

# No. 24 February 2008 Aquaculture Explained Farming of Eurasian Perch Volume 1: Juvenile production



Bord Lascaigh Mhara Irish Sea Fisheries Board

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# Aquaculture Explained

# **Farming of Eurasian Perch Volume 1: Juvenile production**

A BIM publication in association with the European CRAFT Project, PERCATECH.



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Farming of Eurasian Perch

Number 24 February 2008

> Aquaculture Explained Special Publication

Farming of Eurasian Perch Volume 1 - Juvenile Production

Compiled and edited by Damien Toner and Carole Rougeot and produced in conjunction with the Aquaculture Technical Section, Aquaculture Development Division, An Bord Iascaigh Mhara.

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### Foreword

This publication is a timely review of the techniques used in the production of the Eurasian Perch (*Perca fluviatilis*). The manual is a joint publication by Bord Iascaigh Mhara (BIM) and the European CRAFT project on perch (PERCATECH). It seeks to outline the current status, state of the art and practices of juvenile perch production in Europe. Although freshwater aquaculture in Europe faces serious constraints, there are also significant opportunities arising particularly from the growing gap between supply and demand for fish products, resulting both from the stagnation or decline of marine capture fisheries and the increases in demand. As margins associated with the farming of traditional species tighten, producers have sought to diversify into higher value niche species. The European Union has funded research into Eurasian perch through a variety of mechanisms since the 1990's. The cumulative results of this research in tandem with progress made by commercial operators, gives us a wide range of information to disseminate in this publication.

The manual aims to offer practical advice and technical information to those farmers already involved in the production of percids and new entrants to the sector. Whilst detailed and scientific information is necessary in elaborating on the techniques employed in perch culture, we have endeavoured in the spirit of the CRAFT programme to provide content in an easy to read format. Those seeking more detailed information on a particular topic should in the first instance refer to cited papers or contact the editors or authors.

Eurasian Perch is a species which has attracted significant interest in recent years and it is our hope that this publication will facilitate the further development of the percid aquaculture sector in Europe.

Damien Toner & Carole Rougeot Co-editors February 2008.



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### Acknowledgements

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# **Chapter 1: INTRODUCTION**

### Damien TONER, Aquaculture Initiative EEIG, Ireland. Pascal FONTAINE, URAFPA - INRA, University of Nancy, France.

Aquaculture has become an important part of overall world food production. Unlike present day agricultural farming, aquaculture has had to develop whilst competitive hunting and gathering of fish still exists on a large scale. Whilst aquaculture techniques and methodology have mirrored that in other intensive agriculture sectors, aquaculture is still in its infancy. In the northern hemisphere salmonid aquaculture and carp culture dominate the freshwater fish aquaculture sector. Farmers and the investment community are constantly looking to increase value and volume of products as margins decrease and competition increases. The potential of new aquaculture species is constantly under review and advances noted by those seeking to penetrate niche markets with new and innovative products.

Developing aquaculture techniques for novel species is challenging. Perch farming is no different in this regard and to date in Europe, only a handful of companies have mastered commercial production. This manual concentrates on juvenile production and outlines the various life stages of perch which must be successfully manipulated in a hatchery environment to produce a healthy juvenile population for growout. The expertise required to carry out research and development is an essential component in the development of any new species and in this regard, perch culture in Europe would not have progressed to the stage it is at today without the financial support of the European Union through various funding mechanisms. PERCATECH is the most recent of these research measures, funded through the CRAFT Programme.

### **1.1. Percatech Project outline**

The main objective of PERCATECH was to secure the production of Eurasian perch (*Perca fluviatilis*) juveniles (3-5 g), in order to sustain the development of European Small and Medium Sized Enterprises producing this novel species. Presently, juvenile availability is sparse and limited to the annual cycle of the reproductive period, which occurs in early spring. Moreover, the quality of supplied juveniles is very variable. Thus, to support the sustainable development of Eurasian perch production, PERCATECH was initiated.

Several scientific objectives characterised the PERCATECH project (COOP-CT-2004-512629-PERCATECH, October 2004 – September 2006):

- Optimisation of a **reliable protocol allowing the production of out-of-season eggs and larvae**,

- Development of a protocol of cryogenic semen preservation,

<sup>-</sup> Assessment of the possibilities (shortening or delaying) to **extend the natural reproductive period** by the management of environmental conditions,

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- Definition of **optimal conditions of broodstock management** (especially feeding) allowing the guarantee of reproductive performance and diminished mortality rates of broodstock during the reproductive period,

- **Production of a domesticated strain** with improved growth performance and a comparison of the growth performance of three generations (F1, F2 and F3) of captive juvenile Eurasian perch reared under intensive conditions,

- Production of a **stock of sex-reversed completely functional males** and the evaluation of the productivity gain when rearing all-female populations obtained with hormonally sex-reversed male breeders,

- Evaluation of the **cost of production of juvenile** perch according to different production strategies and under different socio-economical contexts.

A total of six SMEs and five RTDs (eleven partners) were involved in the project.

### **SME Partners:**

**Partner 1:** Lucas Perches SARL, Moulin du Cany, 57170 Hampont, France (Key person: *David Vandevoorde*)

**Partner 2:** Bornholms Hatchery Lakseklaekkeri, Øster Flak 2, Nexø, Denmark (Key personnel: *Julia Overton and Helge Paulsen*)

Partner 3: EARL Esox, Plate forme des salins, Promenade J.L. Navarro, 34140 Mèze, France (Key person: *Emmanuel Rezzouk*)

**Partner 4:** Gebr. Dil Import-Export b.v, Kerklaan 40, 1920 AA Akersloot, The Netherlands (Key personnel: *Hein Dil and Tim Eriks*)

**Partner 5:** Rybárství Nové Hrady S.R.O., Stipton 78, 373 33 Nové Hrady, Czech Republic (Key person: *Lubomir Zvonar and Theodor Vondra*)

**Partner 6:** PDS Irish Waters Perch Ltd, Knockaghy, Corlismore, Gowna, Co. Cavan, Ireland (Key personnel: *Philip Simpson (PDS), Damien Toner (AI), Lucy Watson (BIM)*)

### **RTD Partners:**

**Partner 7, Coordinator:** Laboratory of Animal Sciences, Université Henri Poincaré – Nancy 1, 34 rue Sainte Catherine, 54000 Nancy, France (Key personnel: *Pascal Fontaine, Jean-Noël Gardeur, Neil Wang and Fabrice Teletchea*)

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**Percatech Team:** Top- Otomar Linhart, Left to right Damien Toner, Carole Rougeot, Patrick Kestemont, Pascal Fontaine, Emile Henrotte, Henk Van der Mheen, Philip Simpson, Helge Paulsen, Robert Mandiki, Neil Wang, Emmanuel Rezzouk, David Vandervoorde, Julia Lynn Overton, Jiri Musil, Front row: Martin Kocour, Lubomir Zvonar, Theodor Vondra, Tomas Policar. Insert- Charles Mélard (top), Lucy Watson (middle), Hein Dill (bottom).

### 1.2. Bord Iascaigh Mhara (BIM)



BIM is the Irish State agency with responsibility for developing the Irish Sea Fishing and Aquaculture industries. BIM was established under the Sea Fisheries Act 1952. The policies and programmes to pursue this mission are

determined by the Board of BIM and are set out within the framework of the National Development Plan, EU policies and available resources. A primary objective of BIM policy is to expand the volume, quality and value of output from the seafish and aquaculture sectors. BIM's approach is to focus on the opportunities for growth in these sectors while seeking to alleviate constraints that impede development.

BIM provides a range of services including advisory, financial, technical, marketing and training supports to all sectors of the Irish seafood industry. The four development divisions of BIM deliver these services through a number of integrated programmes. The programmes are funded from the Exchequer, the European Commission and

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charges for services. BIM's clients comprise fishermen, fish farmers, processors and all those engaged in marketing Irish seafood. BIM's information service assists students, educators, the media, seafood consumers and the general public. BIM provides grants and technical advice and delivers quality and environment programmes as well as marketing and training supports to promote the development of the fish farming industry in a sustainable manner.

In 1997 BIM published 'Cultivating Perch' by Declan A. Ashe M.Sc. The manual was part of BIM's Aquaculture Explained series. This manual builds on and compliments that publication. Each chapter has been written with contributions from authors with specific expertise in their field. Where possible, reference is made to practices on commercial farms operating perch hatcheries. For a more detailed



study on a specific topic, a complete list of references is to be found in Appendix 1.



# **Chapter 2 : Perch Description and Biology**

Carole ROUGEOT, CEFRA, University of Liège, Belgium. Pascal FONTAINE, URAFPA - INRA, University of Nancy, France. S.M.N. MANDIKI, URBO, University of Namur, Belgium.

### 2.1. Taxonomy and Description

There are 3 species of Perca, which biologically are very similar: *Perca fluviatilis Linnaeus* (Eurasian or European perch), *Perca flavescens Mitchill* (yellow perch) and *Perca schrenki Kessler* (Balkhush perch). Perch belong to the Perciforme order and to the Percidae family. Perch is a Teleostean fish with a laterally compressed body with ctenoides scales. The body coloration ranges from grey-green for the upper body to green-yellow for the abdomen. Flanks are marked with 5 to 9 transversal black bands (Plate 2.1). Pelvic, pectoral and anal fins are generally orange-red. The first dorsal fin and operculum present a spine, which is often raised by the fish when threatened.



Plate 2.1: Eurasian perch, Perca fluviatilis.

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### 2.2. Distribution and Habitat

Perch is widely distributed to the North of Eurasia and Asia. The geographic distribution of perch is limited by water temperature through its effect on the metabolic process. Perch have also been introduced in many countries such as Australia, New-Zealand, South Africa and the Azores Islands (Plate 2.2).

Perch tolerate a wide range of environments, but prefer shallow and moderately productive freshwaters. This is a freshwater species, but because of its euryhaline tolerance, perch is also present in brackish water in the Baltic Sea. Perch prefer lentic river conditions (with a low flow rate) and are also present in deep lakes, up to 40m in depth. Perch tolerate a wide range of temperatures (4-31°C) and that also determines its geographic distribution. Some ecological characteristics are summarised in Table 2.1.



Plate 2.2: Geographic distribution of *Perca fluviatilis* (hatched) and *Perca flavescens* (black), (in Craig, 2000).



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Table 2.1: Ecological characteristics of perch natural habitats.

## 2.3. General Biology

Perch is a carnivorous species and mainly piscivorous in habit. Feeding behaviour occurs mainly at daybreak and twilight. Young larvae first eat algae and zooplankton (rotifers, cladocerans and copepods) and subsequently insect larvae and small fish. Cannibalism is an important characteristic in this species and appears firstly in young fingerlings (1.5cm). Larvae and young juveniles are gregarious whilst adults live a more solitary existence.

In their natural habitat Eurasian perch display a low growth rate compared to other Percid species and they are characterised by a huge weight and size heterogeneity resulting from feeding competition. Growth rate is dependent on season, with a maximum at the end of Spring and in Summer and a minimum, even a cessation of growth, at the end of Autumn and during Winter. The optimal temperature for growth is 23°C. Mean body length range from 20 to 35 cm with a maximum of 51 cm and mean body weight ranges from 0.3 to 2 kg with a maximum of 5 kg. Perch display a sexual growth dimorphism in which female growth is 20% faster than males.

### **2.4. Reproduction**

In Europe, perch reproduce only once a year, during Spring (from March to June) with increasing photoperiod and temperature according to their geographic origin (Table 2.2). To mature, perch need a minimum 160 days chilling period at a temperature less than 8°C. Perch spawn in river shallows or lake shores utilising substrates (plants, branches) for egg laying (Plate 2.3). Perch eggs are held together in a long ribbon like structure ranging from about 10 centimetres to 1.5 meters for the biggest females (>1kg). Fecundity generally ranges from 30,000 to 120,000 eggs per female. The egg size at spawning ranges from 1.0 to 2.0 mm depending on the female size. After fertilisation and hydration their diameter increases to between 1.9 - 2.8 mm.

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Table 2.2: Spa	wning period and	l water temp	perature for	Eurasian	perch repro	oduction
	accor	ding to geo	graphic orig	gin		

Area	Spawning Period	Temperature °C	Reference
<b>Belgium</b> La Gombe	April - June	8.5 - 13.5	Dalimier et al., 1982
<b>England</b> Slapton Ley Windermere	Mid March – early May Mid May – mid June	8-14 9 - 18	Craig, 1974 Guma'a, 1978
<b>Finland</b> Lake Saarlampi	End May	12 – 14	Urho, 1996
<b>France</b> Lake Aydat	Early May	8	Jamet and Desmolles, 1994
<b>Greece</b> Lake Agios Vasilios	Mid March – early April	8	Papageorgiou, 1977
<b>Russia</b> Ivan'kovo reservoir	April - May	7 - 15	Makarova, 1973
Switzerland Lake Zurich	End April – early June	8 - 15	Zeh et al., 1989
<b>Sweden</b> Formarsk and Oskarshamm	Early April – end June	7 - 20	Sandström et al., 1995
Ireland	Early April - Mid May	9-14	CFB (pers comm)

In their natural environment, sexual maturity in perch is reached at 2 years for the males and 3 years for females. In a controlled intensive environment, sexual maturity can be reached one year earlier for both sexes. The sex of perch is difficult to identify from external examination except during the spawning time when females are gravid with a swollen red papilla and males expel milt on handling. Perch gonads are morphologically different between sexes: males present 2 testes whilst female gonads fuse during the development to form a single ovary.



Plate 2.3: perch egg ribbon in vegetation substrate.



### **Chapter 3: Broodstock Management**

Pascal FONTAINE, URAFPA – INRA, University of Nancy, France. Patrick KESTEMONT, URBO, University of Namur, Belgium. Charles MELARD, CEFRA, University of Liège, Belgium.

As with all commercially produced aquaculture species, broodstock management is a challenging but essential part of the production cycle. The subsequent success of any production cycle is directly correlated with the broodstock quality and control of reproduction.

