



**Securing juvenile production of Eurasian perch by
improving reproduction and larval rearing**

Project Acronym: Percatech

Proposal number: 512629

Instrument
Thematic priority

Periodic Activity Report

Period covered: from 1 October 2004 to 30 September 2005

Date of preparation: November 2005

Start date of project: October 2004

Duration: 2 years

Project coordinator: Pascal FONTAINE

Project coordinator organisation name

Université Henri Poincaré - Nancy 1

Laboratoire de Sciences Animales

MAN, 34 rue Sainte Catherine

F - 54000 Nancy

E-Mail: pascal.fontaine@lsa-man.uhp-nancy.fr

CONTENTS

1 - EXECUTIVE SUMMARY	6
A - Description of project objectives	7
B - Work performed and main results obtained during the reporting period.....	7
• B1 – Co-ordination task (WP1).....	7
• B2 – Broodstock management	7
B2.1 – Extension of the natural reproduction period (WP2).....	7
B2.2 - Production of out-of-season spawning with completed control of the reproductive cycle (WP3).....	8
• B3 – Gamete quality and breeders welfare	8
B3.1 – Control of the female and male gamete quality (WP4)	8
B3.2 – Breeders mortality and welfare (WP5).....	9
• B4 – Production of juvenile with genetically improved performances (WP6).....	9
• B5 – Socio-economic study of the juvenile cost production (WP7)	10
• B6 – Transfer of technology (WP8).....	10
2.1 - PROJECT OBJECTIVES AND MAJOR ACHIEVEMENTS.....	11
A - Description of project objectives	11
B - Progress towards objectives during the reporting period	13
• B1 – Co-ordination task (WP1).....	13
• B2 – Broodstock management	13
B2.1 – Extension of the natural reproduction period (WP2).....	13
B2.2 - Production of out-of-season spawning with completed control of the reproductive cycle (WP3).....	14
• B3 – Gamete quality and breeders welfare	14
B3.1 – Control of the female and male gamete quality (WP4)	14
B3.2 – Breeders mortality and welfare (WP5).....	15
• B4 – Production of juvenile with genetically improved performances (WP6)....	15
• B5 – Socio-economic study of the juvenile cost production (WP7)	16
• B6 – Transfer of technology (WP8).....	16
2.2 – WORPACKAGE PROGRESS OF THE PERIOD	17
A - Task 1: Co-ordination action (WP1)	17
• Workpackage objectives and starting point of work	17
• Progress towards objectives	17
• Deviations from the project workprogramme	18
B - Task 2: Broodstock management	18
B.1 - Sub-task 2.1: Extension of the natural reproduction period - early or delayed spawning (WP2).....	18
• Workpackage objectives and starting point of work	18
• Progress towards objectives	18
Materials and methods	19
Broodstock holding facilities	19
Reproduction facilities	19
Measurements.....	19
Results and Discussion.....	19
Broodstock holding facilities	19
Catching breeders	19
Mortality.....	19
Feeding the broodstock	20

Treatment of fungi.....	21
Reproduction	21
Spawning results	21
Conclusions	24
References	24
• Deviations from the project workprogramme	24
B.2 - Sub-task 2.2: Production of out-of-season spawning with completed control of the reproductive cycle (WP3).....	25
• Workpackage objectives and starting point of work	25
• Progress toward objectives	25
Materials and Methods	26
Animals	26
Experimental design	26
Experimental period	26
Results and Discussion.....	27
References	30
• Deviations from the project workprogramme	31
C - Task 3: Gamete quality and breeders welfare	31
C.1 - Sub-task 3.1: Control of the female and male gamete quality (WP4).....	31
• Workpackage objectives and starting point of work	31
• Progress towards objectives	32
a) Determination of the sperm quality and quantity from males cultured in both artificial and natural conditions	32
Materials and methods	32
Quantitative assessment of sperm	32
Qualitative assessment of sperm	32
Results and discussion.....	33
Quantitative and qualitative assessment of sperm during November	33
Quantitative and qualitative assessment of sperm during reproduction season.....	33
Development of a hatching test for experimental condition for WP 3 and 4... ..	34
Development of a qualitative assessment of sperm for WP3 and 4	34
b) Mass semi-artificial propagation of hormonally and non hormonally stimulated perch spawners	34
Materials and methods	34
Artificial and semi-artificial spawning under experimental conditions	34
Evaluation of fecundity in perch females.....	35
Time effort of artificial and semi-artificial spawning	36
Efficiency of egg incubation	36
Economical analysis of the egg production during the experiment	36
Survival rate of broodstock during and after spawning period	36
Semi-artificial spawning with hormonal treatment and natural spawning without hormonal treatment under working conditions	36
Results and discussion.....	37
Artificial and semi-artificial spawning under experimental conditions	37
Evaluation of fecundity in perch females.....	37
Time effort of artificial and semi-artificial spawning	37
Efficiency of egg incubation	37
Economical analysis of the egg production during this experiment	38
Survival rate of broodstock during and after spawning period	38
c) Determination of the freezing conditions to preserve the perch	45

Materials and methods	45
Animals	45
Sperm Collection and Dilution.....	45
Cryopreservation and thawing	45
Experimental Design	46
Hatching Trial	46
Observation and measurement of sperm motility and velocity.....	46
Data analysis	46
Results and discussion.....	47
References	49
• Deviations from the project workprogramme	49
C.2 - Sub-task 3.2: Breeders mortality and welfare (WP5)	50
• Workpackage objectives and starting point of the work	50
• Progress toward objectives.....	50
Materials and Methods	50
Experimental protocol	50
Methods.....	51
Results and discussion.....	51
Body condition and metabolic indicators.....	51
Erythrocyte and lymphocyte numbers.....	53
Respiratory burst and lysozyme activities.....	54
Transaminase activities	55
Stress indicators.....	55
Conclusions	56
References	56
• Deviations from the project workprogramme	57
D - Task 4: Production of juvenile with genetically improved performances (WP6).....	57
D.1 - Sub-task 6.1: Effect of domestication	57
• Workpackage objectives and starting point	57
• Progress towards objectives	57
Material and methods	57
Breeders and quality of eggs	57
Larval resistance and rearing.....	57
Juveniles rearing.....	58
Results and discussion.....	58
Breeders and quality of eggs	58
Larval resistance and rearing.....	59
Juveniles rearing.....	61
Conclusions	63
• Deviations from the project workprogramme	64
D.2 - Sub-task 6.2: Effect of sex-control	64
• Workpackage objectives and starting point	64
• Progress towards objectives and starting point of work.....	64
Material and methods	64
Results and discussion.....	64
Conclusions	65
• Deviations from the project workprogramme	65
D.3 - Sub-task 6.3: Effect of hybridization	65
• Workpackage objectives and starting point of work	65

• Deviations from the project workprogramme	65
D.4 - Sub-task 6.4: Effect of triploidisation	65
• Workpackage objectives and starting point of work	65
• Deviations from the project workprogramme	66
D.5 - Sub-task 6.5: Functional XX-male.....	66
• Workpackage objectives and starting point of work	66
• Progress towards objectives	66
Material and methods	66
Results and discussion.....	66
• Deviations from the project workprogramme	66
D.6 - Sub-task 6.6: Natural spawning of XX male	66
• Workpackage objectives and starting point of work	66
• Progress towards objectives	66
• Deviations from the project workprogrammes.....	67
E - Task 5: Socio-economic study of the juvenile cost production (WP7)	67
• WP objectives and starting point of work	67
• Progress towards objectives	67
Materials and methods	67
a) Autumn adaptation of perch fingerlings from pond into recirculation (controlled) conditions and their following rearing.	67
b) Rearing of advanced fry under pond conditions.	68
c) Pond culturing of juveniles during the whole growing season.	68
Results and discussion.....	70
a) Autumn adaptation of juvenile from pond into recirculation (controlled) conditions and their following rearing.	70
Survival of fingerlings.....	70
Growth of fingerlings	70
b) Rearing of advanced fry under pond conditions.	71
c) Pond culturing of juveniles during the whole growing season	71
Early diet of perch larvae reared in nursing ponds with different zooplankton quality.....	71
Rearing of 0+ perch in ponds under different feeding conditions.....	71
• Deviations from the project workprogramme	80
F - Task 6: Transfer of technology (WP8)	81
• Workpackage objectives and starting point of work	81
• Progress towards objectives	81
• Deviations from the workprogramme	81
G - List of deliverables and milestones	82
2.3 - CONSORTIUM MANAGEMENT	83
2.4 – OTHER ISSUES	86
A - Description of the overall contributions of SMEs, RTDs, OTHs	86
B - Extend to which work of the RTDs has benefited to the SMEs.....	86
C - Balance of work/resources between RTDs & SMEs/OTHS	86
D - Ethical issues.....	86
E - Gender issues.....	87
3. ANNEX - PLAN FOR USING AND DISSEMINATING THE KNOWLEDGE.....	88
A - Exploitable knowledge and its Use	88
B - Dissemination of knowledge.....	88
C - Publishable Results	88

1 - EXECUTIVE SUMMARY

COOP-CT-2004-512629-PERCATECH

Securing juvenile production of Eurasian perch by improving reproduction and larval rearing
--

Type of contract: Co-operative Research Project

Call identifier: FP6-2002-SME-1

Total Cost: 1 782 000 Euros

EU contribution: 764 700 Euros

Duration: 2 years

Starting date: 01 / 10 / 2004

Coordinator: **Pascal FONTAINE**, Université Henri Poincaré - Nancy 1, Laboratoire de Sciences Animales, MAN, 34 rue Sainte Catherine, F – 54000 Nancy

Phone: 00 33 3 83 30 84 47, Fax: 00 33 3 83 32 36 13

E-Mail: pascal.fontaine@lsa-man.uhp-nancy.fr

SMEs Partners:

Partner 1: Lucas Perches SARL, Moulin du Cany, 57170 Hampont, France (*Key persons: David Vandeworde and Bertrand Ritter*)

Partner 2: Bornholms Hatchery Lakseklaekkeri, Øster Flak 2, Nexø, Denmark (*Key persons: 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 persons: Hein Dil and Tim Eriks*)

Partner 5: Rybářství Nové Hrady S.R.O., Štiptoň 78, 373 33 Nové Hrady, Czech Republic (*Key person: Zvonar Lubomir*)

Partner 6: PDS Irish Waters Perch Ltd, Main St, Arvagh, Co. Cavan, Ireland (*Key persons: Philips Simpson and Damien Torner*)

RTD Partners:

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

Partner 8: Facultés Universitaires N.D. de la Paix de Namur, Rue de Bruxelles 61, 5000 Namur, Belgium (*Key persons: Patrick Kestemont, Robert Mandiki and Emilie Henrotte*)

Partner 9: Centre de Formation et de Recherches en Aquaculture, University of Liège, Chemin de la Justice, 4500 Tihange / Huy, Belgium (*Key persons : Charles Mélard and Carole Rougeot*)

Partner 10: The Netherlands Institute for Fisheries Research, Haringkade 1, 1970 AB Ijmuiden, The Netherlands (*Key person: Heen van der Mheen*)

Partner 11: Research Institute of Fish Culture and Hydrobiology, University of South Bohemia, Zátíší 728/II, 389 25 Vodňany, Czech Republic (*Key persons: Otomar Linhart, Tomas Policar and Marek Rodina*)

A - Description of project objectives

The main objective of this project is to secure the production of Eurasian perch (*Perca fluviatilis*) juveniles (3-5 g) in order to sustain the development of European SMEs which have funded in this new way of diversification (production of 10-15 g fillets for consumption markets corresponding to 100-150 g fish). Presently, the juveniles availability is indeed very low and limited to the annual cycle of 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, this R&D project has proposed to help the setting of a hatchery - nursery activity that other SMEs wish to develop.

The research programme is divided into six tasks and eight workpackages or WP. A first part of this R&D project has focused on the optimisation of the broodstock management considering environmental, nutritional and population variables (i) to obtain delayed (extension of the natural reproductive period, **WP2**), and out-of-season spawning (**WP3**), (ii) to improve the control of gametes and larval quantity (**WP4**), and (iii) to reduce breeders mortality during the spawning period (**WP5**). A specific action has been conducted on the cryopreservation of Eurasian perch semen for artificial reproduction (**WP4**). A second part has focused on the development of protocols for the production of populations of juveniles with improved potentialities (females monosex, *Perca fluviatilis* x *Perca flavescens* hybrids, triploids: **WP6**). Finally, an economical study has been realised to define the cost of production of juveniles using different rearing systems (semi-intensive in ponds and intensive in tanks) and considering different socio-economic contexts: **WP7**. The **WP1** and **WP8** correspond to the co-ordination action and the transfer of technology respectively, both involving all partners and supervised by partner 7.

B - Work performed and main results obtained during the reporting period

- *B1 – Co-ordination task (WP1)*

A first meeting occurred in Nancy, France (21-22th October 2004) and a second one in Vodnany, Czech Republic (17-18th August, 2005). The first one mainly focused on the presentation of each partner, the achievement of the consortium agreement, the constitution of the board management (one member from each partner) and the planning of both the collaborations and the work. The second mainly focused on the analysis of the first results obtained during the year.

- *B2 – Broodstock management*

B2.1 – Extension of the natural reproduction period (WP2)

During the first year, research has been focusing on the effect of extending the reproduction period of 'wild' breeders on spawning. In the period of December 2004-April 2005, mature perch were caught and stored in tanks (2 m³, 6°C, LD 15:9) set up in a cold room. Catching of wild breeders proved to be more difficult than expected. Indeed, during cold periods of the year, perch stayed in deep water, from where it was impossible to catch the fish and keep them alive. Thus the first batch of wild breeders became available at 31-12-04. These fish were caught in the river Merwede (The Netherlands). This stock contained 44 males (476 g) and 52 females (626 g). However all breeders died within one month after their arrival. Thus, no reproduction trial could have been conducted with these fish. Any obvious reason for this mortality could be observed. A second batch of breeders was caught on the 15th of March. This batch contained 10 males (85 g) and 13 females (108 g). A third batch of

breeder arrived on April, 1st 2005, and a fourth batch arrived on April 8th. Among these three batches of breeders 28% of the males and 49% of the females died during their stay in the broodstock holding facilities. We conducted six spawning experiments on 15/3/2005, 4/4/2005, 22/4/2005, 3/5/2005, 13/6/2005 and 5/7/2005. Hatching was, according to literature information, expected 14-21 days after incubation (15 ± 0.5 °C), yet in our experiment hatching started 7 days after incubation (17.5 ± 0.5 °C). The results showed that (i) early catching of wild breeders and their storage in tanks and (ii) holding wild breeders under winter conditions for prolonged time have both a negative effect. This results in high mortality of breeders and disease problems, such as fungi and chronic bacterial problems. This study has also shown that it is better to let the wild breeders spawn “naturally” in the tank after the spawning induction period and HCG injections than to practice a stripping. Higher fecundity and fertilization rates were observed at the beginning of the spawning period (March).

B2.2 - Production of out-of-season spawning with completed control of the reproductive cycle (WP3)

A fractional factorial experiment was used to evaluate the effects of 8 environmental (chilling temperature, photophase increase, dawn simulation, handling, light intensity) or nutritional factors (initial nutritional state, food type, feeding rate) on the quality of reproduction. In October 2004, a broodstock, i.e. 1500 fish provided by Lucas Perches (Partner 1) was acclimatized and reared in our structures until it reached sexual maturity. In May, the broodstock was equally divided in two batches. One batch was fed to optimal feeding rate whereas the other one was fed at maintenance rate until March 2005 (Kestemont and Mélard, 2000), in order to obtain breeders with two different initial nutritional states. The experiment began on the 15th of March 2005, spawning was expected in October 2005. Firstly, 12 fish were distributed randomly into each of the 16 tanks distributed in two thermo-regulated rooms for control of the temperature (two different thermal programs were applied). A common program of induction of the reproductive cycle has been applied for nine weeks (Wang *et al.*, submitted). Temperature was decreased from 23°C to 14°C whereas photoperiod was abruptly decreased from 16L:8D to 8L:16D fifteen days before. At the end of this period, a blood sampling was done on all fish for 11 keto-testosterone assay (a typically male hormone) to differentiate males from females. Fish were redistributed to balance the sex-ratio (4 males and 8 females per tank). Then, the different combinations of factors according to a two-levels fractional factorial design given by Planor Software were randomly applied and experiment started (Kobilinsky, 2000). At the end of the chilling phase (20th September 2005), the average mortality was 12% and a blood sampling was realized on all fish to determine (i) plasma cortisol, a stress indicator, (ii) plasma sexual steroids (estradiol, testosterone and 11-keto-testosterone) and (iii) serum ability to lyse *Echerichia coli*, an immune indicator.

- *B3 – Gamete quality and breeders welfare*

B3.1 – Control of the female and male gamete quality (WP4)

The first part of this WP focused on the determination of the sperm quality and quantity from males cultured in both artificial and natural conditions. Quantitative and qualitative assessments of sperm during November and the reproduction season in April were compared. In November, males showed higher concentration of spermatozoa ($50-100 \cdot 10^9$ Spz per mL vs $20-80 \cdot 10^9$ Spz per mL), lower volume of sperm (0.4-0.6 mL vs 0.4-4.5 mL), lower motility level (20-50% vs 80-100%) and lower speed of spermatozoa than in April. The determination of the freezing conditions to preserve the perch sperm has been performed.

Cryopreservation of sperm appears to be a useful and reliable technique not only for conservation of sperm for experimental condition but also for wide-ranging artificial propagation

Another part focused on the optimization of mass semi-artificial or artificial propagation of hormonally and not hormonally stimulated perch spawners. The type of propagation used did not influence the spawning rate (> 80%). Significantly higher number of eggs per 1 ml (454.5 ± 110.5 eggs) and 1 g (598.5 ± 130.2 eggs) was observed in artificial spawned females. Eggs after artificial spawning were less swollen than eggs after semi-artificial spawning (253 ± 80.2 eggs per 1ml and 318.5 ± 132.0 eggs per 1 g). The time from injection to spawning was found statistically longer in case of semi-artificial spawning of females. The best synchronization of spawning was performed during semi-artificial spawning under 15°C. The highest hatching success (62.9 %) was found after semi-artificial spawning, which was performed under 15 °C, the lowest hatching success (26.1 %) being recorded after artificial spawning under 10 °C. During the spawning period it was found a high survival rate of broodstock, whereas low survival rates were found in females from both artificial (32.5%) and semi-artificial spawning (31.9 %) 7 days after the spawning period. Methanol at concentrations of 10 % with 300 mM of glucose yielded the highest velocity in frozen-thawed sperm ($149 \mu\text{m}\cdot\text{s}^{-1}$).

B3.2 – Breeders mortality and welfare (WP5)

The objective of this WP was to answer the question whether high mortality encountered in female Eurasian perch during the reproduction period may be considered as a trade-off between reproduction and immune status and/or as an exhaustion related to winter environmental conditions. Three groups of captive breeders were compared three times during gonad maturation and/or spawning period (January, February and March): captive breeders submitted to constant conditions (no gonad development), captive breeders reared in outdoor tanks under natural nycthemeral variations and forage feeding to ensure an efficient reproductive maturation and, captive breeders submitted to controlled out-season conditions to advance the spawning period (early February instead of mid-April). In addition, wild breeders from the Meuse River were captured before (late March), during (mid-April) and after (late April) spawning for comparison to captive breeders. Overall, the results did not show an important trade-off between reproduction and immune system because different immune activities were unaffected during gonad maturation and spawning in captive breeders. However, a reduction of both lysozyme and respiratory burst activities and a smaller spleen size were found in wild breeders at a similar temperature compared to captive breeders in March. An increase in cortisol release related to environmental conditions but not to reproductive processes may be involved in a such down-regulation even if other measured indicators for stress did not change.

- *B4 – Production of juvenile with genetically improved performances (WP6)*

The main goals of this WP were to compare the growth performance of 3 generations (F1, F2, F3) of captive juveniles and to produce a domesticated strain with improved growth and survival performances. Spawns quality, in term of both fertilization and hatching rates, was significantly increased with the domestication: spawns originated from F3 breeders displaying higher fertilization and hatching rates than spawns originated from F1 and F2 breeders. Nevertheless, the larval quality of the domesticated F4 generation seems to be lower than the wild generation as the malformation rate at hatching and the resistance to the osmotic stress were higher and lower respectively. The growth performances of the F1 larvae were significantly higher than the domesticated strain. Nevertheless, this difference seems to be

related to the difference between the initial stocking density of the batches. After the larval rearing period, the best growth performances in term of final mean body weight, specific growth rate and food conversion ratio, were obtained with the domesticated F4 generation.

The increase of growth performances (30% after 360 days of rearing) and the improvement of food conversion ratio with all-females families will allow obtaining a significant improvement of the productivity of the perch reared under intensive conditions. This result was in concordance with the milestone and the expected result of the sub-task.

- *B5 – Socio-economic study of the juvenile cost production (WP7)*

A model was constructed that is able to calculate production costs for perch fingerlings. The model is now in the process of being adapted to the various production methods used by the project partners. The model is a flexible model: technical data, production data, and costs are variables. To be able to calculate the cost of fingerling production under different management regimes, information is being gathered to feed the model. This data is generated and collected in several field trials. In the mean time data was collected for costs price analysis by intensive or semi-intensive rearing of perch fry under natural feeding pond conditions (partner 5, 6 and 11). During the first year, experiments have focused on the autumn adaptation of perch fingerlings from pond into recirculation (controlled) conditions and their following rearing: rearing of advanced fry under pond conditions, and pond culturing of juveniles during the whole growing season considering the socio-economic environment in Czech Republic (partner 5) and Ireland (partner 6).

- *B6 – Transfer of technology (WP8)*

An intense cooperation between all partners has permitted important transfers of technology linked to existing know-how (from RTD performers to SMEs or between SMEs). A specific website (www.percatech.org) presenting the PERCATECH project has been developed to inform all publics interested by the Eurasian perch culture and to promote the main results obtained in this project. In September 2005, a contact has been officially realized with the General Secretary (Mr C. Hough) to organize a specific workshop on Percids culture during the year 2007. Such an event will ensure the dissemination of the results to the European fish farmer or others interested by Eurasian perch culture.

2.1 - PROJECT OBJECTIVES AND MAJOR ACHIEVEMENTS

A - Description of project objectives

The diversification of activities, productions and products is a major stake to ensure a sustainable development of the European aquaculture. These actions must notably satisfy very strict local demands (indigenous species, consumed forms...). This is already the case for the culture of freshwater species such as roach (*Rutilus rutilus*) in the valley of the river Moselle in Luxembourg, tench (*Tinca tinca*) in Estremadure in Spain, or European catfish (*Silurus glanis*) in the south of Germany. In this way, the breeding of common perch (*Perca fluviatilis*) has emerged and been developed to supply the market of the alpine countries (France, Italy, Switzerland...).

Presently, it appears that an important traditional market exists in these countries with a demand extensively superior to the offer (Ashe, 1997). This market concerns the merchandising of small fillets (10 -15 g), with or without skin. Among these countries, Switzerland is the most important consumer with about 4000 tons of perch fillets per year, of which 90 % are imported (Fontaine *et al.*, 1998). Besides, an investigation demonstrated that this fish was consumed under different forms in several other European regions (Tamazouzt *et al.*, 1993), what brings an additional advantage for this species. Moreover, some additional new markets are likely to emerge in the countries of the Baltic sea and in the west of Europe (France, Belgium). Since these markets are supplied by professional fishing in the alpine, Scandinavian and Baltic lakes and in Eastern Europe as well, the production levels largely depend on the climatic conditions, the productivity and the quality of the fished areas. Therefore, the offer in perch fillets greatly varies in quantity and quality from year to year.

This context has led various partners of the aquaculture industry (wholesale fish merchant, professional fishers, fish farmers, researchers...) to be interested in this species. Thus, research have been performed for these last years on the adaptation of the breeding techniques to the culture of this species, notably in the perspective of regional, national and European research programs (FAIR CT96-1572, FAIR CT98-9241, Σ! 2321 ACRAPEP). These research allowed mainly to characterize the reproductive cycle of *P. fluviatilis* and its environmental determinism, adapt the techniques of larval rearing (feeding protocol, breeding conditions...), determine the nutritional needs (qualitative and quantitative aspects) and define growth performances.

In this context, private investors progressively elaborated projects of periculture. Currently, several growing farms producing common perch have been built in Europe and managed as pilot farms: Flamicell Verd. in Spain, Lucas-Perches and Esox in France, Perch Ltd in Ireland and Bornholms Hatchery in Denmark. The localization of these projects clearly shows the European future of this “young industry”. Besides, the present project has grouped together the main European research centres that have been studying and specializing in the biology and the aquaculture of this species for more than ten years.

The perpetuation and the development of the activities of these farms, that aim to specialize in growing and transformation of fish (filleting), highly depend on the supplying of juvenile of good quality. Currently, the offer in juvenile is virtually inexistent, what leads these farms to solicit the traditional hatcheries of extensive pond farms that are only able to provide eggs or larvae, but not weaned fingerlings. This leads to various problems that must be solved:

- (i) a dependence of the natural reproductive period (mainly in April) what reduces the availability of eggs or larvae and is incompatible with the requirements of a market that must be supplied all the year round and presents a stronger demand in summer,

- (ii) an offer (eggs or larvae) that does not fit with the demand of the growing farms (juvenile of 3-5 g),
- (iii) an absence of selection or improvement of the offer: this part is yet crucial to increase growth performances and reduce growth heterogeneity.

Consequently, the main objective of this project is to increase the availability of juveniles (3-5 g) for growing farms both in quantity and the all year round, and improve the quality of offer, thus permitting a better competitiveness of the growing farm. This objective is attested by the presence, in the syndicate of SME (Partners 1-6), of firms (Esox in France, Petruv Zdar in Republic Czech, Perch Ltd in Ireland) specialized in hatching and larval rearing for pond fishes, distributed in 5 different countries of the European Union (or under adherence). This project also integrates five European laboratories, RTD (partners 7-11), that have been working on the biology and the breeding of the common perch for about ten years.

The research programme is divided into six tasks and eight workpackages or WP, which are:

- assessment of the possibilities (shortening or delaying) of extension of the natural reproductive period by the management of the environmental conditions (**WP2**),
- optimization of a reliable protocol permitting the induction and the control of gametogenesis in Eurasian perch and the production of out-of-season eggs and larvae with increased fertilization rates and viability respectively (**WP3**),
- comparison of the potentialities offered (advantages, inconveniences) by the two strategies of broodstock management evoked above (extension of the natural reproductive period, out-of-season reproductive cycle), (**WP2 and WP3**)
- identification and the classification of the main factors influencing the quality of the reproduction of Eurasian perch, while considering the usual indicators of usual reproduction (relative spawning rate and fecundity, fertilization, hatching rates...), gametes quality and the broodstock welfare as well (**WP4**),
- development of a protocol of cryogenic semen preservation (**WP4**),
- understanding of the biologic mechanisms that leads to the high mortality rates of the broodstock during the reproductive period (**WP5**),
- definition of optimal conditions of broodstock management (especially feeding) allowing satisfying reproductive performances and diminished mortality rates of the broodstock during the reproductive period (**WP5**),
- production of domesticated strain with improved growth performance and a comparison of the growth performance of 3 generations (F1, F2 and F3) of captive juvenile Eurasian perch reared under intensive conditions (**WP6**),
- production of a stock of sex-reversed male completely functional and the evaluation of the productivity gain when rearing all-female populations obtained with hormonally sex-reversed male breeders (**WP6**),
- production of hybrids of Eurasian perch (*P. fluviatilis*) and yellow perch (*P. flavescens*) in order to compare growth performance of hybrid vs. pure species under intensive rearing conditions (**WP6**),
- production of a triploid population in large scale and to evaluate the productivity gain when rearing diploid and triploid populations (**WP6**), and
- evaluation of the cost of production of a juvenile of perch according to the different production strategies and the different socioeconomic contexts (**WP7**).

WP1 and **WP8** correspond to the co-ordination action and the transfer of technology respectively, both involving all partners and supervised by partner 7.

B - Progress towards objectives during the reporting period

- *B1 – Co-ordination task (WP1)*

Responsible: LSA - UHP (Partner 7)

Associated partners

- SMEs: all partners

- RTDs: all partners

The first meeting occurred in Nancy, France (21-22th October 2004) and the second one in Vodnany, Czech Republic (17-18th August, 2005). The first one mainly focused on the presentation of each partner, the achievement of the consortium agreement, the constitution of the board management (one member from each partner) and the planning of both the collaborations and the work. The second mainly focused on the analysis of the first results obtained during the year.

- *B2 – Broodstock management*

B2.1 – Extension of the natural reproduction period (WP2)

Responsible: RIVO (Partner 10)

Associated partners:

- SME: Dil-Vis (Partner 4)

- RTD: FUND (Partner 8)

During the first year, research has been focusing on the effect of extending the reproduction period of ‘wild’ breeders on spawning. In the period of December 2004-April 2005, mature perch were caught and stored in tanks (2 m³, 6°C, LD 15:9) set up in a cold room. Catching of wild breeders proved to be more difficult than expected. Indeed, during cold periods of the year, perch stayed in deep water, from where it was impossible to catch the fish and keep them alive. Thus the first batch of wild breeders became available at 31-12-04. These fish were caught in the river Merwede (The Netherlands). This stock contained 44 males (476 g) and 52 females (626 g). However all breeders died within one month after their arrival. Thus, no reproduction trial could have been conducted with these fish. Any obvious reason for this mortality could be observed. A second batch of breeders was caught on the 15th of March. This batch contained 10 males (85 g) and 13 females (108 g). A third batch of breeder arrived on April, 1st 2005, and a fourth batch arrived on April 8th. Among these three batches of breeders 28% of the males and 49% of the females died during their stay in the broodstock holding facilities. We conducted six spawning experiments on 15/3/2005, 4/4/2005, 22/4/2005, 3/5/2005, 13/6/2005 and 5/7/2005. Hatching was, according to literature information, expected 14-21 days after incubation (15 ± 0.5 °C), yet in our experiment hatching started 7 days after incubation (17.5 ± 0.5 °C). The results showed that (i) early catching of wild breeders and their storage in tanks and (ii) holding wild breeders under winter conditions for prolonged time have both a negative effect. This results in high mortality of breeders and disease problems, such as fungi and chronic bacterial problems. This study has also shown that it is better to let the wild breeders spawn “naturally” in the tank after the spawning induction period and HCG injections than to practice a stripping. Higher fecundity and fertilization rates were observed at the beginning of the spawning period (March).

