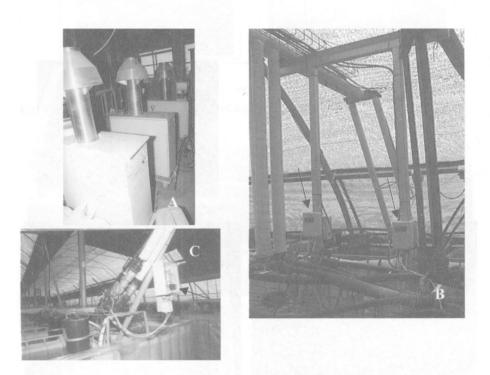


Fig. 4.9. Heating system: A – heaters; B – distribution pipes with individual temperature control (arrow) for each tank; C – distribution pipes with temperature control (arrow) for a line of tanks.

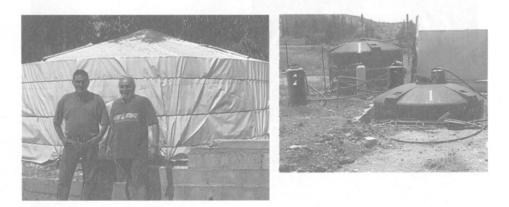


Fig. 4.10. (A) The author Gad Degani (left) and a colleague standing outside the greenhouse where the water supply filters and tanks are located. (B) Containers for storing water before use in fish tanks (1) and carbon filter (2) for removing poisons, especially chlorides.

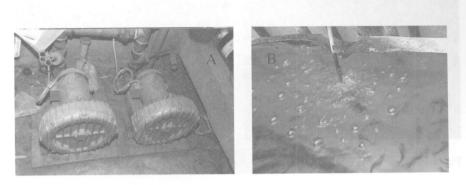


Fig. 4.11. Blowers (A) and air stones (B) supply air to tanks.

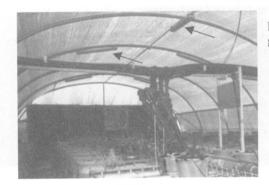


Fig. 4.12. Ceiling lamps (arrow) in the greenhouse.



Fig. 4.13. Emergency generator that automatically operates in case of electrical failure.

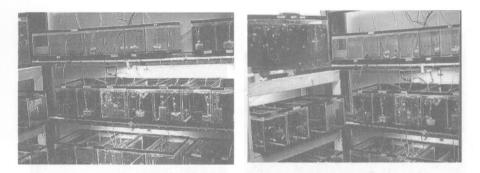


Fig. 4.14. Three rows of aquaria for raising guppies in a laboratory.



Fig. 4.15. Live tiny creatures that serve as a raw food source for fish, including daphnia, female diaptomus with follicles, female cyclops with follicles, Bosminidae, chironomos (red mosquito larvae), corethra (white mosquito larvae), pupa, and culex (black mosquito larvae).

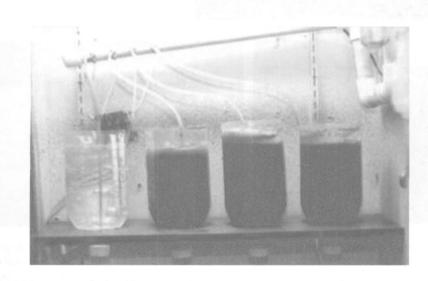


Fig. 4.16. A system for hatching Artemia eggs.

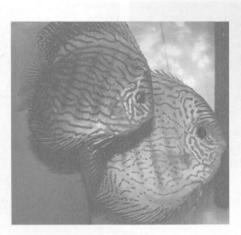
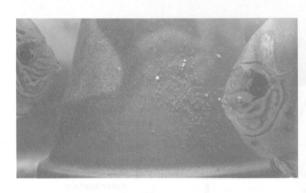


Fig. 5.1. A blue discus couple.



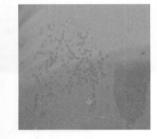


Fig. 5.2. Eggs laid on the pipe in the aquarium. The few spoiled eggs are white.

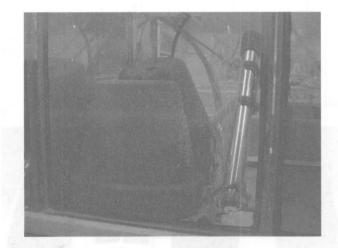


Fig. 5.3. Aquarium for maintaining the eggs until hatching.

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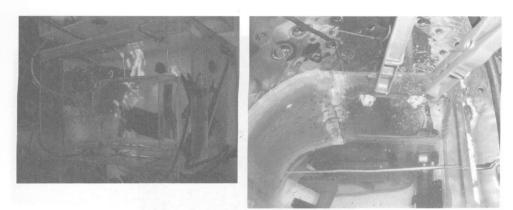


Fig. 5.4. Plastic container within aquarium for larvae before adaptation to food in the nursing aquarium.

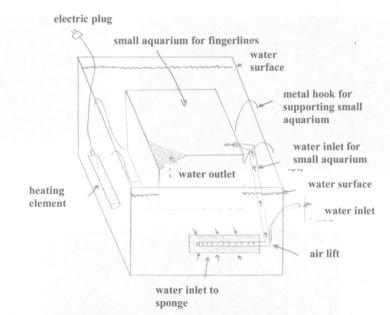


Fig. 5.5. Plastic container within aquarium for larvae (sketch).

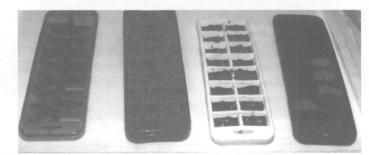


Fig. 5.6. MIGAL meat paddies.

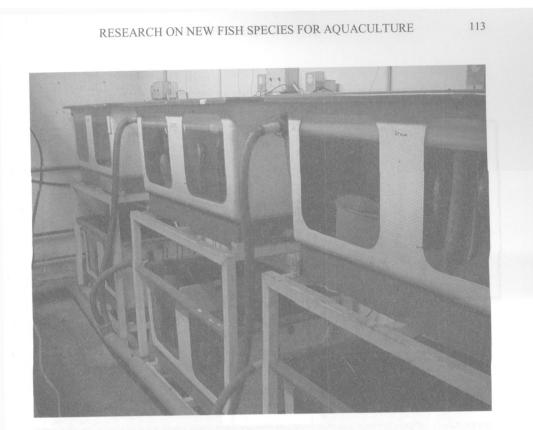


Fig. 5.7. Six treatments in the feed experiment.

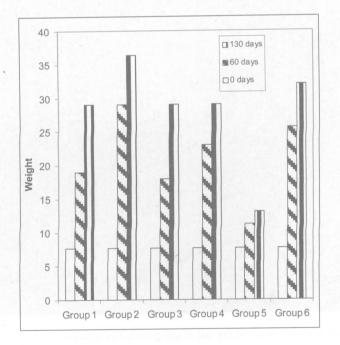


Fig. 5.8. Growth (in g) of discus feed six different diets.

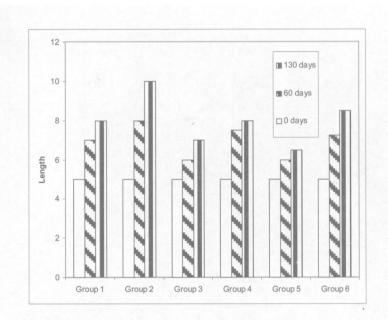


Fig. 5.9. Growth (in cm) of discus feed six different diets.

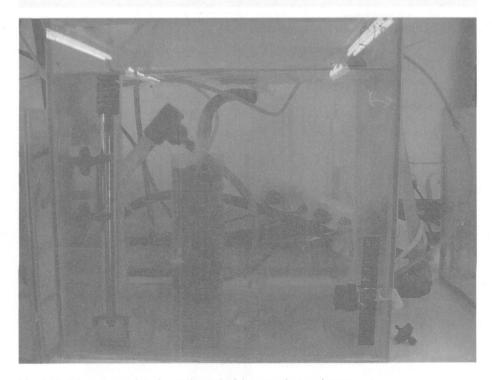
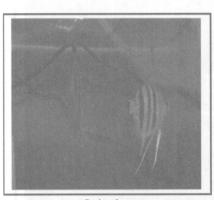
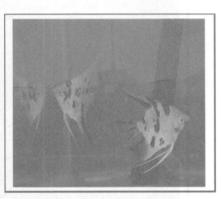


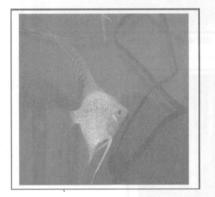
Fig. 5.10. Discus at market size at the end of the growth experiments.



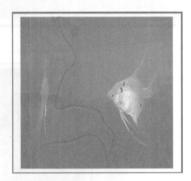
Striped gray



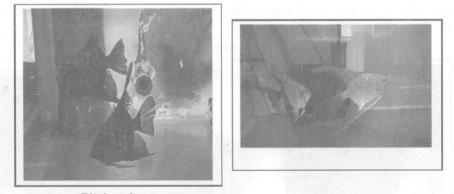
Marble black



Diamond silver



Gold marble



Black coal

Fig. 6.1 Angelfish strains studied in northern Israel.

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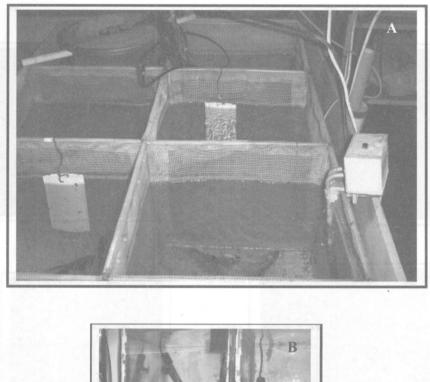




Fig. 6.2 Two methods for holding breeding pairs of angelfish: a container divided into partitions (A) and an aquarium (B).

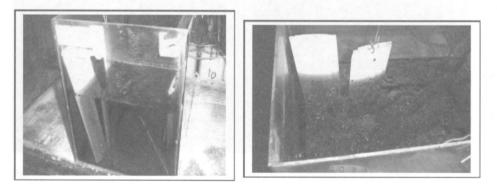


Fig. 6.3. A quarium for hatching scalar eggs with malachite blue (C16H18CIN3H). Eggs stick onto the strips of plastic hanging in the a quarium.