Within the framework of the domestication of a new species, such as Eurasian perch at present, knowledge and control of the reproductive cycle is one of the most important criteria to ensure the development of sustainable production. Control of the reproductive cycle is necessary

- to target the production of larvae with a high and constant quality,
- to obtain the production of out-of-season spawning (numerous reproductive cycles per year),
- to develop breeding programmes in order to improve fish performance in production systems (growth, stress resistance, flesh quality and yield, diseases)

Recent reproductive research on Eurasian perch has focused on the following successive steps:

- a complete description of male and female reproductive cycles as a reference,
- determination of the environmental control of the reproductive cycles,
- development of an artificial programme to induce out-of-season spawning
- and identification and quantification of the effects of contributing factors.

### **3.1. Description of a Natural Reproductive Cycle**

As previously mentioned (Chapter 2), the natural reproductive cycle of perch follows an annual pattern. Spawning occurs in early Spring followed by a sexual resting period until the Autumn. This sexual maturation is best expressed by Gonado Somatic Indices (GSI= gonad weight x 100)/body weight in %) or oocyte (unfertilised egg) diameter (O.D.) in females.

In females, after a sexual resting period observed from May to August (GSI < 1%, OD < 200  $\mu$ m), significant higher GSI and OD are observed in Autumn. This means that oogenesis (oocyte development) starts during the summer period when the water temperature increases then subsequently decreases and the photoperiod (ambient light duration) decreases.

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After the induction of the oogenesis, the GSI progressively increases until mid March (15%), then rapidly until spawning (25%, OD =  $850 \,\mu$ m) which occurs in Spring (10-15°C). In males, GSI reaches its maximum in Autumn (8.5%). At this time, spermatozoa are abundant. During winter, GSI remains stable (5%) until spawning.

Of course, the overall timing of the Eurasian reproductive cycle varies with its large geographic distribution, with some variations (timing of spawning, temperature at spawning) related to climatic changes (Table 2.2, Chapter 2).

### **3.2. Broodstock Facilities**

The maintenance of a healthy broodstock population is a prerequisite to stable and efficient juvenile production. Facilities for holding broodstock tend generally to avail of recirculation systems, with independent controls for manipulation of temperature and light. Such facilities are recommended to be independent in nature utilising strict biosecurity protocols and appropriate husbandry measures. Different strains may be tagged and kept separate to facilitate broodstock selection programmes. Stress to adult perch should be kept to a minimum and the system layout should reflect this. Broodstock can be held up to 20kg/m<sup>3</sup>, with minimum suggested oxygen levels of 80%. Tanks should be a minimum of 1.5m<sup>3</sup> (Plate 3.1). Lighting and temperature control of individual tanks is according to their appropriate reproductive cycle as determined by the farm's requirements. Such systems are generally controlled by a centralised computer system.



Plate 3.1: Outdoor recirculating system for holding broodstock with 1.6m<sup>3</sup> tanks (CEFRA, Belgium).

### **3.3. Environmental Control of the Reproductive Cycle**

As is the case with all temperate fish, the seasonal variations of photoperiod and water temperature synchronise the perch's reproductive cycle. By controlling light duration and intensity, the fish farmer can advance or suppress sexual maturation of broodstock in tandem with adjusting temperature and feeding regimes. With this in mind, research





has been carried out in order to specify the importance of photoperiod variation in the induction of the reproductive cycle and control of the whole cycle. Commercial hatcheries exert total control on broodstock reproductive cycle. Such control is necessary to ensure stable juvenile production (Chapter 4). Control of photoperiod and temperature regime strongly influences Eurasian perch gametogenesis, spawning time, spawning rate, egg quality and broodstock mortality. Hatcheries intending to spawn fish utilising the natural reproductive cycle will hold fish in outdoor systems availing of ambient light and temperature or in indoor systems where natural photoperiod and temperature are artificially simulated.

### 3.4. Production of Out-of-season Spawning

### **3.4.1. Induction of reproductive cycle**

The development of reliable out-of-season spawning is of utmost importance to the aquaculture industry. Market demand places a year round requirement from ongrowing facilities. Consequently, hatcheries are under pressure to provide multiple batches of juveniles throughout the year. Year round production can also be of benefit to hatcheries in optimising production costs. Recent work in this area has concentrated on understanding the primers necessary to properly initiate broodstock maturation and spawning whilst maintaining egg and larval quality. Several factors such as amplitude of photoperiod and temperature decrease, speed of photoperiod decrease and the timing of the photoperiod decrease / temperature decrease control sexual maturation and spawning. In order to improve the rate of breeders response, it is recommended to decrease the photoperiod 15 days before reducing the temperature, which is similar to the natural cycle (Figure 3.1). An initial moderate decrease of temperature from 22°C to  $14^{\circ}C$  (-  $8^{\circ}C$ ) is better than a decrease from  $22^{\circ}C$  to  $6^{\circ}C$  (- $16^{\circ}C$ ). Subsequently after 9 weeks, a second decrease of temperature to  $6^{\circ}C$  is carried out. A 2 hours decrease of photoperiod is enough to obtain the maximum photoperiod stimulation.



Plate 3.2: Gravid female.

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In out-of-season conditioning, some environmental photoperiod conditions can inhibit the onset of the gonadogenesis in male and female Eurasian perch. For example, an increase of photoperiod (+ 3 hours) or a constant photoperiod (17L:7D) during the pre-induction period (15 days prior to induction) completely inhibits the response of breeders. Therefore the fish farmer must take care with photoperiod changes when broodstock are transferred between tanks or systems.

### Summary

The optimal photo-thermal protocol for efficiently inducing gonadogenesis must include the following steps:

- No increase of the photoperiod during the pre-inductive period (before the application of a photo-thermal programme),

- A decrease of the photoperiod (minimum 2 hours of amplitude) 2 weeks before temperature decrease

- A progressive and limited decrease of the temperature (22°C to 14°C, over a 3 week period).

### 3.4.2. Out-of-season spawning

The success of vitellogenesis in females strictly depends on the length of the chilling period. After an 8 month cycle, out-of-season spawning can be obtained, however the rates of gravid females and spawning, and the egg quality are low. When females have achieved their vitellogenesis, a rapid increase of temperature from 6°C to 14°C (over 1 month) is better than a slower one over 2 months to induce spawning. During gametogenesis (after 6 to 9 weeks), photoperiod and temperature should be maintained constant at 6°C (13L:11D or 9L:15D). The application of a long decrease of photoperiod and temperature (16 weeks), as observed in the natural habitat from September to December, is not necessary to stimulate gametogenesis. Using such a protocol, the rates of gravid females and spawning are close to 100% with normal eggs ribbons (Plate 3.3). A long chilling period of 5 months is required to achieve fertilisation rates higher than 50% and an improvement of the fertilisation rate could be reached using an artificial method of reproduction (Chapter 4).

### **Summary**

The optimal photo-thermal protocol to obtain out-of-season spawning with high rates of spawning and fertilisation must include the following points:

- The use of the optimal photo-thermal protocol for inducing the onset of the gametogenesis,

- The application of a long chilling period (5 months at 6°C)

- A rapid final warming-up phase (6°C to 14°C).





Plate 3.3: Out-of-season spawn of Eurasian perch obtained in December 2006 in Nancy, France.

### **3.5 Factors Affecting the Quality of the Reproductive Cycle**

Up to now, the development of Eurasian perch culture has been limited by the supply of high quality eggs, especially from breeders fed commercial dry diets. Indeed, if breeders are reared in recirculating systems under appropriate temperature and day length profiles (see 3.4) and are fed "natural food" (composed of a mixture of live forage fish and frozen chironomid larvae), the maturation of females and males can be obtained successfully, with ovulation rates comparable to or even better than that obtained from breeders held in ponds. Up to 90% of these maturing females can spawn during the normal or simulated reproductive season without hormonal stimulation. If the breeders are fed commercial dry diets (formulated for trout or seabass), the ovulation rates drop to 20-45%, depending on the quality of the diet (standard grow out feed or feed enriched with vitamin E). Aside from the reduction in ovulation rate, the fertilisation and hatching rates of eggs produced by perch fed commercial dry diets are also much lower than from breeders fed "natural prey" food, and frequently range between 0 and 10%. Moreover, the enrichment of grow-out diets with additives such as vitamin E, vitamin C or a mixture of highly unsaturated fatty acids (HUFA) (normally considered as nutritional compounds important in egg quality) can not significantly improve the reproductive performance and the hatching rate of perch larvae.

Recently, experimental dry diets containing different HUFA ratios (namely docosahexaenoic DHA/eicosapentaneoic EPA/arachidonic ARA acids) were formulated and tested on perch breeders. The spawning performances (ovulation rate and, fecundity) as well as the quality of eggs and larvae (fertilisation rate, egg and larval size, hatching rate, biochemical composition of eggs and larvae, survival to

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extended starvation or osmotic shock) of these breeders was compared to those from fish fed a standard commercial diet or live food (forage fish). The trials were conducted with fish held in cage-based ponds or in a recirculating system from August to May, covering thus a complete reproductive cycle. The results demonstrated that a compound diet with an adequate ratio of DHA/EPA/ARA can provide similar spawning performances and larval quality to a diet composed of forage fish, while inappropriate HUFA ratios significantly impaired reproductive performances as well as gamete and larval quality. Fish fed diets containing a ratio of DHA/EPA/ARA with a high proportion of n-3 HUFA (namely 17/12/1 or 14/16/1) produced eggs of poor quality while those fed with DHA/EPA/ARA containing a huge proportion of arachidonic acid (n-6 HUFA) (namely ratio 2/1/1) displayed reproductive performances similar to those of fish fed live preys (forage fish) and produced high quality eggs and larvae, whatever the rearing conditions (in ponds or recirculating system). Based on these results, it may be possible to improve the reproductive performances as well as egg and larval quality of Eurasian perch by manipulating the FA composition of broodstock diet, especially ARA, while this species is not well adapted to a diet containing excess dietary EPA.

### 3.6. Present Bottlenecks and Perspectives

An efficient photo-thermal protocol is now available for fish farmers and allows outof-season spawning with a very high spawning rate (90-100%) and acceptable fertilisation rate (50%). An improvement of fertilisation rate may be achieved utilising artificial methods of spawning (Chapter 4). However further co-operative research with producers (SMEs) is necessary, to optimise the photo-thermal protocol and to solve some remaining bottlenecks. Of course, these further actions must consider the socio-economic constraints of producers.

The current method of out-of-season spawning is a long process (8-10 months), and is consequently expensive. Further research should concentrate on optimising the chilling period and temperature. One major new bottleneck is the high variability of larval quality. Different broodstock successively submitted to an out-of-season protocol (but also in natural conditions), exhibit a high variability in larval quality. Numerous factors (environmental, nutritional and population variables) can impact on larval quality. Finfish File

# **Chapter 4 : Reproduction and Spawning**

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### 4.1. Broodstock Handling

The process of controlled spawning is an essential component in any fish hatchery. The manipulation and control of broodstock is necessary to provide a structured and stable larval supply. Generally, perch broodstock do not tolerate robust handling and it is recommended to use minimal and very careful handling during the spawning period. It is therefore sometimes necessary to use an anaesthetic for the elimination of stress in perch during handling. Common anaesthetics used include:

- 2-phenoxyethanol preparation at a dose ranging from 0.3 to 0.5 ml.l<sup>-1</sup> with expiration in 2 minutes.
- clove oil at a dose 0.03 ml.1<sup>-1</sup> with expiration in 3-4 minutes.

Handling fish should only take place using protective gloves, to prevent skin damage to the fish, which may result in fungal infections. Rooms used for broodstock should be dimly lit and noise disruption kept to a minimum (Chapter 3).

### 4.2. Spontaneous Ovulation and Spawning

Under controlled conditions (in tanks or fibre-glass troughs), perch can spawn naturally (induced ovulation by natural water temperature). A few weeks or days before ovulation, in early springtime, both sexes are stocked into tanks with or without spawning substrate (e.g. dry bushes and tree branches) at a 1:1 ratio. Using branches is a very useful and easy method to improve the condition of breeders under controlled conditions. Presence of spawning substrate creates shelter for broodstock and provides a platform on to which they can lay their egg ribbons (Plate 4.1).



Plate 4.1: Spawning substrate and egg laying in tank with perch broodstock.

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### 4.3. Induced Spawning by Hormonal Treatment

Synchronization of ovulation is one method to control the reproductive cycle in perch. Although artificial propagation using different hormones has been studied in perch, this kind of treatment is still not a common practice. The benefits for the hatchery are that hormone induced spawning can be very predictable offering egg batches of the same age, which facilitates production schedules. Generally, two methods of hormonally induced spawning can be used in perch: artificial and semi-artificial.

### 4.3.1. Artificial induction of spawning

In this method males and females are kept separated during spawning. Hormonal injections are subsequently used on females, allowing the eggs to be manually stripped and artificially fertilised. The advantage of this protocol is the total control of the spawning process in perch.

The first step is to choose suitable breeders for spawning. Fish selected should be mature, displaying good physiology and in a healthy condition. Females and males with a minimum body weight of about 200 g and 100 g respectively are recommended. Smaller males are used because they produce more milt in relation to body weight than larger ones. As mature males release milt naturally after stripping, they are used for artificial reproduction without requiring a hormonal injection. To improve the success of artificial reproduction, it is recommended to use males releasing clean milt. In order to synchronize the spawning, females must be hormonally injected by a single or a more elaborate priming injection. Hormonal injection is usually applied intramuscularly in the dorsal area of the female body (Plate 4.2). Different hormonal treatments at different water temperature have been used to induce the ovulation in perch. Time between injection and ovulation (time of latency) depends on the hormone used and on water temperature (Table 4.1).



Plate 4.2 Perch anaesthesia before handling of the breeders (left) and intramuscular injection of hormones to induce ovulation (right).