B2.2 - Production of out-of-season spawning with completed control of the reproductive cycle (WP3)

Responsible: LSA-UHP (Partner 7)

Associate partners:

- SMEs: Lucas Perches (Partner 1)
- RTD : FUNDP (Partner 8), USB (Partner 11)

A fractional factorial experiment was used to evaluate the effects of 8 environmental (chilling temperature, photophase increase, dawn simulation, handling, light intensity) or nutritional factors (initial nutritional state, food type, feeding rate) on the quality of reproduction. In October 2004, a broodstock, i.e. 1500 fish provided by Lucas Perches (Partner 1) was acclimatized and reared in our structures until it reached sexual maturity. In May, the broodstock was equally divided in two batches. One batch was fed to optimal feeding rate whereas the other one was fed at maintenance rate until March 2005 (Kestemont and Mélard, 2000), in order to obtain breeders with two different initial nutritional states. The experiment began on the 15th of March 2005, spawning was expected in October 2005. Firstly, 12 fish were distributed randomly into each of the 16 tanks distributed in two thermo-regulated rooms for control of the temperature (two different thermal programs were applied). A common program of induction of the reproductive cycle has been applied for nine weeks (Wang *et al.*, submitted). Temperature was decreased from 23°C to 14°C whereas photoperiod was abruptly decreased from 16L:8D to 8L:16D fifteen days before. At the end of this period, a blood sampling was done on all fish for 11 keto-testosterone assay (a typically male hormone) to differentiate males from females. Fish were redistributed to balance the sex-ratio (4 males and 8 females per tank). Then, the different combinations of factors according to a two-levels fractional factorial design given by Planor Software were randomly applied and experiment started (Kobilinsky, 2000). At the end of the chilling phase (20th September 2005), the average mortality was 12% and a blood sampling was realized on all fish to determine (i) plasma cortisol, a stress indicator, (ii) plasma sexual steroids (estradiol, testosterone and 11-keto-testosterone) and (iii) serum ability to lysates *Echerichia coli*, an immune indicator.

- B3 – Gamete quality and breeders welfare

B3.1 – Control of the female and male gamete quality (WP4)

Responsible: USB (Partner 11)

Associate partners:

- SMEs: Bornholms Hatchery (Partner 2), Dil-Vis (Partner 4), NH (Partner 5)
- RTD: LSA-UHP (Partner 7), FUNDP (Partner 8), Ulg (Partner 9)

The first part of this WP focused on the determination of the sperm quality and quantity from males cultured in both artificial and natural conditions. Quantitative and qualitative assessments of sperm during November and the reproduction season in April were compared. In November, males showed higher concentration of spermatozoa (50-100 10⁹ Spz per mL vs 20-80 10⁹ Spz per mL), lower volume of sperm (0.4-0.6 mL vs 0.4-4.5 mL), lower motility level (20-50% vs 80-100%) and lower speed of spermatozoa than in April. The determination of the freezing conditions to preserve the perch sperm has been performed. Cryopreservation of sperm appears to be a useful and reliable technique not only for conservation of sperm for experimental condition but also for wide-ranging artificial propagation

Another part focused on the optimization of mass semi-artificial or artificial propagation of hormonally and not hormonally stimulated perch spawners. The type of propagation used did not influence the spawning rate (> 80%). Significantly higher number of eggs per 1 ml (454.5 ± 110.5 eggs) and 1 g (598.5 ± 130.2 eggs) was observed in artificial spawned females. Eggs after artificial spawning were less swollen than eggs after semi-artificial spawning (253 ± 80.2 eggs per 1ml and 318.5 ± 132.0 eggs per 1 g). The time from injection to spawning was found statistically longer in case of semi-artificial spawning of females. The best synchronization of spawning was performed during semi-artificial spawning under 15°C. The highest hatching success (62.9 %) was found after semi-artificial spawning, which was performed under 15 °C, the lowest hatching success (26.1 %) being recorded after artificial spawning under 10 °C. During the spawning period it was found a high survival rate of broodstock, whereas low survival rates were found in females from both artificial (32.5%) and semi-artificial spawning (31.9 %) 7 days after the spawning period. Methanol at concentrations of 10 % with 300 mM of glucose yielded the highest velocity in frozen-thawed sperm ($149 \mu\text{m}\cdot\text{s}^{-1}$).

B3.2 – Breeders mortality and welfare (WP5)

Responsible: FUNDP (Partner 8)

Associate partners:

- SMEs: Bornholms Hatchery (Partner 2), Dil-Vis (Partner 4)
- RTD: LSA-UHP (Partner 7), Ulg (Partner 9)

The objective of this WP was to answer the question whether high mortality encountered in female Eurasian perch during the reproduction period may be considered as a trade-off between reproduction and immune status and/or as an exhaustion related to winter environmental conditions. Three groups of captive breeders were compared three times during gonad maturation and/or spawning period (January, February and March): captive breeders submitted to constant conditions (no gonad development), captive breeders reared in outdoor tanks under natural nycthemeral variations and forage feeding to ensure an efficient reproductive maturation and, captive breeders submitted to controlled out-season conditions to advance the spawning period (early February instead of mid-April). In addition, wild breeders from the Meuse River were captured before (late March), during (mid-April) and after (late April) spawning for comparison to captive breeders. Overall, the results did not show an important trade-off between reproduction and immune system because different immune activities were unaffected during gonad maturation and spawning in captive breeders. However, a reduction of both lysozyme and respiratory burst activities and a smaller spleen size were found in wild breeders at a similar temperature compared to captive breeders in March. An increase in cortisol release related to environmental conditions but not to reproductive processes may be involved in a such down-regulation even if other measured indicators for stress did not change.

- *B4 – Production of juvenile with genetically improved performances (WP6)*

Responsible: Ulg (Partner 9)

Associated partners:

- SMEs: Lucas Perches (Partner 1), Bornolms Hatchery (Partner 2), NH (Partner 5)
- RTD: USB (Partner 11)

The main goals of this WP were to compare the growth performance of 3 generations (F1, F2, F3) of captive juveniles and to produce a domesticated strain with improved growth and survival performances. Spawns quality, in term of both fertilization and hatching rates, was

significantly increased with the domestication: spawns originated from F3 breeders displaying higher fertilization and hatching rates than spawns originated from F1 and F2 breeders. Nevertheless, the larval quality of the domesticated F4 generation seems to be lower than the wild generation as the malformation rate at hatching and the resistance to the osmotic stress were higher and lower respectively. The growth performances of the F1 larvae were significantly higher than the domesticated strain. Nevertheless, this difference seems to be related to the difference between the initial stocking density of the batches. After the larval rearing period, the best growth performances in term of final mean body weight, specific growth rate and food conversion ratio, were obtained with the domesticated F4 generation.

The increase of growth performances (30% after 360 days of rearing) and the improvement of food conversion ratio with all-females families will allow obtaining a significant improvement of the productivity of the perch reared under intensive conditions. This result was in concordance with the milestone and the expected result of the sub-task.

- *B5 – Socio-economic study of the juvenile cost production (WP7)*

Responsible: RIVO (Partner 10)

Associated partners:

- SMEs: All partners
- RTD: All partners

A model was constructed that is able to calculate production costs for perch fingerlings. The model is now in the process of being adapted to the various production methods used by the project partners. The model is a flexible model: technical data, production data, and costs are variables. To be able to calculate the cost of fingerling production under different management regimes, information is being gathered to feed the model. This data is generated and collected in several field trials. In the mean time data was collected for costs price analysis by intensive or semi-intensive rearing of perch fry under natural feeding pond conditions (partner 5, 6 and 11). During the first year, experiments have focused on the autumn adaptation of perch fingerlings from pond into recirculation (controlled) conditions and their following rearing: rearing of advanced fry under pond conditions, and pond culturing of juveniles during the whole growing season considering the socio-economic environment in Czech Republic (partner 5) and Ireland (partner 6).

- *B6 – Transfer of technology (WP8)*

Responsible: LSA - UHP (Partner 7)

Associated partners

- SMEs: all partners
- RTDs: all partners

An intense cooperation between all partners has permitted important transfers of technology linked to existing know-how (from RTD performers to SMEs or between SMEs). A specific website (www.percatech.org) presenting the PERCATECH project has been developed to inform all publics interested by the Eurasian perch culture and to promote the main results obtained in this project. In September 2005, a contact has been officially realized with the General Secretary (Mr C. Hough) to organize a specific workshop on Percids culture during the year 2007. Such an event will ensure the dissemination of the results to the European fish farmer or others interested by Eurasian perch culture.

2.2 – WORPACKAGE PROGRESS OF THE PERIOD

A - Task 1: Co-ordination action (WP1)

Responsible: LSA - UHP (Partner 7)

Associated partners

- SMEs: all partners
- RTDs: all partners

- *Workpackage objectives and starting point of work*

The co-ordinator, highly helped by a management board, is responsible for:

1. Overall management and co-ordination of all project activities
2. Overall financial control
3. Establishment and maintenance of the project web site
4. Preparation of the reports (progress and final) to the EC
5. Integration of the results of the workpackages into the overall objectives of the project communication with the EC
6. Organization of management meetings (5 meetings during the 2-year period)

- *Progress towards objectives*

A **management board**, which is in fact the real manager of the project, has been constituted during the first meeting in Nancy (October 2004). It is composed by a member of each partner: D. Vandeworde (partner 1), J. Overton (partner 2), E. Rezzouk (partner 3), H. Dil (partner 4), L. Zvonar (partner 5), D. Torner (partner 6), P. Fontaine (partner 7), P. Kestemont (partner 8), C. Mélard (partner 9), H. van der Mheen (partner 10) and O. Linhart (partner 11). The board management gets together at the end of each meeting to assess the progress of the project and to discuss about perspectives, particularly between the future collaborations and the possible action for dissemination. The partner 7, as the coordinator, ensures the financial management (EU grant) of the project following the rules recommended by the EU. At this level, partner 7 is the interface between partners who have specific questions and the EU commission. In November 2004, a consortium agreement has been signed between all SME contractors and RTD performers. To avoid any mistakes in writing the different reports (i.e. progress and final reports; technical, management and financial reports), the coordinator has participated to the workshop “Reporting requirements for FP6 contracts” organized by the EU commission the 3rd June 2005 in Brussels.

A specific website (<http://www.percatech.org>) has been developed to promote the Percatech project and disseminate the main objectives and results obtained within the framework of this project.

Two meetings have been organized during the first year. The first meeting occurred in Nancy (hosted by partners 1 and 7) the 21-22th October 2004. It mainly focused on the presentation of each partner (team, facilities...), the achievement of the consortium agreement, the constitution of the board management (one member from each partner) and the planning of the collaborations and the work. Experimental designs and protocols have been discussed. The second meeting took place at Vodnany (partner 5 and 11) between the 17th and 19th August 2005. This meeting mainly focused on the analysis of the first results obtained during the year, the planning of the experiment during the second year and the preparation of

the progress reports. After the meeting, a visit of the SMEs commercial facilities took place especially to favour exchanges between SMEs partners.

- *Deviations from the project workprogramme*

The second meeting, which was originally planned on March, 2004, was cancelled and delayed to January 2005 due to calendar incompatibilities between partners. To avoid such problem over the second year, the next three meetings have ever already been planned: 26-27th January 2005 in Cavan, Ireland (partner 6), 8-9th June in Montpellier, France (partner 3) and 21-22th September in Nexø, Denmark (partner 2).

B - Task 2: Broodstock management

B.1 - Sub-task 2.1: Extension of the natural reproduction period - early or delayed spawning (WP2)

Responsible: RIVO (Partner 10)

Associated partners:

SME: Dil-Vis (Partner 4)

RTD: FUND (Partner 8)

- *Workpackage objectives and starting point of work*

The overall objective of this task is to extend the spawning period of ‘wild’ common perch from some weeks (generally 3 or 4) to 6 months.

Specific goals are:

- (i) to define the optimal period for harvesting of ‘wild’ breeders,
- (ii) to optimise the photo-thermal program of spawning induction in an extended season and
- (iii) to optimise stocking conditions for harvested ‘wild’ breeders.

- *Progress towards objectives*

During the first year, research has been focusing on the effect of extending the reproduction period of ‘wild’ breeders on spawning. In the period of December-April, mature perch were caught and stored. Every month, fish were induced to spawn by increasing water temperature from ‘natural’ to 12 °C over a period of 2 weeks. The remaining fish were kept under an artificial natural photoperiod and low water temperature. Breeders were stocked at controlled temperature in a closed system. Every month, males and females from each sampling were stored in a separate tank in which water temperature was slowly increased. Fish were induced to spawn by hCG-injection and stripped according to the protocol developed by Kucharczyk *et al.* (1996). Each separated batch was then incubated. Fertilization and hatching rates served as indicators for quality evaluation.

Materials and methods

Broodstock holding facilities

The tanks had a diameter of 160 cm, and a water depth of 100 cm. Total water volume of the tanks was 2 m³. The tanks were made of plastic and had a black colour. The tanks contained pieces of PVC piping and plastic flaps to provide cover material for the fish. The tanks were located in a cold room with a constant air temperature. The water in the tanks was kept at a constant low temperature of 6°C by circulating cold air through the tanks. Water was pumped over a filter, and water exchange was set at 50% per day. The photoperiod in the area was 15hD:9hL, which corresponded to the natural photoperiod in December in the Netherlands. Males and females were kept in separate tanks.

Reproduction facilities

Monthly, randomly selected individuals, males and females, were transferred to the reproduction facilities in another climate control room. The size of these tanks was 100x70x80 cm. In these tanks water temperature was increased over a period of 15 days from 6 to 12 °C, while the photoperiod gradually changed to the photoperiod of April/May (9hD:15hL) (Tab. 1). From day 15, the fish were induced to spawn according to the protocol developed by Kucharczyk *et al.* (1996) (Table 1). The collected eggs were fertilized with pooled sperm of 3 males. Approximately 600 eggs of each female were incubated. Incubation was conducted at 15°C and O₂ >6 ppm. Approximately 300 eggs from each female were incubated in one Petri dish, and two dishes per female. Hatching of the eggs was expected 14-21 days after incubation.

Measurements

- Mortality of breeders
- Fecundity per female: weight of eggs/weight of fish
- Fertilization rate: number of fertilized eggs/total number of eggs
- Hatching rate: number of hatched larvae/number of fertilized eggs

Results and Discussion

Broodstock holding facilities

Water quality in the holding facilities remained at suitable levels during the whole trial. Oxygen level fluctuated from 8.5 to 14.6 mg/l. Temperature remained at 5.5°C, pH remained constant at 7.6, and water replacement was 15% per day.

Catching breeders

Catching of wild breeders proved to be more difficult than expected. During cold periods of the year, perch stayed in deep water, from where it was impossible to catch the fish and keep them alive. The rapid change in water pressure, which occurred when collecting the fish alive proved to have a serious toll on the breeders. The first batch of wild breeders became available at 31-12-04. These fish were caught in the river Merwede. This stock contained 44 males (average weight 476 g) and 52 females (626 g). We caught a second batch of breeders on the 15th of March. This batch contained 10 males (85 g) and 13 females (108 g). A third batch of breeder arrived on the first of April 2005, and a fourth batch arrived on April 8.

Mortality

The first batch of breeders died within one month after arrival. Thus, no reproduction trial could be conducted with these fish. Any obvious reason for this mortality could be observed.

The fish did not show any serious damages or infections, albeit their behaviour was very apathetic and they showed difficulties in their orientation. Of the other batches of breeders 28% of the males and 49% of the females died during their stay in the brood stock holding facilities, and could therefore not be used for reproduction.

Table 1. The protocol in the reproduction facilities.

1	2	3	4
Day	T °C	Photo period	Actions
1	5.5	15D:9L	Transfer of wild breeders from the broodstock holding tank to the reproduction facility.
2	6.0	15D:9L	Maintenance and check facility.
3	6.5	14D:10L	“”
4	7.0	14D:10L	“”
5	7.5	13D:11L	“”
6	8.0	13D:11L	“”
7	8.5	12D:12L	“”
8	9.0	12D:12L	“”
9	9.5	11D:13L	“”
10	10.0	11D:13L	“”
11	10.5	10D:14L	“”
12	11.0	10D:14L	“”
13	11.5	9D:15L	Set-up of incubation system
14	12.0	9D:15L	“”
15	12.0	9D:15L	Priming injection of HCG
16		9D:15L	Stimulator injection of HCG
17		9D:15L	Resolving injection of HCG and putting ♀ and ♂ together.
18		9D:15L	Check Spawning induction system for eggs regularly
19		9D:15L	Check Spawning induction system for eggs regularly
20		9D:15L	Incubation of eggs
21			
22			Estimate fertilization %
23			
24			
25			
26			
27			
28			
29			
30			
31			Estimate hatching %

Feeding the broodstock

We experienced great difficulties with feeding our wild breeders. At the beginning, pieces of fish were tried, but they didn't eat them. Likewise, live goldfish (3 cm) has been tried, but the again fish didn't eat them. Maggots were also not taken, as weren't the pellets (Skretting). After one month the broodstock has started to take some earthworms, yet very cautiously. Males were more aggressive in taking the earthworms than females. Broodstock was fed two-

three times a week depending on how they reacted on the food given, but they didn't eat much and at the end of June the broodstock stopped eating completely.

Treatment of fungi

We experienced many problems with fungi infections, especially in the reproduction facilities. As soon as the temperature increased the fungi appeared massively on the breeders, resulting in mortality of several fish, and very poor conditions of others. Because of severe fungi problems it was necessary to treat the fish. Treatments using salt up to 10ppt, malachite green (5mg/l for 50 minutes) and H₂O₂ (250 ug/l for one hour were tried). Malachite green as used appeared to be deadly for European perch and H₂O₂ hadn't the results required. Salt gave the best results; indeed it appeared that in salt (10 ppt) the fungi couldn't grow out. However, fungi reappeared on the fish within one day after the fish returned to fresh water.

Reproduction

It was originally planned to reproduce the fish according to the protocol developed by Kucharczyk et al. (1996), which included stripping the females. As stripping of the wild breeders appeared to be very difficult (it was tried several times with different females between 12 and 24 hours after the resolving injection) it was decided to let the wild breeders spawn "naturally" in the tank after the spawning induction period and HCG injections. This proved to work very well. Hatching was, according to literature information, expected 14-21 days after incubation (15 ± 0.5 °C) in our experiments hatching started 7 days after incubation (17.5 ± 0.5 °C).

Spawning results

We conducted six spawning experiments on 15/3/2005, 4/4/2005, 22/4/2005, 3/5/2005, 13/6/2005 and 5/7/2005. In May, reproduction was conducted in two separate batches. Results of the reproduction are presented in the following figures 1 – 6.

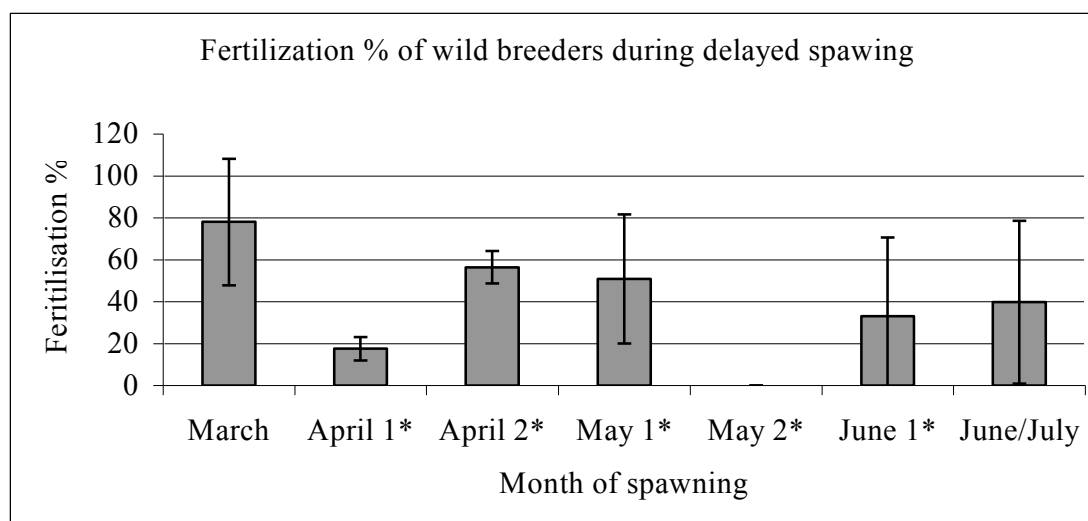


Figure 1. The fertilization % of the wild breeders as estimated during the delayed spawning trails. Legends are explained in the text below.

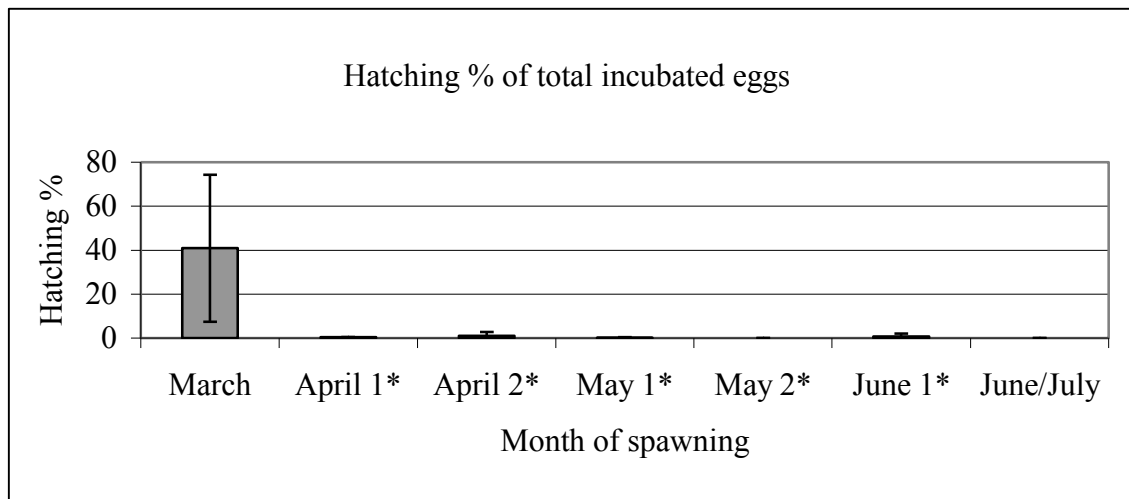


Figure 2. The hatching % regarding the total incubated eggs from the wild breeders as estimated during the delayed spawning trails.

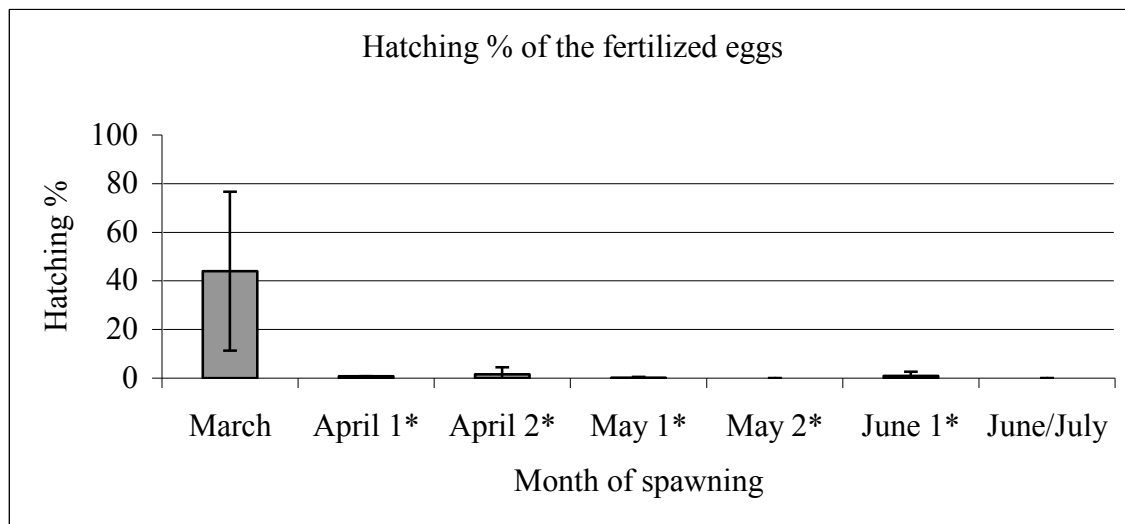


Figure 3. The hatching % of the fertilized eggs from the wild breeders as estimated during the delayed spawning trails.

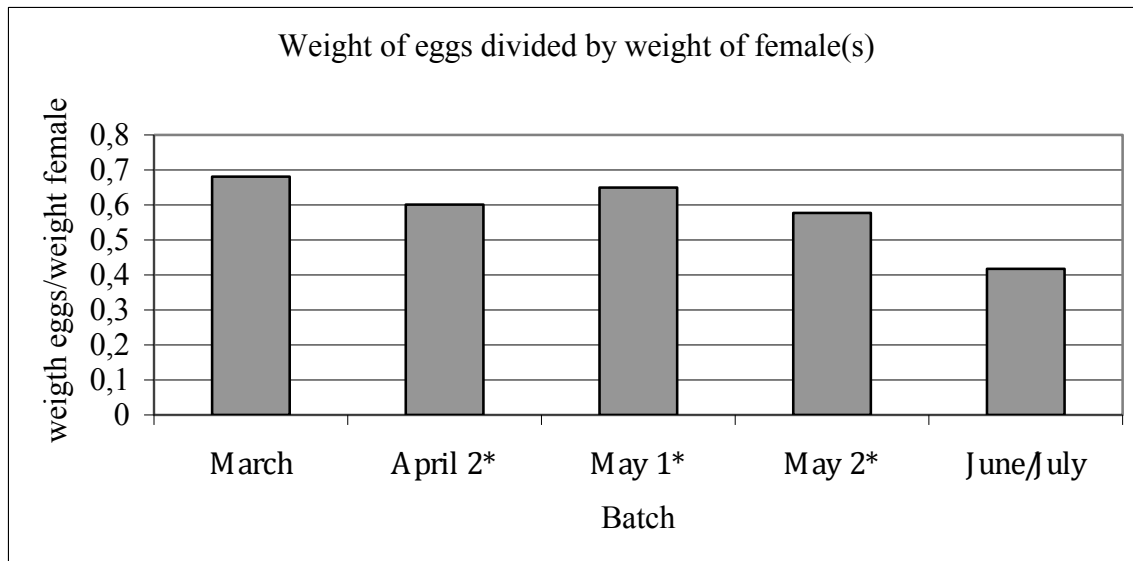


Figure 4. The average fecundity (weight eggs/weight fish) of the different batches. Important to stress is that since the fish spawned “naturally” the weight of the eggs is the weight after collection from the spawning induction tank. From batch April 1* and June 1* the weight of the responsible females is unknown.

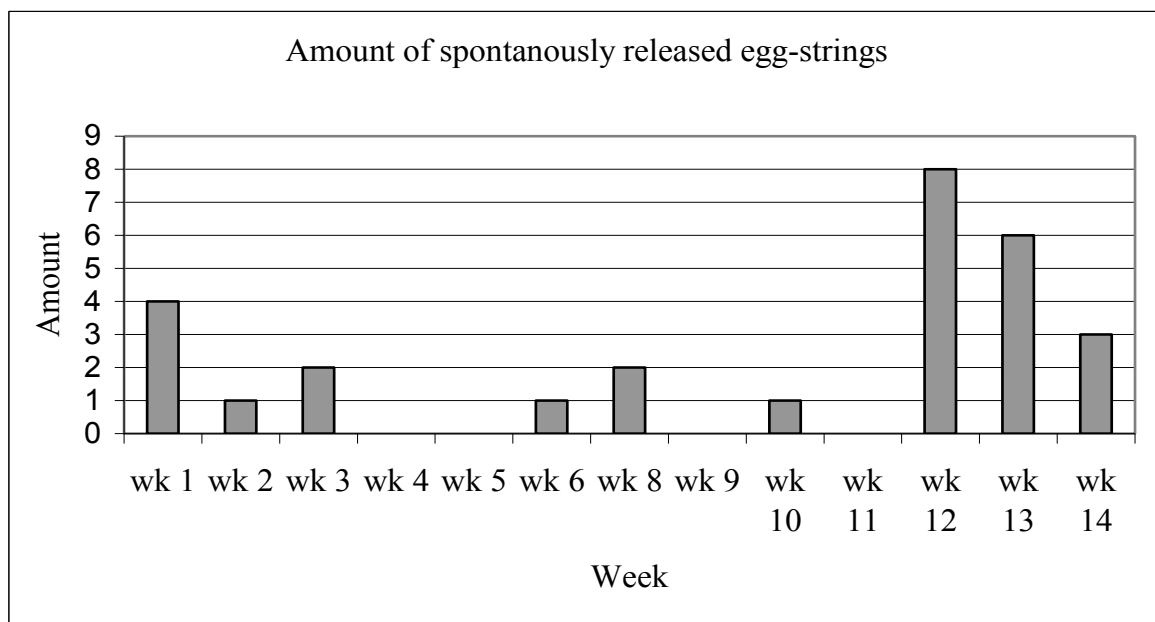


Figure 5. The amount of spontaneously released egg strings per week in the brood stock tank in the period between April and July.