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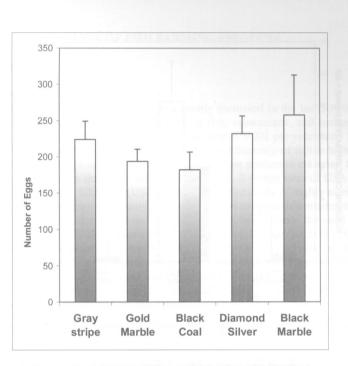


Fig. 6.4. Mean number of eggs per spawning in five strains of scalar.

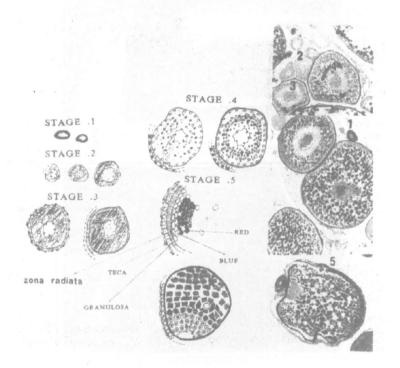


Fig. 6.5. Stages of oogenesis in the ovary of *Pterophyllum scalare*: 1 - chromatin nucleolus stage, 2 - perinucleolus stage, 3 - lipid vesicle stage, 4 - granular stage, 5 - maturation.

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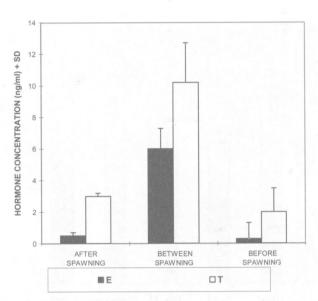


Fig. 6.6. Levels of E2 and T in female plasma during the gonadal cycle, measured by RIA

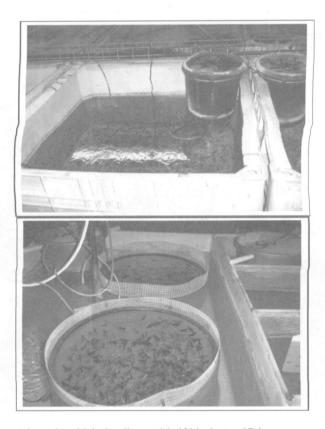


Fig. 6.7. The containers in which the diamond half-black angelfish were grown and a basket of black marble before shipment to market.

### CHAPTER 7 ECONOMIC EVALUATION OF FISH FARMING PROJECTS

### 7.1 Introduction

#### 7.1.1 The value of economic evaluation

The economic significance of aquaculture has greatly increased in the last 30-40 years. The contribution of aquaculture to the world supply of fish, crustaceans, and mollusks reached 29% in 2001, increasing at an average compounded rate of 9.2% per year since 1970 (FAO, 2003). Numerous studies have shown that the biological efficiency of fish is higher than that of land animals (Jolly and Clonts, 1993). Many aquaculture producers are small farms, often "backyard" part-time farms that deal with ornamental fish. Average sales of USA ornamental fish growers in 1998 were \$200,000 (USDA, 2000). The flexibility of highly motivated small and family farms can be helpful in innovation processes. Numerous new market varieties and innovative technologies, often based on multiple research lines, create the need for farmers and researchers to economically evaluate projects at farm level (Yom Din et al., 2002, 2004).

Careful economic evaluation in the fish farming industry is important for the following reasons. Development of aquaculture enterprises is a multistage process that includes, typically, establishment of the business and brood stock, first reproduction cycle and growth stage, additional reproduction and growth cycles, and, possibly, expansion after the first fish sales. As an alternative, purchased fry can replace the reproduction cycles. These stages differ in material and cash flows. Some require investment of the farmer's capital and some require loans. Aquaculture is a risky business, involving production (reproduction success; mortality) and market (seasonal and yearly volatility of prices) risks. The level of risk can be decreased by establishing the farm in phases and/or by growing more than one variety/line of fish. Economic evaluation allows the beginning farmer to place all the above-mentioned factors on a time axis and anticipate cash flows and risks on the same axis.

Economic evaluation can help the aquaculture farmer answer the following questions: What are the required water volume, area, and physical characteristics of the production and auxiliary facilities? Here the planner takes into account needs at different stages of reproduction and growth, pre-sale sorting and packing, quarantine and laboratory facilities, office rooms and equipment, infrastructure such as water and electricity lines. What is the required initial investment in buildings, infrastructure, equipment, and brood stock or fry? What working capital is needed at different stages of the farm's establishment? What capital and loans are needed? What are the expected sales flow, scheduling, and future plans? What is the expected capital return? What is the net present value of the cash flow and how is it sensitive to production and market risks?

Economic evaluation can help potential farmers decide whether to begin a considered business project or test ideas such as whether to reproduce fish with brood stock or purchase fry from other suppliers, how many fish varieties to raise to diminish risks and make the sales flow less volatile, whether to establish the farm in stages, and whether to establish the farm as a sole proprietorship or a partnership.

#### 7.1.2 Making an economic evaluation

Economic evaluation can be divided into stages. In the first stage, basic assumptions are formulated and data is collected. Basic assumptions can include how many fingerlings are produced per female, what survival rate can be expected, how long it takes to reach market size, how many brood stock must be replaced each year, what are realistic sales prices at the farm gate, what buildings and equipment are needed, what capital and working loans are required and at what interest rate, what are the rate of return and grace period of loans. Economic calculations presented in following sections are based on data prevalent in the Upper Galilee.

Next, in processing the data, several economic terms and calculations are used. "Net income" is calculated as the difference between the income of the farm and its expenses. It can be expressed in cash or as a percent of the income, i.e., net income = farm income –

production expenses – amortization – interest payment, or net income = net income/farm income x 100. Net income shows profit before taxes and the ability to return loans.

"Operating cash flow" is the difference between income and payments to suppliers including payments at the investment stage. "Net cash flow" derives from the operating cash flow after adding capital and loans and subtracting payments for loans, principal, and interest. Cash flows, contrary to net income, do not include non-cash charges such as amortization.

"Net present value (NPV)" of the investment derives from the operating cash flow after discounting the latter by the assumed interest rate ("discount rate"). In the following evaluations, the discount rate was assumed to be 10% per year.

Net income, operating and net cash flows, and  $\dot{\text{NPV}}$  can be presented in accumulated form.

"Internal rate of return (IRR)" is defined as the discount rate at which the NPV of the investment equals zero. If the IRR is greater than the discount rate used to calculate NPV, the project is attractive for the investor. In small projects evaluated for relatively short periods, such as is typical for fish farms, NPV and IRR usually produce the same recommendation for the investor.

"Sensitivity analysis" is another name for "what-if". In the following evaluations, the sensitivity of NPV to the main basic assumptions (survival rate and gate price) is analyzed. The survival rate and gate price that result in an NPV of zero are break-even points. Beyond these values, the project is unattractive for the investor.

## 7.1.2 Using electronic spreadsheets

Electronic spreadsheets presented in this chapter were built with MS Excél. There are three methods that are used to develop spreadsheets: chain calculations, spreadsheet functions, and graphic analysis. A chain calculation is a formula, saved in a cell of the spreadsheet. Such a calculation might be used, for example, to calculate the water volume required for a farm. Spreadsheet functions are built into the program and automatically process data entered into a spreadsheet table. As an example, IRR can be calculated by a function in the "Financial" category, IRR(B102:H102), which calculates IRR based on the forecasted cash flow entered in cells B102 through H102. NPV can also be calculated by a spreadsheet function, NPV(0.1,B102:H102), that calculates NPV from the operating cash flow and interest rate. Graphic analysis is available in the 'Chart' menu of Excel and is used to visually present conclusions.

#### 7.2 Economic evaluation of an eel farm

### 7.2.1 Basic assumptions and initial data

A commercial eel farm is a complicated and expensive farm. The total investment cost is over US\$1 million, including the initial cost of fingerlings. Required labor increases from 3.5 positions in the beginning of the project to 6 positions in the fifth year. The growing cycle of the fish entails three periods and five years (Chapter 1). Sex changes in mature fish can affect average farm gate prices since different prices are received for males and females. The sales volume and operating expenses vary from year to year. An additional risk of eel farms is that most facilities need to be established before the end of the first year when eels are moved to large ponds. To simplify the evaluation, the total investment is shown as a one-time payment in the beginning of the first year.

The following basic assumptions were made. The first growth period lasts for 3 months after capture of the glass eels and is characterized by high mortality (40%) and a wide distribution of fish size. Glass eels are raised in special containers and the required water volume is 0.1 liter per fish. In the second period (9 months), elvers are transferred to small ponds where the required water volume is 0.5 liter per fish. The assumed survival is 70%. When eels are raised in high-density conditions, a higher percentage of males develops. Therefore, a special diet containing steroids and hormones is needed. During the third period, gender control and manipulation are very important. The eels are raised in large ponds with 4 liters per fish and survival of 94%. Sales begin at the end of the second year at a weight of

200 g. The percentage of fish sold is affected by the heterogeneous growth of males and females and is anticipated to be 12% of the total fish produced in year 2, 24% in years 3 and 4, and 40% in year 5. The basic assumptions used in this evaluation are shown in Table 7.1. Sales potential (Table 7.2) and required water volumes (Table 7.3) are presented for a single cycle of eel growth and extrapolated to five years.

Table 7.	1.		
Basic assumptions used to analyze	e economics	of an eel	l farm.
Item	Females	Males	Total
Fingerlings (kg)			750
Population ratio	70%	30%	
Coefficient of survival:			
1 <sup>st</sup> period of growth			60%
2 <sup>nd</sup> second period of growth			70%
3 <sup>rd</sup> third period of growth			94%
Weight of fish for sale (g)	400	130	
Farm gate price (US\$/kg)	9.38	7.14	8.71
As a part of the investment require	ed:		
Own capital			50%
Loan for the investment minus	own capital		50%
Loan for the 1 <sup>st</sup> year working ca	pital		15%
Loan for the 2 <sup>nd</sup> year working of	capital		40%
Loan for the 3 <sup>rd</sup> year working ca	apital		33%
Loan for the 4 <sup>th</sup> year working ca	apital		20%
Interest rate of loan and of net pre	sent value		10%
Grace period for the principal pay	ment (years	)	1

Table	7.2.	