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		Table 4	1.1: Substances	used to induce	spawning in Eur	asian perch.	
Active subs tance	Dose	A - artificial, S - Semiartificial	Temperature (°C)	Time of latency (hours)	Ovulation efficiency (%)	Fertilization (%), * - hatching rate	References
Carp pituitary	4000 µg.kg <sup>-1</sup>	А	10-11	14-16	100	62.5	Kucharczyk et al. 1996 in Craig (2000)
hCG	5700 IU. kg <sup>-1</sup>	А	10-11	14 - 84	100	84.5	Kucharczyk et al. 1996 in Craig (2000)
hCG + Carp	500 IU.kg $^{-1}$ and 4000 µg.kg $^{-1}$	А	10-11	14-16	100	60.3	Kucharczyk et al. 1996 in Craig (2000)
pituitary							
GnRH a	5 µg.kg <sup>-1</sup>	A	15.4	94	11	98.5	Kouřil et al. (1997)
	25 µg.kg <sup>-1</sup>	A	15.4	103	27.6	98.6	Kouřil et al. (1997)
	50 µg.kg <sup>-1</sup>	А	15	75	100	38.2*	Policar et al., in press
	100 μg.kg <sup>-1</sup>	A	15	74	100	36.5*	Policar et al., in press
		A	16.1 - 17.0	82 - 105	80 - 100	60 - 95	Kouřil and Hamácková (1999)
		S	13.3 - 17.9	78-120	55-85	60 -> 95	Kouřil and Linhart (1997),
							Kouřil and Hamáčková (1999)
	125 μg.kg <sup>-1</sup>	А	15.4	115	78	94.1	Kouřil et al. (1997)
GnRHa +	3.12 $\mu g.kg^{-1}$ and 6.24 mg. $kg^{-1}$	А	15	85	100	47.5*	Policar et al., 2007
metoclopramide	$6.25~\mu g.kg^{-1}$ and 12.5 mg.kg $^{-1}$	A	15	85	100	45.5*	Policar et al., 2007
	12.5 $\mu g.kg^{-1}$ and 25 mg.kg $^{-1}$	A	15	75	100	45.9*	Policar et al., 2007

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### 4.3.2. Semi-artificial induction of spawning

The second method to induce spawning is semi-artificial. Keeping both sexes together (sex ratio 1:1), an intramuscular hormonal injection is given to females inducing spontaneous spawning with natural egg laying and fertilisation.

### 4.4. Spawning and Post Spawning Mortality of Broodstock

Handling stress during the spawning period can result in up to 10% in mortalities (Plate 4.3). In the Czech Republic during semi-artificial and artificial spawning of wild caught perch, mortality ranged from 0 to 10% for males and from 15 to 17% for females. Higher mortality of broodstock was evident 7 days after the spawning period (Table 4.2). No marked differences in post spawning mortality were found between artificial and semi-artificial spawning in perch broodstock.

Table 4.2: Mortality of wild caught perch broodstock 7 days after artificial and semi-<br/>artificial spawning (%).

	Artificial spawning	Semi-artificial spawning
Females	68	68
Males	22	8

Wild caught broodstock had significantly higher mortality compared to captive broodstock 7 to 90 days after spawning. Little or no mortality of captive stock was observed during the spawning season and this has also been observed by commercial operators in Ireland.



Plate 4.3: Example of necrosis on the skin of Eurasian perch breeder during spawning.





### 4.5. Fecundity

The absolute fecundity (number of eggs per female) in perch ranges from 15,000 to 300,000 eggs under natural conditions. During artificial spawning, average absolute and relative fecundity (number of eggs per kg of body weight) was around 40,000 eggs per female and 102,000 eggs per kg of body weight, respectively, in females with an average body weight of 378 g.

### 4.6. Egg and Larvae Quality

Egg and larval quality vary markedly during the perch spawning period, with usually a marked decrease during the second half of the season. This decrease of quality causes a reduction of fertilisation rate, a change in the aspect and firmness of the egg strands and premature hatching associated with mortality of newly hatched larvae. The hormonal induction of ovulation and spawning of females early during the spawning period would allow commercial operators to avoid the possible ageing of non-ovulated mature oocytes.

### 4.7. Collection and Quality of Sperm

Perch semen can be easily collected without hormonal treatment by applying pressure on the males abdomen and using a syringe to collect the resultant semen. Noncontaminated sperm (no urine or blood) can be used 12 hr post stripping at 2-4°C. For artificial insemination, the testicular sperm (sperm of squeezed testes) is not recommended because of low sperm velocity, motility and fertilising ability. The stripped sperm has the colour of milk with high viscosity and consistency. Sperm volume and density have been measured in a range between 0.5 to 7 ml and 3.5 to 44 x 10<sup>9</sup> sperm ml<sup>-1</sup>, respectively. The motility of sperm is induced by hypo-osmotic pressure in freshwater. The optimal sperm motility was observed when the sperm was first diluted in immobilizing solution containing NaCl 200 mM, NaHCO<sub>3</sub> 2.38 mM (osmolality 380 mOsmol Kg<sup>-1</sup>) in a ratio 1:50. Then, the maximum sperm velocity was observed after dilution in an activation solution containing 2.5 mM Ca<sup>2+</sup>, 50 mM K<sup>+</sup> and sucrose with osmolality 100 mOsmol Kg<sup>-1</sup>.

### 4.8. Cryopreservation of Sperm

Perch sperm cryopreservation can be applied in aquaculture either for efficient utilisation of sperm in highly valuable males or for wide-ranging artificial propagation. Sperm cryopreservation allows the preservation of the genetic resources and maintenance of the genetic variability of fish broodstock. Sperm of perch with 100 % motility stored on ice must be diluted with a 300 mM glucose solution extender at dilution ratio 1:6. DMSO (dimethyl sulphoxide) is added as a cryoprotectant at 10% of final concentration. Diluted sperm is added into 0.5 ml straws and frozen in a Styrofoam box (3 cm above the N<sub>2</sub> level) for 10 min and then transferred into N<sub>2</sub>. Before utilisation, frozen sperm must be thawed in a 40 °C water bath for 8 sec.

Frozen/thawed stripped sperm obtain similar hatching rates when used at a density  $12 \times 10^5$  and  $2.4 \times 10^5$  spermatozoa per egg.

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### 4.9. Fertilisation

Three methods of fertilisation can be used during perch reproduction. The first method is the "dry method" of artificial fertilisation (Plate 4.4.):

- Eggs are stripped manually into a container, taking care not to have any contact with water,
- Sperm is collected: for one egg ribbon it is recommended to use a pooled source of sperm originating from three males,
- The eggs are covered with milt: it is recommended to add approximately 2ml of milt per 100 g of eggs,
- The eggs and sperm are gently mixed,
- Clean water is added to the gametes in order to initiate the fertilisation process.

For artificial fertilisation it is also possible to use the so-called "wet method", where eggs and sperm are added into the water at the same time. Eggs must be fertilised within two minutes after the addition of water as spermatozoa are viable for less than one minute after activation. Use of this fertilisation method ensures a high fertilisation rate (80 - 97%) in perch after artificial spawning.

Semi-artificial spawning with natural fertilisation is the third method, which can be used in perch. In studies, a higher hatching rate was reached after semi-artificial spawning (65%) compared to artificial spawning with the dry fertilisation method (45%). After semi-artificial spawning, fertilisation rate is usually high with a mean of 65 - 70% and a maximum 90 - 100%.



Plate 4.4: On the top: egg stripping and spawn collection (left), stripping collection of sperm with a syringe (right). On the bottom: gentle mixing of eggs and sperm (left), addition of water (right) to induce activation of spermatozoa and the fertilisation of eggs.

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### 4.10. Egg Incubation

Incubation time is very similar for all percid species, ranging from 80 - 160 degree days at a recommended temperature of  $15-16^{\circ}$ C. The temperature at which commercial hatcheries incubate eggs varies from  $13^{\circ}$ C to  $18^{\circ}$ C (14-16^{\circ}C in PDS and Key Water Fisheries Limited in Ireland). Hatching of eggs starts after 14, 7 and 4 days of egg incubation at water temperatures of 13, 17 and 25 °C, respectively.

Fertilised ribbons expand in size over the first few days of their incubation as they hydrate (absorb water). The development of perch eggs is fast and a constant check on the development is necessary to ensure good quality larvae. Commercial hatcheries discard egg batches, which are not developing correctly. The fertilised ribbons are generally moved from the broodstock unit into dedicated hatching tanks, cages, containers or aquariums (Plate 4.5). These can vary in size from 1001 - 40001 and may accommodate single ribbons or groups of ribbons from the same batch (see Chapter 5 on larval rearing).

The practice in Ireland and Denmark is to disinfect the ribbons 3 days after fertilisation with a buffodine solution. This disinfectant prevents the development of viral, bacterial and fungal infections on the eggs. This is especially important to reduce the risk of contamination of newly hatched larvae. The ribbons are placed into the hatching tanks after disinfection and counting is done using a graduated cylinder and volumetric analysis. The eggs are either placed in floating trays in the tanks or draped on wire mesh supports. It is important that the ribbons are spread out and allowed to gently turn by water exchange to ensure that each egg has sufficient oxygen. Water exchange is minimal and just enough to ensure adequate oxygenation. Temperature and other water parameters such as dissolved oxygen must be kept constant. It is important to maintain pH below 8 to avoid calcium deposits on the eggs, which can impair oxygen absorption.



Plate 4.5: Incubation in Dnepr containers (left), in Ruckl-Vacek containers (middle) and floating trays in cylindro-conical tanks (right).

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# 4.11. Hatching

The condition of embryos should be monitored optically at the end of the egg incubation. At the beginning of the hatching, it is very important to have good water quality, oxygenation and a gentle water flow. To improve hatching efficiency and prevent the loss of larvae, it is sometimes necessary to artificially rupture the egg membrane. Egg ribbons are placed into a container and vigorously stirred to release unhatched larvae. After artificial or natural breakdown of the egg membrane, freshly hatched larvae must be separated from the rest of the eggs. Hatched larvae have to be gently siphoned off with a flexible pipe from the hatching tanks, containers or aquaria for transfer into larval rearing tanks.



Plate 4.6: Newly hatched perch larvae. (Ola Öberg)



### **Chapter 5 : Larval and Juvenile Production**

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Three methods of producing Eurasian perch fingerlings are currently practised in Europe: extensive to semi-intensive production in ponds, semi-intensive production in large and shallow tanks (mesocosm system), and intensive production in tanks, usually in recirculation aquaculture systems (RAS). The ultimate goal of fingerling production is to provide feed-trained juveniles for grow out. In this respect, the pond culture system usually requires an additional step in tanks, during which the fingerlings are habituated to artificial feed. This chapter outlines the techniques used in each of these three methods.

### **5.1. Extensive Production in Ponds**

Although pond farming represents a significant part of the wider aquaculture sector, especially in central and eastern part of Europe, Eurasian perch has only recently became a commercially cultivated fish species. Historically, its presence in a pond environment was classified as highly undesirable, mainly due to its feeding competition on zooplankton and macrozoobenthos with major commercial fish species and its predation on their juveniles. The development of pond culture of perch juveniles for commercial ongrowing for the table market has traditionally been carried out in France and more recently in the Czech Republic and in Ireland. In the northeast of France (Lorraine), some pond farmers produce perch either in monoculture in small ponds or in polyculture in large ponds of several hectares. In Ireland, the culture of perch juveniles in ponds is relatively new, in large part due to the limited number of species cultured in this country (predominately salmonids) and the associated lack of knowledge of extensive culture techniques. Juveniles are produced in ponds and they are subsequently feed trained in tanks. Perch juvenile production in the Czech Republic can occur in monoculture or polyculture, with the ongrowing stage also in ponds.

Besides the production of high quality juveniles that can eventually be trained later on artificial food, the rearing of perch in ponds also includes the maintenance of broodstock or the production of highly valuable market fish growing out on natural feeding sources only. Among the advantages of perch grown in ponds is the suitability of these fish for restocking programmes, partly due to a high feed adaptation and non-biased behaviour when released in natural waters.

### **5.1.1. Pond type and size**

The types of ponds used for perch culture differ according to their origin, structure and level of management. Practically, the most common sources of water are surface streams or connecting pond channels in the case of a pond cascade, flowing by gravity into each individual pond. Such a pond includes an overflow system (usually a monk) practically situated close to the corner of the levee. Overall length and width is strongly dependant on the expected water depth and total water dimension in case of floods. Selection of water depth is often based on modelling of pond stratification, pond production and energy balance reflecting the dissolved oxygen level characteristics, and most frequently range from 0.5 to 2m. In Ireland, pond depth is 2 m with approximately 1.5 m of water. The pond design and depth ensures that predation from herons is rarely a problem.



Plate 5.1: Juvenile production pond with harvesting channel (PDS, Ireland).

Pond area for juvenile production varies greatly from farm to farm and country to country. In most cases, however, the size of ponds specifically devoted to the production of perch juveniles ranges from 0.1 to 0.8 hectares. These ponds are equipped with a sump outlet in order to harvest the fish under the levee.

### 5.1.2. Pond water quality and fertilization

Pond management is critical to the successful rearing of juvenile perch in extensive systems. In Ireland, the ponds are drained two months before stocking and treated with a chloros solution or lime to sterilise the pond substrate. This is essential to prevent the development of larger zooplankton populations before perch larvae are ready to feed and to ensure adequate zooplankton succession. Predators such as tadpoles, newts, dragonfly larvae, diving beetles, sticklebacks etc must all be removed to ensure that the ribbons and subsequent larvae suffer as little predation as possible. Every two years the ponds substrate is scraped to remove vegetation.

The water for filling the culture pond may originate from a well, reservoir, surface runoff, or other surface water source; filling procedures vary depending on the water





source. For the production of larvae and early juveniles, feeding first on natural prey, rearing ponds should only be flooded for 7-15 days before stocking the fry to take advantage of the initial abundance of small-sized invertebrates. Longer pre-stocking periods might allow for larger sized invertebrates to become established, resulting in a limited number of suitable-size prey for Eurasian perch fry. Nearly all surface waters naturally contain zooplankton, and, given enough time, less fertile waters, e.g., well water, will be colonized. Because fish usually are cultured at high densities, the densities of their prey, zooplankton, also must be high. For yellow perch reared in north-American ponds, minimum density of suitable-sized zooplankton is 100/L. The application of fertilizers, either inorganic and/or organic, is recommended, the level of fertilization depending on natural water fertility. This fertilization must be applied 7 days prior to fish larval stocking in the case of inorganic fertilizers (e.g. urea or ammonium nitrate 52%-Nitrogen, superphosphate or phosphoric acid 32%-P2O5), and 10 to 15 days in the case of organic fertilizers (e.g. poultry manure). It supports a rapid and durable phytoplankton bloom, followed by a high abundance of zooplankton prey such as rotifers and copepod nauplii (the most important prey for early feeding larvae), and then copepodites and cladocerans. Regular fertilization is recommended after fish stocking, in order to keep high the N/P ratio (20:1), with 600  $\mu$ g N/L and 30  $\mu$ g P/L. In the case of inorganic fertilization, liquid fertilizers are preferred to granular ones because of easy use and faster results.