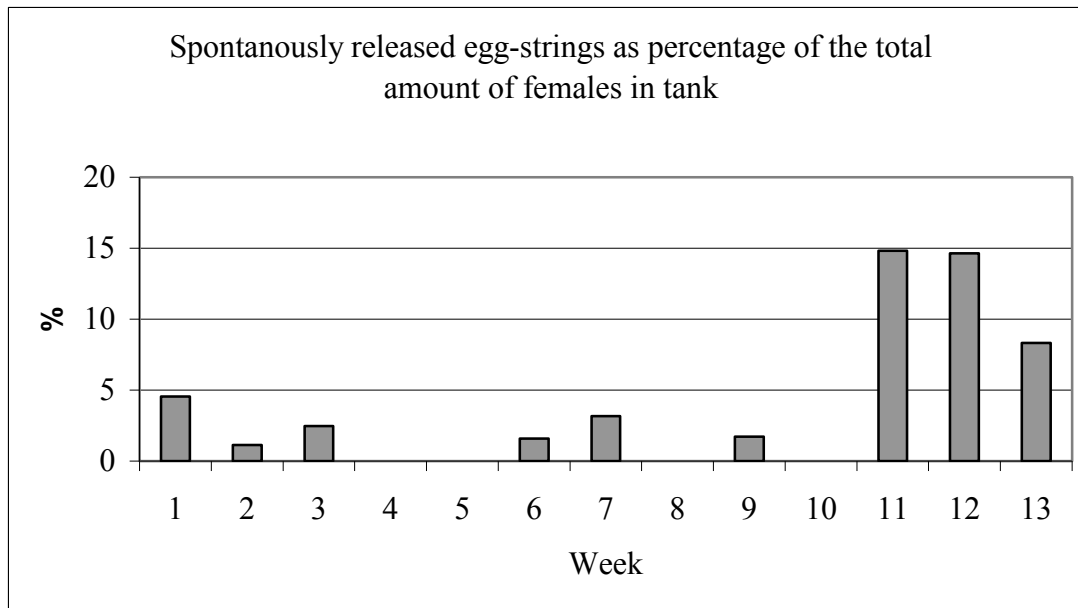


Figure 6. Spontaneously released egg strings per week as percentage of the population in the broodstock tank in the period between April and July.

Conclusions

Egg quality decreased with delaying spawning. This is most obvious for the hatching %. This corresponds to observations made at a pike perch farm (personal comment Erik Philipsen, pikeperch farm). Holding wild breeders under winter conditions for prolonged time has a negative effect on the condition of these breeders. This results in mortality of the breeders and disease problems, such as fungi and chronic bacterial problems. Maintaining winter conditions over a prolonged period was not sufficient to completely suppress the egg release of the breeders. It may have been that gonad development was already progressed to far when we caught out breeders.

Early spawning is most likely a better option than delayed spawning. Another option to be tested could be the way pikeperch are handled. With pikeperch, broodstock are hold at high temperatures, i.e. the optimal growth temperature. To prepare the breeders, they are brought to a lower temperature using a certain ΔT . In that case the breeders can be induced to start gonad development, but are still eating and have probably much better immune defence system. The spawning induction period could be a period were the fish experience a temperature increase of $\Delta T = 10$ over a certain period followed by the same HCG injections.

References

Kucharczyk D., Kujawa R., Mamcarz A., Skrzypczak A, 1996. Induced spawning in perch, *Perca fluviatilis* L. using carp pituitary extract and HCG. *Aquaculture Res.* 27, 847-852.

- *Deviations from the project workprogramme*

This study has been less important as initially foreseen due to (i) a low number of batches of wild breeders caught (because living in deeper waters in winter) and (ii) a high mortality

rate with wild breeders, especially with the first batch (December 2004). It is planned to use cultured breeders during the second year.

B.2 - Sub-task 2.2: Production of out-of-season spawning with completed control of the reproductive cycle (WP3)

Responsible: LSA-UHP (Partner 7)

Associate partners:

SMEs: Lucas Perches (Partner 1)

RTD : FUNDP (Partner 8), USB (Partner 11)

- *Workpackage objectives and starting point of work*

First results obtained prior to the beginning of the CRAFT project on the control of the reproductive cycle of Eurasian perch showed that it was mainly determined by both temperature and photoperiod variations (Migaud *et al.*, 2002, 2003, 2004a,b, 2005). Indeed, these authors had demonstrated that a decrease of temperature and photoperiod was required to induce a reproductive cycle. Then a chilling phase of 5 months at 6°C allowed gametogenesis. At last, the increase of water temperature to 13°C induced spawning. However, the first photothermal programs applied under controlled conditions led to a reproduction of poor quality with low spawning rates (30-50 %), null hatching rates (Migaud *et al.*, 2002, 2003, 2004b) and very high mortality of broodstock during the spawning season (Wang *et al.*, 2003; Migaud *et al.*, 2005). This strongly suggested that other factors (e.g. stress, light quality, food...) must act on the quality of the reproductive cycle. Consequently, the objective of this workpackage is to develop an optimised artificial program for (i) the achievement of the control of the reproductive cycle, (ii) the obtaining of eggs and larvae of good quality and (iii) the understanding of the broodstock mortality in relation with their physiological state. To reach these purposes, the understanding of the determinism of the quality of reproduction is of paramount importance. This implies to determine which factors (environmental, nutritional and populational) applied to broodstock throughout a reproductive cycle have an impact on the quality of reproduction. According to the literature, these factors are potentially numerous. Consequently, a screening of these factors based on the use of fractional factorial designs has been undertaken. Such designs allow testing a great number of factors and their interactions using few experimental units. Besides, one can deduce from the results a classification of these factors by order of importance. In the framework of this workpackage, the screening has been divided in two consecutive fractional factorial experiments planned on two years (the duration of one single reproductive cycle ranges between eight and nine months). The first one deals with the effects of environmental and nutritional factors on the quality of reproduction. The second one includes the effects of populational factors to the significant factors deduced from the first experiment.

At the beginning of the project (October 2004), the broodstock, i.e. 1500 fish at commercial size, provided by the Lucas Perches fish farm (Partner 1) in January 2004 were under breeding in our structures until they reached sexual maturity in January 2005. In May, the broodstock was equally divided in two batches. One batch was fed to optimal feeding rate whereas the other one was fed at maintenance rate until March 2005 according to Kestemont and Mélard (2000).

- *Progress toward objectives*

The first experiment is currently carried out. It began on the 15th of March 2005 and the spawning season is expected for the end of the first year of the project in October 2005. The levels of the eight factors tested are listed in table 2.

Materials and Methods

Animals

A set of 1500 fish (mean weight of 122 g) was purchased from “Lucas Perches” fish-farm (Lorraine, France) in January 2004. It was equally distributed and held in four 6000 L-fiber glass tanks working as independent water-recirculated system (Fontaine et al., 1996). Photoperiod was set at 16L:8D and temperature was maintained around 23 ± 1 °C. Light was provided by incandescent daylight white bulb (Philips TLD 58 W - 86₅). Light intensity was about 250 lux at the water surface. Feeding rates were calculated from Mélard et al. (1996). Fish were fed with commercial food (Bioptimal 4.5 ST, BioMar, France) at optimal feeding rate until May 2004. Then, fish were fed at two different feeding rates until mid-March 2005, in order to test the initial nutritional status of the broodstock on the quality of the reproductive cycle (see Table 1). Half of the fish (two tanks) were fed at maintenance rate (about one third of the optimal rate), while the other half remained fed at optimal rate.

Prior to experimentation, 10 fish per set of batches were sacrificed to characterize the initial state of broodstock. All female and males gonado-somatic indexes were below 1% and 0.2% respectively, showing that fish were still non maturing. Moreover, the fat index of the fish (relative quantity of mesenteric fat) was different between fish fed at maintenance rate (3.8%, LFI) and fish fed at optimal rate (5.3%, HFI), confirming that the two sets of fish were at different nutritional states.

Experimental design

Induction of the reproductive cycle

Firstly, 13 fish were distributed randomly into each of the 16 tanks working as an individual water recirculating system. These tanks were distributed equally in two thermo-regulated rooms for control of the temperature (two different thermal programs were applied). The same optimised program of induction of the reproductive cycle was applied for nine weeks to all the fish (Wang *et al.*, submitted). It was based mainly on temperature and photoperiodic variations. Briefly, temperature was decreased from 23°C to 14°C whereas photoperiod was abruptly decreased from 16L:8D to 8L:16D fifteen days before (Figure 1).

At the end of the inductive program (31st May 2005), 8 fish of each initial nutritional state (one fish per tank) were sampled and killed for morpho-anatomical measurements. All males were maturing whereas all females were maturing in the LFI group but only 66% in the HFI group (table 1).

Then, since it is impossible to differentiate morphologically males from females perch brood fish, a blood sampling was done on all fish for 11 keto-testosterone assay (a typically male hormone). Fish were redistributed to balance the sex-ratio (4 males and 8 females per tank).

Experimental period

Experiment started on the 23rd June 2005 (T0). The different combinations of factors according to a two-levels fractional factorial design (table 3) given by Planor Software were randomly applied (Kobilinsky, 2000). Photothermal programs applied and sampling times are given on figure 1. At the ends of the chilling phase and reproduction phases (T1 and T2 respectively), a blood sampling will be realized on all fish to determine (i) plasma cortisol (stress indicator), (ii) plasma estradiol, testosterone and 11-keto-testosterone (reproductive physiology indicators) and (iii) serum ability to lysates *Echerichia coli*, (immune indicator).

Spawning is expected for October 2005, just after a progressive warming of temperature to 13°C on weeks 38 and 39 of this year (second half of September). Spawning rates, fecundities and egg weight will also be determined.

Two thermo-regulated hatchery structures have been purchased to test egg (fertilization and hatching rates) and larvae (resistance to starvation and osmotic shock) quality. At the end of the experiment, sperm quality (concentration, motility and osmolality) will be assessed.

In addition to the support of the Lucas Perches fish farm for providing the broodstock, this workpackage includes RTD partners for the assessment of gamete quality (USB, Partner 11) and the histological observations (FUNDP, Partner 8).

Results and Discussion

Fore and foremost, fish were non maturing prior to the experiment and the inductive program applied at the beginning of the experiment was efficient enough to induce gonadal recrudescence in almost all broodstock. Moreover, the initial nutritional states of the two sets of broodstock were different prior to the beginning of the inductive program, as shown by the calculation of fat indexes. Fish mortality rate during the chilling period was almost the same (12%) than that observed by Migaud et al. (2003) in a previous experiment on the effect of photoperiod on the quality of reproduction in Eurasian perch (see figure 2). There was no effect of any factor nor interaction on fish mortality during this period.

Results concerning this first experiment will be described and discussed in the next periodic activity report.

Table 1. Morpho-anatomical status of the two batches of broodstock at the end of the inductive phase.

Nutritional State	Sex	Maturing fish (%)	GSI	FI
LFI	Males	100%	4.2 ± 1.8	1.5 ± 1.2
	Females	100%	4.6 ± 0.8	1.9 ± 0.6
HFI	Males	100%	3.9 ± 0.9	3.7 ± 0.8
	Females	66%	2.7 ± 1.7	2.4 ± 1.9

Table 2. Presentation of the factors and levels tested during the experiment.

Factors	Levels
Chilling temperature	Chilling at 8°C for four months
	Chilling at 4°C for four months
Initial nutritional state	High initial Fat Index (HFI, feeding at optimal rate)
	Low initial Fat Index (LFI, feeding at maintenance rate)
Photophase increase	Progressive increase six weeks before spawning (40 min per week)
	None
Dawn simulation	Progressive increase of light intensity at dawn for 35 minutes
	Abrupt switch
Handling	Chasing with a net for 3 minutes per day, five days a week
	No chasing
Food type	Artificial diet
	Forage fish
Feeding rate	<i>Ad libitum</i>
	Alternatively fed <i>ad libitum</i> and starved each second week
Light intensity	100 lux (daylight fluorescent tubes)
	1000 lux (daylight fluorescent tubes)

Table 3. Matrix of the 16 combinations of the experimental design. Comb.: Combination; Temp: Temperature; Ini Nut: Initial Nutritional State; Phot: Photophase increase.

Comb.	Temp	Ini Nut	Phot	Dawn	Handling	Food Type	Feed Rate	Light Intensity
1	4	HFI	8	Simulated	Chase	Artificial	Starvation	100
2	4	HFI	8	None	No Chase	Artificial	<i>Ad libitum</i>	1000
3	4	HFI	13	Simulated	Chase	Prey	<i>Ad libitum</i>	1000
4	4	HFI	13	None	No Chase	Prey	Starvation	100
5	4	LFI	8	Simulated	No Chase	Prey	Starvation	1000
6	4	LFI	8	None	Chase	Prey	<i>Ad libitum</i>	100
7	4	LFI	13	Simulated	No Chase	Artificial	<i>Ad libitum</i>	100
8	4	LFI	13	None	Chase	Artificial	Starvation	1000
9	8	HFI	8	Simulated	No Chase	Prey	<i>Ad libitum</i>	100
10	8	HFI	8	None	Chase	Prey	Starvation	1000
11	8	HFI	13	Simulated	No Chase	Artificial	Starvation	1000
12	8	HFI	13	None	Chase	Artificial	<i>Ad libitum</i>	100
13	8	LFI	8	Simulated	Chase	Artificial	<i>Ad libitum</i>	1000
14	8	LFI	8	None	No Chase	Artificial	Starvation	100
15	8	LFI	13	Simulated	Chase	Prey	Starvation	100
16	8	LFI	13	None	No Chase	Prey	<i>Ad libitum</i>	1000

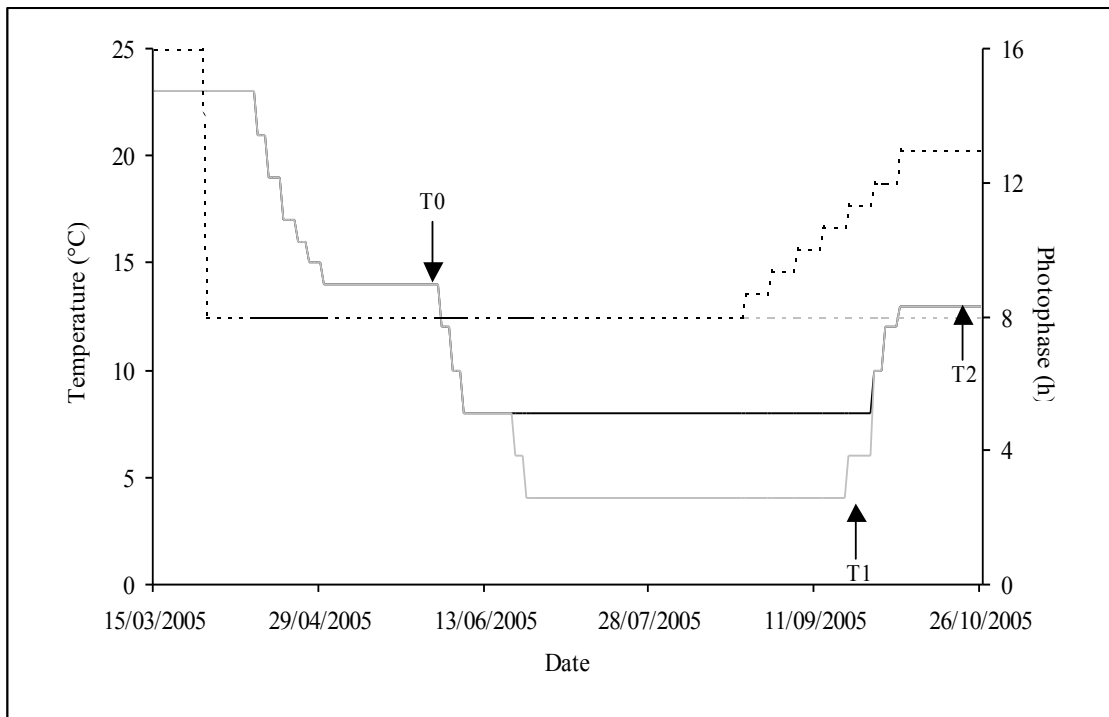


Figure 1. Graphic representation of the two levels of temperature and photoperiod programs applied throughout the experiment. Temperature (solid lines): black line = 8°C treatment; grey line = 4°C treatment. Photoperiod (broken lines): black line = increase to 13h photophase; grey line = no increase.

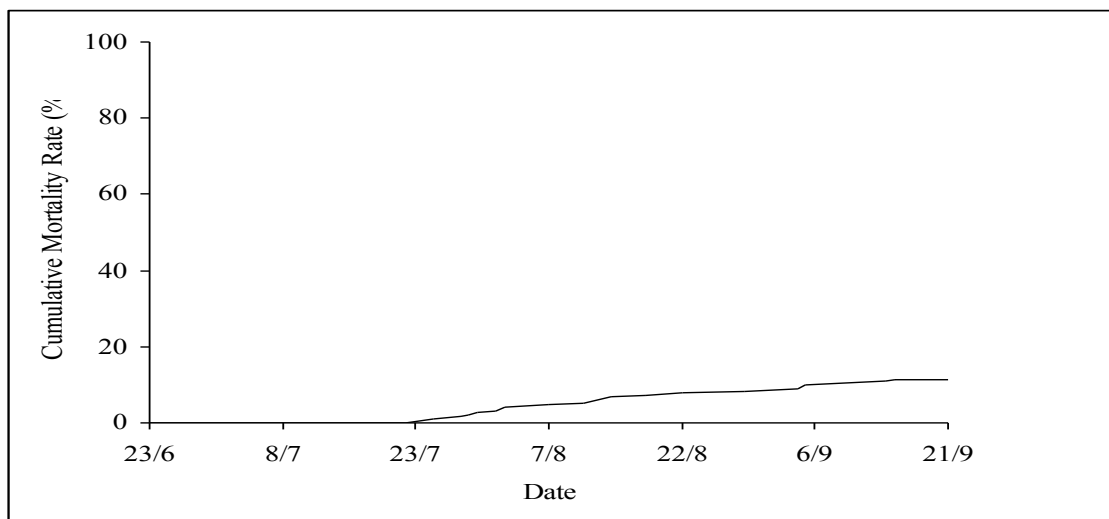


Figure 2. Cumulative broodstock mortality during the chilling phase (between T0 and T1).

References

- Kestemont, P., Mélard, C., 2000. Reproductive biology and husbandry management. In Craig J.F. (Eds), Percids: Systematics and Ecology and Exploitation. *Fish and Aquatic Resources Series 3*. pp 191-224.
- Kobilinsky, A., 2000. Analys and Planor softwares. Unité de Biométrie, INRA.

- Migaud, H., Fontaine, P., Sulisty, I., Kestemont, P., Gardeur, J.N., 2002. Induction of out-of-season spawning in Eurasian perch *Perca fluviatilis*. Effects of rates of cooling and cooling durations on female gametogenesis and spawning. *Aquaculture* 205, 253-267.
- Migaud, H., Mandiki, R., Gardeur, J.N., Kestemont, P., Bromage, N.R., Fontaine, P., 2003. Influence of photoperiod regimes on the Eurasian perch gonadogenesis and spawning. *Fish Physiol. Biochem.* 28, 395–397.
- Migaud, H., Fontaine, P., Kestemont, P., Wang, N., Brun-Bellut, J., 2004a. Influence of photoperiod on the onset of gonadogenesis in Eurasian perch *Perca fluviatilis*. *Aquaculture* 241, 561-574.
- Migaud, H., Gardeur, J.N., Kestemont, P., Fontaine, P., 2004b. Off-season spawning of Eurasian perch *Perca fluviatilis*. *Aquacult. Int.* 12, 87-102.
- Migaud, H., Wang, N., Gardeur, J.N., Fontaine, P., 2005. Influence of photoperiod on reproductive performances of Eurasian perch. *Aquaculture. In press.*
- Wang N., Migaud H., Acerete L., Gardeur J.N, Tort L., Fontaine P., 2003. Mortality and non-specific immune response of Eurasian perch, *Perca fluviatilis*, during the spawning season. *Fish Physiol. Biochem.* 28, 523-524.
- Wang N., Gardeur J.N., Henrotte E., Marie M., Kestemont P., Fontaine P.. Determinism of the induction of the reproductive cycle in female Eurasian perch, *Perca fluviatilis*: effect of environmental cues and permissive factors. *Aquaculture, submitted.*

- *Deviations from the project workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

C - Task 3: Gamete quality and breeders welfare

C.1 - Sub-task 3.1: Control of the female and male gamete quality (WP4)

Responsible: USB (Partner 11)

Associate partners:

SMEs: Bornholms Hatchery (Partner 2), Dil-Vis (Partner 4), NH (Partner 5)

RTD: LSA-UHP (Partner 7), FUNDP (Partner 8), Ulg (Partner 9)

- *Workpackage objectives and starting point of work*

The three objectives of this workpackage during the first year are:

- Determination of the sperm quality and quantity from males cultured in artificial condition and in natural condition (with partners 5 and 7): to determine in 2005 the effect of artificial rearing and natural nursing on the parameters of sperm quality, quantity, including egg fertilization and hatching test with WP 3 and 4.
- Optimization of mass semi-artificial propagation of hormonally and non hormonally stimulated perch spawners (with partner 5) to obtain up to 100 000 viable larvae of perch during the spawning period for WP 3, 6 and 7.
- Determination of the freezing conditions to preserve the perch sperm (with partners 7 and 8): freeze and thaw spermatozoa keeping on their viability with respect to sperm of sex-inverted gynogenic neomales from WP 6. Neomale sperm will be obtained during 2006 from partner 9. A sperm freezing procedure will be developed, leading after thawing to high

motility of spermatozoa as well as to high values of velocity and percentage of sperm motility, fertilization and hatching yield.

- *Progress towards objectives*

a) Determination of the sperm quality and quantity from males cultured in both artificial and natural conditions

Determination of the sperm quality and quantity from males cultured in both artificial and natural conditions (with partners 5 and 7): to determine in 2005 the effect of artificial rearing and natural nursing on the parameters of sperm quality, quantity, including egg fertilization and hatching test for WP 3 and 4.

Materials and methods

We used a group of perch males of the same age originating from the same reproductive period reared until maturation under separated conditions. The first group was in a recirculation system, feed with artificial feeds and under simulated natural photoperiod and temperature regimes. Male's out-of-season spawning of perch was induced by thermal conditions. This part of project was performed in four 400 l tanks located in room equipped with controlled light and temperature. In each tank were stocked 100 one-half year old perch (females and males in rate 1:1) in autumn 2004. Perch were reared in these tanks during six months. During that period, four thermal periods were performed (1. period- normal period, temperature 14-17°C; 2. period – cooling period, temperature 10-8°C; 3. period – chilling period, temperature 3-5°C; 4. normal period, temperature 14 – 15°C). After this thermal regime, males were induced to spawn. Unfortunately there were very low success of male's spermiation and such experiment was cancelled. The second and third groups were in a pond with natural feeding (zooplankton and fodder fish). Quantity and quality of fish sperm was assessed during the normal reproductive period without hormonal administration. The second group of mature perch were reared in pond condition and during November, 30 males were acclimatized during a period of 10 days from 6 °C up to 15 °C, and males were finally striped individually with individual assessment of sperm quantity and sperm quality. The third group about 100 males was proportionally striped 4 times during full reproductive season of perch. Quality and quantity of sperm were evaluated and values of sperm's quality was compared to sperm's quality of perch from out-of-season stripping, i.e. from November.

Quantitative assessment of sperm

The sperm was collected one time from 12 males per each condition, 4 times during a period of 1 month (April in Czech Republic). Sperm concentration was counted with Thoma cell hemocytometer under Olympus microscope BX 41 (400x) and mean number was expressed per 20 squares of Thoma cell. The concentration of sperm is expressed as billions of spermatozoa per ml of sperm. Volume of sperm per male and number of sperm per male, volume of sperm per kg of male body weight (b.w.) and number of sperm per kg of male b.w. are expressed as billions of spermatozoa per male and billions of spermatozoa per kg of b.w., respectively according to the methods described by Linhart et al. (2000b).

Qualitative assessment of sperm

Sperm motility was evaluated for the percentage of motile spermatozoa and for velocity after sperm activation. Measurements of motility parameters were carried out using dark field microscopy. For testing the swimming ability of spermatozoa, the fresh sperm was pre-diluted

1:20000 with immobilizing medium and then 0.5 μl of diluted sperm was directly mixed with a 49.5 μl drop of swimming medium (SM = hatchery water or a special medium), placed on a glass slide previously settled on the microscope stage; immediately after mixing it was examined under 200x magnification. Motile spermatozoa were video recorded within 8 s after activation for measurement of velocity and percentage of actively swimming spermatozoa. The 10 $\mu\text{m}\cdot\text{s}^{-1}$ was the minimal value of velocity used as threshold, below which motility was considered as zero. The movements of spermatozoa was recorded on a tape recorder (SONY S-VHS) using a CCD video camera (SONY) set on a dark-field microscope (Olympus BX 41), including record of the time elapsed since initiation of movement (Linhart et al., 2002). The successive positions of the recorded sperm heads was measured from successive video frames using video-recorder (SONY SVHS, SVO-9500 MDP) and analyzed at 10 s after activation and each time using 5 successive frames by Micro Image Analysis (version 4.0.1. for Windows by Olympus, with a special application from Olympus C&S, Czech Republic, Linhart et al., 2000a) for velocity and percentage of moving spermatozoa. The immotile sperm (velocity = 0) were not taken into account in the velocity curve, i.e. only motile sperm can be plotted.

Results and discussion

Yet, based on preliminary observations, it could be concluded that:

Quantitative and qualitative assessment of sperm during November

- high concentration of sperm, i.e. about $50\text{-}100 \times 10^9$ spermatozoa per male,
- low volume of sperm, i.e. about 0.4-0.6 ml per male,
- high osmolality: 350-450 mOsmol/kg-1,
- low motility level: 20-50 %,
- low speed of spermatozoa,
- it was not possible to dilute sperm by immobilizing solution for short-term storage at 0°C and finally freeze the sperm

Quantitative and qualitative assessment of sperm during reproduction season

Quantitative and qualitative assessment of sperm during reproduction season (4 times collection of sperm in April, see Tab. 1):

- lower concentration of sperm: $24\text{-}83 \times 10^9$ spermatozoa per male,
- high volume of sperm: 0.4-4.5 ml per male,
- high osmolality: 350-450 mOsmol/kg-1,
- good motility level: 80-100 %,
- good speed of spermatozoa,
- it was possible to dilute the sperm with an immobilizing solution, short-term storage at 0°C and finally freeze it

Table 1. Quantitative assessment of sperm during April, 2005 (detail results will be presented in future report).

	Body weight of male (g)	Sperm concentration (109.ml)	Volume of sperm (ml)	Sperm osmolality (mOsmol. kg-1)	Number of spermatozoa per 100g of male B.W	Volume of sperm per 100g of male B.W.
mean value	78.29	45.08	1.30	319.92	108.53	2.74
SD	98.14	12.47	1.01	63.01	73.39	1.50
min	16.90	24.81	0.40	262.00	21.54	0.66
max	427.00	82.50	4.50	507.00	269.79	5.83

Development of a hatching test for experimental condition for WP 3 and 4

- Put two g of eggs (760 eggs per g) into a 50 ml dish;
- Add 15 µl of fresh not diluted sperm (400000 spz per egg),
- Add 10 ml of hatchery water;
- Mixed together for 1 minute on a shaking table set to 300 rpm and 10 mm deflection;
- Then placed into a special incubator cage of 200 ml supplied with UV-sterilized recirculated tap water at 15°C;
- Hatching yield including malformations was evaluated after hatching,
- With this technique we observed about 70-95 % percent of hatching success

Development of a qualitative assessment of sperm for WP3 and 4

- For testing the swimming ability of spermatozoa, the fresh sperm was pre-diluted by KUROKURA (containing 220 mmol of NaCl) medium 1:200.
- Then 0.5 µl of diluted sperm was mixed with a 49.5 µl drop of 30 mM NaCl +0.1 % BSA.
- The movements of spermatozoa were recorded on a tape recorder and recorded sperm heads was measured from successive video frames.
- *Sperm viability test* : LIVE/DEAD spermatozoa was measured by DNA staining in fluorescence microscopy

b) Mass semi-artificial propagation of hormonally and non hormonally stimulated perch spawners

Optimizing mass semi-artificial propagation of hormonally and non hormonally stimulated perch spawners during 2005 (with partner 5): to obtain up to 100 000 viable larvae of perch during the spawning period for WP 3, 6 and 7:

- Artificial and semi-artificial spawning in perch under experimental conditions
- Semi-artificial spawning with hormonal treatment and natural spawning without hormonal treatment under working conditions:

Materials and methods

Artificial and semi-artificial spawning under experimental conditions

Perch broodstock were caught from ponds in farm Nové Hradý (SME partner 5) during the autumn harvest season. In total, 550 perch broodstock (total biomass of 104.5 kg) were caught and transported to Vodňany. Fish were divided into two equal groups and stocked into two

ponds after their transport. Before stocking perch, prey fish (mainly topmouth gudgeon: *Pseudorasbora parva*, 50 kg. ha⁻¹) were stocked into these ponds. Perch broodstock overwintered under pond conditions. The first pond, where perch broodstock were reared, was harvested on 1st April 2005. Mean water temperature was 9.9 °C in that period. In total, 240 broodstock (120 females and 120 males) were caught. Health and nutrition conditions of perch broodstock were checked after their catching. Then, perch broodstock were measured, weighed, marked by elastomers under skin (for separation artificial and semi-artificial spawned broodstock) and classified according sex. The mean total body length of females was 215.8 ± 57.0 mm (min. 145 mm and max. 338 mm) and the total body weight 190.2 ± 156.4 g (20.4-684.9 g). The mean total body length of males was 194.4 ± 37.2 mm (134-302 mm) and the total body weight 91.4 ± 36.3 g (26.8-375.0 g). Only females were hormonally treated for induction of spawning. Males released milt without hormonal injection. The hormonal injection by preparation of Supergestran was used intramuscularly in all groups of females. The preparation Supergestran contains 25 µg lecorelinum (active substance of preparation) per 1ml of preparation. The dose used was 50 µg lecorelinum.1000g⁻¹ (body weight) of female perch. It means that we used 2 ml of preparation .1000g⁻¹ bw of female perch. After measure and hormonal treatment, perch was separated into 12 groups (2 type of spawning: semi-artificial and artificial, 2 different water temperature (10°C and 15°C) and each group had three replications). Each replications of group was composed of 20 fish (10 females and 10 males). Females and males for semi-artificial spawning were stocked together (10 females and 10 males per group) into 6 tanks. Broodstock for artificial spawning were stocked separately according to sex. Females were stocked into 6 tanks (groups), 10 females per group. All males for artificial spawning (60 individuals) were stocked into two tanks, where two different water temperatures were thermoregulated (10 and 15 °C). The whole experiment was performed in the fish facilities of RIFCH Vodňany (RTD partner 11). Perch broodstock were stocked into 14 tanks of two recirculation systems (6 tanks for semi-artificial spawning, 6 tanks for artificial spawning and 2 tanks for males of artificial spawning). Total volume of tanks was 600 litres. The first control of spawning was performed 24 hours after stocking of broodstock. Other controls were performed in six hours interval (6 p.m., 12 p.m., 6 a.m. and 12 a.m). During control of spawning, females for artificial spawning were caught and artificial spawning was performed. Ovulation of eggs was tested by pressure of fingers of fisherman on the ventral part of female body. Released eggs were collected in dry saucer. Gained eggs were fertilized by milt which was gained from males specified for artificial spawning according to Kouřil and Hamáčková (1999). Spawned female was identified (according to size of females and mark) and removed from tank into earth pond. Time of spawning was registered in each spawned female. The presence of eggs were followed on the bottom of tanks in groups of semi-artificial spawning, only (without handling with broodstock). Gained eggs were collected and then assigned to definite spawned females in tank. Time of spawning was registered down for each spawned female.