·	Project y	ear				
	Months 1-3	Months 4-12	2	3	4	5 onwards
For one cycle						
Coefficient of survival	60%	70%	94%	94%	94%	94%
Growth period (months)	3	9	6	12	12	12
No. fish at end of period (thousands)	1,364	955	803	567	345	
Sales (tons)			30	60	60	103
Fish for sale (thousands)			94	188	188	324
Farm gate price of fish (US\$)			2.90	2.90	2.90	2.90
Sales (thousand US\$)			273	546	546	942
Sales of multicycle farm (thousand US\$)			273	820	1,366	2,308

The required investment (Table 7.4) is based on the required number of containers, small ponds, and large ponds, estimated from the required water volume. Costs were taken from data of the model farm in the Upper Galilee (Chapter 1) and may be greater in areas with less favorable climatic conditions. To enable analysis of project sensitivity to changes in survival, expenses, or number of fish grown, expenses (Table 7.5) are divided into fixed and variable. Fixed expenses are "fixed" in relation to the number of fingerlings or fish in each stage and do not change up to a certain point (say, by 20%). "Variable" expenses vary in relation to the number of fingerlings/fish and depend, for example, on survival. For example, labor expenses grow from the second year to the fifth. Variable expenses account for 64% of the total production expenses, similar to the ratio for guppy and discus farms (65%; below).

Wa	Ta ter volume re	able 7.3. quired for	the eel f	arm.		
	Project year Months 1-3		2	3	4	5 onwards
	containers	small ponds	large p	onds		
For one cycle						
No. fish at start of period (thousands)	2,273	1,364	955	803	567	345
Water per fish (liters) Additional for control and losses	0.1	0.5	4	4	4	4
Volume required for 1 cycle (m <sup>3</sup> )	341	1,023	5,727	4,819	3,402	2,069
Total volume required for farm (m <sup>3</sup> )		1,364	7,091	11,910	15,312	17,381

Table 7.4.

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Item	Investment	Period of	Amortization
		amortization	(annual)
Buildings	·		
Greenhouse (400 m <sup>2</sup> )	160.0		
Containers (23 units)	36.8		
Small ponds (12 units)	29.0		
Large ponds (28 units)	233.1		
Total buildings	458.8	15	30.6
Equipment			
Greenhouse			
Electrical system	9.1		
Water circulation system	58.0		
Water pipes	19.1		
Heating system	10.0		
Control	4.5		
Small ponds			
Water pipes	35.2		
Electrical system	18.2		
Large ponds			
Water pipes	221.3		
Electrical system	20.5		
Oxygen device	77.3		
Control devices	11.4		
Total equipment	484.5	7	69.2
Fingerlings	132.0		
Total investment	943.3		99.8
Total investment + fingerlings	1,075.3		

Item	Proje	ct year			
	1	2	3	4	5 onwards
Fixed:					
Fingerlings		132	132	132	132
Electricity	5	24	54	79	103
Fuel	3	12	27	39	51
Oxygen	2	9	22	32	42
Miscellaneous	24	66	143	205	278
Total fixed	34	244	378	486	606
Variable:					
Medications and disposables	3	12	27	39	52
Packaging and sales expenses	0	32	147	276	460
Food	20	90	210	300	400
Water	0	2	3	4	4
Labor	82	98	114	130	146
Total variable	100	222	477	712	1,010
Total production expenses	134	466	855	1,198	1,616
Amortization	100	100	100	100	100
Total expenses + amortization	234	566	954	1,298	1,716

Table 7.5. Expenses of the eel farm (thousand US\$

#### 7.2.2 Economic evaluation of the project

Because of the five-year production cycle, an eel project needs thorough loan planning (Table 7.6). Four loans during the first four years will be needed, assuming that the owners' capital represents 50% of the investment. The first year loan will be used to finance 50% of the investment costs and 100% of the working capital for that year (15% of the investment costs). Loans in the second, third, and forth years are used to finance 40%, 33%, and 20% of the working capital, respectively. All loans are planned for a 7-year return at an interest rate of 10% with payment of the principal beginning in the second year (one-year grace period). The total interest paid in all four loans is US\$652,600, or 61% of total investment including fingerlings.

In the forecasted profit and loss (Table 7.7) and cash flow (Table 7.8), it is assumed that a new production cycle begins every year. The farm profit (net income prior to taxes) becomes positive in the fifth year after which it grows because of the decreasing interest payments on loans. The forecasted net cash flow in the first four years is slightly positive, due to the loans. The NPV is negative in the first three years when sales are relatively small. The accumulated net cash flow equals investment costs in the eighth year when the accumulated NPV becomes positive (Fig. 7.1), indicating a much longer "return period" than for the guppy and discus projects (3 and 5 years, respectively).

The IRR is close to 20%. This is greater than the assumed 10% discount rate, as in the guppy and discus projects, but smaller than the more attractive IRR evaluated for guppy (61%) and discus (78%) projects. Money invested in the larger eel farm with a five-year production cycle works slower.

In the sensitivity test (Table 7.9), the coefficient of survival was assumed to be 60% in the first growth stage, 70% in the second, and 94% in the third. The farm gate price was assumed to be US\$9.38/kg for females and US\$7.14/kg for males. Based on the basic assumptions, the NPV is US\$786,000, also shown in Table 7.8 where the average price used to calculate income was US\$8.71/kg and 70% of the fish were female. The sensitivity analysis shows critical changes in NPV resulting from combined changes in the coefficient of survival and average farm gate price. The combinations corresponding to zero values of NPV form an iso-line in Fig. 7.2. Combinations that allow keeping the NPV equal to US\$786,000 are presented as another iso-line. Point A corresponds to no change in average farm gate price and a decrease of 3% in coefficient of survival in all three growth stages. Point B corresponds

 Table 7.6.

 Principal and repayment of loans (at 10% yearly interest for 7 years with a grace period of 1 year) needed to establish the eel farm (thousand US\$).

	Year 1	_		Year 2			Year 3	_		<u>^</u>			
Year of repayment	\$613,10	)	\$377,300				\$311,300			\$188,700			
	Interest	Principal	Total	Interest	Principal	Total	Interest	Principal	Total	Interest	Principal	Total	
1	61.3	0.0	61.3	37.7	0.0	37.7	31.1	0.0	31.1	18.9	0.0	18.9	
2	54.9	79.5	134.3	33.8	48.9	82.7	27.8	40.3	68.2	16.9	24.5	41.3	
3	47.7	87.4	135.2	29.4	53.8	83.2	24.2	44.4	68.6	14.7	26.9	41.6	
4	39.9	96.2	136.1	24.6	59.2	83.7	20.3	48.8	69.1	12.3	29.6	41.9	
5	31.3	105.8	137.1	19.3	65.1	84.4	15.9	53.7	69.6	9.6	32.5	42.2	
6	21.9	116.3	138.2	13.5	71.6	85.1	11.1	59.1	70.2	6.7	35.8	42.5	
7	11.4	128.0	139.4	7.0	78.8	85.8	5.8	65.0	70.8	3.5	39.4	42.9	
Total	268.5	613.1	881.6	165.2	377.3	542.5	136.3	311.3	447.6	82.6	188.7	271.	

 Table 7.7.

 Forecasted profits and losses of the eel farm (thousand US\$).

Item	Project year										
	1	2	3	4	5	6	7	8	9	10	
Income		273	820	1,366	2,308	2,308	2,308	2,308	2,308	2,308	
Production expenses + fingerlings	271	478	880	1,236	1,670	1,670	1,670	1,670	1,670	1,670	
Amortization	100	100	100	100	100	100	100	100	100	100	
Interest payment	61	93	113	116	97	76	53	28	13	4	
Net income prior to taxes	-432	-397	-273	-86	441	461	484	510	525	534	
Net income as % of sales			-33%	-6%	19%	20%	21%	22%	23%	23%	

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	Forecaste	d cash now	for the ee	i iarm (inc	usand U	<u>33).</u>				
Item	Project	year								
	1	2	3	4	5	6	7	8	9	10
Income		<b>2</b> 73	820	1,366	2,308	2,308	2,308	2,308	2,308	2,308
Cash payments:										
Suppliers and labor	139	478	880	1,236	1,670	1,670	1,670	1,670	1,670	1,670
Equipment	943									
Total payments	1,083	478	880	1,236	1,670	1,670	1,670	1,670	1,670	1,670
Operating cash flow	-1,083	-205	-61	130	637	637	637	637	637	637
Own capital	538									
Loan	613	377	311	189						
Interest + principal payment	61	172	249	306	331	333	336	198	128	43
Net cash flow	7	1	2	12	307	304	301	439	509	594
Net cash flow (accumulated)	7	7	9	21	328	632	934	1,373	1,882	2,476
Net present value	-984	-169	-45	89	396	360	327	297	270	246
Net present value (accumulated)	-984	-1,153	-1,199	-1,110	-714	-355	-28	270	540	786

 Table 7.8.

 Forecasted cash flow for the eel farm (thousand US\$).

Sensitivity of NPV to changes in the coefficient of survival or market price Coefficient of survival and assortment												
Farm gate price of fish	-20%	-15%	-10%	-5%	0%	5%						
-20%	-2,942	-2,780	-2,428	-1,835	-942	324						
-15%	-2,690	-2,504	-2,116	-1,473	-510	849						
-10%	-2,438	-2,228	-1,805	-1,111	-78	1,374						
-5%	-2,186	-1,952	-1,493	-749	354	1,899						
0%	-1,934	-1,676	-1,182	-386	786	2,424						
5%	-1,682	-1,401	-870	-24	1,218	2,949						

to no change in coefficient of survival and a decrease of 9% in the average farm gate price. Analysis of the dashed iso-line leads to a similar conclusion: every 1% change in coefficient of survival can be balanced by a 3% change in price in the opposite direction.