Physico-chemical quality of pond water must be controlled regularly, particularly during unsuitable climatic conditions, in order to maintain it within the limits recommended (Table 5.1). High water temperature associated with several sunny days can provoke a strong development of algal biomass, inducing an increase of water pH and an elevated risk of anoxia during early morning or cloudy periods. It is important for the farmer to monitor on a daily basis the oxygen concentrations and if necessary to take remedial action such as the introduction of paddle aerators or air lifts. Oxygen concentrations can vary from over 200% during the day to less than 50% at night if left unchecked. Such low oxygen levels will cause large mortalities in young fish larvae. In general, all considerations provided in cyprinid culture can be applied to the pond culture of perch.



Plate 5.2: in pond aeration to maintain oxygen levels (PDS, Ireland).

Variables	Recommended limits
pH Ammonium NH <sub>3</sub>	6 - 9 < 0.02 ppm
NO <sub>3</sub>	< 1 ppm
NO <sub>2</sub>	< 0.1 ppm
Dissolved oxygen	> 4 mg/l
CO <sub>2</sub>	5-10 ppm
Suspended matter Chloride Chlorine	< 80 ppm > 4 ppm > 0.003 ppm

 Table 5.1: Recommended limits of water characteristics for juvenile production of Eurasian perch.

### **5.1.3.** Larval stocking, survival and growth in ponds

Pond stocking is usually done in April, with newly hatched larvae, but also with fertilised eggs at the eyed stage or one to two days prior hatching. Eyed eggs are placed in small floating cages  $(0.4 \times 0.4 \times 0.4 \text{ m})$  with small mesh sizes (1.5 mm) retaining the eggs but allowing the passage of hatched larvae. Fertilised ribbons can also be draped over supporting branches laid throughout the pond. Stocking density is highly variable, and ranges from 10,000 to 60,000 eyed eggs per are  $(100 \text{ m}^2)$ .

Several studies and farmer experience have shown that it is not easy to produce over 1,000 to 5,000 juveniles (0.5 to 1.5 g) per are regardless of stocking density, representing a survival rate of 5-20 %. It is important that ponds are stocked with ribbons or larvae, which are close in age. Within ribbons there can be a difference of up to three days between the first larvae hatching and the last. This can contribute to large size heterogeneity at a later stage and cannibalistic tendencies. High stocking density induces a rapid depletion of natural food resources, and, as a consequence, the emergence of cannibalistic behaviour. If stocking density is adequate, growth of perch fry is rather homogenous, at least during the first 6-8 weeks. A regular assessment of zooplankton populations allows for identification of harvest times whereby fingerlings are harvested before complete zooplankton population declines, resulting in fewer instances of in-pond cannibalism. If the harvest is postponed to September, the survival of juveniles is strongly reduced, and the fish size heterogeneity very high (ranging from 2 to 40g).

Studies conducted in central Europe demonstrated that perch culture might be designed very similarly to pikeperch (*Sander lucioperca*) culture, using a prey fish in association with perch juveniles. The use of a sufficient biomass of either broodstock (stocked together with advanced fry of perch) or optimal prey fish (multiple cyprinid spawner) and regular prey fish addition in some cases might be of interest. Nevertheless, such a culture requires a high degree of food availability control due to rapid differences in the growth rates of piscivorous and non-piscivorous individuals, with direct effects on the perch intra-cohort survival and dynamics.



In the USA, the habituation of yellow perch juveniles on artificial diets is directly applied in some farms and results in rather good survival rates. When the fingerlings reach approximately 17.0–20.0 mm TL, they are fed frequently throughout the day with a commercial trout starter diet (mixed with krill). As they grow, the fish are then fed with increasingly larger food sizes until they are harvested in the autumn. In addition to feeding the fish on a daily basis, adding lights has proven to be effective in getting this fish to feed on commercial diets. Yellow perch smaller than 50 mm (30-45 days old) exhibit a strong photopositive behaviour, due to UV-sensitive cones in their eyes. These UV-sensitive cones disappear when the fish attain about 50 mm TL. Automatic feeders can be installed near the perimeter of the pond adjacent to the submerged lights. At night, the lights concentrate large numbers of fish in the vicinity of the feeders. The feeders then disperse food frequently throughout the night. This modification increases the percentage of fish that initially accept the formulated food and reduces total food use. The disadvantage of this method is that water quality can deteriorate and it facilitates the development of cannibalistic tendencies due to size heterogeneity. Thus the practice in Ireland is to remove the perch juveniles at a smaller size (< 0.5g) for feed training in tanks.

### 5.1.4. Tandem Pond/tank Culture

In this method, perch larvae are initially stocked into fertilized production ponds where they feed on natural food. Once the fish reach approximately 0.3-1g, usually within 8 weeks post hatch (mid to late June) and exhibit a shoaling behaviour, they are harvested and stocked into tanks where they are trained to accept artificial feeds. A key advantage to this behaviour is that shoals contain fish of the same size so an initial grading is not required. Once removed from the pond the juveniles are placed in a saline solution of 12ppt for 20 minutes. This salting helps clean the gills and reduces stress associated with netting and transportation. In Ireland (PDS farm), the weaning facility for pond based juveniles consists of 15 tanks of 650 litres each constructed of gel coated fibreglass. The tanks are circo-cylindrical in shape, which aids the removal of uneaten food.

Fish are placed directly in the tanks at 17°C at a density of 10 fish/L for weaning. They are weaned on a 700 mm to 1 mm cod diet (DanEx 1562). Weaning at the required density is important for reducing cannibalism and facilitating the transfer from natural to artificial diets. The fish wean quickly with most fish accepting the diet with 5 days. Once weaned, the fish can be moved to the nursery system (e.g. 5 m<sup>3</sup> tanks in RAS and equipped with automatic feeders). Once weaned the fish remain in this system until ready for transfer to ongrowing farms. It is of paramount importance to grade the fish regularly to limit cannibalistic tendencies and associated mortalities. Fish are graded on bar graders (size range 1.5mm – 5mm).

The principal advantages of this method are that large numbers of small size fish can be produced in ponds at low cost compared with the tank culture method. Normally, 70–90% of the fingerlings of this size harvested from ponds can be habituated to formulated feeds and tank culture conditions. Skeletal and other deformities

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associated with nutritional deficiencies or imbalances often observed in tank-cultured fry and early fingerlings are rarely observed in perch reared initially in ponds. There are some disadvantages associated with the tandem pond/tank method. Fingerlings subjected to excessive harvesting stress can be difficult to train to formulated diets and are more susceptible to disease and in particular parasitic infection, (Costia outbreaks are not uncommon). Moreover, the tank rearing stage of the tandem strategy requires frequent feeding, tank cleaning, and other animal husbandry works.



Plate 5.3: Pond feeder combined with light to improve the training of yellow perch fry to accept dry diet directly in ponds, in Wisconsin, USA (photo from J. Held, diagram courtesy of J. Malison,).

### 5.2. Semi-intensive Production in Mesocosms

### 5.2.1. Design of the culture system

To date, semi-intensive production of perch in mesocosms has not been performed in a full commercial operation but it has been experimentally done on a pilot scale in rectangular concrete outdoor tanks (10  $m^2/5m^3$ , 50 cm depth) (Figure 2) at the Aquaculture Education and Research Centre of the University of Liège (CEFRA, Tihange, Belgium). There is growing interest in this method of production from commercial operators given the potential for cheaper large scale juvenile production. Some trials have been carried out at the PDS site in Ireland and initial indications are that the mesocosm method of production as developed at CEFRA may become more mainstream in the future. Mesocosm systems utilise the benefits of extensive cultivation but in an intensive environment thus providing controlled natural conditions for the fish. At CEFRA each tank is supplied with a mixture of heated water, river water (River Meuse in Belgium) and/or well water. Tanks are equipped with an air diffuser, a gas stripping column and a heat exchanger (supplied with heated water from a nuclear power plant or from natural geothermal source). Temperature is controlled by an automatic regulation system. A PVC or plastic sheet covers the tank in order to reduce the light incidence and prevent the growth of filamentous algae.


#### **5.2.2. Fertilization process**

About one week before the stocking of perch eggs into the mesocosm, water is fertilized with 1.5 kg of chicken manure (in the form of pellets). Water temperature is maintained between 23 and 27°C in order to stimulate the production of phytoplankton and small zooplankton (protozoa, rotifers) naturally present in the water. Under appropriate temperature and sun light conditions, the rotifer concentration ranges between 2,000 and 6,000 ind/L. There is no water renewal during the fertilization process.



Plate 5.4: Mesocosm system for larval rearing of perch in semi-intensive conditions.

#### **5.2.3. Culture Conditions**

The success of various stocking densities (ranging from  $400-6,000 \text{ eggs/m}^2$  of mesocosm) has been investigated at different water temperatures  $(17 - 23^{\circ}C)$ . Despite large year to year variations, mainly due to factors such as climatic conditions and their impact on phyto- and zooplankton dynamics, egg quality, water quality and presence of parasites, the growth of perch is clearly enhanced at high temperatures, mean body mass ranging from 370-860 mg at 23°C and from 190-280 mg at 17°C (Figure 5.1). However, the higher temperature negatively affects survival rate (18.7% after 44 days at 23°C vs 38.5% at 17°C), due to an increase in size heterogeneity and, consequently, a higher proportion of cannibals, as well as an increase of pathogens (bacteria and parasites) (Figure 5.1). Regardless of temperature, high stocking density produces significantly more homogenous growth within the cultured stocks, which decreases the overall mortality rate and the emergence of cannibals. Greater densities of cannibals are usually observed at the highest densities, e.g. from 2.6 to 14.7 cannibals per m<sup>2</sup> of mesocosm at 500 and 4,000 fish/m<sup>2</sup>, respectively, but, proportionally, the impact of cannibalism is reduced with increasing stocking density. All in all, a stocking density of 4,000 eggs/m<sup>2</sup> and a rearing temperature of 17°C can be recommended for the fingerling production of perch in a mesocosm

One day before the stocking of fertilised eggs (eyed embryos), water is cooled down to 17°C. Perch ribbons with eyed embryos (6 days post-fertilization at 15°C) are

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placed on a tray suspended in the mesocosm. During the first 2-3 days after perch hatching (occurring usually 1-2 days after stocking), there is no water renewal. Then, well water is progressively added and the tank is 80% covered by a plastic sheet. Optimal temperature of rearing is 17°C and oxygen level is maintained over 6 ppm during the whole larval rearing period. The nitrite and ammonia concentrations are maintained below 0.2 and 0.5 mg/L, respectively. In order to secure the larval rearing process, temperature and oxygen are monitored and controlled every day, with ammonia and nitrite concentrations measured twice per week.

On day 3 post-hatching, *Artemia* nauplii are provided, as a complement to the natural zooplankton which is rapidly depleted. During the first 6 days of feeding (day 3 to day 8 post hatching), live *Artemia* nauplii is the sole exogenous food, distributed 5 times a day, usually in the same place, in the uncovered area. On day 9 post-hatching, artificial food formulated for larvae (45-56% crude proteins, 18-20% lipids) is provided during the daytime with an automatic belt feeder. *Artemia* and artificial food are concomitantly distributed until day 23 post-hatching (Figure 5.2). From day 24 onwards, perch juveniles are fed artificial food only, *Artemia* nauplii being completely suppressed within one day. The particle size of the artificial food is regularly adapted according to the growth of larvae and young juveniles, from 200-300  $\mu$ m during the second week post-hatching to 300-500  $\mu$ m during the third week and 500-700  $\mu$ m from week 4 to week 6 post-hatching.

After 44 days of rearing, juveniles are harvested from the mesocosm. At this time they are all conditioned to ingest dry feed. The water height is progressively decreased from 50 cm to 25 cm and the heat exchanger removed from the tank. The larvae are gently calmed by adding 3 g/L of salt into the water. Perch are harvested using a small mesh net. Cannibal fish (usually 2.5 larger than the mean size fish) are removed by hand and stocked in a separate tank while the weaned juveniles are transferred into the ongrowing system.



Figure 5.1: Survival rate (%), mean body weight (mgX10) and cannibals (%) of weaned perch juveniles harvested from mesocosm after 44 days of rearing.







#### **Summary**

Optimal temperature for mesocosm fertilization	23-27°C
Optimal temperature during perch rearing	17°C
Dissolved oxygen	> 6 ppm
Ammonia concentration	< 0.2 mgL <sup>-1</sup>
Nitrite concentration	< 0.5 mgL <sup>-1</sup>
Optimal initial stocking density	4000-6000 larvae/m <sup>2</sup>
Water renewal (during feeding)	1.0-1.8m <sup>3</sup> /h
Natural food only	Day 1 – day 2 post-hatching
Feeding with Artemia nauplii	Day 3 – day 23 post-hatching
Feeding with artificial food	Day 9 – day 44 post-hatching
Duration of juvenile production	44 days
Duration of juvenile production	44 days

#### **5.3. Intensive Production in Recirculation System**

Intensive culture of fry has many advantages, e.g. stable culture conditions, rearing of fry produced by out-of-season spawning, more predictable production of juveniles and easier monitoring and control of cannibalism compared to culture in ponds and mesocosms. However, there are several critical factors affecting the success of Eurasian perch intensive culture including the small mouth gape (although 60-70% of the larvae are able to ingest *Artemia* nauplii as first food), the dependence on live food organisms and the cannibalism. Despite the availability of high quality feeds for small larvae, mainly formulated for marine species, the acceptance, growth and survival of perch fed formulated diets as starting food are still highly variable and rather unsatisfactory.