Evaluation of fecundity in perch females

Weight and volume of gained eggs were recorded in each spawned female. Samples of eggs were collected from each female for assessment of egg number per 1 ml and 1 g of egg mass. Fecundity (total number of eggs, total weight of eggs, variation of fecundity on the total length of females and variation of fecundity on the total weight of females) was measured for each spawned female. Number of eggs per 1 ml and 1 g was compared between females spawned by different type spawning under 2 different water temperature using ANOVA (Statgraphics, Tukey test, P< 0.01).

Time effort of artificial and semi-artificial spawning

Time between injection and spawning (in hours, days and degree-days) were followed during spawning test. Synchronization of spawning was evaluated for 2 different type of spawning under 2 different water temperatures. Effect of water temperature and type of spawning on the time effort of spawning was also evaluated. Time between injection and spawning (in hours, days and degree-days) was compared between artificial and semi-artificial spawning under 2 different water temperature using ANOVA (Statgraphics, Tukey test, $P < 0.01$).

Efficiency of egg incubation

After spawning, samples of eggs (approximately 1 g of eggs) were gained from all spawned females for assessment of hatching success. Samples were then separately incubated in special cage from each female. Cages for incubation of eggs were placed in two troughs of recirculation system with total volume of water 1000 litres. Water temperature (mean 15.0 ± 1.03 °C), oxygen content (7.5 ± 0.45 mg O₂.l⁻¹) were measured in six hours interval. Rest of eggs from all females was incubated in others four troughs, which were part of two identical recirculation systems. Efficiency of egg incubation was compared between artificial and semi-artificial spawning under two different water temperature conditions using ANOVA (Statgraphics, Tukey test, $P < 0.01$).

Economical analysis of the egg production during the experiment

All operation costs were recorded during the whole experiment. Total production of larvae were compared with all operation cost. Operation cost per 1000 larvae was estimated.

Survival rate of broodstock during and after spawning period

All females and males of artificial and semi-artificial spawning were differently marked by elastomers for their identification. The survival and mortality rate of females and males were recorded during artificial and semi-artificial spawning. After spawning, females and males were stocked into earthen pond (with prey fish). Fish stayed there during seven days. The survival of broodstock was recorded after seven days, when broodstock was caught, transported and stocked into the pond. The next checking of broodstock survival was made after 90 days, i.e. after spawning season.

Semi-artificial spawning with hormonal treatment and natural spawning without hormonal treatment under working conditions

This part of the study was performed in the Nové Hradky farm (SME partner 5). In total, 180 females (the mean total body length of 259.8 ± 60.2 mm and the mean total body weight of 248.2 ± 166.8 g) and 90 males (241.2 ± 47.2 mm and 189.5 ± 52.7 g) were used during spawning season. Broodstock were reared under pond conditions before spawning season. Perch broodstock were caught on 14th April. After catching of perch, broodstock were divided into two groups. The first group of fish was used for semi-artificial spawning with hormonal treatment of females. The second group of fish was used for natural spawning without hormonal treatment. Each group of perch contained 135 fish (90 females and 45 males). All perch were measured and weighted. Females used for semi-artificial spawning were hormonal injected as females in the first experiment. Then all fish were stocked into two tanks with flow of water (total volume 4000 litres per tank). After stocking of perch, spawning was checked for 24 hours. Other checking of spawning was performed in 24 hours interval. The total produce of eggs, synchronization of spawning and survival rate of broodstock were followed during the spawning period. After spawning, gained eggs were incubated in special incubators (originally used for incubation of grass carp eggs, capacity of water 255 litres) under different density of eggs. Eggs were incubated in six incubators under six densities of eggs (375 000,

750 000, 800 000, 1 200 000, 1 400 000, 1 600 000 eggs per incubator). The efficiency of egg incubation was evaluated by hatching success at the end of the incubation of eggs. The economical analysis of this spawning and incubation eggs was evaluated.

Results and discussion

Optimizing mass semi-artificial propagation of hormonally and non hormonally stimulated perch spawners.

Artificial and semi-artificial spawning under experimental conditions

In total, 83.3 % (50 ex.) and 80 % (48 ex.) females were artificially spawned under water temperature at 15°C and 10 °C, respectively. Similar results were obtained during semi-artificial spawning, when 86.7 % (52 ex.) and 80 % (48 ex.) females were spawned under 15 °C and 10°C, respectively. Overall, all females produced 3 026 700 ex. fertilized eggs. However, only 600 000 ex. larvae hatched after egg incubation.

Evaluation of fecundity in perch females

The mean value of fecundity of females, with minimum and maximum values is presented in the Tab. 1. During the spawning period, very unstable fecundity of females was recorded. No significant difference was found between fecundity of artificially and semi-artificially spawned females under different water temperature (Tab. 1). The effect of the type of spawning on the fecundity of females was not evidenced. The high level of correlation ($r = 0.89$ and 0.84) were found for dependence of fecundity on total body length and weight of females, respectively. Total weight and length of females notably influenced their fecundity (Fig. 1 and 2). Significantly higher number of eggs per 1 ml (454.5 ± 110.5 eggs) and 1 g (598.5 ± 130.2 eggs) was observed in artificial spawned females. Eggs after artificial spawning were less swollen as eggs after semi-artificial spawning (253 ± 80.2 eggs per 1ml and 318.5 ± 132.0 eggs per 1 g) (Fig. 3 and 4).

Time effort of artificial and semi-artificial spawning

The time from injection to spawning was found statistically longer in case of semi-artificial spawning of females. The time from injection to spawning in case of semi-artificial spawning was 187.4 ± 20.7 hours (7.8 ± 0.86 days, 78 ± 8.6 degree days) and 98.5 ± 17.2 hours (4.1 ± 0.7 days, 61 ± 10.7 degree days) under 10 °C and 15 °C water temperature, respectively. The time from injection to spawning in case of artificial spawning was 129.8 ± 25.9 hours (5.4 ± 1.1 days, 54 ± 10.8 degree days) and 84 ± 18.3 hours (3.5 ± 0.76 days, 52 ± 11.4 degree days) under water temperature of 10 °C and 15 °C, respectively. Semi-artificial spawning under water temperature of 15 °C appeared later than artificial spawning under same water temperature, about 14.5 ± 1.1 hours (0.6 ± 0.3 days, 9 ± 0.7 degree days). The semi-artificial spawning under water temperature of 10 °C appeared later than artificial spawning under same water temperature - about 57.6 ± 5.2 hours (2.4 ± 0.24 days, 24 ± 2.2 degree days) (Fig. 5, 6 and 7). The best synchronization of spawning was performed during semi-artificial spawning under 15°C. In total, 86.7 % females were spawned for three days. During semi-artificial spawning under 10 °C, altogether 80 % females were spawned for three days. In total, 83.3 % females were spawned during artificial spawning under 10°C and 15 °C for four days (Fig. 8).

Efficiency of egg incubation

In general, very low hatching success (from 26.1 % to 62.9 %) was found after incubation of all eggs. Statistically the highest hatching success (62.9 %) was found after semi-artificial

spawning, which was performed under 15 °C. Statistically the lowest hatching success (26.1 %) was recorded after artificial spawning under 10 °C (Fig. 9).

Economical analysis of the egg production during this experiment

Total operation cost (2310 Euro) was counted for the whole spawning activity. Operation cost was calculated as: costs for broodstock (800 €), wintering pond (200 €), Supergestran preparation (100 €), personal costs (800 €), energy costs (200 €) and depreciation of fixed assets (210 €). It means that the producing of 1000 larvae costs 385 Euro.

Survival rate of broodstock during and after spawning period

During the spawning period it was found a high survival rate of broodstock. Male survival rate under semi-artificial spawning (91.7 %) was lower than male survival rate during artificial spawning (100 %). Generally, female survival rate (83.3 % and 85.0 % during semi-artificial and artificial spawning, respectively) was lower than male survival rate during the spawning period. A low survival rate was found in females from artificial (32.5%) and semi-artificial spawning (31.9 %) 7 days after spawning period. Survival of males was higher than survival of females 7 days after spawning period. No mortality rate of males was recorded 7 days after artificial spawning. The mortality rate of males (8.3 %) was found 7 days after semi-artificial spawning. The very low survival rate of females (1.1 % after artificial and semi-artificial spawning) and males (7.9 and 15.3 % after artificial and semi-artificial spawning, respectively) were found 90 days after spawning period (Fig. 10).

Semi-artificial spawning with hormonal treatment and natural spawning without hormonal treatment under working conditions

During semi-artificial spawning, total production of fertilized eggs was 4 500 000 eggs from 54 females (60 %). All this females spawned during a 6 days spawning period. Females of natural spawning produced only 500 000 fertilized eggs. In total, only 7 females (7.7 %) spawned during a 6 day period. The survival rate of broodstock was very low in both groups during spawning. In total, survival rate of females was 5.4 and 4.3 % in semi-artificial and natural spawning, respectively. Very high mortality rate was obviously caused by great manipulation of fish during their transport from pond to hatchery (Fig. 11). During the incubation of eggs, very low hatching success was found, from 7.0 to 16.1 %. Correlation coefficient $r = 0.98$ was found between the effect of egg density and the hatching success. It means that a higher density of egg caused a lower hatching success during egg incubation of eggs (Fig. 12). During this spawning activity 1125 Euro was counted as total operation cost. Operation cost was calculated as follows: costs for broodstock (600 €), wintering of broodstock (100 €), Supergestran preparation (100 €), personal costs (275 €) and energy costs (50 €). It means that production of 1000 larvae costs 231 Euro.

Table 1. The fecundity of females (in total number of eggs).

Type of spawning	Mean	Standard deviation	Maximum	Minimum
<i>Artificial (15°C)</i>	30 721.2 a	28 795.9	92 003	5 234
<i>Artificial (10°C)</i>	26 615.6 a	18 600.6	75 354	3 545
<i>Semi-artificial (15°C)</i>	42 215.9 a	40 128.2	253 687	4 567
<i>Semi-artificial (10°C)</i>	22 194.7 a	14933.2	71 319	8 060

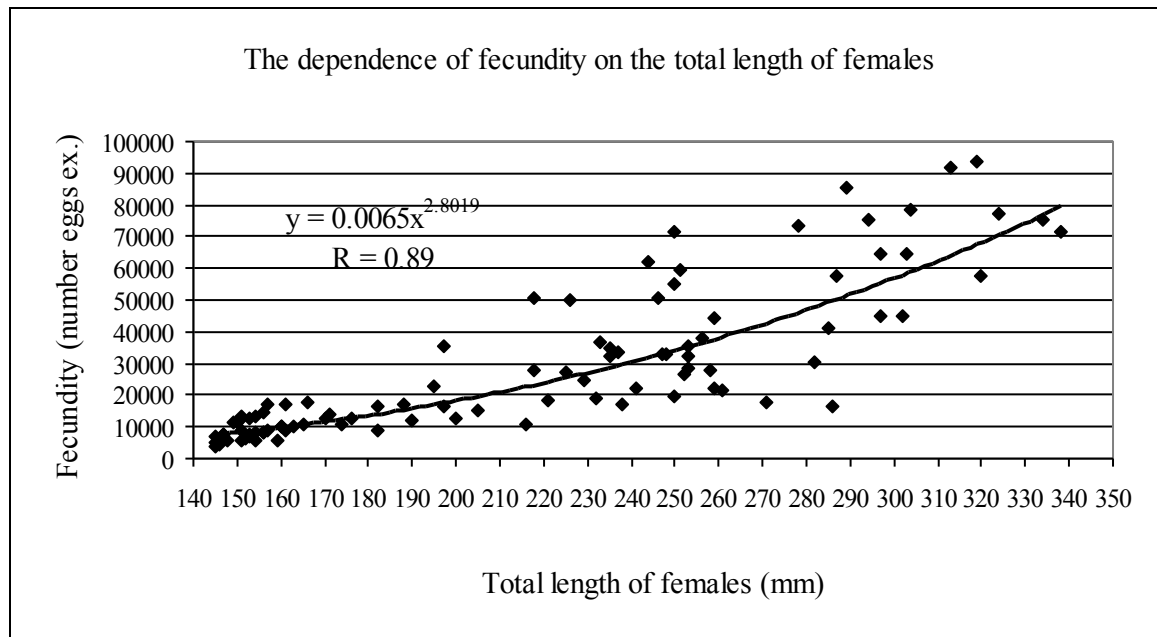


Figure 1. The dependence of fecundity on the total length of females.

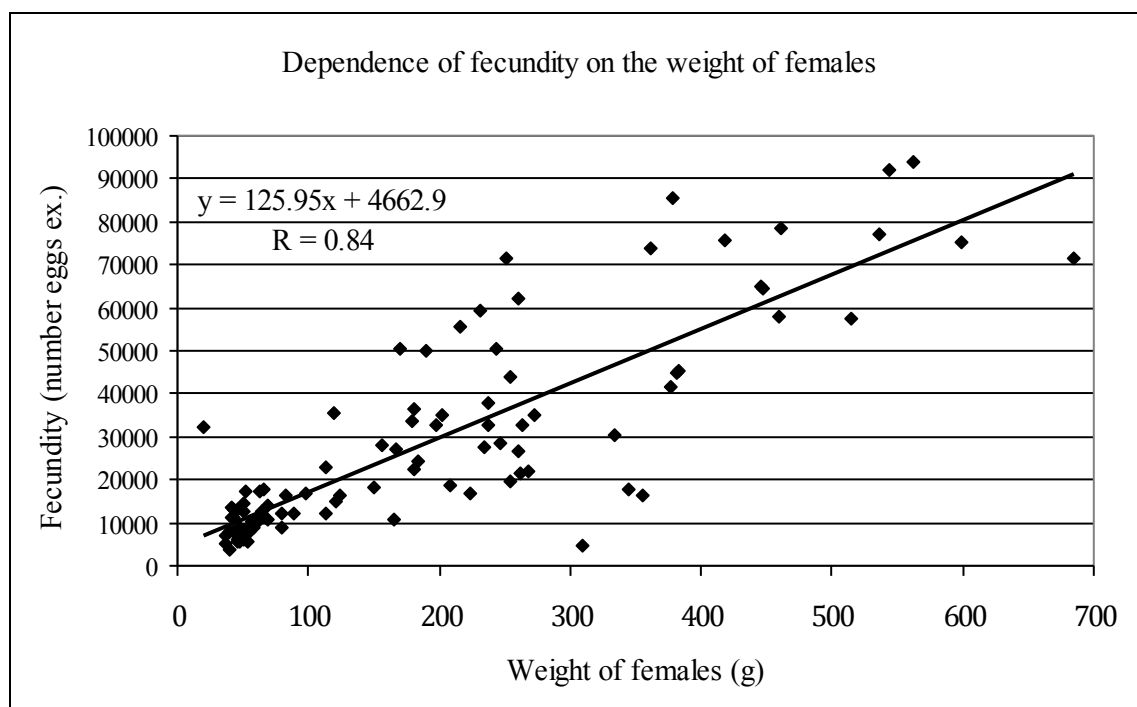


Figure 2. Dependence of fecundity on the weight of females.

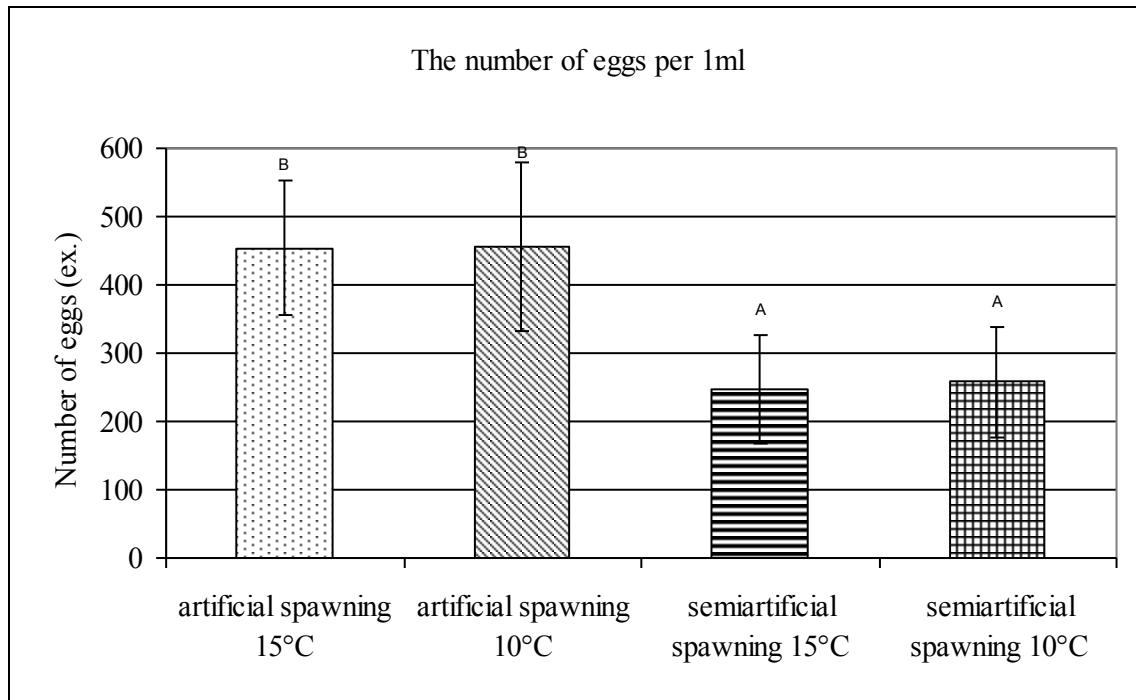


Figure 3. Number of eggs per 1 ml.

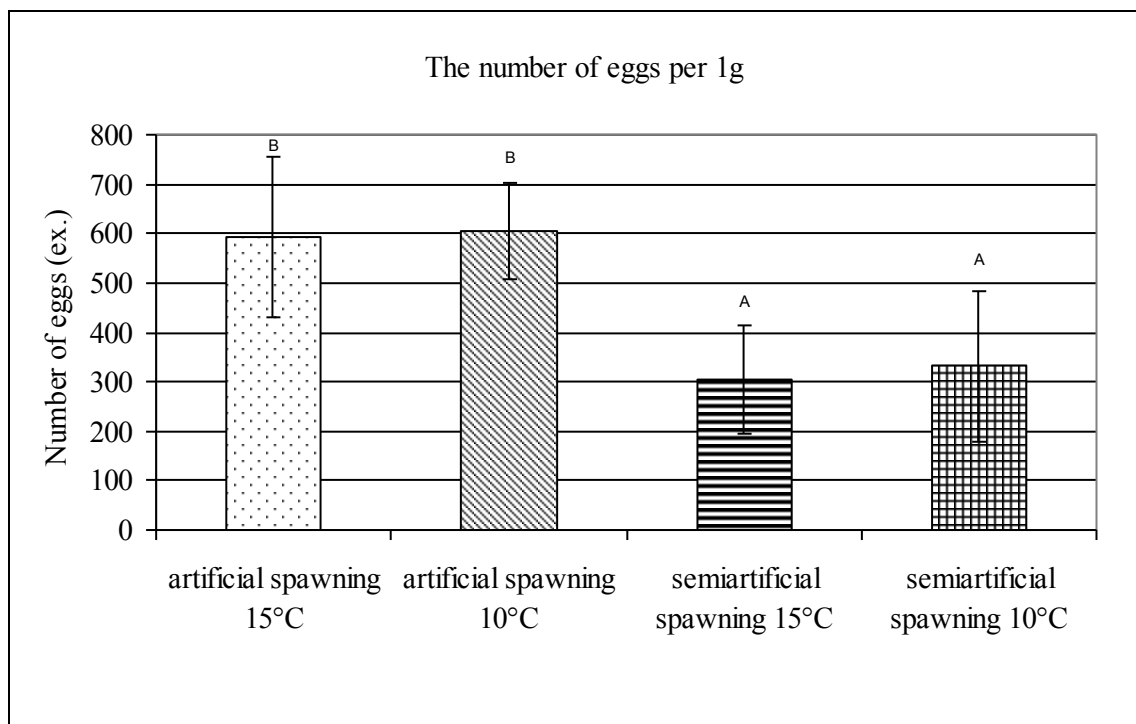


Figure 4. The number of eggs per 1 kg.

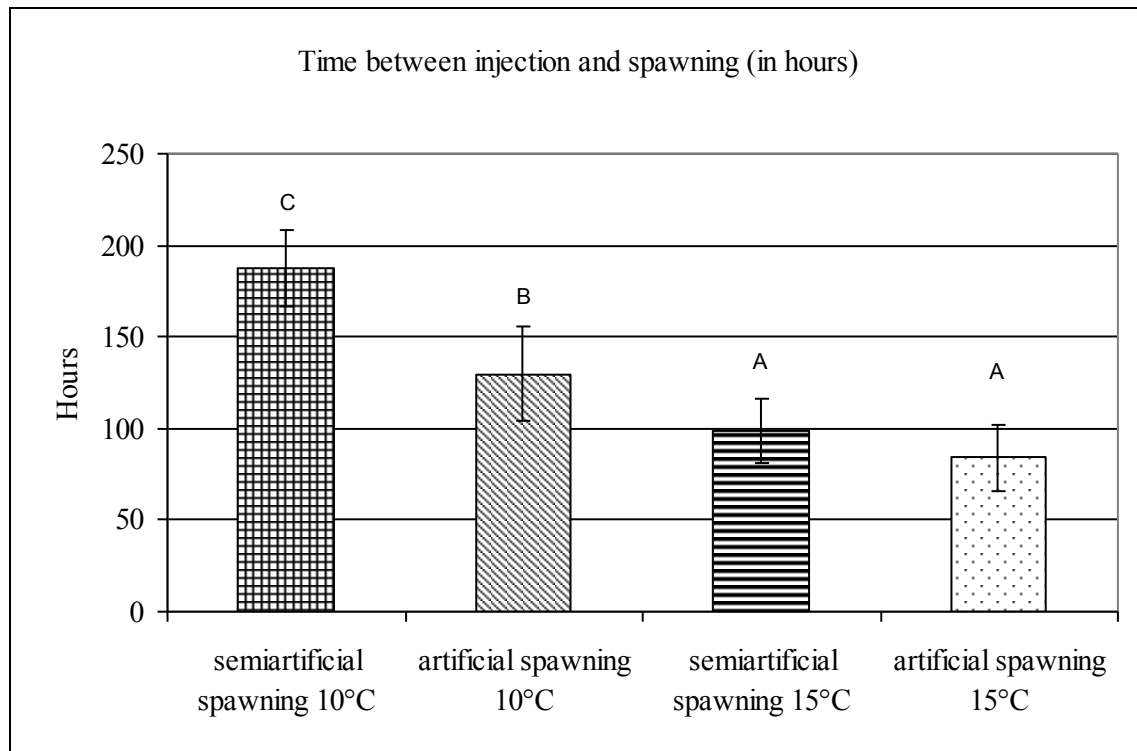


Figure 5. Time between injection and spawning (in hours).

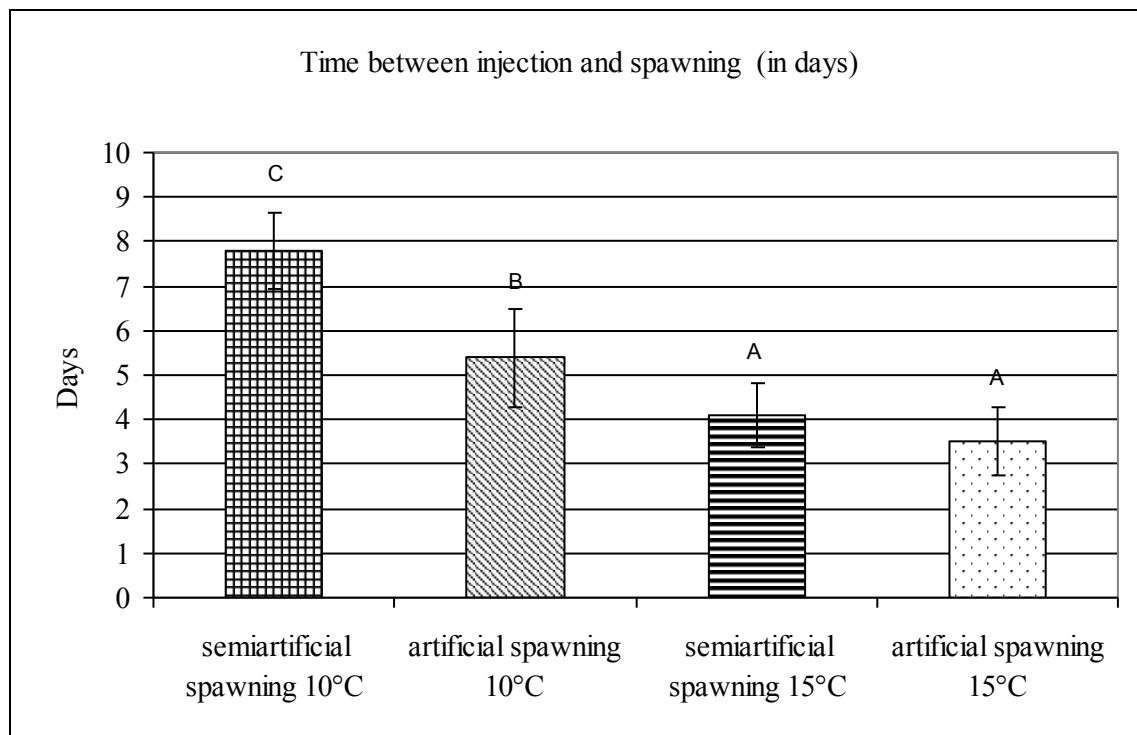


Figure 6. Time between injection and spawning (in days).

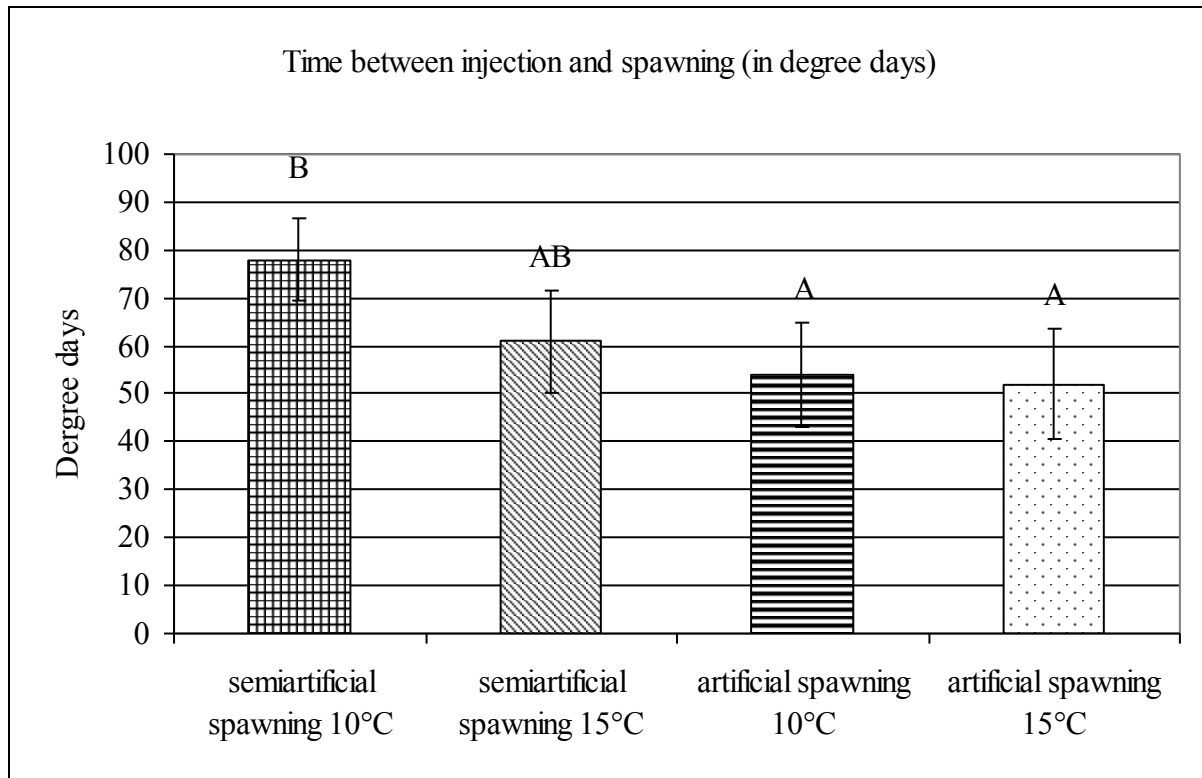


Figure 7. Time between injection and spawning (in degree-days).