The high sensitivity of the NPV to changes in coefficient of survival prompted Degani and Gallagher (1995) and Northern R&D (2000) to develop technological innovations for eel farms. Yom Din et al. (2004) developed a theoretical model for economic evaluation of those innovations that could analyze multiple research lines combined in the same innovation. Their model was based on a general model of induced innovations in agriculture that includes multiple research lines (Sunding and Zilberman, 2001)

7.2.3 Economic evaluation of innovations

The price of females is approximately 30% higher than the price of males. Hormonally increasing the percentage of females is one innovative technology developed for eel farms to obtain improved economics. Fig. 7.3 shows the results of changing the percentage of females and recalculating the NPV. Point A corresponds to the basic assumptions that 70% of an eel population is female and that NPV equals US\$786,000. Point B reflects a 5% increase of females (i.e., 73.5%) and results in an NPV of US\$1.014 million (an increase of 29%). In other words, every 1% increase in females leads to a 5.8% increase in NPV.

The model for economic evaluation enables much more detailed calculations than that presented in Fig. 7.3. Several research lines can be evaluated at the same time such as 3-4 temperature levels, dietary fat and protein levels, fish densities, and hormone concentrations. The number of possible combinations of these research lines at different levels of intensity, and for all three growth stages, is 216,000.

The example in Table 7.10 shows application of the model to research lines that were selected as the most profitable: improved diet, increased density, and use of hormones to increase the percentage of females (Degani and Gallagher, 1995), with other factors remaining as described in Table 7.9. A computer program quantified the effects of the considered combinations of research lines to find the farm's most profitable innovative program and characteristics in different growth stages (Table 7.11). All possible combinations were compared. The last two columns reveal significant changes in those biological characteristics that are crucial for profitability. That is, survival increases by 37% as a result of improved diet, increased density, and a water exchange rate of 24 times daily but increased survival of glass eels and higher density reduce the growth rate by 44% in the first growth stage. Nevertheless, the lower growth rate in this stage is compensated for in stage 2 when it increases by 17% and stage 3 by 46%.

## 7.3 Economic evaluation of a guppy farm

#### 7.3.1 Basic unit

The purposes of establishing a basic guppy unit are to gain experience in producing and marketing ornamental live breeding fish and to achieve economic success that can serve as a basis for expansion of the farm. The basic assumptions of the economic evaluation appear in Table 7.12.

Within 6-9 months from the introduction of brood stock to the system, a farm can market 15,000 live breeders per month, depending on the size of the initial breeding school. In the evaluated case, six months of sales are planned for the first year and 12 months from the second year onwards (Table 7.13).

The capital investment is based on the required water volumes of aquaria for brood stock maintenance, breeding, growing, and control (Table 7.14). About 60% of the costs for equipment and infrastructure of the basic unit (Table 7.15) is sufficient for an expanded farm.

Farm expenses are shown in Table 7.16. The estimated amortization of buildings and equipment was based on the required investment and known periods of amortization. Fixed expenses account for 45% of the total expenses.

Net income before taxes (Table 7.17) includes the farmer's own labor, calculated as 0.5 day, for operating the unit. From the second year onwards, US\$45,000 is the planned

		E	ffect of techno	logical ir	Table 7.		al and growth r	ate.		
Growth stage	Innovation	No. research lines	Coefficient	of surviv	al		Growth rate (g/day)			
			Farm level	Chang- intensi		studied	Farm level	Chang intens	-	studied
				min	avg	max		min	avg	max
1	Diet	5	0.60	0.67	0.70	0.77	0.004	0.001	0.005	0.013
	Density	2	0.60	0.37	0.70	0.96	0.004	0.001	0.009	0.015
2	Hormones	5	0.70	0.70	0.70	0.70	0.040	0.004	0.021	0.051
	Diet	3	0.70	0.70	0.70	0.70	0.040	0.008	0.051	0.113
3	Hormones	2	0.94	0.94	0.94	0.94	0.240	0.264	0.326	0.383
•	Density	2	0.94	0.94	0.94	0.94	0.240	0.240	0.268	0.321

\*Density is measured in kg/m<sup>2</sup> for the first growth stage and kg/m<sup>3</sup> in the third. Concentration of hormones is measured in mg/kg diet (wet weight).

Characteristics of the	e most profita	ble innovation	s.			
Characteristic	Thousand US\$	Part additional profit (%)	of	Change coefficient survival	in of	Growth rate
Farm profit before innovation	2,367					
Farm profit after innovation	6,307					
Additional profit	3,940	100.0%				
Additional profit by growth stages and research lines Stage 1						
Diet 10% chicken oil, water 25°C	1,307	33.2%				
Density 10 kg/m <sup>2</sup> , water changed 24 times daily	140	3.6%				
Total stage 1	1,448	36.7%		37%		-44%
Stage 2						
Hormone insulin 40 ppm	32	0.8%				
Diet 20% lipids, water 25°C	136	3.4%				
Total stage 2	167	4.3%		0%		17%
Stage 3						
Hormone human chorionic gonadotropin in isolated	1,504	38.2%				
groups						
Density 60 kg/m <sup>3</sup> , water recirculation system	820	20.8%				
Total stage 3	2,324	59.0%		0%		46%
Total for the innovation	3,940	100.0%				

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Table 7.11. cteristics of the most profitable innovations.

Table 7.12.	
Basic assumptions used in the economic evaluation	of a guppy farm.
Fingerlings per female (monthly)	10
Coefficient of survival and assortment	75%
Exchange of parents	33.3%
Farm gate price of fish (US\$)	0.25
As a part of the investment required	
Own capital	50%
Loan for investment minus own capital	50%
Loan for first year working capital	10%
Interest rate of loan and net present value	10%
Grace period for the principal payment (years)	1

Table 7.13.
Potential sales of the basic guppy unit.

	First year	Second year onwards
Parent fish (males and females)	2,400	2,400
Females	2,000	2,000
Fingerlings per female (monthly)	10	10
Coefficient of survival and assortment	75%	75%
Introduction of parental schools (months)	2	
Growth period (months)	4	4
Annual no. fish for sale	90,000	180,000
Farm gate price of fish (US\$)	0.25	0.25
Sales (US\$)	22,500	45,000

Table 7.1	4.
Required water volume and floor sp	bace in the basic guppy unit.
No. fish per growth period	60,000
Water per fish (liters)	1
Additional volume for control	10%

71

142

Water per parent fish (liters)

Total volume required (m<sup>3</sup>)

Paved floor space  $(m^2)$ 

income.	The	only	variable	component	in	the	net	income	calculation	is	interest	on	loans.
Profitab	ility c	of the	basic unit	grows to 60	%	by tł	ne se	venth ye	ar.				

The forecasted cash flow (Table 7.18) includes payments for brood stock in the first year, renewal of brood stock in the second year onwards, equipment, and loans, but does not include amortization. Net cash flow and NPV can be calculated from the forecasted cash flow. The year in which net cash flow equals the investment or NPV becomes positive is an indicator of the "return period". The total investment is US\$67,800 including payments for brood stock. The net cash flow indicates a return period of 3 years while NPV indicates 33 months, both relatively short periods of return (Fig. 7.4). The IRR, calculated from the operating cash flow, equals 61% - much greater than the 10% discount rate, indicating that investment in the project is attractive.

Table 7.19 shows the sensitivity of the project to changes in the coefficient of survival or farm gate prices. The combined changes, presented as iso-lines in Fig. 7.5, show that a 53% lower survival or 44% lower farm price lead to an NPV of zero. The difference in

Item	Cost	Meets the needs of an extended (249 m <sup>3</sup> ) farm?	Period of amortization (years)	Amortization (annual)
Buildings				
Greenhouse (1,000 m <sup>2</sup> )	3.0	yes		
Reinforced plastic covering	2.0	yes		
90% shade net	1.2	yes		
Concrete floor w/drainage	6.9	yes		
Electrical system	6.0	yes		
Plumbing	3.3	yes		
Heating system	6.0			
Machinery room	7.5			
Total buildings	36.0		15	2.4
Equipment				
50' fan	0.8	yes		
Cultivation pools (71 m <sup>3</sup> )	13.0	yes		
Automatic backup generator	7.7			
Blowers	2.7			
Quality control aquarium	0.3			
Freezer for food	1.3			
Electronic scale	0.5			
Oxygen meter	1.0			
pH meter	0.3			
Fish nets	0.3	yes		
Styrofoam for floor isolation	0.2	yes		
Biological filters	0.7	yes		
Total equipment	28.8		7	4.1
Parents (initial school)	3.0	yes		
Total investment	64.8			6.5
Total investment + parents	67.8			

Table 7.15.

Table 7.16.

Expenses of the basic guppy unit (thousand US\$).						
Item	First year	Second year onwards	Comments			
Fixed						
Exchange of parents		1.0	33%			
Electricity	1.2	1.2				
Fuel	1.7	1.7				
Miscellaneous	0.8	0.8				
Total fixed	3.8	4.8				
Variable						
Medications and disposables	0.3	0.4				
Transportation to the marketplace	1.1	2.1				
Food	0.8	1.2				
Water	1.6	2.0	6,362 m <sup>3</sup>			
Total variable	3.8	5.8				
Total production expenses	7.6	10.6				
Amortization	6.5	6.5				
Total expenses + amortization	14.1	17.1				

va promo							
Project y	year			_		-	
1	2	3	4	5	6		
22.5	45.0	45.0	45.0	45.0	45.0	45.0	
10.6	10.6	10.6	10.6	10.6	10.6	10.6	
6.5 4.1	6.5 3.6	6.5 3.2	6.5 2.6	6.5 2.1	6.5 1.4	6.5 0.8	
1,300 6%	24,300 54%	24,700 55%	25,300 56%	25,800 57%	26,500 59%	27,100 60%	
	Project y 1 22.5 10.6 6.5 4.1	Project year 1 2 22.5 45.0 10.6 10.6 6.5 6.5 4.1 3.6 1,300 24,300	Project year         3           1         2         3           22.5         45.0         45.0           10.6         10.6         10.6           6.5         6.5         6.5           4.1         3.6         3.2           1,300         24,300         24,700	Project year $1$ $2$ $3$ $4$ $22.5$ $45.0$ $45.0$ $45.0$ $10.6$ $10.6$ $10.6$ $10.6$ $6.5$ $6.5$ $6.5$ $6.5$ $4.1$ $3.6$ $3.2$ $2.6$ $1,300$ $24,300$ $24,700$ $25,300$	Project year $1$ $2$ $3$ $4$ $5$ $22.5$ $45.0$ $45.0$ $45.0$ $45.0$ $45.0$ $10.6$ $10.6$ $10.6$ $10.6$ $10.6$ $10.6$ $6.5$ $6.5$ $6.5$ $6.5$ $6.5$ $4.1$ $1,300$ $24,300$ $24,700$ $25,300$ $25,800$ $570/$ $570/$ $570/$ $570/$ $570/$	Project year $1$ $2$ $3$ $4$ $5$ $6$ $22.5$ $45.0$ $45.0$ $45.0$ $45.0$ $45.0$ $45.0$ $10.6$ $10.6$ $10.6$ $10.6$ $10.6$ $10.6$ $6.5$ $6.5$ $6.5$ $6.5$ $6.5$ $6.5$ $4.1$ $3.6$ $3.2$ $2.6$ $2.1$ $1.4$ $1,300$ $24,300$ $24,700$ $25,300$ $25,800$ $26,500$ $5.90'$ $5.90'$ $5.90'$ $5.90'$ $5.90'$ $5.90'$	

Table 7.17. Forecasted profits and losses of the basic guppy unit (thousand US\$).