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#### 5.3.1. Tank culture design

The rearing of perch in tanks is usually performed in recirculation aquaculture systems (RAS) in order to efficiently control the water characteristics and to avoid the presence of pathogens. Larval rearing tanks are of various shapes and materials, squared or cylindro-conical, or made of cages (with 250-300  $\mu$ m mesh size) placed in a large concrete tank (Plate 5.5). Regardless of shape, they must be rather large, ranging from 300 L to several m<sup>3</sup>. In small tanks, perch larvae cling to the tank walls and display a non feeding behaviour. In the case of PVC or fibreglass tanks, darker walls are recommended in order to improve the contrast between the feed (live prey or inert food) and the background, facilitating its detection and ingestion by the young larvae. The water parameters (dissolved oxygen, ammonia, nitrites) must be checked on a daily basis as the stocking density of larvae is rather high, inducing rapid accumulation of ammonia and nitrites and water quality degradation. All tanks or cages should be equipped with individual water and air supply.



Plate 5.5: Clockwise from top: cylindro-conical tanks and tank-based cages used for the experimental culture of perch larvae in recirculation aquaculture system (CEFRA, University of Liège, Belgium). squared tanks for production of weaned juveniles used at Bornholms Hatchery (Denmark). cylindro-conical tanks for incubation and early rearing of perch.



#### 5.3.2. Culture conditions

As in mesocosm culture systems, perch are transferred into the larval rearing tanks at the stage of eyed eggs, in order to reduce the mortalities induced by handling newly hatched larvae. Eggs can be suspended on trays one day before hatching, initially at a temperature close to that of incubation. Within one day, the temperature is raised to 20°C, and then maintained constant between 19-23°C (dependant on strain) throughout the larval rearing phase, up to the time of transfer of juveniles to ongrowing tanks. Because perch larvae are visual feeders daylength is fixed at LD 16:8 (or even at 24:0 if a continuous food supply is ensured) and light intensity between 90 and 400 lux at the water surface. Non inflation of gas bladder (NIGB) occurs sometimes during the larval rearing phase, but Eurasian perch is much less sensitive than pikeperch. Spray flow, avoiding the accumulation of an oil film at the water surface, can be used to reduce the risk of NIGB and, consequently the rate of skeletal deformities (scoliosis). Surface skimmers and degassers may also be employed. Gas saturation levels are monitored to avoid nitrogen supersaturation, which can result in larval mortality.

Initial stocking density usually ranges between 20 and 50 larvae/L. Higher densities, up to 100 newly hatched larvae/L have been successful, but fish density must be reduced after the weaning phase, as the fish grow. Cannibalism constitutes a major cause of mortality during the early life stages of perch. Many variables have been investigated in order to mitigate its impact on the survival and overall production of perch larvae and juveniles. At the larval stage, survival is improved at high stocking density (100 fish/L) compared to low density (10 fish/L) because of a significant reduction of the cannibalism, while, at the post-larval stage (200 mg body mass), stocking density (from 10 to 1 fish/L) negatively affects the survival rate, due to a strong increase of cannibalism.



Plate 5.6: Grading 1g perch fry (PDS, Ireland)

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One to two days after hatching, the larvae are fed small size Artemia nauplii (350-380  $\mu$ m) over 3 days (Plate 5.6), in order to maximise the proportion of larvae able to ingest the live prey. Then, the larvae are fed regular size Artemia nauplii (420-450  $\mu$ m). During the first week, a daily ration of 500 to 1000 nauplii per larvae is provided, distributed very frequently by hand or with a peristaltic pump during the daylight period. From the end of week 1 to the end of week 3, the daily ration (calculated on the dry matter basis of Artemia) can be reduced from 35 to 10% of fish biomass. On day 21 post-hatching (body mass of 50 mg), they can be trained to accept dry feed, by replacing progressively the live prey with a high quality compound feed (300-500  $\mu$ m) within 4 days. First-feeding with rotifers (either the brackishwater species Brachionus plicatilis or the freshwater species B. calyciflorus) has also been carried out. The size of these prey (100-150  $\mu$ m) is more suitable for the small mouth gape size of the newly hatched perch larvae, securing higher survival rates. However, the use of rotifers before or in combination with Artemia nauplii constitutes an additional step in the feeding programme, inducing extra costs. At PDS (Ireland) the procedure used is to cover the newly hatched larvae for the first three days to allow them absorb their yolk sac fully. Freshwater rotifers from a continuous culture system are then added to the larval tanks for a further one to three days (dependant on larval size) before Artemia are introduced.



Plate 5.7: Artemia incubation at PDS (Ireland)

#### Summary

Optimal temperature at hatching	17-20°C
Optimal temperature during perch rearing	20-23°C
Dissolved oxygen	> 6 ppm
Ammonia concentration	< 0.2 mgL <sup>-1</sup>
Nitrite concentration	< 0.5 mgL <sup>-1</sup>
Optimal initial stocking density	50-100 larvae/L
Feeding with small size Artemia nauplii	Day 2 – day 4 post-hatching
Feeding with regular size Artemia nauplii	Day 5 – day 20 post-hatching
Co-feeding Artemia-dry feed	Day 21 - day 24 post-hatching
Feeding dry feed only	Day 25 onwards
Duration of juvenile production	44 days
Body mass at harvest	500 mg

### **Chapter 6 : Genetic Improvement of Growth**

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Among the different problems related to Eurasian perch aquaculture, the poor growth rate can be considered as a major limiting factor in the commercialization of the species. The production of marketable 80-100 g fish (Swiss market) reared at the optimal temperature of 23°C in recirculating aquaculture systems takes at least one year starting from larvae. Under a natural temperature regime (cage rearing), the minimal market size is obtained in more than 800 days. Due to the slow growth, intensive perch culture is impaired by low production rate (350-400 g m<sup>-3</sup> d<sup>-1</sup>), even at high stocking biomass (60-80 kg m<sup>-3</sup>). Growth rate heterogeneity is another major feature of perch rearing. Fast growing fish grow twice as fast as slow growing ones. Several techniques based on genetic characteristics including strain selection, domestication, all-female populations and hybridisation have been developed to improve the growth rate of Eurasian perch in culture conditions.

#### **6.1. All-female Production**

Eurasian perch, *Perca fluviatilis*, as with the American yellow perch, *P. flavescens*, display a sexual growth dimorphism in which females grow around 20 % faster than males (Plate 6.1). Therefore, the improvement of the productivity of perch culture systems can be attained using this sexual growth dimorphism and all-females populations.



Plate 6.1: Female (top) and male (bottom) 365 day old perch.

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All female populations can be obtained in different ways, either through gynogenesis, which is a restricting technique with low results, or hormonal sex reversal, which is the most useful method for the production of monosex populations in fish. Hormonal sex reversal treatment can be used directly, by feeding fish with the feminising hormones 17a-ethynylestradiol (E2) or indirectly by producing hormonally sex-reversed males that will give rise to 100% female progenies when crossed with a normal female. Because of ethical and legal issues, direct hormonal treatment should be avoided in fish production destined for human consumption. Therefore, production of all-female population is carried out within two generations : production of hormonally sex-reversed males breeders in the first generation and a cross between these sex-reversed males and normal females in the second generation (Figure 6.1).

#### 6.1.1. Obtaining sex-reversed XX male breeders

Obtaining sex-reversed males breeders takes place in 3 steps:

1. Year 1: Masculinisation of a standard progeny which will induce an all-male juvenile population with 50% of juveniles displaying a male genotype (XY) and 50% a female (XX) genotype.

2. Year 2: crosses between masculinised males and normal females and analysis of the sex ratio of the progenies:

- If the progeny display a normal sex-ratio (50% males and 50% females), the male breeder is therefore a normal male and not useful.
- If the progeny is all-female, any male breeders are therefore sex-
- reversed males with a female genotype (XX).

3. Year 3: Crosses between sex-reversed male breeders and females and masculinisation of the progenies in order to produce a stock of sex-reversed males breeders (Figure 6.1).





Figure 6.1: Flow diagram illustrating the different steps in the production of sex-reversed male breeders in the first generation and the production of an all-female population in the second generation.

The success of hormonal sex reversal treatments depends on three major factors: moment of application, doses and duration. The time of application is the first factor to be examined, as treatments applied outside of the so-called hormonsensible period have no effect or an effect lower than expected. Even though the treatment is applied at the right time, low doses and short duration are prone to produce incomplete or no sex reversal, whereas excessively high doses and/or treatment duration are likely to produce sterile fish, or individuals developing no gonoducts. In Eurasian perch, parameters tested to date include:

- moment of application: 45 mg (32 days post-hatching at 17°C), 70 mg (36 dph), 150 mg (42 dph) and 205 mg (dph);
- 17 MT doses: 5, 10, 20, 30, 40, 60 and 80 mg kg<sup>-1</sup> food
- Duration: 30 and 80 days
- Masculinising hormone: 17a-méthyltestosterone (MT, Sigma)
- Treatment form: MT dissolved in 95% ethylic alcohol (600 ml kg<sup>-1</sup> food), incorporated into formulated feed for larval fish 24h before feeding in order to allow the evaporation of the ethyl alcohol. Food was delivered in excess.

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Complete sex reversal (100% male progenies) was obtained when the hormonal treatment was applied in fish initially ranging from 40 to 71 mg. Treatments applied later gave rise to variable proportions of males (45-62%), females (0-29%), sex reversed males (16-40%), fish with ovotestis (0-18%), and sterile individuals (0-27%, Picture 6.2). Long duration treatment (80 days) and high hormonal doses (> 40 mg kg<sup>-1</sup> food) induced sterility in 25% of fish. A 40 mg kg<sup>-1</sup> food MT treatment induced 100% sex reversal (100% males) but with only 15% functional males (with a spermduct), whereas treatments with lower hormonal doses (5 and 10 mg kg<sup>-1</sup> food) induced 80% functional sex-reversed males. All sex-reversed males used for reproduction gave between 95 and 100% female in the progenies.



Plate 6.2: Gonads of perch progenies after feeding with 17a-methyltestosterone. a) double testes from a normal male, b) abnormal fused testis from sex-reversed males (genetically female), c) ovotestis from a female not completely sex-reversed, d) ovary from female.

#### Summary

Sex-reversed males breeders production						
Hormone:	17a-methyltestosterone, 17MT					
Initial mean body weight:	70 mg					
Initial age:	36 dph (at 17°C)					
Dose:	5 or 10 mg kg <sup>-1</sup> food					
Duration:	30 days					
Temperature	23°C					
Sex reversal	100%					
Functional sex-reversed breeders:	80%					



#### 6.1.2. Improvement of growth with all-female populations

All-female families were obtained either by natural reproduction in tanks between females and functional hormonally sex-reversed males or by artificial reproduction with hormonally sex-reversed males, selected based upon the morphology of the gonad (Plate 6.2). Only experimental data exists on comparative growth between all-female and mixed-sex populations in a recirculating system (tanks :  $0.5m^3$ ;  $23^{\circ}C$ ,  $O_2 > 6ppm$ , initial stocking density : 1000 fish.m<sup>-2</sup>). When fish reached a mean body weight of 30g (170 days of rearing), all-female families grew faster than mixed-sex families (Figure 6.2). After 360 days of rearing, all-female families displayed a mean body weight 30% higher (143g) than mixed-sex families (103g, Plate 6.1). The market size (100g) was reached within 250 days with all-female families as compared to 300 days with mixed-sex families. The food conversion ratio was also better for all-females than for mixed-sex families (1.5 vs 1.7). The increase of growth performance (30% after 360 days of rearing) and the improvement of food conversion ratio with all-female families will allow a significant improvement in the productivity of perch reared under intensive conditions.



Figure 6.2: Growth of all-female and mixed-sex juveniles reared under intensive conditions in 0.5m<sup>3</sup> tank in a recirculating system (23°C) at an initial stocking density of 2000 fish.m<sup>-3</sup>.

#### Summary

All-female rearing	
Improvement of growth	30%
Marketable size	In 250 days

#### **6.2. Triploidisation**

Current interest in polyploidy induction is almost entirely due to its potential application in fish farming, for the production of triploid and tetraploid fish. Induced triploidy is used to produce partially or completely sterile fish, whose three chromosome sets impair the meiotic division involved in germ cell formation. As triploids are partially or completely sterile, production of triploid female perch should prevent sexual maturation and it avoids the negative effects of gonadal development (mean gonadosomatic index : 20 %) on growth, survival, and flesh quality. In warm water culture, Eurasian perch do not mature, so the need for triploidisation lies mainly in the production of Eurasian perch in temperate waters (from 4 to 25°C).

A variety of treatments have proven to be effective in inducing triploidy, including thermal (cold or heat), chemical (colchicine or cytochalasin B) or hydrostatic pressure shocks. Even if pressure shock led generally to high survival and/or triploidisation rates, temperature shock is preferred for the achievement of 100% triploid fish. This is especially so for situations in which a large volume of eggs need to be treated, because of the cost implications of a pressure shock system. Triploid fish can also be produced by crossing tetraploid with diploid breeders.

Triploidy must be induced after artificial reproduction. Semen is collected in a syringe by stripping mature males and kept on ice until fertilisation. Egg ribbons from each mature female are manually extracted by stripping. After dry mixing with semen, water at 16-17°C is added to induce fertilisation. Eggs are rinsed 2 or 3 times. Heat shocks are applied by immersing eggs into a thermostatic water bath maintained at  $30^{\circ}$ C. After shock administration, eggs can be transferred into the hatchery, with water maintained at  $16-17^{\circ}$ C (O<sub>2</sub>  $\ge$  6 ppm).

In summary low intensity (28 and 30°C) out – long duration (10 and 25 minutes) shocks give a significantly higher survival ( $40 \pm 5\%$ ). The best triploidisation rate ( $100 \pm 0\%$ ) with a yield of triploids of  $43 \pm 34\%$  is obtained at 30°C, when heat shock is applied 5 minutes post-fertilisation for 25 minutes duration.

Different techniques can be used to assess the degree of ploidy in the progeny (annex 1):

- **Caryology:** which relies on the count of chromosomes observed with an optical microscope: performed for small sample sizes, but excessively time-and money-consuming for large samples (about one hour per fish examined)
- Analysis of the nucleole organising regions (NORs): combines rapidity and low cost, but its accuracy is compromised by the variability of the number of NORs in different cells, and the technical difficulty of performing homogenous staining with silver nitrate
- Flow cytometry analysis: which measures the amount of DNA in a cell from its fluorescence: combines reliability and rapidity, as about 20-25 fish can be examined per working hour.