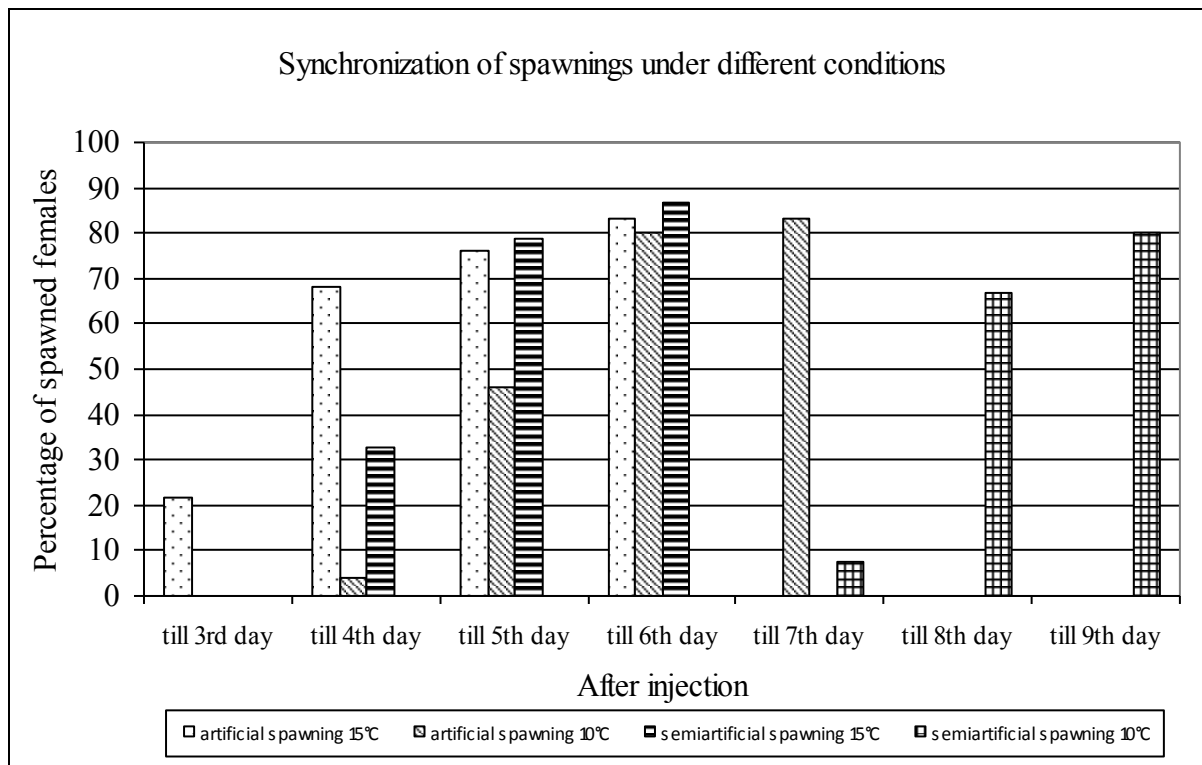


Figure 8. Synchronization of spawning under different conditions.

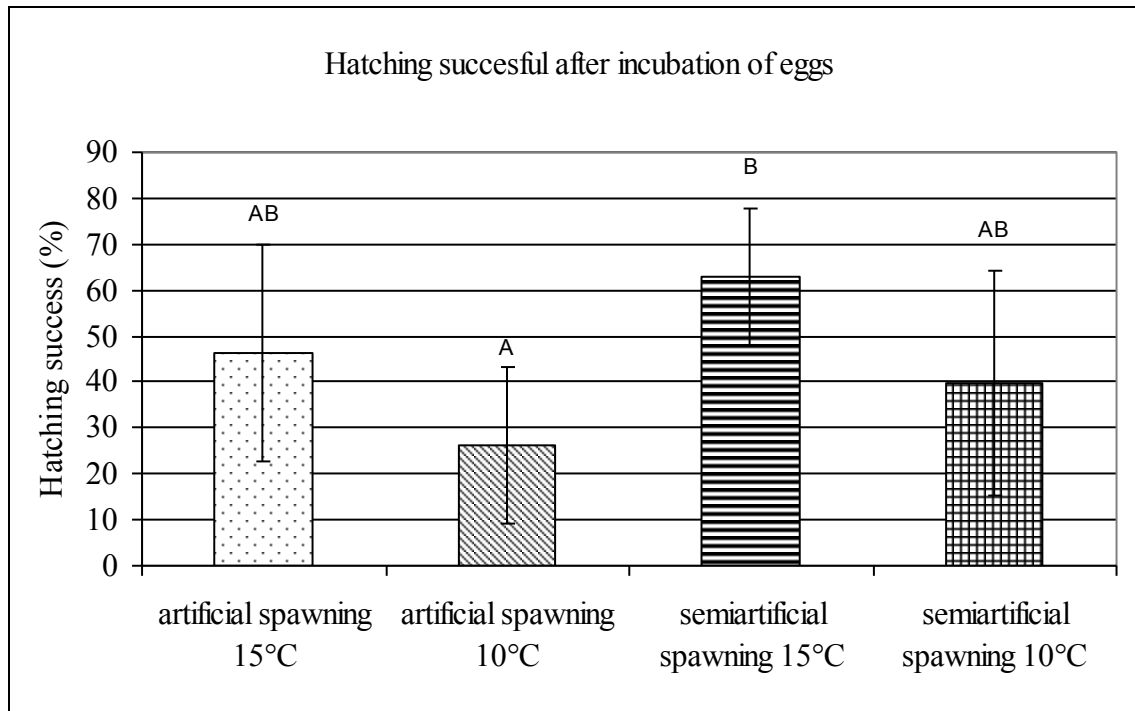


Figure 9. Hatching success after incubation of eggs.

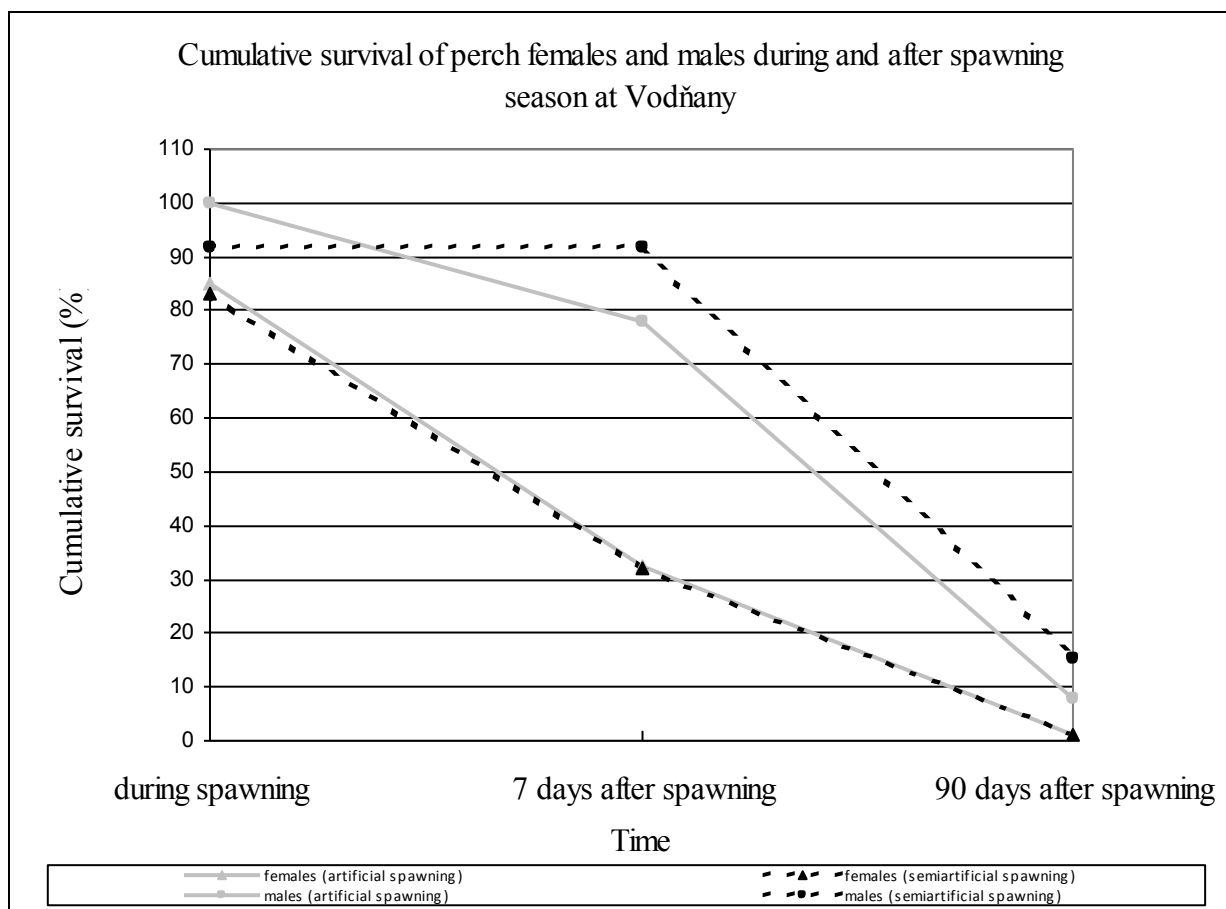


Figure 10. Cumulative survival of perch females and males during and after spawning season at Vodnany.

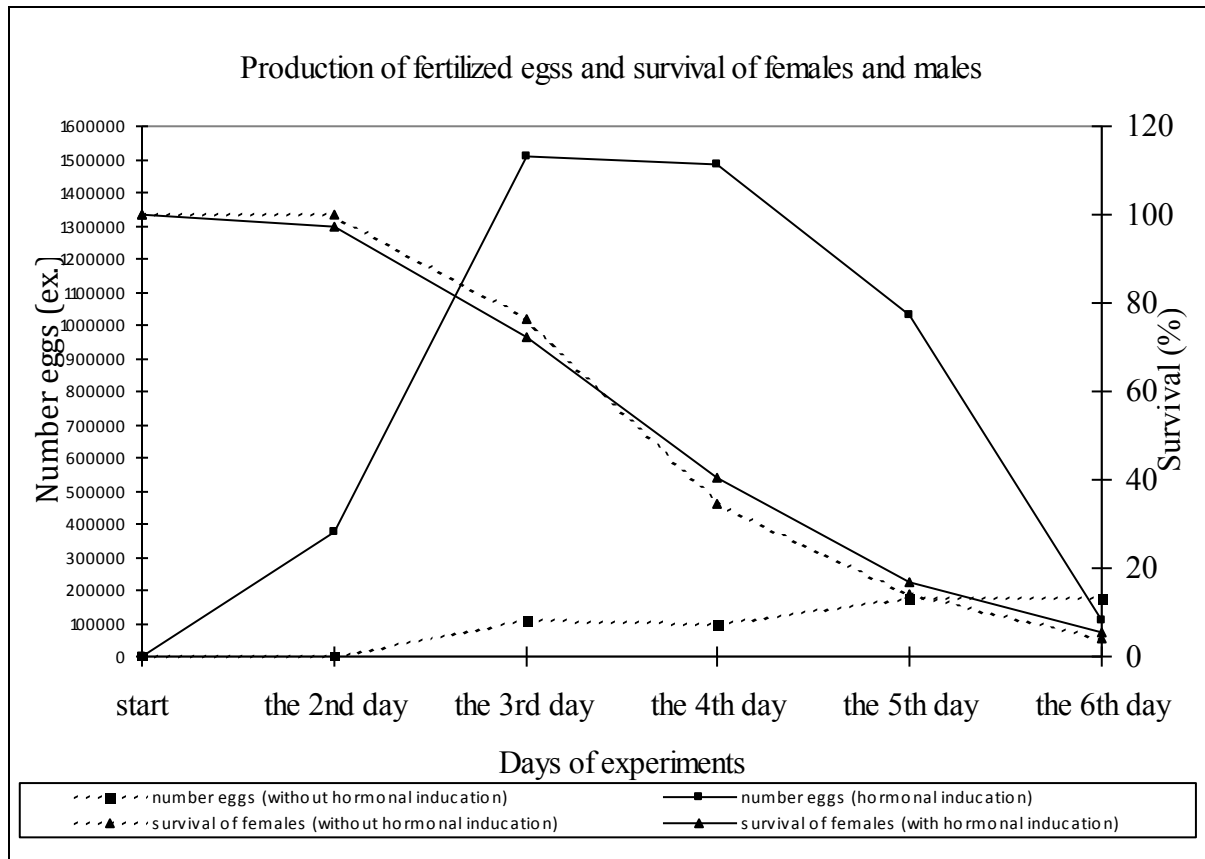


Figure 11. Production of fertilized eggs and survival of females and males.

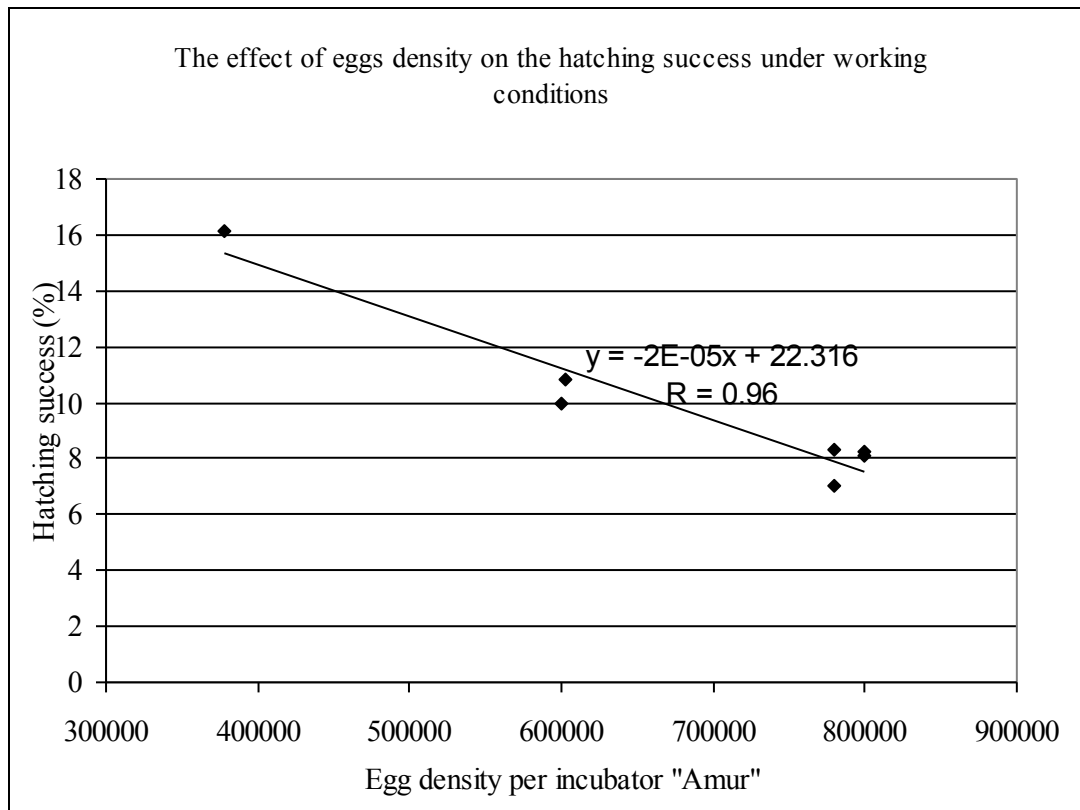


Figure 12. The effect of eggs density on the hatching success under working conditions.

c) Determination of the freezing conditions to preserve the perch

During 2005, the determination of the freezing conditions to preserve the perch sperm (with partners 7 and 8) has been performed: freeze and thaw spermatozoa keeping on their viability with respect to sperm of sex-inverted gynogenic neomales from WP 6. Neomale sperm will be obtained during 2006 from partner 9. A sperm freezing procedure will be developed, leading after thawing to high motility of spermatozoa as well as to high values of velocity and percentage of sperm motility, fertilization and hatching yield.

Materials and methods

Animals

The experiments were performed at the experimental hatchery of the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, Department of Fish Genetics and Breeding at Vodnany, Czech Republic. The broodfish were cultivated and prepared for reproduction according to pond condition. Individual broodfish suitable for stripping were selected in April and kept isolated in 1000 litre tanks with recirculation water of 0.2 l.s^{-1} at 15°C . The ovulation was stimulated by GnRH α injected intramuscularly at doses of $100 \mu\text{g}$ per kg of body weight. Sperm was collected without treating of males. Ovulated oocytes were stripped from individual females. Eggs from 3 females were not pooled, short-term stored at a temperature of 15°C and directly used for experiments. Prior to the fertilization experiments, three batches from each female of approximately 0.2 g unfertilized eggs were weighed to the nearest 0.0001 g and fixed in 4 % formaldehyde for later counting and determination of mean egg weight. On this basis, the number of eggs in a sample was expressed as the weight of the sample (g) multiplied by 763. Before each injection and gamete collection, the males and females were anaesthetized in a solution of 2-phenoxyethanol (1:1000).

Sperm Collection and Dilution

Perch sperm was individually collected during April 2005 in plastic syringes containing sperm and air (from 1:5 in volume, sperm: air) and stored under aerobic condition at 4°C for two to four hours. The quality of the extended sperm was checked for the percentage of sperm motility from video records. Ten males showed spermatozoa motility 100%; their sperm was pooled and used for cryopreservation. Pooled sperm concentration was counted in a Thoma cell haemocytometer under an Olympus microscope BX 41 (400x) and the value was expressed as the mean number of spermatozoa counted in 20 squares of the Thoma cell. Sperm was diluted 1:6 in a extender of different immobilizing solution (Kurokura solution, glucose 300 mM or 400 mM) and equilibrated for 40 min at 4°C .

Cryopreservation and thawing

Ten % of pure cryoprotectant (methanol – Fluka no.65543; DMSO (Me_2SO) – Sigma kat. no D-8779) were added to extended sperm and every 0.5 ml of mixture was transferred to 0.5 ml straws, equilibrate for 10 minutes and freeze in styrofoam box with liquid nitrogen on 3-cm-high floating frame for 3 minutes and latter plunged into liquid nitrogen. The spermatozoa were thawed in water bath temperature at 40°C for 8 s. A sample of frozen pooled of 10 males was checked in triplicate for percentage of sperm motility, sperm velocity and hatching yield. A part of the pooled sperm was kept on ice ($0-4^\circ\text{C}$) for 5 hours until used as an unfrozen control.

Experimental Design

One pool of sperm and eggs of three males was used for all experiments. For each treatment four straws were cryopreserved and the following parameters were evaluated after 5 h storage for each: velocity of sperm, % of sperm motility, % of hatching. The same parameters were measured in the control sample of unfrozen fresh sperm. The effect of two immobilizing solution and two cryoprotectants of sperm cryopreservation were studied. The cryoprotectants were DMSO (Me₂SO) at final concentrations of 10 % and methanol at final concentration of 10 %. Half ml of cryoextended sperm was frozen in straws of 0,5 ml.

Hatching Trial

Two grams of eggs (763 eggs per 1 g) were placed into a 50 ml dish; an accurate volume of sperm (thawed or non frozen as control) with 400,000 spermatozoa per one egg was dropped on them from a micropipette. The dish was then placed on a shaking table set to 300 rpm and 10 mm deflection. Ten ml of hatchery water at 15°C was added. Two minutes later, all eggs were placed into a special incubator cage of 200 ml supplied with UV-sterilized recirculated tap water at 23°C, 9 mg.l⁻¹ O₂. For each experiment, the procedure was repeated three times. After hatching (approximately 11 days after fertilization); the number of larvae was counted in each cage as well. The percentage of hatching rate (H_r) was calculated for each cage from the number of hatched larvae (H_l) and from the total number of eggs placed in the cage (E_l) as follows: $H_r = (H_l/E_l) \times 100$

Observation and measurement of sperm motility and velocity

Dark field microscopy and a Sony camera were used as described by Billard et al. (1997), Cosson et al. (2000) and Linhart et al. (2002). Percentage motility and velocity was examined at 200x magnification, with sperm pre-diluted 1:200 with KUROKURA solution containing 220 mM of NaCl and immediately after mixing 0,5 µl of pre-diluted sperm with 49 µl of 30 mM NaCl swimming medium supplemented with 0.1% BSA on a glass slide prepositioned on the microscope stage. The final dilution was 1:20000. BSA was added to prevent sperm heads from sticking to the glass slide. Sperm motility was recorded on video tape from 0 to 1 min after activation. The focal plane was always positioned near the glass slide surface. Video records were made with a S-VHS (SONY, SVO-9500 MDP) video recorder at 50 half frames.s⁻¹ using a CCD video camera (SONY, SSC-DC50AP) mounted on a dark-field microscope (Olympus BX 50) and visualized on a video monitor illuminated with a Strobex stroboscopic lamp (Chadvick-Helmut, 9630, USA) with a frequency of 50 Hz. Velocity and motility were assessed at 10 s after activation: the successive positions of the video - recorded sperm heads were analyzed from video frames by means of Olympus Micro Image software (Version 4.0.1. for Windows with a special macro by Olympus C&S). The velocity and percentage motility were measured by evaluating spermatozoa head positions on five successive frames with three different colours (frame 1 - blue, frames 2 - 4 - green and frame 5 - red). The analyses were repeated 3 times from 3 records at 10s after activation, i.e. frames 1-5. Thirty to forty spermatozoa were evaluated from each frame. Motile spermatozoa were visible in three colours, while non-moving spermatozoa were white. Percentage of motile spermatozoa was easily calculated from white versus red coloured cells (green was not used). Velocity of spermatozoa was calculated as µm.s⁻¹ based on the length traces of sperm heads (blue-green-green-green-red) of the motile spermatozoa after calibration of magnification.

Data analysis

Mean values of the investigated parameters and SD were calculated 3 and 10 replicates for hatching and velocity and percentage of sperm motility, respectively. Statistical significance was assessed using multiple analysis of variance (ANOVA, Statgraphics version 5), followed

by Tukey HSD multiple range test comparisons. Probability values < 0.05 were considered as significant.

Results and discussion

Control sperm had good quality (Fig. 1) with hatching rate of 72 %. In experiments the sperm quality changed depending on treatment with range from 50 to 78 %. The best hatching rates of 78 % (not significantly) were obtained when sperm was freezing in IS of 300 mM glucose with 10 % of methanol. These results were not significantly different from the control sample (Tab. 1). The percentage motility in frozen/thawed sperm ranged from 19 to 74 %. The percentage motility in the fresh sperm (control) was 100 %. Ten % methanol with 400 mM glucose not significantly yielded to the highest percentage motility in post-thawed sperm (74 %). These results were not significantly different from the control sample (Tab. 2). The velocity of frozen-thawed spermatozoa ranged from 48 to 149 $\mu\text{m}\cdot\text{s}^{-1}$. Methanol at concentrations of 10 % with 300 mM of glucose yielded the highest velocity in frozen-thawed sperm (149 $\mu\text{m}\cdot\text{s}^{-1}$) which was still significantly lower to the velocity of the control (186 $\mu\text{m}\cdot\text{s}^{-1}$, Tab. 3). Cryopreservation of sperm appears to be a useful and reliable technique not only for conservation of sperm for experimental condition but also for wide-ranging artificial propagation (see Fig. 4).

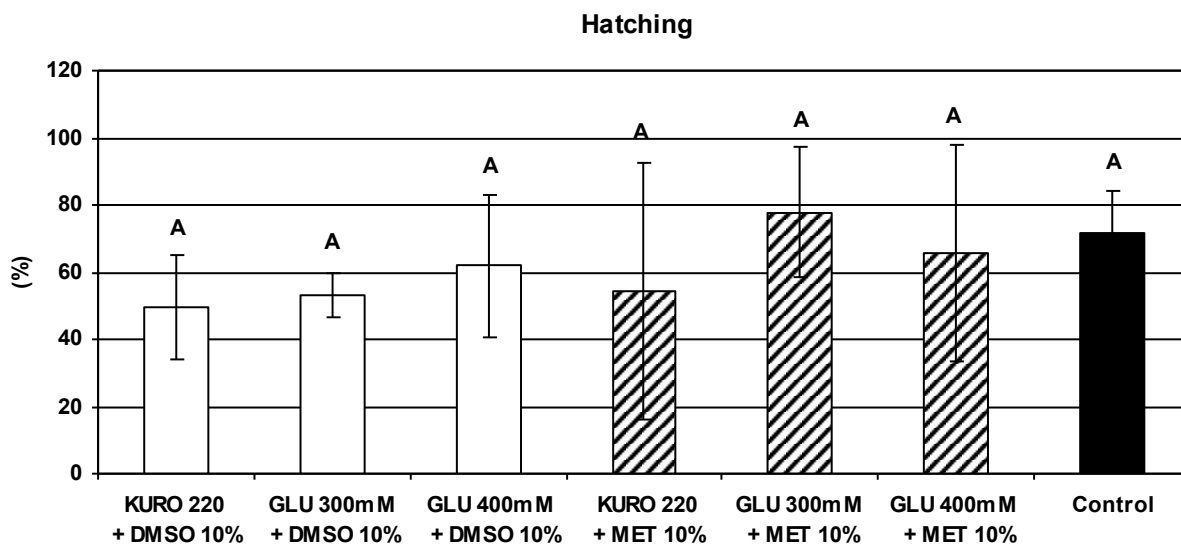


Figure 1. Effect of two different cryoprotectants and three different immobilizing solutions on the percentage of hatching of frozen/thawed sperm. Mean values of 3 replicates are shown with SD. Groups with a common superscript for each parameter do not differ significantly ($P < 0.05$).

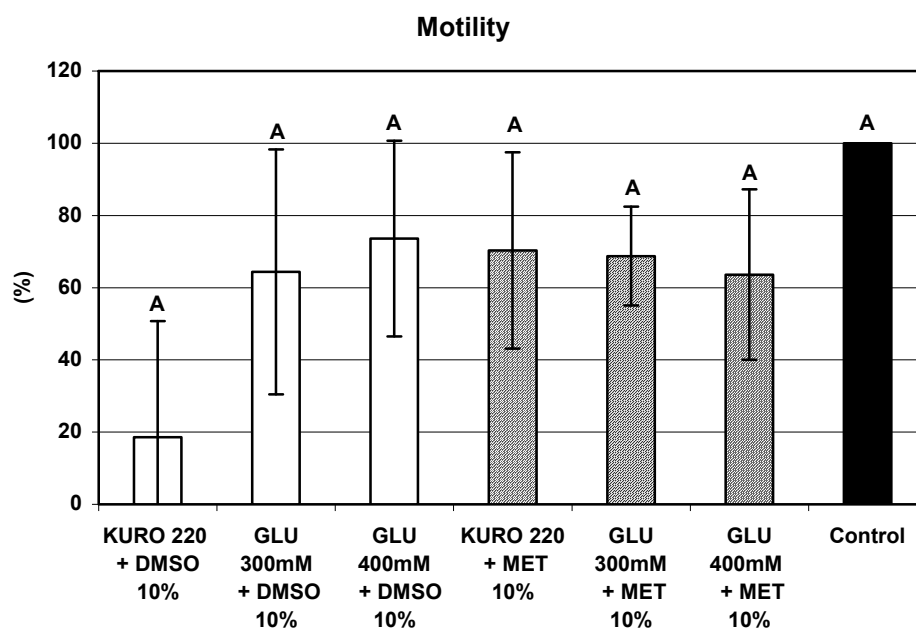


Figure 2. Effect of two different cryoprotectants and three different immobilizing solutions on percentage of sperm motility of frozen/thawed sperm. Measurements were made 10 s after activation. Mean values of 3 replicates are shown with SD. Groups with a common superscript for each parameter do not differ significantly ($P < 0.05$).

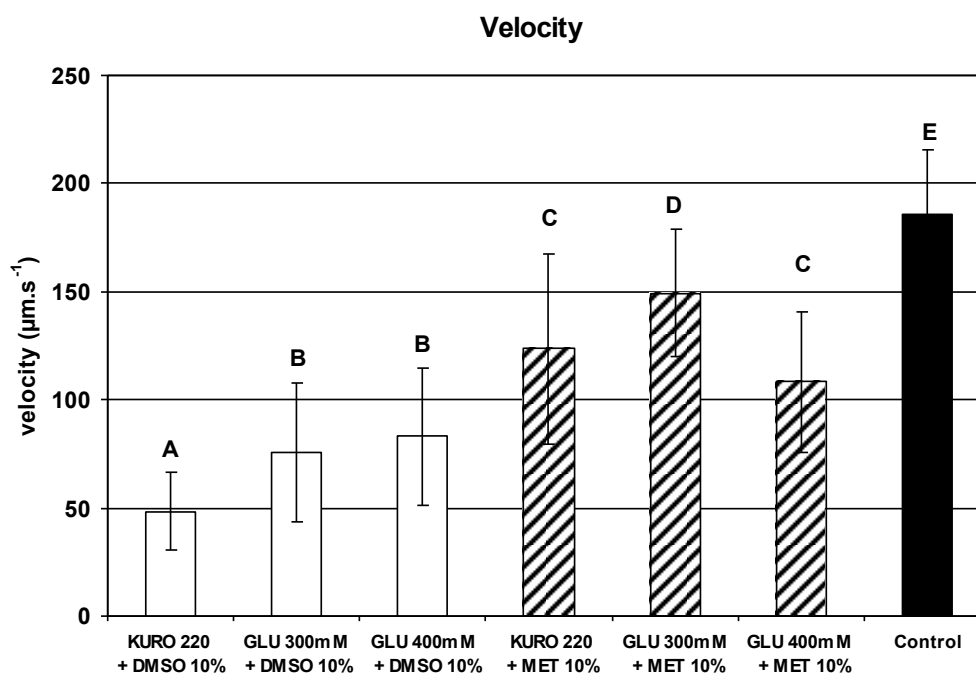


Figure 3. Effect of two different cryoprotectants and three different immobilizing solutions on sperm velocity ($\mu\text{m}\cdot\text{s}^{-1}$) of frozen/thawed sperm. Measurements were made 10 s after activation. Mean values of 10 replicates are shown with SD. Groups with a common superscript for each parameter do not differ significantly ($P < 0.05$).