\* after taking into consideration half day self-labor

fish

Table 7.18. Forecasted cash flow for the basic guppy unit (thousand US\$	)
Project year	(

Item	Project year									
	1	2	3	4	5	6	7			
Income	22.5	45.0	45.0	45.0	45.0	45.0	45.0			
Cash payments Suppliers	10.6	10.6	10.6	10.6	10.6	10.6	10.6			
Equipment Total payments Operating cash flow	64.8 75.4 -52.9	10.6 34.4	10.6 34.4	10.6 34.4	10.6 34.4	10.6 34.4	10.6 34.4			
Own capital Loan	32.4 40.7	0								
Interest + principal payment Net cash flow	4.1 16.1	8.9 25.5	9.0 25.5	9.0 25.4	9.1 25.3	9.2 25.3	9.2 25.2			
Net cash flow (accumulated) Net present value	16.1 -48.1	41.6 28.4	67.1 25.9	92.4 23.5	117.8 21.4	143.0 19.4 70.5	168.2 17.7 88.18			
Net present value (accumulated)	-48.1	-19.7	6.2	29.7	51.1	70.5	00.10			

Table 7.19. Sensitivity of NPV to changes in survival and farm gate prices. Farm gate price of Coefficient of survival and assortment 10% 0% -30% -20% -10% -50% -40% -60% -31.0 -27.2 -35.1 -39.5 -44.1 -59.6 -54.2 -49.0 -60% -5.3 -17.2 -11.1 -51.6 -44.2 -37.1 -30.2 -23.6

-50%	-51.6	-44.2	-37.1	-30.2	-23.6	-17.2	-11.1	-5.3	0.2
-40%	-43.7	-34.3	-25.2	-16.3	-7.7	0.7	8.7	16.5	24.1
-30%	-35.8	-24.4	-13.2	-2.4	8.2	18.5	28.6	38.4	47.9
-20%	-27.8	-14.4	-1.3	11.5	24.1	36.4	48.5	60.2	71.7
-10%	-19.9	-4.5	10.6	25.4	40.0	54.3	68.3	82.1	95.6
2010	17.7	5.4	22.5	39.3	55.9	72.2	88.2	103.9	119.4
0%	-4.0	15.4	34.4	53.2	71.8	90.0	108.0	125.8	143.2
10%			46.3	67.1	87.7	107.9	127.9	147.6	167.1
20%	4.0	25.3	40.5	07.1	01.1	101.2			

sensitivity to these two factors is explained by the drop in variable expenses that results from lower survival.

The positive economics of the basic guppy unit make it reasonable to consider expanding the farm.

20%

-23.6

0.2

## 7.3.2 Expanded farm

The results of the previous section show that a basic guppy unit can be suitable for a "backyard" part-time farmer. Expansion of this small unit to a 275 m<sup>3</sup> farm makes a profitable project that can stand on its own. In the expanded project, an additional variety of live breeding ornamental fish, the green swordtail, is added. The green swordtail is projected as 30% of fish population of the expanded farm (Table 7.20). The green swordtail requires about 40% more water than guppies. Together with increased guppy production, the total water volume of the expanded farm is about four times that of the basic unit (Table 7.21) but the investment cost is only twice that of the basic unit (Table 7.22) because about 60% of the facilities of the basic unit meet the needs of the expanded farm. Expenses of the expanded farm (Table 7.23) include the cost of a half-day hired worker while the farmer will be engaged a full day. In the first year of expansion, sales of the additional guppies are projected for 6 months and for the green swordtails for 5 months (Table 7.24). From the second year onwards, sales are planned for 12 months for both varieties.

The cost of the farmer's labor is included in the net income prior to taxes (Table 7.25). Profitability of the expanded farm grows to 49% during the first seven years. The IRR is about 68% - much greater than the 10% discount rate. The return period (Table 7.26) is 56 months according to the accumulated cash flow and 39 months according to the NPV (Fig. 7.6).

The NVP of the expanded farm is sensitive to a 65% change in the coefficient of survival or 48% change in fish price (Table 7.27) whereas sensitivity in the basic unit was 53% and 44%, respectively. The differences in sensitivity are explained by decreased variable expenses when the coefficient of survival decreases. The iso-line for NPV = 0 of the expanded farm is lower than that of the basic unit (Fig. 7.7). For example, at point A, where the change in the coefficient of survival is -60%, the fish price for the basic unit must increase by 15% to maintain NPV at zero. At point B, where the change in the coefficient of survival also equals - 60%, even if the price for the extended farm decreases by 10%, the NPV is still positive.

	Guppy	Green swordtail	Total
Population ratio	70%	30%	
Fingerlings per female (monthly)	10	15	
Coefficient of survival and assortment	75%	75%	
Exchange of parents	33.3%	33.3%	
Farm gate price of fish (US\$)	0.25	0.36	
As a part of the investment required			
Own capital			0%
Loan for investment minus own capital			100%
Interest rate of loan and net present value			10%
Grace period for principal payment (years)			1

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Water volume require	d for the exp	anded guppy farm.		
	Guppies	Green swordtail	Total	
No. fish per growth period	120,000	56,300		
Water per fish (liters)	1	1.4		
Additional volume for control	10%	10%		
Water per parent fish (liters)	2	2.8		
Total volume required (m <sup>3</sup> )	141.6	90.0	231.6	
Paved floor space (m <sup>2</sup> )	283.2	180.0	463.2	

Item	Invested in	for the expanded guppy far Additional investment		Ammortization	Annual
	basic unit	for expanded farm	investment	(years)	amortization
Buildings	. <u></u> d				
Greenhouse (1,000 m <sup>2</sup> )	3.0		3.0		
Reinforced plastic covering	2.0		2.0		
90% shade net	1.2		1.2		
Concrete floor w/drainage	6.9		6.9		
Electrical system	6.0		6.0		
Plumbing	3.3		3.3		
Heating system	6.0	13.7	19.7		
Machinery room	7.5	16.9	24.4		
Total buildings	36.0	30.6	67	15	4.4
Equipment		,			
50' fan	0.8		0.8		
Cultivation pools (70 m <sup>3</sup> )	13.0		13.0		
Automatic backup generator	7.7	17.5	25.2		
Blowers	2.7	6.1	8.8		
Quality control aquarium	0.3	0.7	1.0		
Freezer for food	1.3	2.9	4.2		
Electronic scale	0.5		0.5		
Oxygen meter	1.0		1.0		
pH meter	0.3		0.3		
Fish nets	0.3	0.7	1.0		
Styrofoam for pool floor isolation	0.2		0.2		
Biological filters	0.7		0.7		
Office equipment		1	1.0		
Total equipment	28.8	28.9	57.7	7	8.2
Parents (initial school)	3.0	6.0	9.0		
Total investment	64.8	59.5	124.3		12.7
Total investment + parents	67.8	65.5	133.3		

 Table 7.22.

 Investment required for the expanded guppy farm (thousand US\$).

Item	First year	Second year onwards	Comment	
Fixed				
Exchange of parents		3.0	33%	
Electricity	4.0	4.0		
Fuel	5.6	5.6		
Miscellaneous	2.7	2.7		
Total fixed	12.3	15.3		
Variable				
Medications and disposables	1.0	1.2		
Transport to market	3.9	5.9		
Food	2.4	3.6		
Water	4.8	5.9	$6,362 \text{ m}^3$	
Hired labor	12.2	12.2	half-day	
Total variable	24.2	28.9		
Total production expenses	36.6	44.2		
Amortization	12.7	12.7		
Total expenses + amortization	49.3	56.9		

 Table 7.23.

 Expenses of the expanded guppy farm (thousand US\$).

7.3.3 Summary of the economics of a guppy farm

The required water volume is  $71 \text{ m}^3$  for the basic unit and  $275 \text{ m}^3$  for the expanded farm, consisting of 168 m<sup>3</sup> for guppies and 107 m<sup>3</sup> for green swordtail.

Initial investment is US\$67,800 for the basic unit, including the cost of brood stock. For the expanded farm, the additional investment is around US\$65,500.

Annual projected sales are 180,000 guppies for the basic unit and 360,000 guppies plus 135,000 green swordtails for the expanded farm.

Net income prior to taxes, after deducting the owner's own labor, is 60% for the basic unit and 49% for the expanded farm. The expected annual net cash flow is about US\$25,000 for the basic unit (including a half-day labor of the farmer) and US\$62,000 for the expanded farm (including a full day of labor of the farmer).

For a basic unit, the NPV is positive after 33 months, and it is sensitive to a 53% change in the coefficient of survival or 44% change in fish price. For an extended farm, NPV is positive after 39 months (including the two years of the basic unit) and it is sensitive to a 65% change in survival or 48% change in price.