#### Summary

Triploidisation	
Shock temperature:	30°C
Time of initiation:	5 min. post-fertilization
Duration:	25 min.
Triploids:	100%
Yield of triploids:	43%

#### 6.3. Hybridisation

Hybridisation is often used in production to improve the survival rate and growth performances of the produced species. Hybridisation trials have been made between *P. fluviatilis* females x *P. flavescens* males in order to enhance the growth in intensive rearing systems. Hybrids were obtained by artificial fertilisation (Chapter 4) and their growth performances compared to purebred *P. fluviatilis*. The growth performances were compared in a recirculating rearing system supplied with water at 23°C. After 230 days of rearing, hybrids displayed an increase of growth performance when compared to purebred fish (Figure 6.3). The market size of 100 g was reached in 11 months with hybrids vs 12 months for purebred P. fluviatilis. After 800 days of rearing, the difference of growth rate is more than 40%. The mean survival rate is also enhanced with hybridisation.



Figure 6.3: Comparative growth of purebred P. fluviatilis and hybrids *P. fluviatilis* female x *P. flavescens* male reared in a recirculating system at 23°C.

Despite the improvement of growth performances and survival with hybridisation, there is still no information on flesh quality of hybrids. Moreover, production of hybrids has to be done according to the authorisation of the countries authorities regarding the importation of exogenous species. Finally, hybrids must be reared in a controlled environment in order to avoid escape of hybrids into the natural environment.

### Summary

Hybridisation	
Improvement of growth	40%
Marketable size	In 320 days

#### **6.4. Strain Selection**

Research conducted on growth of several Eurasian perch wild strains originating from different regions of Europe reared in R.A.S at 23°C suggested marked differences between strains: at day 200 starting from larvae, body weights of Belgian and North-East France strains were 56% and 76% larger respectively than in South-West France and North Italy strains (Figure 6.4). Starting from 4-5 g fingerlings, survival was also higher (60%) in Belgian and North-East France strains than in South-West France and North Italy strains.



Figure 6.4: Growth of 4 different strains of Eurasian perch reared under intensive conditions in a recirculating system at 23°C, two progenies / strain.

The identification of the best strain adapted to intensive culture conditions is the first step in starting a selection program.

#### **6.5.** Domestication

The domestication process, as exists at present in Europe, consists of rearing wild breeders in intensive conditions from year to year and reproducing them from one generation to the next. There are limited selection programs. The growth potential of domesticated progenies (F1, F2 and F4) is significantly improved compared to wild progenies from the same strain.





In Belgium, a domesticated strain of perch originating from the river Meuse have been held captive for many years in CEFRA – ULG they are now at the fourth captive generation. Each new generation is obtained after natural reproduction in tanks. Rearing domesticated strains allows significant improvement in the growth performance of perch. As an example, after 340 days of rearing at 23°C in 1.6m<sup>3</sup> in a recirculating system (CEFRA-ULg), F2 and F4 generation display a final mean body weight 1.6-fold higher (184 and 196g) than the wild strain (117g)(table 6.1). Specific growth rate (SGR) as well as individual growth have been shown to be better with domesticated strains. Finally, food conversion ratio (FCR) is also better for the domesticated strain (1.2) compared to the non domesticated one (1.55, Table 6.1).

Table 6.1: Example of growth parameters [Initial and final mean body weight, heterogeneity (CV, %), survival (%), specific growth rate (SGR, %), growth (g.ind<sup>-1</sup>.d<sup>-1</sup>) and food

conversion ratio (FRC)] obtained with domesticated strain of perch (F1, F2 and F4) reared in 1.6m<sup>3</sup> tank in a recirculating system (t<sup>o</sup> = 23°C,  $O_2 > 6$  ppm) at an initial stocking density of 3125 fish.m<sup>-3</sup> during 340 days from 54 to 394 days post-hatching.

	Initial body weight (g)	Final body weight (g)	Initial CV (%)	Final CV (%)	Survival (%)	SGR (%)	Growth (g. ind-1 .d-1)	FCR
F1	0.6	120	30.8	36.7	57	1.5	0.3	1.5
F2	0.4	200	33.4	35.4	62	1.9	0.6	1.2
F4	0.4	185	31.7	46.2	61	1.8	0.5	1.2

With the domesticated strains (F2 and F4) it was possible to reach the marketable size  $(\pm 100g)$  within 240 days, whereas wild strains reached this size after one year of rearing in the same conditions (Figure 6.5).



Figure 6.5: Growth of 3 domesticated (F1, F2 and F4) strains of juveniles reared under intensive conditions in 1.6m<sup>3</sup> tanks in a recirculating system at 23°C and  $O_2 > 6$ ppm.

#### Summary

**Domestication** Improvement of growth Marketable size

35% In 240 days

All-female rearing, hybridisation, strain selection and domestication, alone or in combination, can induce a positive response in the short-term, to enhance the growth of Eurasian perch (minimum 20%). The use of these methods in intensive production should allow a shortening in the duration of production and the attainment of market sized fish in a shorter time. An improved growth rate combined with lower FCR should significantly contribute to an increase in productivity conditions leading to a decrease the production costs of Eurasian perch in intensive culture.



## **Chapter 7 : Fish Health**

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The maintenance of a healthy fish population is a pre-requisite to a successful aquaculture enterprise and Eurasian perch are no exception. The fish health issues associated with percids are not greatly different to that of any other species cultured in intensive conditions. As in all aquaculture, the presence or appearance of disease is normally associated with a lapse or failure in fish husbandry and management. It is however likely that even the best run facilities will at one time or another have to deal with fish health issues. Perch are prone at different stages in their lifecycle to certain threats and a well managed hatchery will be aware of this and take preventative and prophylactic measures.

This chapter will seek to identify the most common fish health issues faced by a perch farmer. It must be remembered that different treatments are recommended and regulated across the EU and that different member states have differing approaches. Of paramount importance therefore is for the perch farmer to develop a good relationship with the local fish veterinarian and regulatory authorities. It is in discussion with these professionals that the best course of treatment for any fish health issue will be decided.

Generally perch have a good resistance to disease and parasites as long as they are not weakened by bad husbandry, unsuitable food, lack of oxygen, abuse of temperature or other stress inducing incidents. In this regard the planning of a new facility is very important. Taking account of optimum fish requirements must be an ever present consideration in the planning and building of a new fish holding unit. Whilst perch are relatively tolerant of low oxygen in the wild, they are no less tolerant of low oxygen in intensive systems than salmonids. Well finished tanks with adequate lighting, sufficient water volume and exchange, in tandem with appropriate feeding and above all high water quality should be to the forefront of a well designed perch facility. If properly planned and well designed facilities are in place, the job of limiting fish health issues is made much easier for the farmer.

The farming of perch (*Perca fluviatilis* and *Perca flavescens*) is developing in both Europe and North America and as the aquaculture of these species develops, so too are some of their health challenges encountered, such as infectious (viral, bacterial, parasitic and fungal) and non-infectious (genetic, congenital, environmental, nutritional) diseases. Some disease conditions have to date been associated only with wild perch, such as Epizootic Haematopoietic Necrosis (EHN), however, it is to be expected that as production of perch increases, some of these disease conditions from wild fish will be observed in the farmed sector.

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#### 7.1. Viral Diseases

Viruses are infectious agents of very small dimensions, which pass through filters that retain bacteria. Although some of the largest ones form particles that can be seen by optical microscopes, the exact shapes and dimensions can only be determined by means of an electron microscope. Epizootic Haematopoietic Necrosis (EHN) virus causes mortalities in wild perch in Australia and has also been encountered in trout farms, however, this Ranavirus has never been reported in Europe, although closely related viruses affect catfish and sheatfish in France and Germany. Clinical signs in perch include lethargy, sometimes with spiral swimming and hundreds of fish head standing on the bottom of affected waterways. No vaccine is available and as with other viral diseases in fish there are no medical treatments.

Perch Fry Rhabdovirus (PFRv) has been recorded in wild and farmed perch throughout Northern Europe and can cause high mortalities in juvenile perch. Clinical signs of the disease in farmed perch in Ireland include abnormal swimming, congestion at fin bases, ascites and scale extension (Plate 7.1). Histopathology associated with this virus includes multifocal necrosis in the haematopoietic tissue in the kidney and spleen, scattered necrotic hepatocytes, necrosis in the lamina propria in the intestine, endocardial cell proliferation and congestion in the meninges. Careful disinfection of egg strings helps prevent apparent vertical transmission of this virus. At hatchery sites in Ireland and in Denmark, an iodine based solution such as Buffodine<sup>TM</sup> is used. The practice at PDS (Ireland) is to disinfect the eggs when they are around 3 - 4 days old.



Plate 7.1: Juvenile perch affected by PFRv exhibiting congestion at the bases of the pectoral fins as well as some scale extension in the lower fish.

Walleye Dermal Sarcoma Virus (WDSV) has been associated with fibrosarcoma in one captive yellow perch, *P. flavescens*, in aquaria in New York State, however, it remains to be established if the virus was the aetiological agent of the tumour.

#### 7.2. Bacterial Diseases

Bacteria are micro-organisms of very small dimensions; usually between 0.5 and  $10\mu$  m. Bacterial infections in fish may occur in the internal organs, in the muscles and in the skin, including the fins. Fish that are not weakened by bad conditions, or by infections with other parasites, generally have a great resistance to bacterial infections. This is due to the high amounts of bactericidal substances in the blood, which helps them to overcome an infection. But if they are wounded, or their resistance is decreased by other causes, bacterial infections may not be conquered easily. Consequently, as mentioned previously, the best precaution against the occurrence of illness amongst perch is the culture of them under optimum conditions.

*Flavobacterium psychrophilum* is the causal agent of rainbow trout fry syndrome (RTFS) and cold water disease in salmonids. It is also encountered in perch farming where it can give rise to elevated mortalities and can present in juvenile fish as a dissolving or necrotic jaw syndrome (mouth rot, Plate 7.2) or in some cases as a systemic disease. Improved environmental conditions and treatment with oral broad spectrum antibiotics are usually successful in controlling this condition. The bacterium has also been detected in otherwise healthy wild perch.



Plate 7. 2: Juvenile perch infected with *Flavobacterium psychrophilum* exhibiting necrosis of the mandible and perforation of the adjacent epithelial and dermal layers.

Aeromonas spp. have been isolated from wild and farmed perch. They include Aeromonas hydrophila, which was implicated in the dramatic perch kills in Lake Windermere, England in the 1970s and A. salmonicida, which is more commonly associated with furunculosis of salmonids. A. sobria has been demonstrated as a significant pathogen for net pen farmed perch during winter months in Switzerland where it resulted in mortalities of 1% total farm stock per day at the peak of the outbreaks. In addition to Aeromonas sp., Streptococcus sp. have also been associated with farmed perch. Wild perch in Finland have been reported to carry Yersinia ruckeri, the causal agent of Enteric Redmouth Disease, however, no clinical disease with this

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bacteria has been reported in farmed fish. A study carried out in 1994-1995 tracked mortality rates in perch following infection with *Aeromonas* bacteria. Infection was rapid and caused significant mortalities especially in the first 10 days. (Figure 7.1).

Figure 7.1. Daily mortality following infection by *Aeromonas veronii in* cultured *Perca fluviatilis* (mean body weight: 8g, 23°C, stocking density 1500 m<sup>-3</sup>, 0.5 m<sup>3</sup> square tanks supplied with water from River Meuse).

In comparison to some other farmed fish species, perch appear vulnerable to skin and tail damage and if the mucus layer is removed, they are then very susceptible to secondary bacterial and fungal infections. In perch farming there is a requirement for frequent grading to prevent cannibalism and the physical stresses this imposes on the fish means that great care must be taken during these procedures.

Epitheliocystis, caused by a chlamydia-like organism, has been observed in juvenile farmed perch in Ireland and also in farmed *P. flavescens* in Pennsylvania, USA (Plate 7.3) and in both cases was associated with gill hyperplasia, respiratory distress and elevated mortalities. Due to the intracellular nature of this organism antibiotic treatment can be of limited benefit, however, oral oxytetracycline (75mg/kg body weight/day for 10 days) has been used with success in some outbreaks.







Plate 7.3: Histopathological section of gills from *Perca flavescens* affected by epitheliocystis. Chlamydia-like organisms present as blue colonies in the gill epithelia, and there is associated hyperplasia (H & E x400).

#### 7.3. Parasitic Diseases

Many of the common protozoan ectoparasitic diseases of fish are seen on juvenile farmed perch skin and gills and these include *Ichthyobodo necator* (costia), which can cause high mortalities in perch fry, *Trichodina spp.* and *Icthyophthirius multifilius* (white spot). These ectoparasites can be controlled through the use of formalin baths or flushes and/or sodium chloride, although perch fry appear more vulnerable to formalin than salmonids and the dosages used may need to be 0.3 to 0.5 times the standard salmonid dose levels.

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Plate 7.4: *Trichodina* infection on a fish gill (Dr. Thomas L. Wellborn Jnr)

*Costia* outbreaks have occured in Ireland when feed training pond juveniles in intensive conditions. Outbreaks can happen quickly and constant monitoring and, if necessary, treatment should be carried out to prevent mortalities. Reproduction of *Costia* takes place by simple transverse division. The tempo of subsequent divisions may be very rapid so that in one to two weeks a fish can be completely covered over its whole skin with specimens of this parasite which can encyst.



Plate 7.5: Electron microcope photo of fish skin covered in Costia (Dr. Thomas L. Wellborn Jnr).

Endoparasitic protozoans recorded in farmed perch include the myxosporidean Myxobolus neurophilus, which has been a particular problem for some yellow perch (*P. flavescens*) farms. This parasite invades the central nervous tissue and can cause nervous signs and high mortalities. Post mortems of affected fish may reveal small white nodules (1-2mm) on the surface of the brain and Giemsa stained impression



smears will reveal the characteristic spores (Plate 7.6). In the absence of any treatment for this parasite, high levels of biosecurity and parasite screening of any incoming livestock will reduce the risk of infection entering a farm. In wild perch there are some other myxosporidean species that have been associated with health problems and these include *Henneguya creplini* reported to cause gill pathology in Finland and *M. sandrae* associated with skeletal deformities in Scotland. Coccidia such as *Eimeria* spp. have been observed in the intestines of farmed and wild perch, however, their clinical significance remains to be determined.