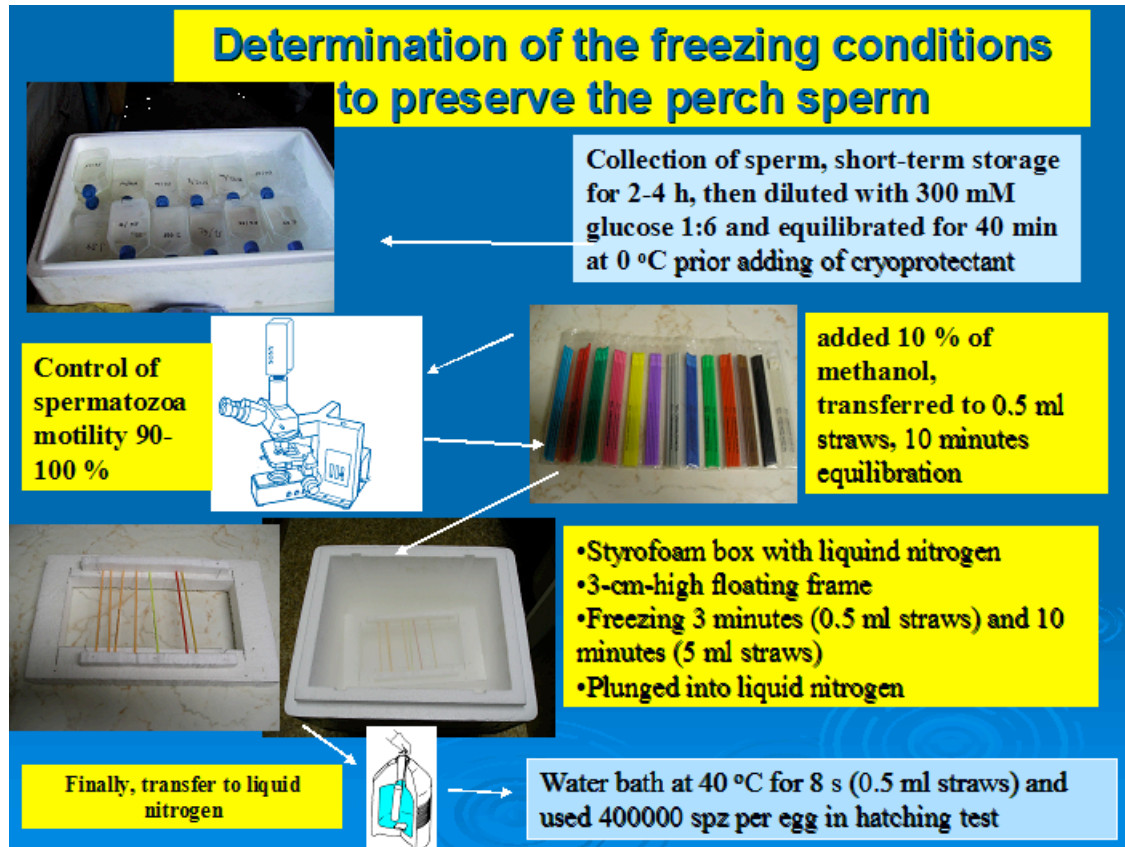


Figure 4. Scheme of cryopreservation procedure of perch sperm.

References

- Billard, R., Linhart, O., Fierville, F and Cosson, J., 1997. Motility of *Silurus glanis* spermatozoa in the testis and in the milt. *Pol. Arch. Hydrobiol.*, 44: 115-122.
- Cosson J., Linhart, O., Mims S.D., Shelton W.L. and Rodina M., 2000. Analysis of motility parameters from paddlefish (*Polyodon spathula*) and shovelnose sturgeon (*Scaphirhynchus platyrhynchus*) spermatozoa. *J. Fish Biology*, 56, 1348-1367.
- Kouril J., Hamackova J., 1999. Artificial propagation of european perch (*Perca fluviatilis*) by means of a GnRH analogue. *Czech J. An. Sc.* 44, 309-316.
- Linhart O., Rodina M. and Cosson J., 2000a. Cryopreservation of sperm in common carp *Cyprinus carpio*: Sperm motility and hatching success of embryos. *Cryobiology*, 41, 241-250.
- Linhart, O., Mims S.D., Gomelsky B., Hiott, A.E., Shelton W.L., Cosson J., Rodina, M. and Gela, D., 2000b. Spermiation of paddlefish (*Polyodon spathula*) stimulated with injection of LHRH analogue and carp pituitary extract. *Aquatic. Liv. Res.*, 13(6), 1-6.
- Linhart, O., Cosson J., Mims S.D., Shelton W.L., Rodina, M., 2002. Effects of ions on the motility of fresh and demembrated paddlefish (*Polyodon spathula*) spermatozoa. *Reproduction*, 124, 713-719.

- *Deviations from the project workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

C.2 - Sub-task 3.2: Breeders mortality and welfare (WP5)

Responsible: FUNDP (Partner 8)

Associate partners:

SMEs: Bornholms Hatchery (Partner 2), Dil-Vis (Partner 4)

RTD: LSA-UHP (Partner 7), Ulg (Partner 9)

- *Workpackage objectives and starting point of the work*

This workpackage is dealing with two main objectives:

- understand whether high mortality encountered in female Eurasian perch during the reproduction period may be related to a trade-off between reproduction and immune status and/or to stress exhaustion related to winter environmental conditions,
- define a feeding strategy for the improvement of reproductive performances and survival rates of breeders.

High mortality rate (see WP 2 and 4 sections) during the reproduction period is one of the major constraints for the development of perch culture. The imposed factors for a high morbidity during the reproduction period has not been investigated in Eurasian perch, but previous reports in some fish species indicate a trade-off between reproduction processes and immune defense (Slater and Schreck, 1998; Kortet et al., 2003). As for mammalian immune system, such phenomenon may be related both to an energy re-allocation for reproductive processes and to seasonal environmental changes via the neuroendocrine system. The increase in sex hormone releases may affect the immune system in various ways. It has been reported that high oestradiol and 11-ketotosterone levels induce stimulation and inhibition of lymphocyte proliferation in rainbow trout (Harris and Bird, 2000). Moreover, high release of androgens may affect the number of antibody-producing cells in fish and this inhibitory effect is enhanced by stress hormones, especially cortisol, which affects directly activities of head kidney and leukocytes (Weyts et al., 1998; Harris and Bird, 2000). The specific objective in the first year was to characterize immunological status of wild broodstock and captive breeders under different stocking and feeding conditions in order to determine to what extent rearing conditions affect immune system during the reproduction period.

- *Progress toward objectives*

The specific objective in the first year was to characterize immunological status of wild broodstock and captive breeders under different stocking and feeding conditions in order to determine to what extent rearing conditions affect immune system during the reproduction period.

Materials and Methods

Experimental protocol

To characterize an eventual trade-off between reproduction processes and immune system, three groups of captive breeders were compared three times during gonad maturation and/or spawning period (January, February and March):

- captive breeders were submitted to constant conditions of temperature (23°C) and photoperiod (12L:12D) which promotes growth in Eurasian perch but not gonad development and maturation = CC group,
- captive breeders were reared in outdoor tanks under natural nycthemeral variations and forage feeding to ensure an efficient reproductive maturation = CN group,

- captive breeders submitted to controlled out-season conditions to advance the spawning period (early February instead of mid-April). Such manipulated rearing conditions allowed a comparison before and after spawning = CDK group.

In addition, wild breeders from the Meuse River were captured before (late March), during (mid-April = Wgroup) and after (late April = Wp group) spawning for comparison to captive breeders. Wild females were not checked during gonad development because we felt to catch fish by nets or electrofishing between December and February. For each sampling period, 6 to 10 fish were checked.

Methods

Different parameters were measured, namely:

1. Body condition and metabolic indicators
 - gonado-somatic index GSI (100 x gonad weight/body weight),
 - spleen-somatic index SSI (100 x spleen weight/body weight),
 - Plasma triiodothyronine (T3)
2. Immunological parameters by haematimetric counting and spectrophotometric methods:
 - Erythrocyte and lymphocyte number,
 - Plasma lysozyme activity,
 - Plasma transaminase enzymes (GOT and GPT),
 - Spleen respiratory burst (by NBT test).
3. Stress and metabolic indicators:
 - Plasma cortisol, chloride and glucose,
 - Osmolality.

Fish were blown just after capture from tanks to avoid any effect of anaesthetic on measured parameters. Then, blood samples were made in maximum five minutes before dissections. After centrifugation (4500 RPM, 20 min, 10°C), plasma was stored at -20°C until assayed. For wild breeders, net fishing was placed over the night, and captured fish were sampled as soon as possible in the following morning. Stress indicators were not checked for these wild fish because of an eventual effect of capture fishing on cortisol release. Data were compared by a two-way-ANOVA followed by a Sheffe post-hoc test, and significance taken at $p < 0.05$.

Results and discussion

Body condition and metabolic indicators

As expected, data concerning gonado-somatic index show that no gonad maturation was attained for captive breeders under constant conditions of rearing (Fig. 1). By contrast, captive breeders submitted to natural nycthemeral variations showed an efficient gonad development with a GSI comparable to wild females in March. Apart from high values in late April for wild breeders, no changes were observed with the time because gonad development was already established since January when we started the experiment. Spleen-somatic index was not affected by gonad development between January and March because values were comparable between fish under constant conditions than fish in natural conditions (Fig. 2). Also, values did not differ before and after spawning in wild females in late April, however captive fish had a significant bigger ($P < 0.05$) spleen than wild breeders. In teleost fish, a large spleen has been reported as indicating an enhanced capacity to respond effectively to infection or increased immunological activity from already established infections, therefore the decrease in spleen size during breeding season in some fish species has been considered as a potential indicator of a decline in immune functions (Kortet et al., 2003). Plasma

triiodothyronine level indicate that metabolic activity was temperature dependent with the highest values in fish submitted to intensive culture conditions at 23°C (Fig. 3). T3 values did not significantly differ between groups of breeders under natural temperature conditions (CN, CDK and Wp), but decreased during the induced spawning period in February in fish under out-season conditions (CDK).

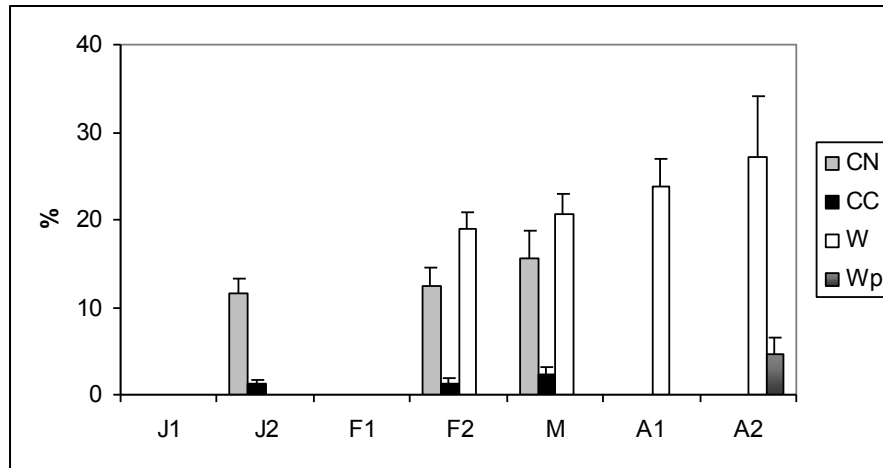


Figure 1. Changes in gonado-somatic index according to the time and environmental conditions. CN = breeders submitted to natural conditions of temperature and photoperiod; CC = breeders under intensive culture (23°C); W = wild breeders captured in mid-April; Wp = wild breeders captured in late April after spawning.

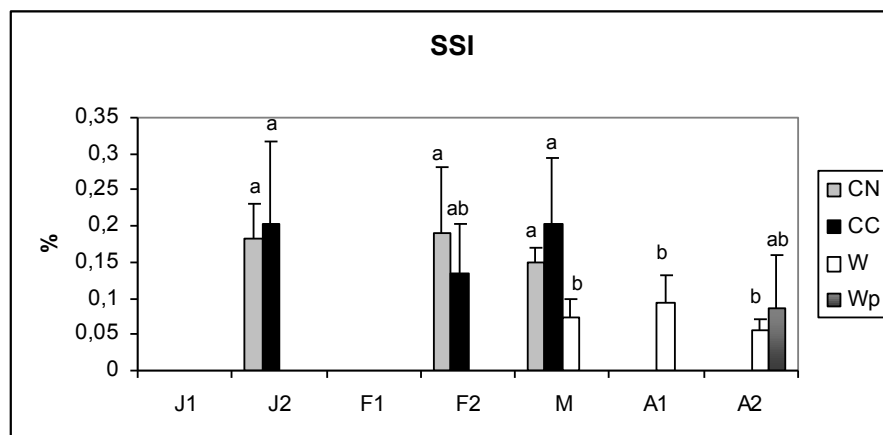


Figure 2. Changes in spleen-somatic index according to the time and environmental conditions. Groups are compared as in Fig. 1.

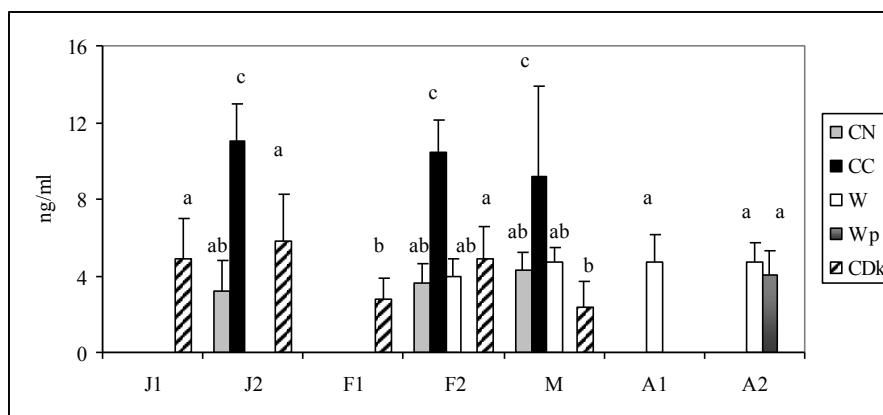


Figure 3. Changes in plasma triiodothyronine level according to the time and environmental conditions. Groups are compared as in Fig. 1.

Erythrocyte and lymphocyte numbers

No significant differences were observed between captive and wild females indicating a same haematopoietic status whatever the living conditions. Moreover, no changes were observed with the time for captive breeders indicating that haematopoietic process was not affected by reproductive events. Lymphocyte number (Fig. 4) was higher ($P < 0.05$) in captive fish reared under natural conditions than fish in intensive culture. This may indicate a higher defence capacity in breeders with efficient gonad maturation since the same was observed in wild fish even lymphocyte number did not differ before and after spawning in the latter group.

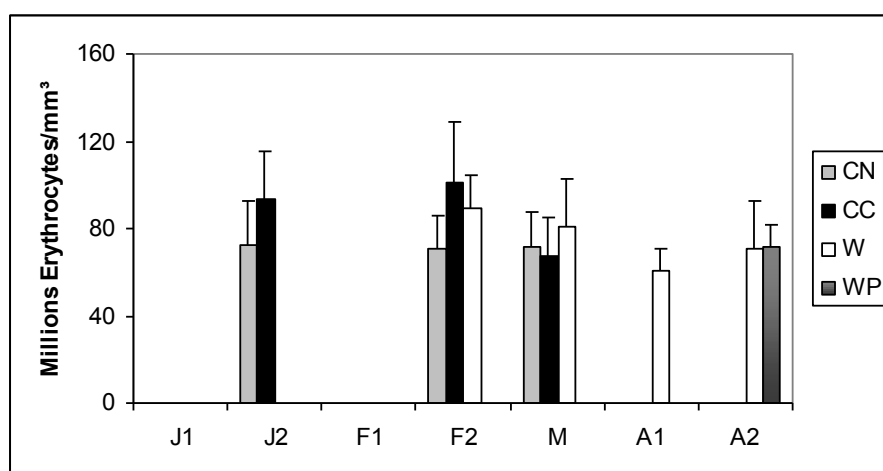


Figure 4. Changes in erythrocyte number with the time and environmental conditions. Groups are compared as in Fig. 1.

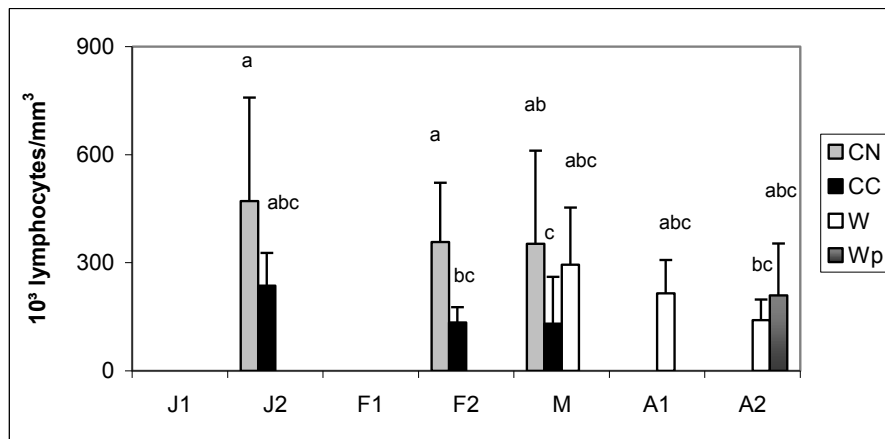


Figure 5. Changes in lymphocyte number with the time and environmental conditions. Groups are compared as in Fig. 1.

Respiratory burst and lysozyme activities

Spleen production of oxidative radicals was significantly ($P < 0.05$) higher in breeders under natural conditions (CN) than in fish under intensive rearing (CC, Fig. 6). This may be related to gonad maturation process as wild breeders had lower respiratory burst than CN group at the same temperature in March. A high respiratory burst activity has been reported in other fish species as an investment in non-specific defence as an adaptation to low temperatures during autumn and winter or to changes in physiological status (Secombes, 1996 in Kortet et al., 2003). Lysozyme activity interfered with temperature conditions with the highest values at 23°C in captive fish under constant conditions but values were comparable in the three groups in March. In wild breeders, values decreased significantly ($P < 0.05$) during spawning and increased after, as an indication of immune trade-off.

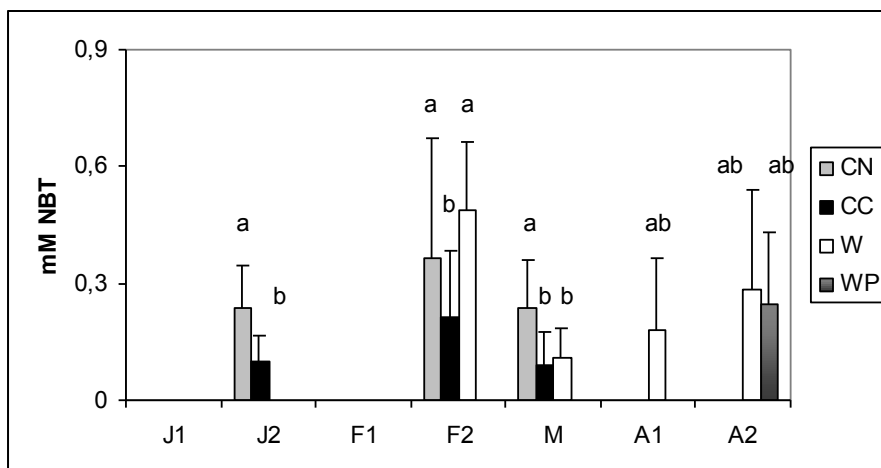


Figure 6. Changes in spleen production of oxidative radicals with the time and environmental conditions. Groups are compared as in Fig. 1.

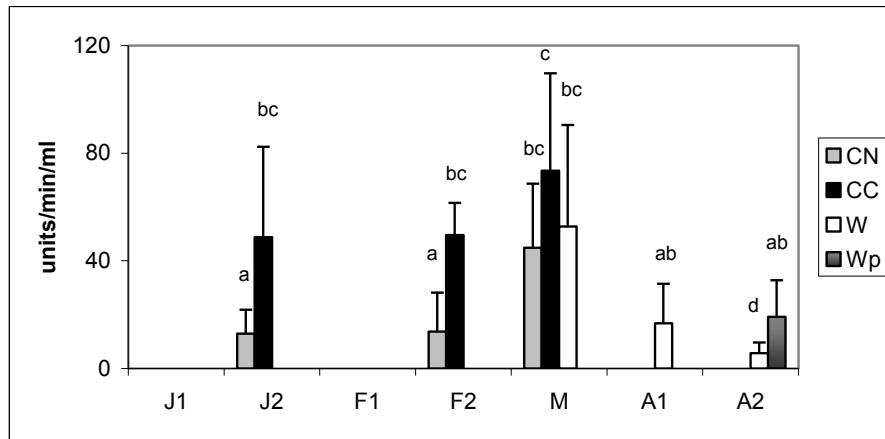


Figure 7. Changes in lysozyme activity with the time and environmental conditions. Groups are compared as in Fig. 1.

Transaminase activities

Changes in transaminase levels, which are usually related to variations in hepatic oxidation show the same profiles for glutamic oxalic acid transaminase - GOT (Fig. 8) and glutamic pyruvic acid transaminase - GPT (Fig. 8). Values fluctuated with the time, and were comparable between captive breeders and wild fish in March or before and after spawning in April, indicating no significant trade-off between reproduction events and hepatic oxidation processes.

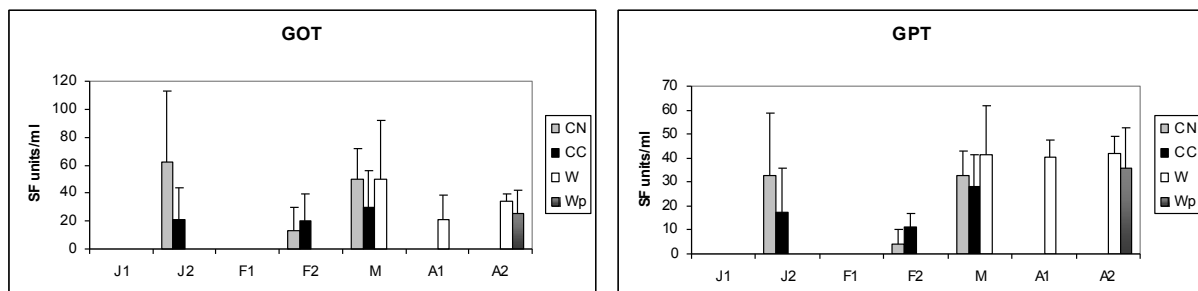


Figure 8. Changes in transaminase activities with the time and environmental conditions. Groups are compared as in Fig. 1.

Stress indicators

Most of the measured parameters (plasma glucose and chloride levels and osmolality) for stress evaluation did not indicate a stressfully status during the gonad maturation for captive breeders, as compared to values in others fish species. Plasma cortisol and glucose levels did not significantly differ in breeders under natural conditions (10.2 ± 7.9 ng/ml, 670.53 ± 338 μ g/ml) and in intensive culture (15.43 ± 9.23 ng/ml, 822.34 ± 259 μ g/ml). Nevertheless, plasma cortisol increased significantly before spawning in February for breeders in out-season conditions (Fig. 9), such a high cortisolemia may affect some compounds of immune system.

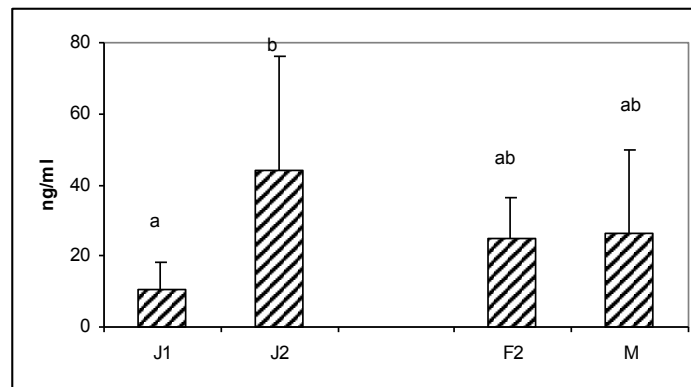


Figure 9. Changes in plasma cortisol level with the time and environmental conditions in captive fish under out-season conditions. Groups are compared as in Fig. 1.

Conclusions

The objective of the present study was to answer the question whether high mortality encountered in female Eurasian perch during the reproduction period may be considered as a trade-off between reproduction and immune status and/or as an exhaustion related to winter environmental conditions. Overall, the results did not show an important trade-off between reproduction and immune system because different immune activities were unaffected during gonad maturation and spawning in captive breeders. However, a reduction in lysozyme and respiratory burst activities and a smaller spleen size were shown in wild breeders at a similar temperature as captive breeders in March. An increase in cortisol release related to environmental conditions not to reproductive processes may be involved in a such down-regulation even if other measured indicators for stress did not change. To complete the analytical aetiology of high mortality rate of breeders during the reproduction period, some immunological parameters will be made during the 2nd year of the project using samples from protocols of WP3 dealing with extension of natural reproduction period (partner 10). As described in the workplan of this project, a second experiment will be carried out in order to define the optimal dietary level of essential fatty acids and the feeding strategy for improving disease resistance in perch during the breeding season.

References

- Harris J. and Bird D.J., 2000. Modulation of the immune system by hormones. *Veterinary Immunology and Immunopathology* 77: 163-176.
- Kortet R., Taskinen J., Sinisalo T. and Joskinen I., 2003. Breedin-related changes in immunocompetence, health state and condition of the cyprinid fish, *Rutilus rutilus*, L. *Biological Journal of the Linnean Society* 78:117-127.
- Slater C.H. and Schreck C.B., 1998. Season and physiological parameters modulate salmonid leucocytes androgen receptor affinity and abundance. *Fish and Shellfish Immunology* 8: 379-391.
- Weyts F.A.A., Verburg-van Kemenade B.M.L. and Flik G., 1998. Characterisation of glucocorticoid receptors in peripheral blood leukocytes of carp, *Cyprinus carpio* L. *Gen. Comp. Endocrinol.* 111:1-8.

- *Deviations from the project workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

D - Task 4: Production of juvenile with genetically improved performances (WP6)

Responsible: Ulg (Partner 9)

Associated partners:

SMEs: Lucas Perches (Partner 1), Bornolms Hatchery (Partner 2), NH (Partner 5)

RTD: USB (Partner 11)

This workpackage is divided in six different points.

D.1 - Sub-task 6.1: Effect of domestication

- *Workpackage objectives and starting point*

The objectives of this sub-task are to compare the growth performance of 3 generations (F1, F2, F3) of captive juveniles and to produce a domesticated strain with improved growth and survival performances.

- *Progress towards objectives*

Material and methods

Breeders and quality of eggs

Three generations of breeders (wild, first captive generation – F1 and third captive generation – F3) were reared in 1.6 m³ tank in a recirculating aquaculture system, with a natural thermal cycle (ranging from 4 to 25°C, Fig. 1). Breeders (55 fish per batch) were fed with forage fish. At the end of March 2005, spawn substrates (boughs) were placed into each tank. During the reproductive period (from early April until mid-May), spawns ribbon were collected each morning, weighted (± 0.1 g) and a sample of eggs (± 500 mg) counted in order to estimate the total number of eggs in the spawn. Some spawn were also collected from the wild (Robertville Lake, Belgium). Strands were placed into a hatchery at 15°C. Four to 5 days later, fertilization rate were estimated on a sample of eggs and calculated as the number of eyed embryos relative to the total number of eggs into the sample. For each ribbon, another sample of eggs (± 10 g) was isolated in order to estimate the hatching rate as the number of hatched larvae relative to the number of fertilized eggs. One day before hatching, fertilized spawns were transferred into the larval rearing system.

Larval resistance and rearing

Larval quality was estimated with both an osmotic and a thermal stress resistance tests. Two-days post-hatching larvae in duplicate were placed in a 9% saline solution during 90 minutes for the osmotic test or at 30°C during 90 minutes for the thermal test. Dead larvae were removed every 15 minutes. Larval were reared in 10 m² fertilized (chicken manure) outdoor tanks at 17°C and up to 6 ppm O₂. Two days after hatching larvae were fed with *Artemia* nauppli during the first 6 days and with *Artemia* nauppli and larval food from day 7 to day 21. After 22 days of rearing, larvae were weaned. Individual body weight (± 0.1 mg) was

measured two days after hatching and every week on 50 fish. At each control, the number of malformations (lordosis and scoliosis) was estimated on 50 fish.

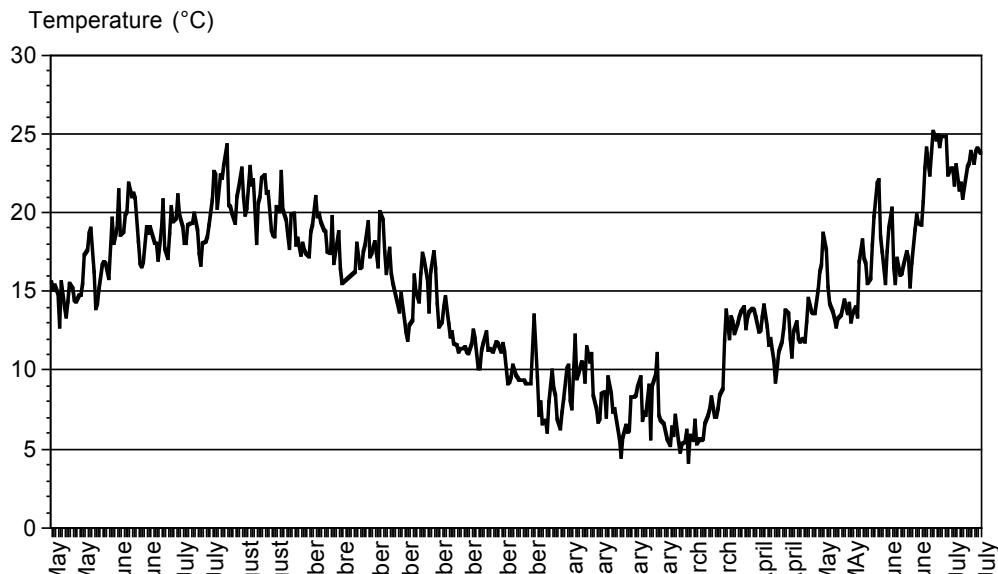


Figure 1. Thermal cycle of the breeders in a recirculating system.

Juveniles rearing

After 54 days of rearing, juveniles were transferred into 1.6m³ tanks in a recirculating system at 23°C and O₂ > 6ppm. F2 (one family) and F4 (four families) juveniles were reared in duplicate at an initial stocking density of 3125 individual m⁻³. Only one batch of the wild generation (two families) was reared at the same initial stocking density. Fish were fed at the maximum ration $R_{max} (\%) = [7.6 W(g)^{-0.31}]$ and food was delivered with an automatic belt feeder during 12 hours. Every two weeks, mean body weight was estimated on 500 juveniles and the total biomass estimated in order to adapt the food ratio.