The evaluation shows that economic performance is better in the expanded farm, built after operating the basic unit for two years, than for the basic unit alone. An expanded farm is preferable, also because of better results in the sensitivity analysis. However, the liability of the expanded farm is higher. For the basic unit, the farmer invests US\$32,400 of his own capital and borrows US\$40,700 (equal to US\$58,500 including interest payments). Repayment of loans accounts for 90% of the total cost of the buildings and equipment in the basic unit. For the expanded farm, the same investment is needed in the first stage (basic unit) and a second loan of US\$65,500 (US\$94,200 including interest) is required for the expansion. The total loan repayment of US\$152,700 represents 115% of the total cost of the buildings and equipment in the expanded farm.

### 7.3.4 The Hefetz model fish farm

The Hefetz ornamental fish farm at Korazim in the lower Galilee was established in 1999. Investment in the project was divided into two stages (Table 7.27a). Stage 1 concentrated on development of a 200-m<sup>3</sup> basic unit on a paved floor area of 500 m<sup>2</sup>. After stage 1 came into full and efficient operation, the farm was expanded to 550 m<sup>3</sup> (water volume) on 1,000 m<sup>2</sup>. For some items (electrical installation, lighting, electronic scale), the investment for stage 1 fully met the needs of the stage 2. Establishment costs for the basic unit were equivalent to

	First year			Second year onwards		
	Guppies	Green swordtail	Total	Guppies	Green swordtail	Total
Parent fish (males and females)	4,800	1,200	6,000	4,800	1,200	6,000
Females	4,000	1,000	5,000	4,000	1,000	5,000
Fingerlings per female (monthly)	10	15		10	15	
Coefficient of survival and assortment	75%	75%		75%	75%	
Introduction of parental schools (months)	2	2				
Growth period (months)	4	5		4	5	
Annual fish for sale	270,000	56,300	326,300	360,000	135,000	495,000
Farm gate price of fish (US\$)	0.25	0.36		0.25	0.36	
Sales (US\$)	67,500	20,300	87,800	90,000	48,600	138,600

Table 7.24. Sales on the expanded guppy farr

Item	Project year Basic unit		Extended farm				
	1	2	3	4	5	6	7
Income	22.5	45.0	87.8	138.6	138.6	138.6	138.6
Production expenses + parents	10.6	13.6	45.6	53.2	53.2	53.2	53.2
Amortization	6.5	6.5	12.7	12.7	12.7	12.7	12.7
Interest payment	4.1	3.6	9.7	8.5	7.2	5.7	4.1
Net income prior to taxes*							
(US\$)	1,300	24,300	19,800	64,200	65,500	67,000	68,600
% of sales	6%	54%	23%	46%	47%	48%	49%

\* includes half-day self-labor for 2 years, full day from third year onwards

Item	Project year						
	1	2	3	4	5	6	7
	Basic	unit	Extende	ed farm			
Income	22.5	45.0	87.8	138.6	138.6	138.6	138.6
Cash payments							
Suppliers and hired labor	10.6	13.6	45.6	53.2	53.2	53.2	53.2
Equipment	64.8	0.0	59.5				
Total payments	75.4	10.6	105.1	53.2	53.2	53.2	53.2
Operating cash flow	-52.9	34.4	-17.3	85.4	85.4	85.4	85.4
Own capital	32.4						
Loan	40.7		65.5				
Interest + principal payment	4.1	8.9	15.5	23.4	23.5	23.7	23.9
Net cash flow	16.1	25.5	32.6	62.0	61.8	61.7	61.5
Net cash flow (accumulated)	16.1	41.6	32.6	94.6	156.5	218.1	279.6
Net present value	-48.1	28.4	-15.8	70.6	64.1	58.3	53.0
Net present value (accumulated)	-48.1	-19.7	-15.8	54.8	118.9	177.2	230.2

 Table 7.26.

 Forecasted cash flow for the expanded guppy farm (thousand US\$).

Sensitivity of NPV to changes in survival and/or gate price in the expanded guppy farm.										
Coefficient of survival and assortment										
Farm gate price of fish	-60%	-50%	-40%	-30%	-20%	-10%	0%	10%	20%	
-60%	-95.0	-85.7	-77.7	-70.8	-65.1	-60.6	-57.3	-55.1	-54.1	
-50%	-75.8	-61.8	-48.9	-37.3	-26.8	-17.5	-9.4	-2.4	3.4	
-40%	-56.6	-37.8	-20.2	-3.7	11.5	25.6	38.6	50.3	60.9	
-30%	-37.5	-13.9	8.6	29.8	49.9	68.8	86.5	103.0	118.4	
-20%	-18.3	10.1	37.3	63.3	88.2	111.9	134.4	155.7	175.9	
-10%	0.9	34.0	66.1	96.9	126.5	155.0	182.3	208.4	233.4	
0%	20.0	58.0	94.8	130.4	164.9	198.1	230.2	261.1	290.9	
10%	39.2	82.0	123.6	164.0	203.2	241.3	278.2	313.9	348.4	
20%	58.4	105.9	152.3	197.5	241.5	284.4	326.1	366.6	405.9	

Тε	able	7.	.27.		

US\$489/m<sup>3</sup> water volume. After completing the expanded farm, the overall establishment costs equaled US\$263/m<sup>3</sup>.

Establishment costs of the Hefetz model guppy			)
Item (required for stage 1)	Basic unit	Expanded farm	Total
	$(200 \text{ m}^3)$	$(550 \text{ m}^3)$	
Reinforced plastic sheeting (2,150 m <sup>2</sup> )	6,265		6,265
Wood for construction	843		843
Construction - labor costs	963		963
Exterior 90% shade net "Aluminet"	2,650		2,650
Interior 90% black shade net	1,686		1,686
Opening mechanism for interior net	602		602
Interior black net - construction costs	481		481
Ramp paving	9,156		9,156
Activated charcoal filters for water main (2)	361	361	722
Underwater pumps (4)	289	289	578
Water control valves (17)	72	48	120
Water storage tanks (40 m <sup>3</sup> )	1,445	1,445	2,890
Biological filters (plumbing, containers, Perlon,	2,048	2,048	4,096
Polygal)			
Water drainage channels on the floor	2,409	1,807	4,216
Air blowers $(4)$ + plumbing for forced air conduction	4,819	4,337	9,156
Heating ovens (2; 350,000 kcal), plumbing, electric	13,253	7,228	20,481
valves/ thermostats/sensors (30)			
Scale + refrigerator + freezer	2,120		2,120
Emergency generator (20 kWh)	7,228		7,228
Electrical installation + neon lighting	6,506		6,506
Plumbing	482		482
Oxygen tanks + apparatus for sorting and packing	2,168	1,686	3,854
Quality control system for export (aquarium + stands)	3,614	2,409	6,023
Operational apparatus for fish	2,891	1,928	4,819
pH meter	241		241
Work tables	1,204		1,204
Cultivation tanks (570 m <sup>3</sup> )	21,686	21,686	43,372
Adaptation of the existing packinghouse	2,409	<i>.</i>	2,409
Computer + printer	-	1,445	1,445
Total	97,891	46,717	144,608

Table 7.27a.

## 7.4 Economic evaluation of a discus farm

### 7.4.1 Basic unit

The reasons for establishing a basic discus unit are to gain experience in production and marketing of a difficult variety of egg-laying ornamental fish and to achieve economic success that can serve as the basis for expansion of the farm. The basic assumptions of the discus unit are given in Table 7.28. It is anticipated that within 6-9 months after stocking the brood stock in the unit, it will market 15,000 live breeders per month. Sales are projected for 6 months in the first year and 12 months in the second year onwards (Table 7.29). The required water volume and floor space (Table 7.30) is much less than required for the guppy project.

About 33% of the equipment and infrastructure of the basic unit will suffice for the expanded farm (Table 7.31); this is less than in the guppy project (60%). The amortization of buildings and equipment for the discus unit is about 12% of the total cost of investment (Table 7.32), greater than for the guppy unit (10%). The structure of expenses for a discus unit is similar to that of a guppy unit: 50% fixed, 50% variable expenses.

Table 7.28.

Basic assumptions for establishment of a basic discus unit						
Eggs per female (monthly)	300					
Coefficient of survival and assortment	27%					
Exchange of parents	33.3%					
Farm gate price of 5 cm fish (US\$)	3.00					
As a part of the investment required:						
Own capital	50%					
Loan for investment minus own capital	50%					
Loan for first year working capital	20%					
Interest rate of loan and net present value	10%					
Grace period for the principal payment (years)	1					

Table 7.29.	
Projected sales schedule for the basic discus unit.	

	First year	Second year onwards
Parent fish (males and females)	40	40
Females	20	20
Reserve parent fish	20	20
Eggs per female (per month)	300	300
Coefficient of survival and assortment	27%	27% ·
Introduction of parental schools (months)	2	
Growth period (months)	4	4
Annual no. fish for sale	9,700	19,400
Farm gate price of fish (US\$)	3.00	3.00
Sales (US\$)	29,200	58,300

#### Table 7.30.

Water volume and floor space required for a basic discus unit.

volume and moor space required for a	busie diseus
No. fish per growth period	6,500
Water per fish (liters)	3.16
Additional volume for control	10%
Water per parent fish (liters)	5
Water for replacement needs (m <sup>3</sup> )	1
Incubators (m <sup>3</sup> )	0.5
Total volume required (m <sup>3</sup> )	24.3
Paved floor space (m <sup>2</sup> )	48.6

As with the guppy unit, net income before taxes includes the cost of the farmer's labor, i.e., a half-day needed to operate the unit (Table 7.33). From the second year onwards, income is projected at a fixed level of US\$ 58,300 and the only variable component is payment of interest on loans. Profitability grows to 68% at the end of 7 years. Loan payments (interest + principal) account for 36% of the accumulated net cash flow (Table 7.34), close to that of the guppy unit (35%). The IRR calculated on the basis of operating cash flow equals 78%, greater than that of the guppy unit (61%), making the discus project attractive to an investor. Both the net cash flow and the NPV indicate a return period of 29 months (Fig. 7.8), shorter than in the guppy unit (3 years for net cash flow and 33 months for NPV).

The sensitivity of the project was tested for changes in the coefficient of survival and farm gate price (Table 7.35). Fig. 7.9 shows that a 60% decrease in survival or 56% decrease in fish price leads to NPV = 0. The difference in sensitivity is explained by the decreased variable expenses resulting when the coefficient of survival decreases.