Although the monogeneans *Gyrodactylus* sp. and *Dactylogyrus* sp. have been observed in farmed perch, there have been no reports of these species causing serious impacts on farmed perch. The digenean, *Diplostomum* sp., which causes severe cataracts in farmed trout, has been observed in perch eyes (vitreous humour and lens), however, it has not been reported as a clinical problem for farmed perch. Crustacean parasites such as *Argulus* sp. and *Ergasilus* sp. have also been observed on wild fish but no clinical outbreaks in farmed fish have been recorded.



Plate 7.6: Impression smear of brain nodules from *P. flavescens* showing *Myxobolus neurophilus* spores (Giemsa x 1000).

#### 7.4. Fungal Diseases

Fungus is one of the commonest diseases affecting fresh water fish. It is characterised by the growth of thin threads of a more or less dirty white or grey colour on the skin or fins. If fungus growth is abundant it may resemble tufts of cotton wool. Fungus is due to a mould, which attacks fish that have been weakened by other parasites or bad conditions. Saprolegniosis, caused by the ubiquitous fungus *Saprolegnia parasitica* is frequently encountered in farmed perch, usually following some physical challenge to the fish or recent movement of livestock and can affect all ages of fish as well as eggs. Fungal hyphae rapidly develop in gills or skin and are often seen with concomitant ectoparasitic infections such as costia or *Trichodina* sp.

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Plate 7.7: Perch egg showing signs of *saprolegnia* infection, Larvae is unaffected (Anders Asp)

A recent study in perch eggs, showed limited fungal growth by *Aphanomyces* and *Saprolegnia* spp. especially *S. diclina*, occurred within dead eggs but did not spread to adjacent live eggs. Perch eggs exposed to parasitic challenge by *Saprolegnia parasitica*, *S. dieclina* (type III) and *S. ferax*, under fluctuating temperature regimes replicating spring water temperatures, did not have significantly greater mortality than did unchallenged controls. These observations suggest that perch eggs have some antifungal properties which usually prevent the spread of fungus throughout the egg mass and that under normal temperatures there should be negligible fungal infection in perch egg masses. Repeated formalin or salt baths with frequent removal of any affected fish has proven effective in controlling this disease.

#### 7.5. Non-infectious Diseases

In perch fry production there are many non-infectious disease hurdles to successful aquaculture and these include failure to inflate the swim bladder, over inflation of the swim bladder and bacterial proliferation in the live feed which can give rise to bacterial enteritis and toxaemia. Use of water skimmers, careful control of water gas saturation levels and cleaning and high levels of hygiene in live feed production can all reduce the risk of these problems appearing, however, constant vigilance is required at the crucial early life stages. Fin erosion has been recorded in wild perch and has been associated with effluent from pulp mills, however fin erosion is also observed in farmed perch but is usually considered secondary to physical challenges which then allow in opportunistic bacterial and fungal infections

Pathogenic agent	Treatment	Concentration	Method of Administration					
	Fertillis	ed eggs						
Saprolegnia sp	Formalin	25 ppm	In recirculating system					
Parasites (Costia,	<u>}</u>							
Trichodina,)	Hydrogen Peroxide	1.000 ppm (suggested)	15 minutes bath (possibly to repeat every-other-day)					
		100 ppm active iodine (= 10m1	· · · · · · · · · · · · · · · · · · ·					
Virus and bacteria	Inorganic iodine	iodine 1% / liter of water;	15 minutes bath					
(prevention)	e	suggested)						
u /								
Larvae and Juveniles (0,5 to 1g)								
Trichodina sp.	Formalin	100 ppm	Flow through					
	Salt	5 to 7 ‰	Bath (30 min.)					
Streptococcus sp.	Oxytetracycline	7.5g / kg food	Feeding 1% biomass/ day					
		(=75mg / kg fish / day)	during 10 days					
	Oxytetracycline / salt	50 ppm / 1,5 ‰	Bath (90 min.) 3 times at 24					
Aeromonas sp.		** ´	hour intervals					
Flexibacter sp.	Chloramine T	25 ppm	Flow through					
Saprolognia sp	Formalin	100 ppm	Flow through					
Branchiomyces sp		100 ppm						
Brunchiomyces sp.	Invánilos	(1 to 40g)						
Hatayon alayia sp	Salt	(10000)	Oui alt din (2 min)					
neteropolaria sp.	San Formalin / salt	30 / 00 (-g / 1) 200 ppm / 3 %-	Quick dip (2 min.) Bath (30 min.)					
	Folilialili / Salt	200 ppm / 5 /00	Dath (30 min.)					
Twicheding	Formalin / salt	200 mm / 2 %	Dath (20 min.)					
Trichoatha sp.	Formarin / sait	200 ppm / 5 700						
	Salt	30 %	Quick dip (2 min.)					
Aeromonas sp.	Oxytetracycline / salt	50 ppm / 1,5 ‰	Bath (90 min.) 3 times at 24					
Flexibacter sp.		10 (1.5.0)	nour intervals					
	Trimetoprim – sulphadrug:	10 ppm / 1,5 ‰	As for oxytetracycline bath					
	150 mg $1  metoprim + 750  mg$	5g/kg tood	F 1: 10/1: /1					
	Sulphadiazine / g	(=50  mg / kg fish / day)	Feeding 1% biomass/ day					
			during 10 days					
	Quinolones:							
	Enrofloxacin 10%	20 ml / kg	Feeding 2.5% biomass / day					
			during 10 days					
	Chloramine T	25 ppm	Long bath (180 min) possible					
Saprolegnia sp	As for larvae		torepeut					
Supi ologinu op	Broodstoo	ck (>40g)	1					
Heteropolaria sp	Salt	8-10 %	Bath (3-5 days)					
	Salt	30 %	Quick din (2 min)					
	Suit	50 700	Quick up (2 min.)					
	Hydrogen Peroxide	100 ppm	Bath (30 min.)					
	Formalin / salt	300 ppm / 3 ‰						
Trichodina sp.	Formalin	300 ppm	Bath (30 min.)					
Gyrodactylus sp.	Organophosphates:							
_	Trichlorfon	4 ppm	Long bath (180 min.)					
	Hydrogen Peroxide	100 ppm	Bath (30 min.)					
	Chloramine T	25 ppm	Long bath (180 min.)					
			possible to repeat					
Aeromonas sp.	As for juveniles							
Saprolegnia sp	Iodine solution	Cleaning lesions with a swab						

Table 7.1: Principal Treatments used in the control of fish health issues of *Perca fluviatilis*at different life stages. (CEFRA, Belgium, amended 2007).

N.B These are suggested treatments only and will vary depending on the numbers of fish, the season, etc. Please note that some treatments are not licensed in some jurisdictions. Antibiotics have to be used after an antibiogram laboratory test. Consultation with the local fish health veterinarian should take place before embarking on any course of treatment.

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At the time of writing there are no authorised medicines or vaccines for the treatment or prevention of infectious disease in farmed perch destined for food. Under the veterinary cascade mechanism there is a very limited availability of medicines licensed for other food fish species, and this amounts to less than a handful of products. The emphasis in all farming is towards prevention and management of the health challenges rather than "silver bullet" remedies and perch farming is no exception. Careful biosecurity, egg disinfection, improved diagnostics and screening of stocks prior to movement will reduce the risks of pathogen spread and allow this aquaculture sector to evolve in a sustainable and productive manner.

### **Chapter 8 : Economics of Juvenile Production**

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Aquaculture production is the fastest growing sector in international food production amounting to approximately one third of the value of world production of fish and shellfish. A decrease in wild fishery resources with a resultant increase in prices is progressively encouraging the farming of new species and the adjustment of markets to new trends and away from historic supply channels and traditional seasonal fluctuations. New markets offering attractive prices, coupled with a reduction in production costs due to improved technology, are stimulating the farming of new species. Current trends indicate that a diverse range of aquaculture species will increasingly become available over the coming years.

In recent years high market prices facilitated and encouraged the commercial development of species such as seabass, sea bream and turbot in particular. High market expectations resulted in significant investment in research and the subsequent rapid commercialisation of these species. Research and development initiatives were undertaken by State-funded institutions or large diversified companies or a combination of both. Such initial research was very often motivated by factors other than economic objectives and indeed the benefits accrued to a wider group than the original pioneer investors. The process of commercialisation is one which is lengthy and expensive as clearly demonstrated by experience to date and a return on investment can only occur after the completion of the research phase.

Whilst the methods of production of Eurasian perch outlined in this publication may encourage a fish farmer or entrepreneur to embark upon producing the species, access to finances ultimately dictates the success or failure of any such venture. The economics of perch culture are not yet fully understood given the current low volume of production and the pilot scale nature of many of the farms involved. At present two farms in Ireland, one in Switzerland and one in France could be said to be fully commercial perch operations. The further development of the sector is currently curtailed by lack of juvenile availability. It is likely that most significant bottlenecks to juvenile perch protection will be overcome in the short term and the full viability of perch production will become apparent.

The CRAFT project PERCATECH was about assisting SME's to secure the production of perch juveniles. A key element of securing juvenile production is a sufficiently low cost base to enable the hatchery to operate economically. Juvenile production is not secure unless hatcheries are economically sustainable. Therefore the financial aspects of juvenile perch production are as important as the technical and biological aspects. An important element in securing juvenile production is the evaluation of the cost price of current hatcheries. Such information can however be commercially sensitive. What we have endeavoured to do in this chapter is to outline likely capital and operating costs in a hatchery and provide guidance aimed at

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enabling a farmer to calculate production costs. Such information is vital for future expansion and investment within the industry.

#### 8.1. Hatchery Design

The design of a farm or hatchery is crucial to the operation of any aquaculture venture. Whilst we have already discussed in other chapters, the role design plays in optimising farm husbandry, fish health, production techniques etc, farm design plays no less a role in facilitating the economic success of a venture. If properly planned and executed a hatchery can provide the optimum efficiencies in terms of production costs. Hatcheries designed inefficiently can have higher running costs associated with excessive power consumption, higher labour costs, and higher depreciation than those, which have been carefully thought through. Unfortunately it is not always easy to plan ahead and visualise what is required not only now, but in five or ten year's time. Consideration of the future production load of a facility should be made. In this regard it is always best to load up capital costs in favour of reducing operating costs. Items such as back up electrical generators, oxygen generation equipment, graders etc should be sufficient to handle up to a 50% increase in current usage. These economies of scale will ultimately be to the farms benefit.

Different system designers will have a different approach to a hatchery design. Ultimately the hatchery should be a work friendly and safe environment for employees allowing access to the appropriate equipment and tools for the job required. Energy efficiency and economies of scale will ultimately ensure the lowest possible production costs.



Plate 8.1: Perch ribbons in hatching tanks at Mahurangi Technical Institute, New Zealand. Note the raised floor gives workers easy accessibility to the tanks and tanks and fittings are constructed with durable materials (T. Kurwie)



#### 8.1.1 Energy efficiency

Any new hatchery or existing farm will be conscious of the cost of energy as an overall part of the production cost. There is little point in developing an elaborate integrated energy system without first addressing energy leaks and loss. Commonly farmers operate a 'Do It Yourself' modus operandi to electrical maintenance. Wires fused together and wrapped in insulating tape, thermostats left to seize up and water pumps operating at inefficient levels all combine to reduce the efficient use of energy. It has been estimated that these losses can add 10% to a farms electricity bill. Many aquaculture operations still use equipment and machinery that they established their business with. While this loyalty is admirable it is increasingly misplaced. Advances in technological design and energy efficiency mean that change is not only necessary for depreciation purposes but also necessary to make an operation more cost effective. High volume pumps for example now have loadings as low as 0.75Kw in comparison to the 2.2Kw pumps in standard use in the recent past. Modern control switches and thermostatically controlled apparatus means that equipment is in use for the minimum time necessary. The benefits of choosing these technologies generally more than compensates for any additional cost. The availability of European and government funding to the sector specifically aimed at the purchase of new capital items means that there has seldom been a better time to update your farms equipment.

The importance of effective design and planning can not be over stressed. Whilst many aquaculture operations have to adapt their current systems to become more energy efficient, a new entrant or the expansion of an existing site gives the designer a blank canvas. It is always tempting to select the cheapest equipment because of budgetary restrictions but this rarely works out in the long run. In general consideration should be taken of the equipment's running cost over 5 years. Regular maintenance and servicing of equipment ensures longevity and efficiency. This is particularly important in the aquaculture sector where the environment is harsh on equipment and machinery as well as fittings and fixtures.



Plate 8.2: The construction of a new Perch broodstock facility at PDS, Ireland. Three layers of insulation provides very efficient heat retention, thereby reducing energy costs.

### 8.2. Capital Costs

Agricultural economists define capital as the monetary representation of the physical inputs used in agricultural production, in addition to financial assets. As such, capital is more than liquid savings such as cash and balances in checking and savings accounts. An aquaculture farmer's capital also includes the monetary value of productive resources such as broodstock, stock of feed, machinery, ponds, buildings, on-farm roads and land. Capital therefore can be liquid and easily converted into purchasing power or very illiquid. Farming as a business requires adequate capital. Capital is necessary to create, maintain, and expand a business, increase efficiency, and to meet seasonal operating cash needs. Generally speaking, commercial farmers can get capital through savings (own equity), borrowing or through a combination of both. The most common external source of funding to provide capital for commercial ventures is grant aid and borrowing, mainly from banks.

The Institute for Marine Resources & Ecosystem Studies based at Wageningen IMARES University in the Netherlands has developed a series of excel based models for calculating investment and operating costs for both Pikeperch and Eurasian Perch. The models consist of linked data sheets which can be manipulated by farmers to give a greater degree of accuracy and visibility on their production costs. The model includes production, feeding and growth, hatchery design, investments and costs including depreciation. Data collected during the PERCATECH project from three hatcheries forms the basis for the assumptions in Tables 8.1 & 8.2. The model is available directly from Wageningen and the tables presented in this publication are intended to serve as a guide rather than an actual template for investment.