Results and discussion

Breeders and quality of eggs

Survival rate of the breeders during all the experimental period ranged from 10.3 (F1 breeders) to 81.3 % (wild breeders) with a differential mortality (95% of dead fish were females). Most of the losses occurred during the chilling period (from October 2004 to February 2005, Fig. 2) except for the wild breeders which displayed a mortality rate of 18.8 % during and after the reproductive period, suggesting that they were more sensitive to intensive rearing conditions during the reproductive period.

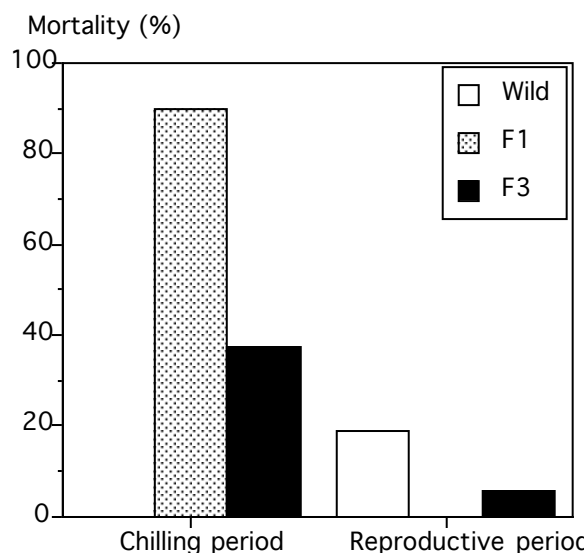


Figure 2. Mortality of the breeders during the chilling process and during the reproductive period.

The spawning rate (number of spawn relative to the number of females) ranged from 60.0 % for the wild breeders to 100% for the F1 breeders (Tab. 1). Nevertheless for this generation, only one female survived and we cannot consider the results obtained for this generation as significant. Spawns originated from the F3 breeders displayed a mean fertilization rates (69.2%) two-fold higher than those obtained during previous years with less domesticated breeders (31.2 and 36.9 % for F1 breeders and 26.3 and 49.1 % for F2 in 2003 and 2004 respectively). Hatching rates for the 3 generations ranged from 39.1 to 58.9 %. Eggs originated from the F3 breeders displayed a mean hatching rate (44.3%) two to ten-fold higher than those obtained during previous year with less domesticated breeders (4.9 and 1.5 % for F1 breeders and 25.8 and 5.9 % for F2 in 2003 and 2004 respectively).

Table 1. Number of spawns, fertilization and hatching rates of eggs from the 3 strains of breeders.

Breeders	Number of spawns	of Spawning rate (%)	Fertilization rate (% , mean \pm SE)	Hatching rate (% , mean \pm SE)
Wild	6	60.0	90.7 \pm 4.8	39.1 \pm 11.0
F1	1	100.0	77.8	58.9
F3	36	67.1	69.2 \pm 6.7	44.3 \pm 3.9

Larval resistance and rearing

Initial mean body weight of 2-days post-hatching larvae ranged from 0.98 to 1.06 mg with the F1 strain displaying the lowest initial mean body weight (Tab. 2). The F4 generation displayed an important initial malformation rate (34.5%) 3.8-fold higher than the F1 generation, suggesting that the larval quality of the F4 generation was lower than for the F1. The F1 generation displayed a higher resistance (LD50 : 42min) to the osmotic stress than the 2 other generations (LD50 : 35 and 36 min for F2 and F4 respectively, Fig. 3), suggesting a higher quality of this progenies. Generally, larvae were less resistant to the thermal choc, with a LD50 ranging from 30 to 34 minutes (Fig. 4).

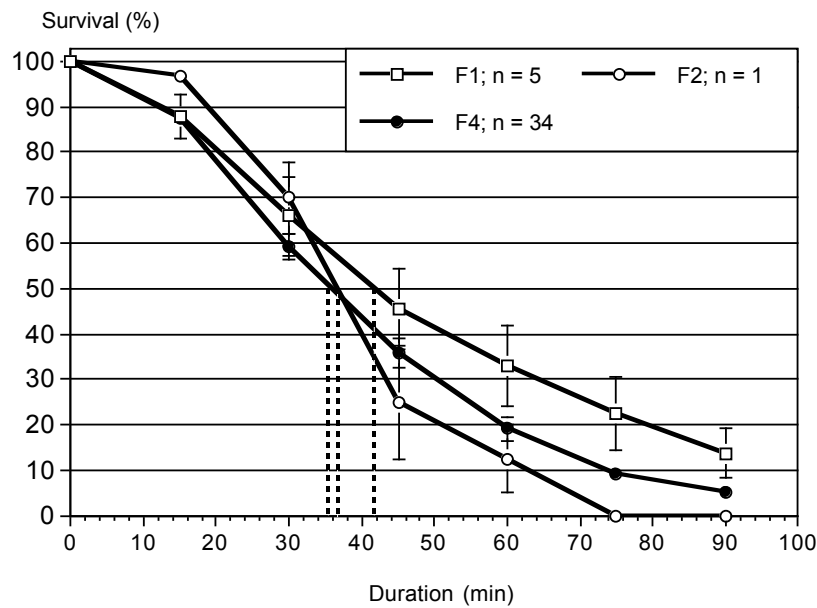


Figure 3. Larval resistance to an osmotic choc (9% NaCl), applied on 2-days post-hatching larvae during 90 minutes. n = number of batches.

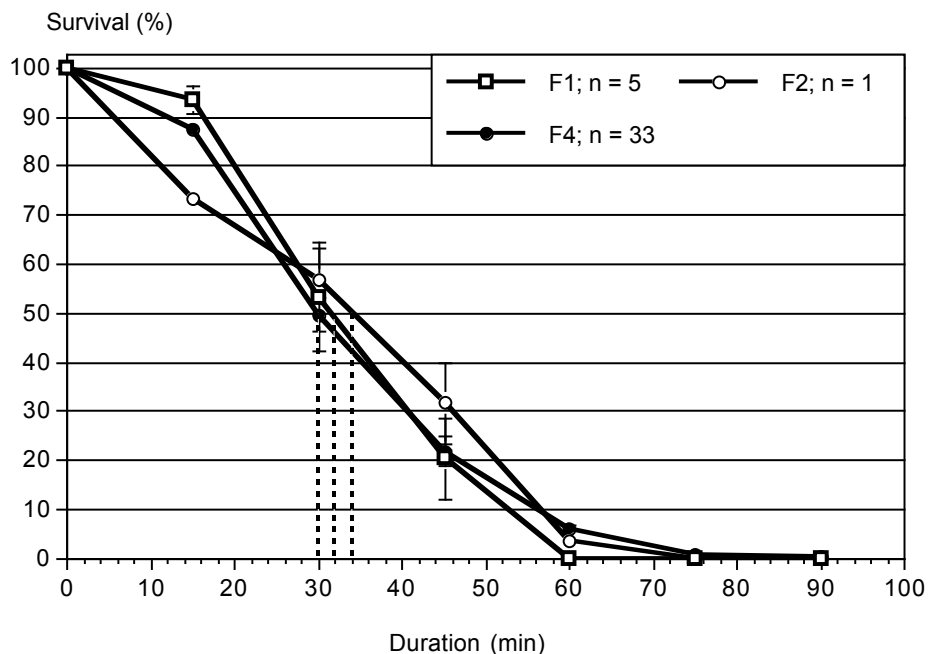


Figure 4. Larval resistance to a thermal choc at 30°C, applied on 2-days post-hatching larvae during 90 minutes. n = number of batches.

The initial stocking density were 2300 larvae.m⁻² for the F2 and F4 generation in duplicate and 1580 larvae.m⁻² for the F1 generation, with only one batch (Tab. 2). Initial mean body weight ranged from 0.98 to 1.06 mg with the F1 strain displaying the lowest mean body weight (Tab. 2). During all the larval rearing period, F1 larvae grew faster than the 2 other strains (Fig. 5) and at the end of the larval rearing period, F1 strains display the highest final

body weight (517.6 mg). This huge difference of mean body weight (3-fold) was probably due to the difference between the initial stocking density which was 1.5-fold lower in the F1 batch. At the end of the larval rearing period, the malformations rates ranged from 1.0 to 2.0% for all the generations, suggesting that the majority of the distorted larvae died during the larval period (probably cannibalized).

Table 2. Survival, initial and final body weight and malformations rates and percentage of cannibal of the 3 strains of perch larvae reared in 10m² tanks in a semi-intensive system at 17°C during 46 days.

	N	Initial Number of larvae	Survival (%)	Body weight (mg)		% cannibal	Malformations rates (%)	
				Initial	Final		Initial	Final
F1	1	15876	34.0	0.98	517.6	0	9.0	2.0
F2	2	22614	35.6±3.1	1.05±0.0	224.2±11.6	0.55±0.05	20.0±0	1.0±1.0
F4	2	23648	55.8±9.2	1.06±0.6	176.7±11.3	0.45±0.15	34.5±0.5	2.0±2.0

Juveniles rearing

Survival rates ranged from 70.3 (F1) to 74.1% (F2, Tab. 3), wild juveniles being more sensitive to bacterial diseases than the domesticated strain. On the contrary, F1 strain displayed the lowest number of cannibal (0.02% vs 0.59 and 1.52% for F2 and F4 respectively).

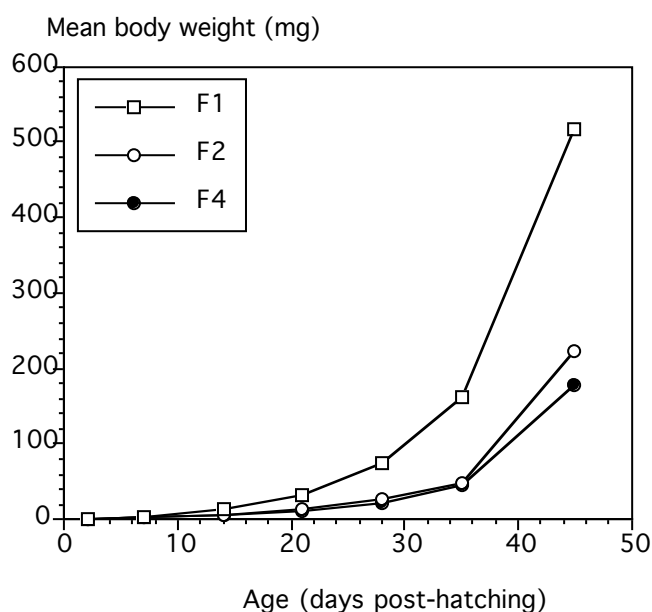


Figure 5. Growth of the 3 strains of domesticated larvae (F1, F2 and F4) reared under semi-intensive conditions (1580 and 2300 larvae.m⁻² for F1 and F2-F4 respectively) in 5m³ tank at 17°C during 46 days. Values are means of two replicates for F2 and F4 generations.

Table 3. Initial and final mean body weight ($n = 200$ fish) and CV, survival, % of cannibal, specific growth rate (SGR), growth and food conversion ratio (FCR) of the 3 generations of perch juveniles reared in 1.6m^3 tank in a recirculating system ($t^\circ = 23^\circ\text{C}$, $\text{O}_2 > 6$ ppm) at an initial stocking density of 3125 fish. m^{-3} during 80 days. Values of survival, % of cannibals, SGR and FCR are mean of two replicates.

	Initial body weight (mg)	Final body weight (g)	Initial CV (%)	Final CV (%)	Survival (%)	Cannibal (%)	SGR (%)	Growth (mg. ind ⁻¹ .j ⁻¹)	FCR
F1	588.4±21.3	17.2±0.5	30.8	29.9	74.0	0.60	4.39	205.1	0.77
F2	360.5±12.1	19.8±0.5	33.4	33.8	72.2	1.52	4.94	240.0	0.80
F4	405.0±12.8	22.7±0.7	31.7	46.1	70.3	0.02	5.22	289.5	0.89

Growth of the 3 strains of juveniles is represented in Fig. 6. Initial mean body weight of wild juveniles was significantly ($P < 0.05$) higher (588.4mg) than the initial mean body weight of the two domesticated strains (360.5 and 405.0 mg for F2 and F4 juveniles respectively, Fig. 7). Until 120 days post-hatching, the F1 generation (wild) displayed a higher mean body weight than the domesticated one, but from 120 days post-hatching, the domesticated generation grew faster than the wild one. The final mean body weight of the F4 generation (22.7g) was significantly ($P < 0.05$) 12.5% and 25% higher than the final mean body weight of the F2 (19.8g) and the F1 (17.2g) generations respectively (Fig. 8).

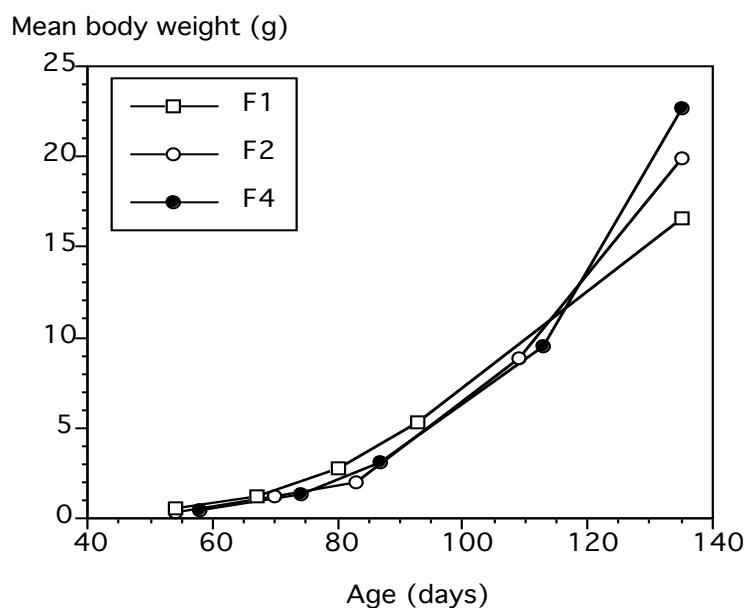


Figure 6. Growth of the 3 strains of domesticated juveniles (F1, F2 and F4) reared under intensive conditions (3125 ind. m^{-3}) in 1.6m^3 tank in a recirculating system at 23°C and $\text{O}_2 > 6$ ppm. Values are means of two replicates for F2 and F4 generations.

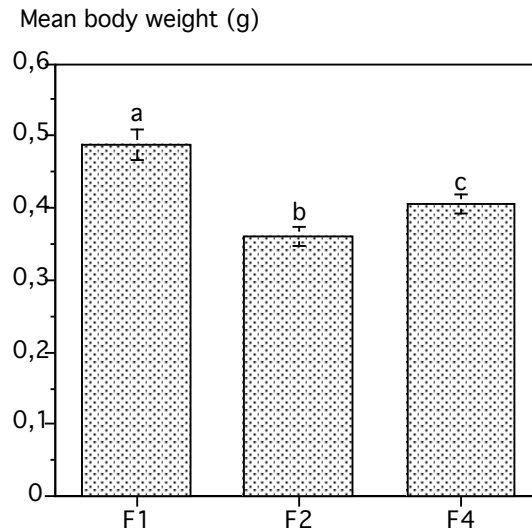


Figure 7. Mean body weight (values \pm SE; $n = 100$) of the 3 strains of juveniles perch at 54 days post-hatching at the beginning of the experimental period of juvenile growth (initial stocking density : 3125 individual m^{-3}). Bars with different letter are significantly ($P > 0.05$) different.

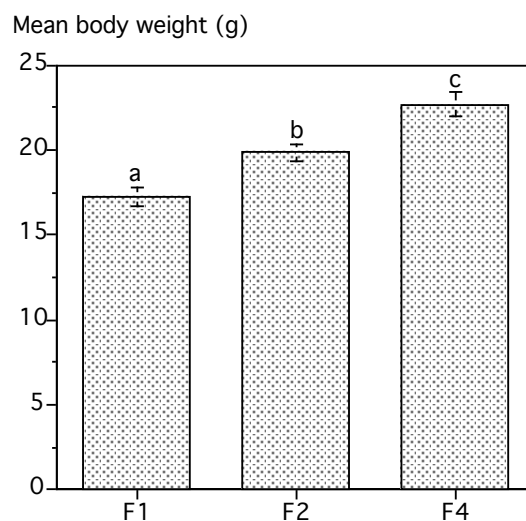


Figure 8. Mean body weight (values \pm SE; $n = 100$) of the 3 strains of juveniles perch at 135 days post-hatching (R.A.C at 23°C, initial stocking density: 3125 individual m^{-3}). Bars with different letter are significantly ($P > 0.05$) different.

The F4 generation displayed the best specific growth rate (5.22%) compared to the two other strain (Tab. 3). The food conversion ratio was also better for the 2 domesticated strain (0.77 and 0.80 for F2 and F4 respectively) compared to the F1 strain (0.89, Tab. 3).

Conclusions

Spawns quality, in term of fertilization and hatching rates, was significantly increased with the domestication, spawn originated from F3 breeders displaying higher fertilization and

hatching rates than spawns originated from F1 and F2 breeders. Nevertheless, the larval quality of the domesticated F4 generation seems to be lower than the wild generation as the malformation rate at hatching and the resistance to the osmotic stress was higher and lower respectively. The growth performances of the F1 larvae were significantly higher than the domesticated strain. Nevertheless, this difference seems to be related to the difference between the initial stocking density of the batches. After the larval rearing period, the best growth performances in term of final mean body weight, specific growth rate and food conversion ratio, were obtained with the domesticated F4 generation, which was in concordance with the milestone and expected results of the sub-task. The comparative growth experiment of juvenile will continue until the end of March 2006. The experiment on eggs and larval quality and larval growth will be also realized during the second year of the project in order to confirm the results obtained during this first year.

- *Deviations from the project workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

D.2 - Sub-task 6.2: Effect of sex-control

- *Workpackage objectives and starting point*
- *Progress towards objectives and starting point of work*

The objective of this sub-task is to produce all-female populations and to compare the growth performances of all-female and mixed-sex fish.

Material and methods

Eggs ribbons were obtained by abdominal stripping of the F1 female and divided into two equal batches. One batch was fertilized with XX male sperm (determined based on the gonad morphology after sacrifice) and the other batch was fertilized with normal male sperm (control group). After larval rearing as described in sub-task 6.1, fish were transferred in 0.5m³ tanks (in duplicate) at an initial stocking density of 1000 fish.m⁻² in a recirculating aquaculture system at 23°C and up to 6 ppm O₂. Fish were fed at the maximal ration and mean body weight and total biomass were measured every two weeks.

Results and discussion

Survival rates of the batches were 69.8 and 71.1% for the control and the mixed-sex respectively. When fish reached a mean body weight of 30g (170 days of rearing), all-female families grew faster than mixed-sex families (Fig. 9). After 360 days of rearing, all-females families displayed a mean body weight 30% higher (143g) than the mixed-sex families (103g). The market size (80g) was reached within 250 days with all-females families against 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).

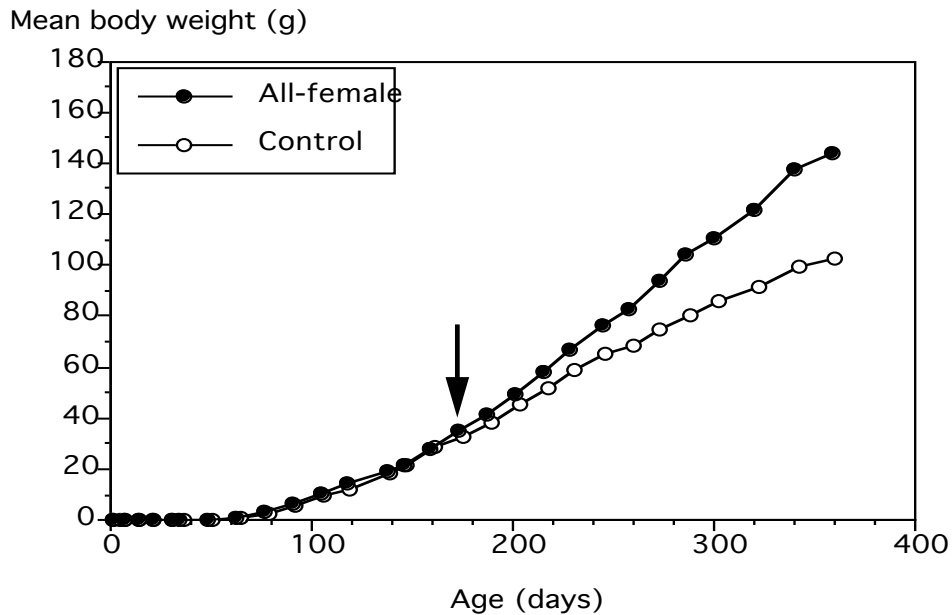


Figure 9. Growth of all-female and mixed-sex juveniles (F2 generations) reared under intensive conditions in 0.5m³ tank in a recirculating system (23°C) at an initial stocking density of 2000 fish.m⁻³ and a final stocking density of 400 fish.m⁻³.

Conclusions

The increase of growth performances (30% after 360 days of rearing) and the improvement of food conversion ratio with all-females families will allow to significantly improve the productivity of the perch reared under intensive conditions. This result was in concordance with the milestone and the expected result of the sub-task.

- *Deviations from the project workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

D.3 - Sub-task 6.3: Effect of hybridization

- *Workpackage objectives and starting point of work*

The objective of this sub-task was to compare growth performances and survival of the hybrids and the pure species.

- *Deviations from the project workprogramme*

In accordance with all others partners, this sub-task was removed from the WP6 after the first Percatech meeting.

D.4 - Sub-task 6.4: Effect of triploidisation

- *Workpackage objectives and starting point of work*

The objectives of this sub-task were to produce a triploid population in mass scale and to evaluate the productivity gain when rearing diploid and triploid populations in a growth test.

- *Deviations from the project workprogramme*

In accordance with all others partners, this sub-task was removed from the WP6 after the first Percatech meeting.

D.5 - Sub-task 6.5: Functional XX-male

- *Workpackage objectives and starting point of work*

The aim of this sub-task is to produce completely functional XX male breeders.

- *Progress towards objectives*

Material and methods

Five batches of 1000 sexually undifferentiated juveniles (initial mean body weight : 40 mg) originated from the F4 strain were constituted in 0.5m³ tank in a recirculating system at 23°C. Fish were fed during 30 days with 5 doses (5, 10, 20, 30 and 40mg kg⁻¹ food) of masculinizing steroid (17 α -methyltestosterone – 17MT) incorporated into food. The 17MT was dissolved in 95% ethyl alcohol and mixed to the food 24h00 before feeding in order to allow the evaporation of the ethyl alcohol. Fish were fed at the maximal ratio $R_{\max} (\%) = [7.6 W(g)^{-0.31}]$ which was delivered with an automatic belt feeder during 12h00. After 6 months of rearing (mean body weight = 30 g, October 2005) 100 fish per batch will be sacrificed and sex ratio determined based on the gonad morphology (a double gonad for the male, a single gonad for the female and a single twisted gonad for the sex-reversed male).

Results and discussion

Results of the hormonal treatment (percentage of sex reversal) and the presence of the sperm duct will be present in the second progress report.

- *Deviations from the project workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

D.6 - Sub-task 6.6: Natural spawning of XX male

- *Workpackage objectives and starting point of work*

The objective of this sub-task is to produce all-female population by natural reproduction in tanks using completely functional sex-reversed males.

- *Progress towards objectives*

This sub-task will be realized during the second year of the project as it was previously planned into the program.

- *Deviations from the project workprogrammes*

For the moment, all scheduled experiments have been carried out in the corresponding delay.

E - Task 5: Socio-economic study of the juvenile cost production (WP7)

Responsible: RIVO (Partner 10)

Associated partners:

SMEs: All partners

RTD: All partners

- *WP objectives and starting point of work*

The overall objective of this work package is the assessment of the production costs of perch fingerlings in relation to:

- The rearing system employed
- Different socio-economic contexts
- Out-of-season production
- Other management options
- Options for future research

- *Progress towards objectives*

During the first year of the project, a model was constructed that is able to calculate production costs for perch fingerlings. The model is now in the process of being adapted to the various production methods used by the project partners. The model is a flexible model: technical data, production data, and costs are variables. To be able to calculate the cost of fingerling production under different management regimes, information is being gathered to feed the model. This data is generated and collected in several field trials.

In the mean time data was collected for costs price analysis by intensive or semi-intensive rearing of perch fry under natural feeding pond conditions (Partner 5, 6 and 11). During the first year, experiments have focused on the:

- Autumn adaptation of perch fingerlings from pond into recirculation (controlled) conditions and their following rearing.
- Rearing of advanced fry under pond conditions.
- Pond culturing of juveniles during the whole growing season

Materials and methods

a) Autumn adaptation of perch fingerlings from pond into recirculation (controlled) conditions and their following rearing.

Typical natural ponds are used for fish aquaculture in the Czech Republic. Common carp with other supplemental fish (e.g. tench, silver carp, grass carp, perch, pike perch, pike and cat fish) had been reared under pond conditions each year. A lot of fingerlings of perch have been produced from ponds, where carp with perch of marked size have been reared, during autumn

harvest season. During autumn harvest season we obtained 26 000 fingerlings (mean total body length 72.8 ± 3.07 mm and mean total body weight 3.63 ± 0.47 g) of perch from ponds of Nové Hradky farm. These fingerlings were transported into RIFCH Vodňany (Partner 11). Fingerlings were stocked into two earthen ponds for short acclimatization. One day after their transport, health and nutrition conditions of fingerlings were checked. Next days (2nd October) fingerlings were measured, weighted and stocked into six tanks of recirculation system (600 litres per one tank). Fingerlings were divided into two groups with three replications (4000 ex. per replication). Fingerlings were fed by two different artificial fodders (“Asta” made by the Institute of Ichthyobiology and Aquaculture Golysz in Poland and “Karpico” made by the Coopens firm). The whole experiment (the autumn adaptation and the following rearing of perch) lasted 124 days (from 3rd October 2004 to 4th February 2005). Perch fingerlings were transferred from outdoor conditions (10°C). At first, fish were stocked into six tanks (controlled conditions) under water temperature of 10 °C. During 10 days, water temperature was gradually increased up to 20°C and recirculation system was opened. The perch started to receive special wet mix feed during that period (temperature adaptation). After temperature adaptation, the feeding adaptation started. At the beginning, fish were fed by special wet mix feed (content: 50 % flash of big head carp, as attractant, and 50 % flour of fodder) during 10 days. Subsequently, food of perch was created from special wet mix (50%) and fodder (50 %) during the next 10 days. In the next 10 days, perch food was created from 100% fodder. The food ratio was 7% of body weight of fingerlings during that period. At the end of the feeding adaptation, most of perch (more than 90% surviving fingerlings) received artificial food. During the temperature and feeding adaptation, growth and survival rate of fingerlings were observed. Growth and survival rate were compared between groups fed by different artificial feeding (Asta and Karpico) using ANOVA (Statgraphics, Tukey test, $P < 0.01$). After temperature and feeding adaptations, the rearing of fingerlings started under controlled conditions. This rearing of fingerlings was distributed in four 21 days periods. The weight and total length of perch, survival rate, specific growth rate (SGR), food coefficient ratio (FCR) and survival rate were checked at the end of each period. These characteristics were compared between Asta and Karpico groups of perch at the end of the each period of rearing using ANOVA (Statgraphics, Tukey test, $P < 0.01$). The experiment was finished at the end of the fourth period, because results of economical analysis were very uneconomic.

b) Rearing of advanced fry under pond conditions.

After incubation of eggs in Vodňany (Partner 11), gained larvae were stocked into four experimental ponds (total area of each pond was 0.08 ha) in experimental area of RIFCH Vodňany. Larvae were stocked under two different densities with two replications (500 000 and 1000 000 larvae per hectare) on 26th April 2005. Temperature of water (17.6 °C), oxygen content ($7.1 \text{ mg O}_2 \cdot \text{l}^{-1}$) and amount of plankton was observed during pond rearing of fry. Pond rearing of fry was finished two weeks after appearance of absence of plankton, on 20th July 2005. All perch were caught from ponds (mean total body length 38.0 ± 3.2 mm and mean total body weight 0.58 ± 0.16 g) and total produce of advanced fry was recorded.

c) Pond culturing of juveniles during the whole growing season.

Appropriate food supply is, particularly during the onset of exogenous feeding, crucial in rearing fish in aquaculture. With the present restrictions on pesticides use (Soldep etc.) in the countries of the European Union simple and effective method of controlling zooplankton quantity and especially quality became inapplicable and in general higher attention need to be paid to proper preparation of ponds towards a required zooplankton community. Therefore,

above all, we aimed our interest to the importance of different zooplankton species (rotifers vs. nauplii and copepodite stages of copepods, and cladocerans) in the diet of perch larvae during the first days of its exogenous feeding to evaluate any exceptional food demands.

Latter in 0+ perch diet zooplankton and macroinvertebrates are the main food sources, although even piscivory was reported. Growth of 0+ fish increases significantly when fish switch from feeding on zooplankton or benthic invertebrates to piscivory, but may cause especially in a European perch case increasing of intra-cohort mortality due a cannibalism. Therefore, in the second chapter of our study ontogenetic diet changes of perch are evaluated under different feeding conditions using a small cyprinid species (stone moroco, *Pseudorasbora parva* Temminck & Schlegel) in a ponds culture.

Four ponds (Tab. 1) of different zooplankton quality, but similar in other aspects were selected for rearing perch larvae. Two of them with higher share of rotifers (SZ1, SZ2) and two with higher share of nauplii and copepodite stages of copepods (LZ1) and/or copepodite stages of copepods and cladocerans (LZ2, Fig. 7). Free swimming larvae still with rest of oil globule (14-17 days post hatch) were stocked in ponds on April 27 in afternoon hours in density of 120 000 per hectare at minimum level derived from prior estimation of ponds trophic capacity and respecting the whole-season ongrowing period.