The positive results of the economic evaluation of the basic unit make it reasonable to consider investing in expansion.

Investment required	for a basi		(thousand US\$)	
Item	Cost	Meets the needs of the expanded farm?	Period of amortization (years)	Amortization (annual)
Buildings				
Building (50 m <sup>2</sup> )	19.6			
Infrastructure (electricity, water, sewage)	5.4	yes		
Planning and other	3.6	yes	10	0.4
Total buildings	25.0		15	1.7
Aquariums	18.9		10	1.9
Production equipment	28.2		7	4.0
Laboratory and packing equipment	6.6	yes	7	0.9
Miscellaneous	10.0	yes	7	1.4
Total equipment	63.7	-		
Parents (initial school)	3.6	yes		
Total investment	88.7	-		10.3
Total investment + parents	92.3			

	Table 7	.31.	
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Table 7.32.

Expenses of a basic discus unit (thousand US\$).						
Item	First year	Second year onwards	Comment			
Fixed						
Exchange of parents		1.2	33%			
Electricity	0.8	0.8				
Fuel	0.6	0.6				
Miscellaneous	0.8	0.8				
Total fixed	2.2	3.4				
Variable						
Medications and disposables	0.3	0.4				
Transport to market	1.1	2.1				
Food	0.2	0.3				
Water	0.6	0.7	2,177 m <sup>3</sup>			
Total variable	2.2	3.5				
Total production expenses	4.4	6.9				
Amortization	10.3	10.3				
Total expenses + amortization	14.7	17.3				

Table 7.33.

Item	Project year							
	1	2	3	4	5	6	7	
Income	29.2	58.3	58.3	58.3	58.3	58.3	58.3	
Production expenses	4.4	6.9	6.9	6.9	6.9	6.9	6.9	
Amortization	10.3	10.3	10.3	10.3	10.3	10.3	10.3	
Interest payment	6.5	5.8	5.0	4.2	3.3	2.3	1.2	
Net income prior to taxes*:								
Thousand US\$	8.0	35.3	36.0	36.9	37.8	38.8	39.9	
% of sales	27%	60%	62%	63%	65%	66%	68%	

Forecasted profits and losses of a basic discus unit (thousand US\$).

Item	Projec	t year					
	1	2	3	4	5	6	7
Income	29.2	58.3	58.3	58.3	58.3	58.3	58.3
Cash payments							
Suppliers	4.4	6.9	6.9	6.9	6.9	6.9	6.9
Equipment	88.7						
Total payments	93.1	6.9	6.9	6.9	6.9	6.9	6.9
Operating cash flow	-64.0	51.4	51.4	51.4	51.4	51.4	51.4
Own capital	44.4						
Loan	64.6						
Interest + principal payment	6.5	14.2	14.2	14.3	14.5	14.6	14.7
Net cash flow	38.6	37.2	37.1	37.0	36.9	36.8	36.7
Net cash flow (accumulated)	38.6	75.8	112.9	150.0	186.9	223.7	260.4
Net present value	-58.2	42.5	38.6	35.1	31.9	29.0	26.4
Net present value (accumulated)	-58.2	-15.7	22.9	58.0	89.9	118.9	145.3

Table 7.34. Forecasted cash flow for a basic discus unit (thousand US\$).

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Sensitivity of NPV to changes in survival and gate price of a basic discus unit.									
Farm gate price	Coeffi	cient of s	survival	and asso	rtment				
of fish	-60%	-50%	-40%	-30%	-20%	-10%	0%	10%	20%
-60%	-61.5	-52.8	-44.0	-35.3	-26.6	-17.9	-9.2	-0.5	8.3
-50%	-51.2	-39.9	-28.6	-17.3	-6.0	5.3	16.6	27.9	39.2
-40%	-40.9	-27.0	-13.2	0.7	14.6	28.4	42.3	56.2	70.0
-30%	-30.6	-14.2	2.3	18.7	35.2	51.6	68.1	84.5	100.9
-20%	-20.3	-1.3	17.7	36.7	55.8	74.8	93.8	112.8	131.8
-10%	-10.0	11.6	33.2	54.8	76.4	97.9	119.5	141.1	162.7
0%	0.3	24.5	48.6	72.8	97.0	121.1	145.3	169.4	193.6
10%	10.6	37.3	64.1	90.8	117.5	144.3	171.0	197.8	224.5
20%	20.9	50.2	79.5	108.8	138.1	167.5	196.8	226.1	255.4

#### 7.4.2 Expanded farm

A basic discus unit can provide a net cash flow of US\$37-38,000 to a half-day grower, 55% higher than the net cash flow of the basic guppy unit. Assuming that the basic unit produced positive results during two years of operation, the discus farm can be expanded to 97 m<sup>3</sup> (water facilities) and the grower to a full-day of labor. Expansion of a discus unit requires a much greater additional investment than expansion of a guppy unit. The expansion produces greater returns, but requires a longer return period.

The basic assumptions upon which the expansion is planned appear in Table 7.36. As for the guppy expansion, it is projected that all necessary capital for the expansion will be borrowed. The economic evaluation of the expanded farm is based on the same productivity level and fish price as the basic unit. In the first year after expansion, only 6 months of additional sales are projected because the first additional reproduction cycle will end only 6 months after the beginning of the year (2 months of parental school plus 4 months of reproduction and growing). From the second year onwards, 12 months of additional sales are planned (Table 7.37).

The total water volume needed for the expansion is four times greater than for the basic unit (Table 7.38). Investment costs are 3.3 times higher than that of basic unit (Tables 7.39) because only 32% of the facilities of the basic unit meet the needs of the extended farm. (For the guppy farm, this percent was 60%.) Similar to the guppy project, the expenses of the expanded farm (Table 7.40) include the cost of a half-day hired worker in addition to a full-day

labor for the farmer. The cost of the farmer's labor is included in net income before taxes (Table 7.41). Profitability of the expanded farm reaches 54% during the first seven years, slightly higher than that of the guppy project (49%).

The IRR, calculated on the basis of operating cash flow (Table 7.42), equals about 70%, much higher than the 10% discount rate but smaller than the IRR for the basic unit (78%). Accumulated cash flow indicates a return in 56 months while NPV indicates 47 months (Fig. 7.10), both longer than for the basic unit (29 months).

The sensitivity of the project to changes in survival and farm gate prices (Table 7.43) shows that a 52% change in the coefficient of survival or 45% change in fish price lead to a zero NPV for the project. The difference in sensitivities is explained by the decreased variable expenses resulting from decreased survival. The combined changes, presented as iso-lines in Fig. 7.11, show that the basic unit is less sensitive than the expanded farm. Point A shows that when the coefficient of survival equals -60% in the basic unit, the fish price need not change to maintain an NPV of 0. Point B shows that for the same coefficient of survival, the fish price must increase by least in 18% to maintain a non-negative NPV of the expanded farm.

Table 7.36.	
Basic assumptions used to plan an expansion of	of a discus farm.
Eggs per female (per month)	300
Coefficient of survival and assortment	27%
Exchange of parents	33.3%
Farm gate price of 5 cm fish (US\$)	3.00
As a part of the investment required:	
Own capital	0%
Loan for the investment minus own capital	100%
Interest rate of loan and net present value	10%
Grace period for the principal payment (years)	1

Projected sales of the expand	ed discus farr	n.
	First year	Second year onwards
Parent fish (males and females)	160	160
Females	80	80
Reserve parent fish	80	80
Eggs per female (monthly)	300	300
Coefficient of survival and assortment	27%	27%
Introduction of parental schools (months)	2	
Growth period (months)	4	4
Annual fish for sale	48,600	77,800
Farm gate price of fish (US\$)	3.00	3.00
Sales (US\$)	145,800	233,300

Table 7.37.	
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Table	1.38.

Water volume required for the expanded	l discus farm.
Fish per growth period	25,900
Water per fish (liters)	3.16
Additional volume for control	10%
Water per parent fish (liters)	5
Water for replacement needs (m <sup>3</sup> )	4
Incubators (m <sup>3</sup> )	2
Total volume required (m <sup>3</sup> )	97.3
Paved floor space (m <sup>2</sup> )	194.6

Item	First year	Second year onwards	Comment
Fixed			
Exchange of parents		4.8	33%
Electricity	3.8	4.8	
Fuel	2.7	3.4	
Miscellaneous	3.9	4.9	
Total fixed	10.4	17.8	
Variable			
Medications and disposables	1.9	2.3	
Transport to market	9.9	12.4	
Food	1.2	1.6	
Water	3.2	4.0	6,362 m <sup>3</sup>
Hired labor		12.2	Half-day
Total variable	16.2	32.5	2
Total production expenses	26.6	50.3	
Amortization	29.3	29.3	
Total expenses + amortization	56.0	79.7	

Table 7.40.

Table 7.41.

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Forecasted profit	s and losses	of the ex	panded di	iscus farm	n (thousan	d US\$).	
Item			P	roject yea	r		
	1	2	3	4	5	6	7
	Basic unit Expanded farm						
Income	29.2	58.3	145.8	233.3	233.3	233.3	233.3
Production expenses	8.0	10.5	41.0	64.7	64.7	64.7	64.7
Amortization	10.3	10.3	29.3	29.3	29.3	29.3	29.3
Interest payment	6.5	5.8	27.1	24.0	20.5	16.7	12.5
Net income prior to taxes*:							
Thousand US\$	4.4	31.7	48.3	115.2	118.7	122.5	126.7
% of sales	15%	54%	33%	49%	51%	53%	54%

\* after considering half-day self-labor

# 7.4.3 Summary of the economics of a discus farm

The water volume needed for the basic unit is 24  $m^3$  while the expanded farm requires four times as much, i.e., 97  $m^3$ .

The initial investment is US\$92,300 for the basic unit including brood stock. An additional US\$220,900 is needed for the expansion.

Expected annual sales are about 19,000 fish for the basic unit and 80,000 for the extended farm.

Net income prior to taxes, including self-labor, is 68% for the basic unit and 54% for the expanded farm. The expected annual net cash flow is about US\$37,000 for the basic unit, including a half-day of labor by the farmer, and US\$104,000 for the expanded farm including a full day of labor by the farmer.