The capital cost of building a new perch hatchery or farm can be substantial. Access to an existing facility or land already in the control of the promoter can reduce significantly the investment required but does not reduce the novel and risky nature of the enterprise viewed through a financial institutions eyes. As elaborated on in the previous section, a good hatchery design coupled with a good site (access to high quality water, infrastructure etc) can minimise the overall end production cost, nevertheless the capital investment will be a substantial outlay. The European Union funds aquaculture ventures through a variety of mechanisms in member states and grants can provide valuable assistance in reducing the overall cost to the promoter. The capital costs in establishing a Eurasian perch hatchery are comparable to most other freshwater hatcheries with the added cost of a live feed facility. Table 8.1 indicates the likely capital costs for a generic perch hatchery in Europe.

Description	Amount	Unit	€/Unit	Subtotal	%	Total
Land Lease	233	m²	3,50	816	0	€814
Permits/Licences				2,500	1	
Electricity Connection				2,000	1	
Phone Connection				350	0	
Water Connection				1,000	0	
Sewer Connection				2,000	1	
Well Drilling				7,000	2	€14,850
Building	155	m²	1,000	155,000	44	
Groundwork	155	m²	20	3,100	1	€158,100
Heating	155	m²	10	1,550	0	
Lighting	155	m <sup>2</sup>	15	2,325	1	
Electricity	12	kW	10	120	0	€3,995
Broodstock Tanks	10	m³	100	1,000	0	
Incubators	2.64	m³	12,225	32,274	9	
First FeedingTanks	2.47	m³	600	1,482	0	
Fingerling Tanks	54,71	m³	600	32,826	9	
Artemia System	213	litres	20	4,260	1	€71,842
Piping	78	m²	50	3,900	1	
Drum Filter	81	m³/hr	150	12,150	3	
Pumps	81	m³/hr	25	2,025	1	CO0 575
Filter Material	10	m°	250	2,500	1	€20,575
Power aggregate	1.21	kW	400	484	0	
Measurements & Controls	78	m	25	1,950	1	
Alarm Contin Tonk				1,000	0	
Seplic Tank Ecoding Equipment	15 no	of tanks	100	4,000	1	
Weighing Equipment	15110	.01 101165	100	1,500	0	
Sorting Equipment				7,000	2	
Cooler/Freezer				7,000	2	
High Pressure Cleaner				3,000	1	
Office				5,000	1	€29,934
Out of season facilities						
Polytunnel	1		7.000	7,000	2	
Tanks	1		5,000	5.000	1	
Piping	1		600	600	0	
Pumps	1		1,500	1,500	0	
Filters	1		2,500	2,500	1	
Miscellaneous	1		1,500	1,500	0	€18,100
Subtotal						
Unforeseen	10%			31,821		€31,821
Total Initial Investment						€350,031
Relative Investment (€/Juvenile)						€0.44

Table 8.1: Indicative capital costs of establishing a perch hatchery

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The main capital costs associated with establishing a perch hatchery can be categorised as follows:

**Land:** A substantial investment if already not in the ownership of the promoter, land is nevertheless a valuable asset in that it may have other potential uses should the enterprise fail. There are alternatives available to land ownership. A promoter may lease or rent land. A site might also be utilised as part of an existing business (i.e power stations). In whatever format a site is obtained it is crucial to the overall success of the venture. A good aquaculture site must have access to sufficient quantities of clean water, to infrastructural services such as electricity, gas, phone services and be easily accessible from main transport arteries.

**Premises:** The capital cost of suitable premises for a hatchery is an important outlay. Buildings must be suitable for the general purpose required. The requirements of a live feed unit, broodstock facility and larval rearing unit can be very different and the building must reflect this. In recent years, hatcheries have been established at facilities previously used in the mainstream agricultural sector and it is worth a promoters time to search their local area for suitable premises before embarking on a build at a green field site.

**Equipment:** Modern hatcheries have numerous types and sizes of culture tanks ranging from 10 l for algal, rotifer and artemia stocks to 20,000 l for juvenile nursery systems. Water treatment facilities make up a substantial element of overall costs and a promoter must spend time assessing the suitability and efficiency of different designed systems before installation and commissioning begins.



Plate 8.3. Well constructed fish tanks are an expensive but necessary capital item

Ancillary capital requirements: Husbandry and grading equipment, back up electrical generators, oxygen generators, workshop tools etc should not be overlooked or underestimated in terms of overall costs. The most significant capital costs are likely to be building construction, water treatment equipment, tanks, live feed equipment and husbandry equipment such as graders and counters. Whilst pre-used equipment can sometimes be utilised, the resultant depreciation can often make this a false economy in particular where grant aid is available.

### 8.3. Operating Costs

The key operating costs associated with perch aquaculture are the same as with any other hatchery operation. Labour, feed, energy and maintenance make up the largest percentage of operating costs and ultimately decide the profitability or not of an operation. A significant operating cost incurred by perch hatcheries, unlike salmonid farms, is live feed production. At the height of a production cycle artemia and rotifer costs can run into hundreds of euro per day. Table 8.2 shows the generic running costs for a perch hatchery producing 800,000 juveniles per annum.

Production (#/Y) Description		800000 Amount €	€/pc	Subotal	%	Total
Feed						
	Rotifers	46	0.00	46	0	
	Artemia	4,567	0.01	4,567	1	
	Dana Start 100	660	0.00	660	0	
	Dana Start 300	3,058	0.00	3,058	2	
	Weanex 500	1,654	0.00	1,654	1	€9,985
Other Inputs						
-	electricity	12,614	0.02	12,614	9	
	oxygen	927	0.00	927	1	
	chem., med., etc.	4,000	0.01	4,000	3	€17,541
Other Company Costs						
	maintenance	7,003	0.01	7,003	5	
	insurance	1,050	0.00	1,050	1	
	general costs	18,000	0.02	18,000	13	€26,053
Labour						
	high	40.000	0.05	40.000	28	
	average	20,000	0.03	20,000	14	€60,000
Depreciation	_	5.004		5 00 /		
	5-year	5,694	0.01	5,694	4	
	10-year	2,163	0.00	2,163	2	
	20-year	4,722	0.01	4,722	3	647 746
	building	5,167	0.01	5,167	4	€17,740
Interest						
	2/3 investment	11671	0.01	11671	8	€11,671
Subtotal			0.18			
Total Initial Investment Production (Juveniles/Ye	ear)					€142,996 800,000

Table 8.2. Indicative operating costs for a perch hatchery

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The main capital costs associated with establishing a perch hatchery can be categorised as follows:

**Labour:** Modern hatcheries employ skilled staff in key positions. Areas such as live food production, broodstock management and larval rearing reqire a range of skills. Two or more people on a farm may carry out these tasks. In a large hatchery producing in excess of 800,000 juveniles per annum, the staff compliment may be 3-4 persons. Staff rotas are required to provide adequate cover 24 hours a day seven days a week. Staff employed have generally been educated in third level institutes and therefore expect good salaries. In the model presented in Table 8.2 , labour accounts for 42% of overall operating costs.

**Feed:** The provision of sufficient volumes of quality live feed and weaning diets is an expensive element of overall operating cost. Artemia and rotifer production is expensive and time consuming. Modern weaning diets can cost as much as  $\in$  1000 per kg.

**Energy:** The cost of heating and chilling water, pumping water, lighting, running oxygen generators etc all adds to a farms total energy usage. Power supply is generally electricity provided from mainline grids. Back up electrical generators are an essential component of a modern hatchery but are uneconomical to run on a full time basis. Gas may be used to heat water, as may a number of new methods such as geothermal heat pumps, solar, wind etc.

**Maintenance** is an ongoing cost on farms and is associated with the high upkeep of systems, which in many cases run constantly during a production cycle. Investment in appropriate and durable equipment can help reduce maintenance costs.





#### **8.4.** Cost of Production

In Table 8.2, the cost of production is assumed to be  $\in 0.18$  per juvenile based on the production of 800,000 juveniles per year (4 cycles of 200,000). The use of out of season spawning is essential to the profitability of a modern hatchery. A hatchery which produces juveniles once per year, produces less juveniles for the same capital investment. Key staff are unlikely to work part time and capital equipment lies unused for a large part of the year. It is calculated in the model that an additional capital investment of  $\in 18,100$  is required to develop out of season facilities. Whilst the operating costs are higher for producing fish out of season (greater heating requirement) the additional production more than compensates. Table 8.3 outlines the effect an increase in numbers of juveniles produced has on cost price.

Table 8.3: Effect of number of juveniles produced annually at a perch hatchery, on the investment, operating costs and cost price per juvenile.

Juvenile production (#/yr)	200,000	400,000	600,000	800,000	1,000,000	1,200,000
Investment (€/pc)	1.75	0.88	0.58	0.44	0.35	0.29
Total costs (€/yr)	120,000	128 ,0000	135,000	143,000	151,000	158,000
Cost price (€/yr)	0.60	0.32	0.23	0.18	0.15	0.13

Costs of production of juveniles of other species can be a useful reference. Table 8.4 provides a breakdown of costs for seabass, pikeperch and perch. Seabass hatchery technology can be considered as relatively well established and therefore largely optimised. Pikeperch juvenile production on the other hand is relatively new as is perch juvenile production and therefore likely to be not fully optimised yet. Objective comparison between these three hatchery productions may not be possible due to differences in production scale and socio-economic conditions such as labour costs. However, such comparison can still provide useful in the current status of perch hatcheries and the potential room for improvements. From Table 8.4 it appears that the production costs of seabass juveniles and perch juveniles are not very different despite the fact that seabass hatchery technology is fully established and perch hatchery technology is not. Both hatchery technologies are comparable in terms of live food requirement, which is reflected by the similar costs for this item. The main difference is the total costs for labour. It is however not known whether this difference results from differences in labour demand per juvenile or differences in costs of labour. Based on the comparison between costs of production for perch and seabass it seems that the projected cost price for perch juveniles is not unrealistically low at  $\in 0.18$  and can be expected to decrease with further optimisation of hatchery technology.

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Cost item	Seabass	Pikeperch	Perch
Interest + Depreciation	0.03	0.15	0.03
Feed	0.06	0.04	0.02
Labour	0.03	0.11	0.08
Other inputs & Costs	0.03	0.10	0.05
Total	0.15	0.40	0.18

Table 8.4: Comparison of the breakdown of costs (€/pc) between 2g seabass juveniles (France), pikeperch (Netherlands) and perch (Ireland

The small harvest size of perch for the Swiss market may be of benefit in terms of production scheduling but it brings added financial pressure to both the ongrower and hatchery. If we compare another niche aquaculture species such as Arctic Charr we can see clearly that the small harvest size places additional demands on the grower in terms of volume of juveniles required and most importantly price (Table 8.5).

Table 8.5: Comparative juvenile costs between perch and charr in a 100 tonne recirculation

Samples	Unit Size (t/annum)	Harvest size of fish	Number of Juvenules required per annum	Cost per Juvenile (current)	Total cost of Juveniles	Juvenile cost expressed as % of final sale price
Artic Charr	100 tonne	1.0 Kg	100,000	€0.40	€40,000	5.7%
European Perch	100 tonne	100g	1000,000	€0.15	150,000	21%

Whilst the juvenile costs are significantly higher for perch, there are a number of caveats. Firstly it assumes the harvest size to be 100g. This is the common size of perch sold into the Swiss market but there are markets for larger size fish in Germany, Italy, Sweden and other European countries. The key cost apart from harvest size is undoubtedly juvenile costs, associated with live feeding. One advantage perch does have is the relatively large number of eggs and larvae that can be produced. The key for the hatchery therefore is to reduce costs and increase production so that the cost per juvenile is reduced. Producing juveniles for  $\in 0.10$  each or less reduces the juvenile cost as a % of final sale price to 14%. Selling harvest ready fish at 200g reduces the juvenile cost to 7%. Perch farmers and hatcheries will have to work together to enable this cost model to become more efficient and profitable and therein lies the challenge over the short to medium term. If this can be accomplished, the future for perch farming in Europe can be a bright one.
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Annex to chapter 6 : Techniques to assess the degree of ploidy

#### Caryology

Caryology relies on the count of chromosomes during the metaphase stage on microscopic mounts, is the most frequently utilised technique. Larvae were soaked in a colchicine solution (anti-mitotic agent), then into an hypotonic solution, then tissues were dissociated, mounted on a microscopic glass mount, stained with Giemsa, and observed with an optical microscope.

#### **NORs** analysis

Analysis of the nucleole organising regions (NORs), where are formed the rRNA proteinic complexes, and they can be stained with silver. The number of NOR is proportional to the degree of ploidy (i.e. 1, 2 and 3 in haploid, diploid and triploid cells, respectively. After sacrification, larvae are preserved in an acetic acid – methanol solution. Upon analysis, they are hydrated again in an acetic acid solution, homogenised with a vortex, and the resulting cellular suspension is poured on a microscopic glass mount and stained with silver nitrate (Howell and Black, 1980). At least 80 cell nuclei should be examined under the optical microscope to provide a reliable estimate of the degree of ploidy.

#### Flow cytometry analysis

Flow cytometry analysis measures the amount of DNA in a cell from its fluorescence. Live larvae are placed individually in Eppendorf® tubes where are added sequentially: trypsine (for digesting tissues), an inhibitor of trypsine, RNAse (for removing RNA, which also is fluorescent), and an organic dye, propidium iodide, which binds to DNA and forms a highly fluorescent compound. Samples are analysed with a flow cytometer, and the degree of ploidy is obtained from fluorescence spectra. The ploidy of embryos (6 days post-fertilization) was assessed by flow cytometry, which measures the fluorescence of a specific fluorophore bound to target molecules within the cell, in this case nuclear DNA. Embryos were first digested in trypsine citric buffer solution. After 10 minutes of incubation, a trypsin inhibitor, a nucleus stabilisator (spermidine) and RNAse were added to the first solution. After 10 minutes of incubation, the resulting nuclar suspension was stained with propidium iodide (Cycle Test®, Becton Dickinson). Analysis was performed with a Becton Dickinson FACSTAR PLUS®. Unshocked diploid embryos were used as a diploid standard for the calibration of the cytometer, with a relative fluorescence set at "200" value, which allows clear separation between triploid and diploid nuclei. A minimum of 20 embryos per batch were analysed except when the number of survivors was lower (in 10 batches the number of embryos analysed was 11 to 18).

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