For the basic unit, NPV is positive after 2.5 years. It is sensitive to a 60% change in the coefficient of survival and 56% change in fish price. For the expanded farm, NPV is positive after 47 months (including the two year basic unit stage) and sensitive to a 52% change in survival and 45% change in fish price.

Item	v for the expanded discus farm (thousand US\$) Project year							
	1	2	3	4	5	6	7	
	Basic	unit 🛛		Extended farm				
Income	29.2	58.3	145.8	233.3	233.3	233.3	233.3	
Cash payments								
Suppliers	8.0	10.5	41.0	64.7	64.7	64.7	64.7	
Equipment	88.7		220.9					
Total payments	93.1	6.9	262.0	64.7	64.7	64.7	64.7	
Operating cash flow	-64.0	51.4	-116.2	168.5	168.5	168.5	168.5	
Own capital	44.4							
Loan	64.6		220.9					
Interest + principal payment	6.5	14.2	36.3	62.7	63.2	63.6	64.1	
Net cash flow	38.6	37.2	68.4	105.8	105.4	104.9	104.4	
Net cash flow (accumulated)	38.6	75.8	144.2	250.0	355.4	460.3	564.8	
Net present value	-58.2	42.5	-87.3	115.1	104.6	95.1	86.5	
Net present value (accumulated)	-58.2	-15.7	-103.0	12.1	116.8	211.9	298.4	

Table 7.42. Forecasted cash flow for the expanded discus farm (thousand US\$).

IRR is greater for the basic unit (78%) than for the extended farm (70%). According to NPV, IRR, and the sensitivity analysis, the basic unit performs better than the extended farm. The extended farm entails greater liability for the farmer. For the basic unit, US\$44,400 is invested by the farmer and US\$64,600 is borrowed (US\$92,000 including interest payments). Repayment of loans accounts for 104% of the total cost of the buildings and equipment in the basic unit. For the expanded farm, the same investment is needed for the basic unit stage and a second sum of US\$220,900 (US\$317,700 including interest) is borrowed. The sum of both loans is US\$410,600, representing 130% of the total cost of the extended farm's buildings and equipment.

#### 7.5 Comparison of the guppy and the discus farms

In this section, four alternatives for a potential investor in an ornamental fish farm are compared. The comparison is made for two varieties of fish (guppies and discus) and for two project sizes (a basic unit and an expanded farm).

In Table 7.44, four indicators are used to compare the alternatives. The first is the capital needed to establish the farm. It partially reveals the level of financial risk. The annual interest and principal payment is another indicator of financial risk. The period needed for the NPV to accumulate to zero – the return period – is an indicator of the attractiveness of the investment and is important not only to the farmer but also to the financial institution considering making loans to the project. The annual net cash flow in year 7, calculated after deducting expenses (suppliers, hired labor, loan payments), shows the 'salary' of the farmer.

As an example of application of the economic analysis, the required capital for the expanded discus farm is five times higher than that of the basic guppy unit. As a result, loan payments are seven times higher. The period needed for NPV to accumulate to zero is 40% longer in the expanded discus project than in the basic guppy project (47 months instead of 33). But the 'salary' of the farmer is 4.15 times the salary in the basic guppy unit, achieved at the 'expense' of working only twice as long (a full day instead of a half).

The four alternatives differ from one another by level of loans needed to finance the establishment of the farm, attractiveness of the investment, and 'salary' for the farmer. Clearly, in addition to this economic evaluation, farmers should take into account equally important factors such as technological and market risks. Raising discus is more risky for the beginning farmer than guppies, especially in the reproduction stage. On the other hand, prestigious varieties of ornamental fish such as discus are easier to market. The farmer should weigh all

Item	Investment in basic unit	Additional for	Total	Annual amortization	
		expansion		(years)	(US\$)
Buildings:					
Building (110 m <sup>2</sup> )	19.6	58.9	78.5		
Infrastructure (electricity, water, sewage)	5.4	9.7	15.1		
Planning and other	3.6		3.6		
Total buildings	28.6	68.6	97.2	15	6.5
Aquariums	18.9	56.8	75.8		
Production equipment	28.2	84.7	112.9		
Laboratory and packing equipment	6.6		6.6		
Miscellaneous	10.0		10.0		
Total equipment	63.7	141.5	205.2	7	29.3
Parents (initial school)	3.6	10.8	14.4		
Total investment	92.4	210.1	302.5		
Total investment + parents	96.0	220.9	316.9		

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 Table 7.39.

 Investment required for the expanded discus farm (thousand US\$).

Sensitivity of NPV to changes in survival and farm prices.											
		Coefficient						of survival and assortment			
Farm gate price of fish	-60%	-50%	-40%	-30%	-20%	-10%	0%	10%	20%		
-60%	-206.5	-188.9	-171.2	-153.6	-136.0	-118.3	-100.7	-83.0	-65.4		
-50%	-179.9	-155.6	-131.3	-107.0	-82.7	-58.4	-34.1	-9.8	14.5		
-40%	-153.3	-122.4	-91.4	-60.5	-29.5	1.4	32.4	63.3	94.3		
-30%	-126.7	-89.1	-51.5	-13.9	23.7	61.3	98.9	136.5	174.1		
-20%	-100.1	-55.9	-11.6	32.6	76.9	121.1	165.4	209.6	253.9		
-10%	-73.5	-22.6	28.3	79.2	130.1	181.0	231.9	282.8	333.7		
0%	-46.9	10.6	68.2	125.7	183.3	240.9	298.4	356.0	413.5		
10%	-20.3	43.9	108.1	172.3	236.5	300.7	364.9	429.1	493.3		
20%	6.3	77.1	148.0	218.9	289.7	360.6	431.4	502.3	573.2		

Table 7.43.

	Basic guppy unit	Basic discus unit	Expanded guppy farm (including 2 year basic unit)	Expanded discu farm (including 2 year basic unit)
Required capital (own and borrowed including interest)	100	151	204	501
Interest and principal payment	100	159	259	695
Period in which NPV accumulates to become positive	100	87	117	141
Annual net cash flow at end of 7 years	100	146	244	415

factors and choose the option that suits his access to capital, professional skills, and tolerance to risk.

# 7.6 References

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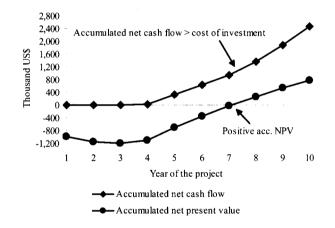


Fig. 7.1. Accumulated cash flows for the eel farm.

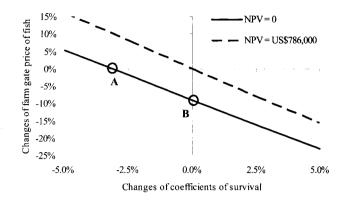


Fig. 7.2. Sensitivity of NPV to changes in survival and farm gate prices.

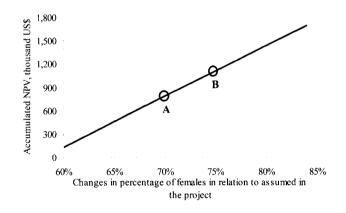
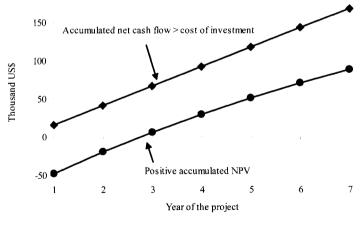


Fig. 7.3. Sensitivity of NPV to an increase in the percentage of females.



----- Accumulated net cash flow ------ Accumulated net present value

Fig. 7.4. Accumulated cash flows for the basic guppy unit.

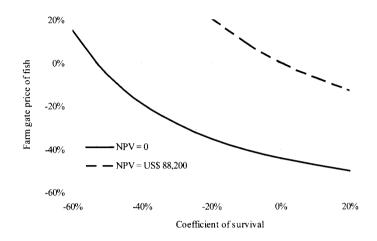


Fig. 7.5. Sensitivity of NPV to changes in survival and farm gate prices for a basic guppy unit.

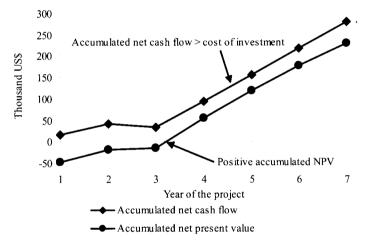


Fig. 7.6. Accumulated cash flows and NPV for the extended guppy farm.

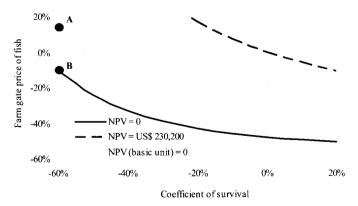


Fig. 7.7. NPV sensitivity of the extended guppy farm, compared to the basic unit.

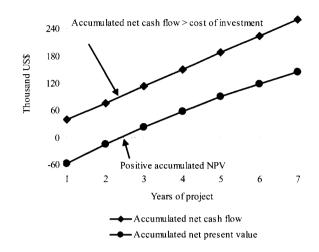


Fig. 7.8. Accumulated cash flow for the discus basic unit.

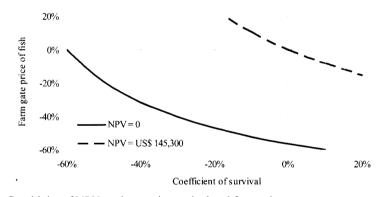
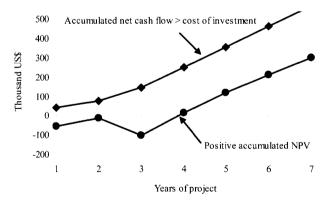


Fig. 7.9. Sensitivity of NPV to changes in survival and farm price.



Accumulated net cash flow — Accumulated net present value Fig. 7.10. Accumulated cash flows for the expanded discus farm.

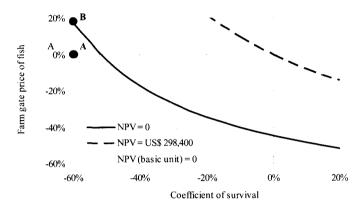


Fig. 7.11. Sensitivity of NPV of the extended discus farm, compared to the basic unit.