During the next six days larvae were collected daily in afternoon hours (15.00-19.00) using circular ichthyoplanktonic net (70cm in diameter) and immediately preserved in formalin solution for further laboratory analysis. Zooplankton samples were collected paralelly with fish samples using circular plankton net (24 cm in diameter, 40µm, two surface tows pooled) and preserved in formalin. In laboratory zooplankton samples were counted and determined into genera level using binocular microscope. From each fish sample 20 larvae were measured (from the tip of the snout to the end of the chorda dorsalis) and dissected under the binocular microscope and the gut contents were identified into the genera level and number of prey counted. For graphical presentation of the stomach content data, Amundsen, Gabler & Staldvik (1996) modification of Costello method was used. This method relates the frequency of occurrence (F_i – the share of predators in which prey i occurs from all predators with filled digestive tract) to prey-specific abundance (P_i – percentage a prey i comprises of all prey items in only those predators in which prey i occurs) and enables to easily explore the prey importance. Prey taxa close to 1% occurrence and 1% abundance are negligible in the diet, conversely prey species approaching the upper right corner (100% occurrence and 100% abundance) are considered as the most important. Points close to 1% occurrence and 100% abundance are considered as a specialization on certain prey taxa by some predators, points close to 100% occurrence and 1% abundance indicate generalized diet of most predators.

Since the early larval feeding period was done, fish were sampled just monthly from June until the end of September between 10 a.m. and 6 p.m. using a beach seine net. In June 17, total biomass of 40 kg per ha of cyprinid spawners were introduced just into two of experimental ponds (A) to test the ability of induced piscivory.

In the field, abundance and length of perch and of prey fish are recorded. Fish are sorted by species on deck and individual body length (SL) is measured to the nearest 1mm. Approximately 60 specimen sorted by size (like a smallest, middle and biggest fish) are preserved in 4 % formalin solution for further laboratory analysis which we assume to be strongly related to the size of fish.

The monitoring of basic environmental parameters (physical and chemical) such as temperature ($^{\circ}\text{C}$), dissolved oxygen (mg.l^{-1}), pH, conductivity and transparency including habitat description as water depth (cm) are conducted at the same time like fish samplings except of temperature measured daily and water chemistry analysed in laboratory latter. Monitoring of potential food web of post-larvae and juveniles – zooplankton, zoobenthic community and prey fish is conducted monthly. Zooplankton samples are collected in a way

like was mentioned above (larval feeding) modified using a bigger- mesh sized net (100 μ m, 39 cm in diameter) thus each sample represents 760.5 l pond water. Quantity and quality of zoobenthonic animals are also evaluated. Phytophilous macroinvertebrates mean animals attached to the aquatic macrophytes (above-ground parts or roots) or swimming among them. For the animals living in the above-ground biomass of aquatic macrophytes a mesh cage 50 x 50 cm is used. All plants biomass with attached animals is removed, the rest of animals are sieved through the sieve with mesh size 500 μ m. Animals are separated manually from the plants. Dry biomass of each of aquatic plants is determined at 105 °C.

Plastic tube (15 x 6.5 cm) is used for sampling animals from the root system of aquatic plants inside the mesh cage as well for typical bottom-benthic animals collected outside the mesh cage. Prey fish, its relative abundance and size-distribution (length frequency) respectively, are recorded for indirect estimation of expected perch predation pressure and its subsamples preserved in 4% formalin solution are taken to evaluate potential feeding competition with perch stock.

Note: this study has begun in 2005 and it is not finished yet, thus only some of objectives are achieved today.

Results and discussion

a) Autumn adaptation of juvenile from pond into recirculation (controlled) conditions and their following rearing.

Survival of fingerlings

The high survival rate of perch fingerlings was recorded in both groups (Asta group 93.6 % and Karpico group 94.5 %) during temperature adaptation. However, low survival rate of perch fingerlings (Asta group 12.6 % and Karpico 20.5 %) was found during feed adaptation. Most of mortality was recorded during final period of feed adaptation, when perch learnt to receive artificial fodder. No significant difference was found between survival rates of both groups during periods of feed adaptation, when wet mix was used. Statistically higher survival rate was recorded in Karpico group (20.5 %) compared with Asta group (12.6%) during final period of feed adaptation. The same statistically differences were found during all periods of following rearing of perch fingerlings. At the end of the rearing, survival rate of fingerlings were found 11.2 % and 15.7 % in Asta and Karpico group, respectively (Fig. 1).

Growth of fingerlings

Perch, which received Asta fodder, grew statistically faster than perch in Karpico group. At the end of the rearing, perch achieved mean body weight 23.3 and 12.7 grams in Asta and Karpico group, respectively. However, perch didn't grow during temperature adaptation period, when mean body weight of perch decreased slightly (Fig.2).

Perch in Asta group achieved statistically greater total and mean gain of biomass during all periods of perch rearing under controlled conditions, except the third period (Fig.3 and 4).

Specific growth rate was between 0.3 - 2.4 % \cdot d⁻¹ without statistically differences, except the fourth period, when SGR of perch in Karpico group was significantly higher than perch in Asta group. Perch in Asta group had higher SGR during all periods of rearing, except the third period (Fig. 5).

Feed conversion ratio was from 1.7 to 24.9 g \cdot g⁻¹ without significantly differences between both groups of perch, except fourth period of rearing. During fourth period, feed conversion ratio was very high in Karpico group, because perch had very low specific growth rate (Fig. 6).

At the end of the fourth period of rearing, all operation costs were counted and economical analysis was performed. Total operation costs (3150 €) was created by costs for personnel costs (1500 €), stocking material of perch (800 €), artificial feedings (50 €), energy costs (500 €) and depreciation of fixed assets (300 €). Total produce of perch biomass (total gain of biomass) was 9 kg of perch (total number of fish 1 076 ex. with mean total body length 112.1 ± 14.9 mm and mean body weight 17.8 ± 8.35 g) for the whole experiment. It means that produce of 1 kilo of perch biomass cost 3150 Euro.

b) Rearing of advanced fry under pond conditions.

This total product of advanced perch fry was found at the end of the pond rearing fry, on 20th July 2005:

- 1st pond (stocking density: 500 000 larvae.ha⁻¹) - 10 000 fry (125 000 fry ha⁻¹)
- 2nd pond (stocking density: 500 000 larvae.ha⁻¹) - 8 000 fry (100 000 fry ha⁻¹)
- 3rd pond (stocking density: 1 000 000 larvae.ha⁻¹) - 4 000 fry (50 000 fry ha⁻¹)
- 4th pond (stocking density: 1 000 000 larvae.ha⁻¹) - 5 000 fry (62 000 fry ha⁻¹)

Lower product of fry was recorded under higher stocking density of larvae. We recommend to use lower stocking density of perch larvae for rearing of advanced fry, about 100 000 larvae per 1 hectare. Perch fry received pond plankton as food during their rearing, only. Finish of this rearing was performed two weeks after appearance of absence of plankton. Most of mortality rate of fry was found during this period, when perch had lack of food in ponds. We recommend finish the rearing of advanced fry immediately after appearance of absence of plankton in the pond.

c) Pond culturing of juveniles during the whole growing season

Early diet of perch larvae reared in nursing ponds with different zooplankton quality

Preliminary analysis of digestive tracts contents revealed copepod nauplii as generally the most important prey taxon (Fig. 8) over the first days of exogenous feeding. However, in the case of ponds SZ1 and LZ2 this result is not very convincing, because most of the larvae (60-95 %, Tab. 2) had oil globule present and were still not feeding. On the other hand, comparing the digestive tract contents of larvae from pond LZ1 and SZ2, where shares of non feeding fish were not so high as in the ponds SZ1 or LZ2 and were similar in both ponds, it can be clearly seen (Fig. 8) that in the case of pond LZ1 nauplii were without any doubts the most important prey taxon over the two days of exogenous feeding and then, very early (day 3), the perch larvae came to larger prey – copepodites of copepods, both cyclopoid and calanoid. This had positive effect on the growth of perch larvae, as the fish in pond LZ1 were the largest at the end of our investigation (Tab. 2). In the pond SZ2, where rotifers numerically dominated, these played only particular role, primarily the genus *Polyarthra*, and similarly as in LZ1, copepod nauplii were the most important prey over the most of the days of the exogenous feeding (Fig. 8).

Our results suggest that specific preparation of nursing ponds towards high abundance of rotifers and on the other hand low abundance of arthropod zooplankton is not necessary in rearing of perch, but in some respect rather contra productive.

Rearing of 0+ perch in ponds under different feeding conditions

Growth of 0+ perch (Fig.10) varied slowly already at the beginning of the second study (May 10) until perch feeding conditions were rapidly changed by prey fish stocking (June 17). Since one month latter (July 25), significantly different growth rate (Tukey HSD test: $p < 0.001$)

between ponds with and without prey fish was observed. Length-frequency distribution (Fig.9) shows trends more in detail and at last presented sampling date (August 28) development of bimodality, but just in a one experimental pond, it is already possible to see. It is probably due to the fact that perch-structured population may form different aggregated cohorts with different time and space use and especially size of sapling area may significantly bias our results. Presentation of mortality rate just indirectly expressed here like a mean number (Fig.11) per unit effort – CPUE units (one beach seine net) must be taken into the account with the respect to various environmental (vegetation) and pond size conditions among ponds (Tab. 1).

Our early results suggest that induced piscivory realized by prey fish availability has an effect on perch stock structuring, although make a definitive conclusion for its efficiency is not possible yet and estimation of such features as ratio of piscivorous to non-piscivorous fish (gut analysis) and ponds harvesting data set are necessary to finish the second chapter of our study. Therefore, the whole study should be available in the next report in 2006.

Tables and figures:

Table 1. Characteristics of ponds.

Locality	Area (ha)	Stocking	Harvesting	
		Density (thousand ind.)	Biomass (kg)	Density (ind.)
Dvorčák pond	0.48	65	X	X
Kamenný pond	1.54	187	X	X
Hejškův pond	0.88	106	X	X
Kudla pond	0.36	44	X	X

Table 2. Average length of perch larvae, percent of non-feeding larvae and percent of larvae with still present oil globule on 1st, 2nd, 3rd and 5th day of exogenous feeding.

pond	average SL [mm] / % of non-feeding fish / % of fish with oil globule			
	day 1	day 2	day 3	day 5
LZ1	6.95 / 35 / 25	7.2 / 75 / 5	7.55 / 55 / 0	8.44 / 22 / 0
LZ2	5.98 / 95 / 100	6.23 / 60 / 90	6.58 / 30 / 10	7.33 / 0 / 0
SZ1	6.03 / 95 / 75	6.05 / 95 / 40	6.28 / 90 / 45	6.95 / 40 / 0
SZ2	6.4 / 30 / 50	6.33 / 85 / 45	7.15 / 45 / 10	7.32 / 45 / 0

Cumulative survival of fingerlings during and after autumn adaptation

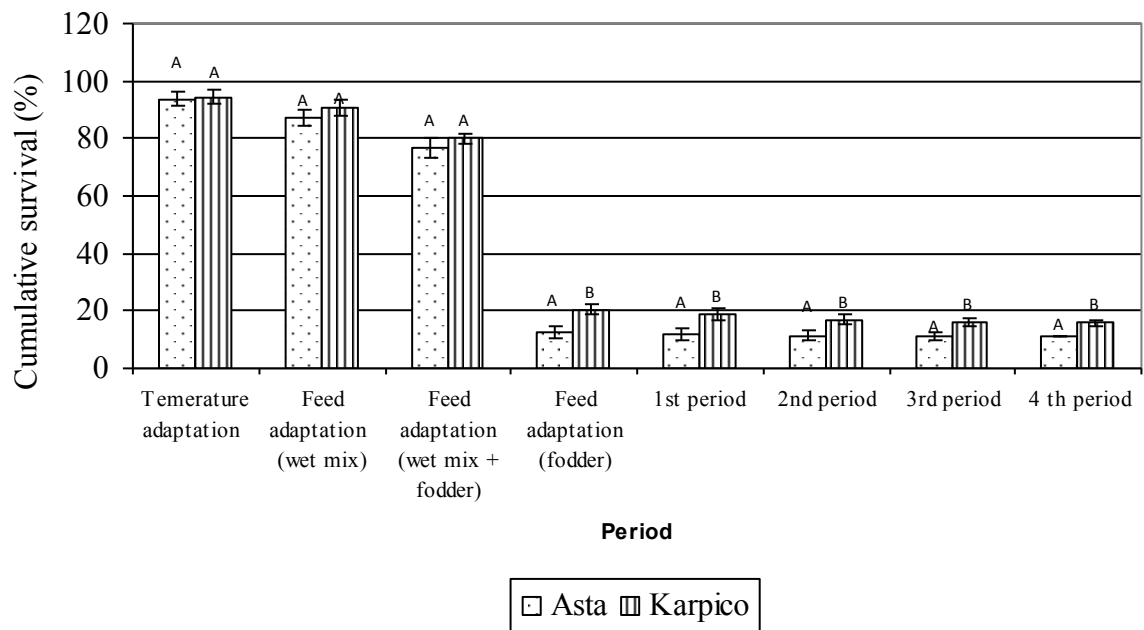


Figure 1. Cumulative survival of fingerlings during and after autumn adaptation.

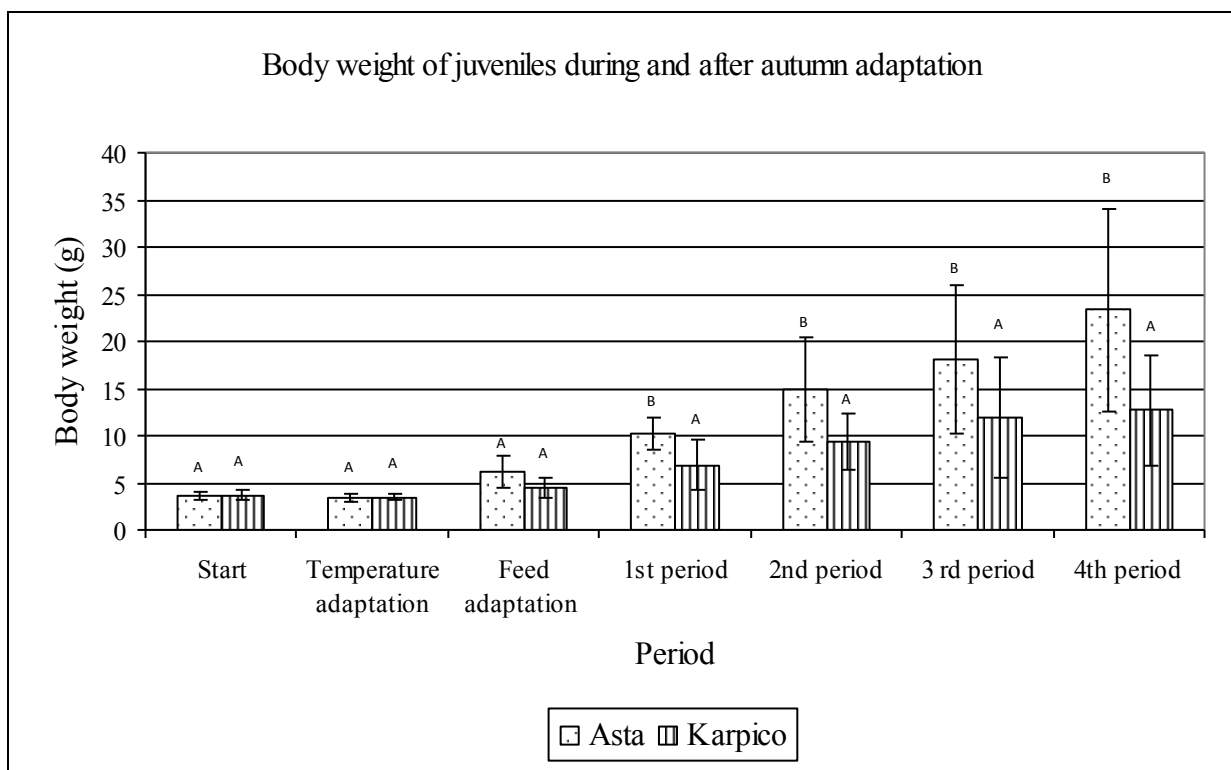


Figure 2. Body weight of juveniles during and after autumn adaptation.

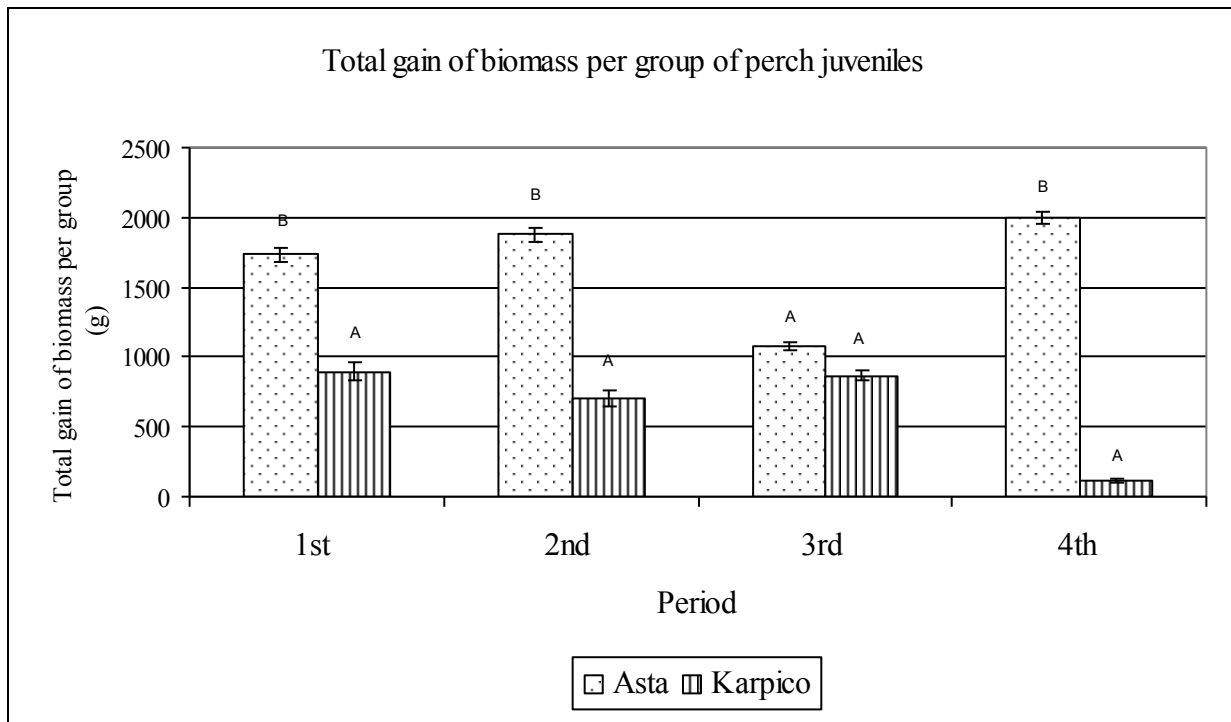


Figure 3. Total gain of biomass per group of perch juveniles.

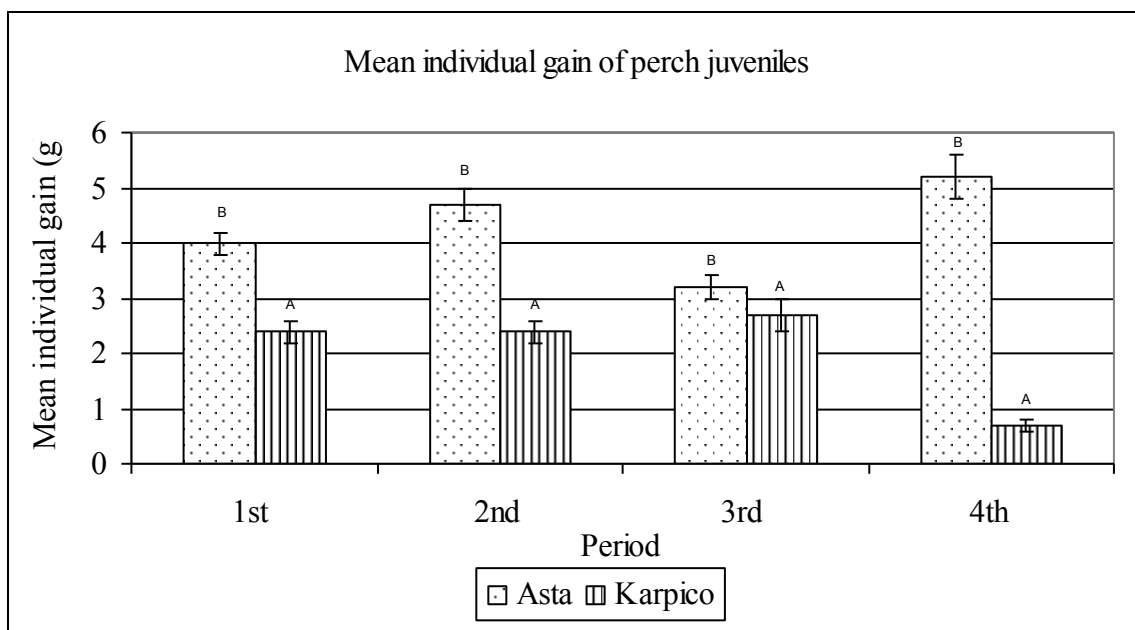


Figure 4. Mean individual gain of perch juveniles.

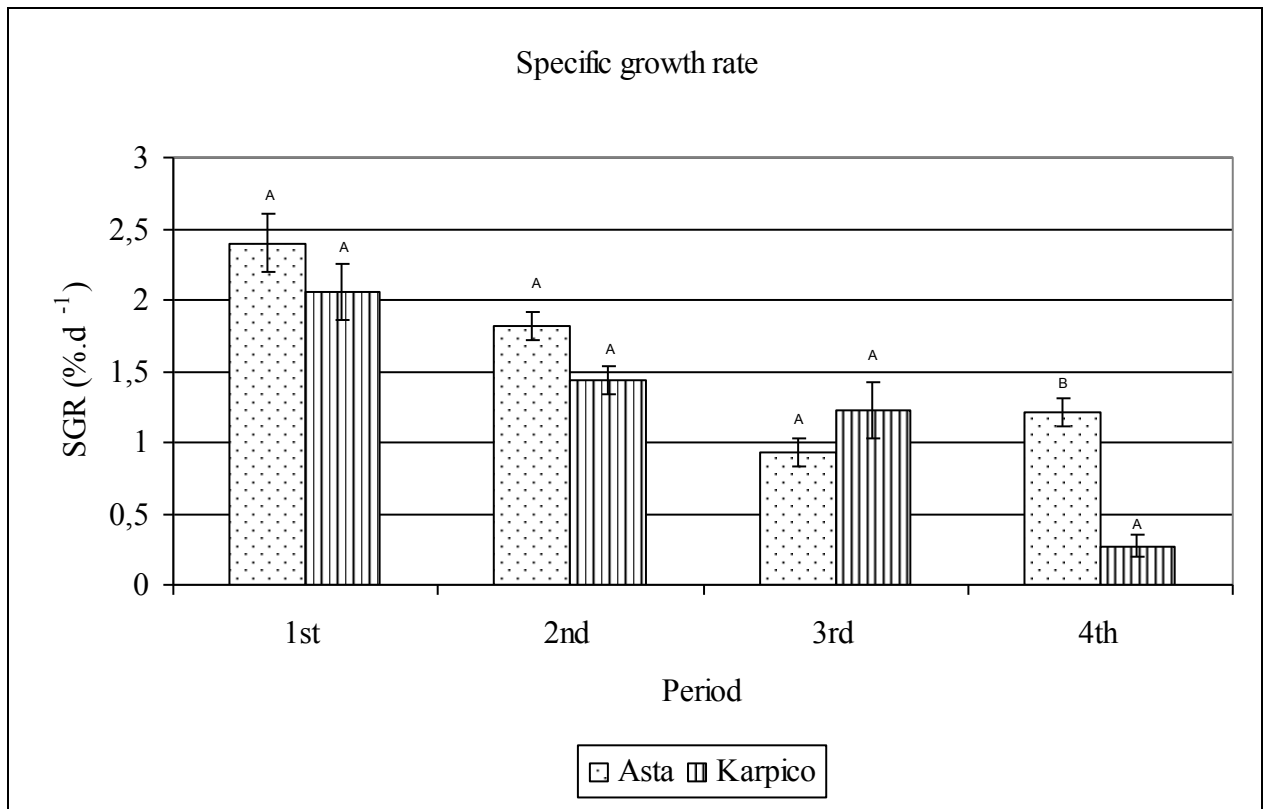


Figure 5. Specific growth rate.

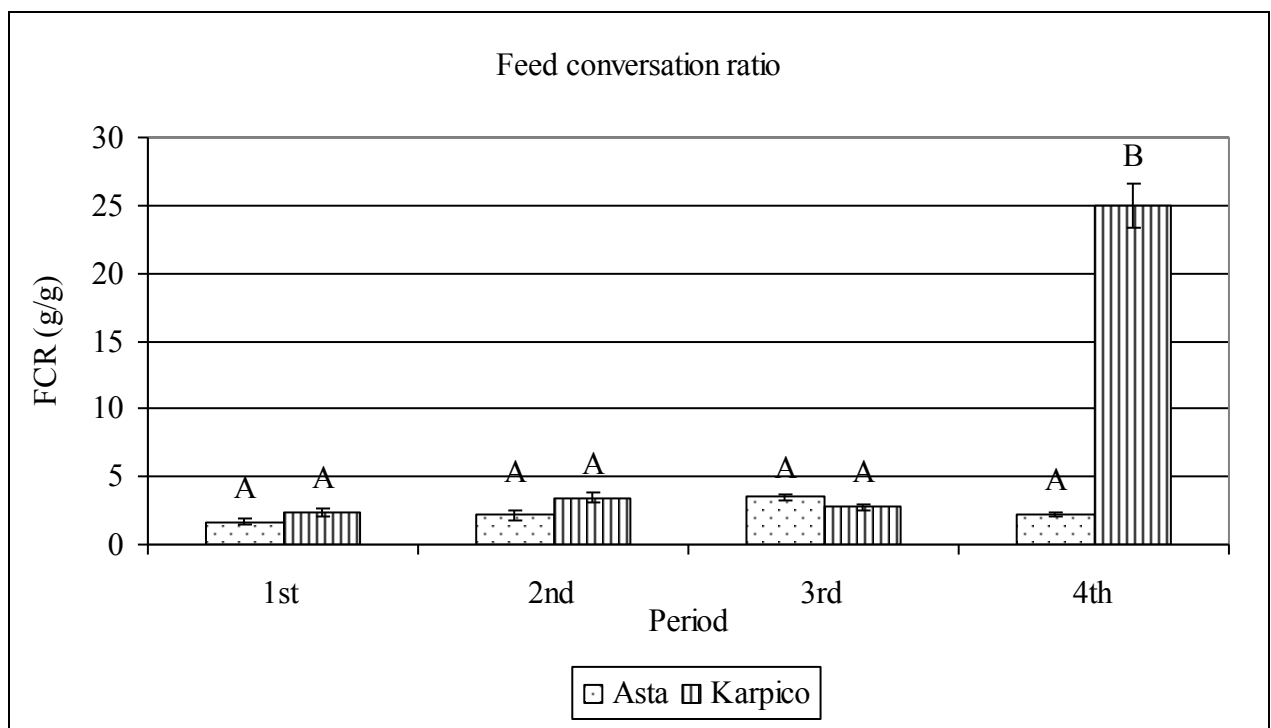


Figure 6. Feed conversion ratio.

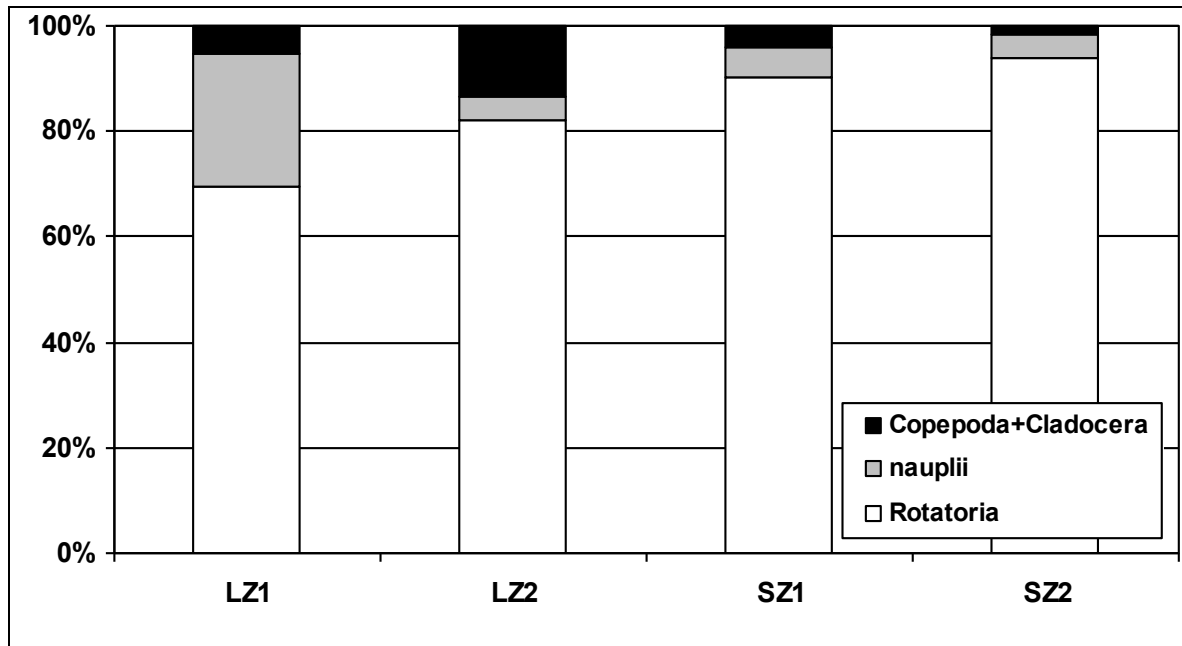


Figure7. Relative share of different zooplankton groups in nursing ponds on day 1 (April 28), numbers above bars indicate zooplankton density in inds.l⁻¹.

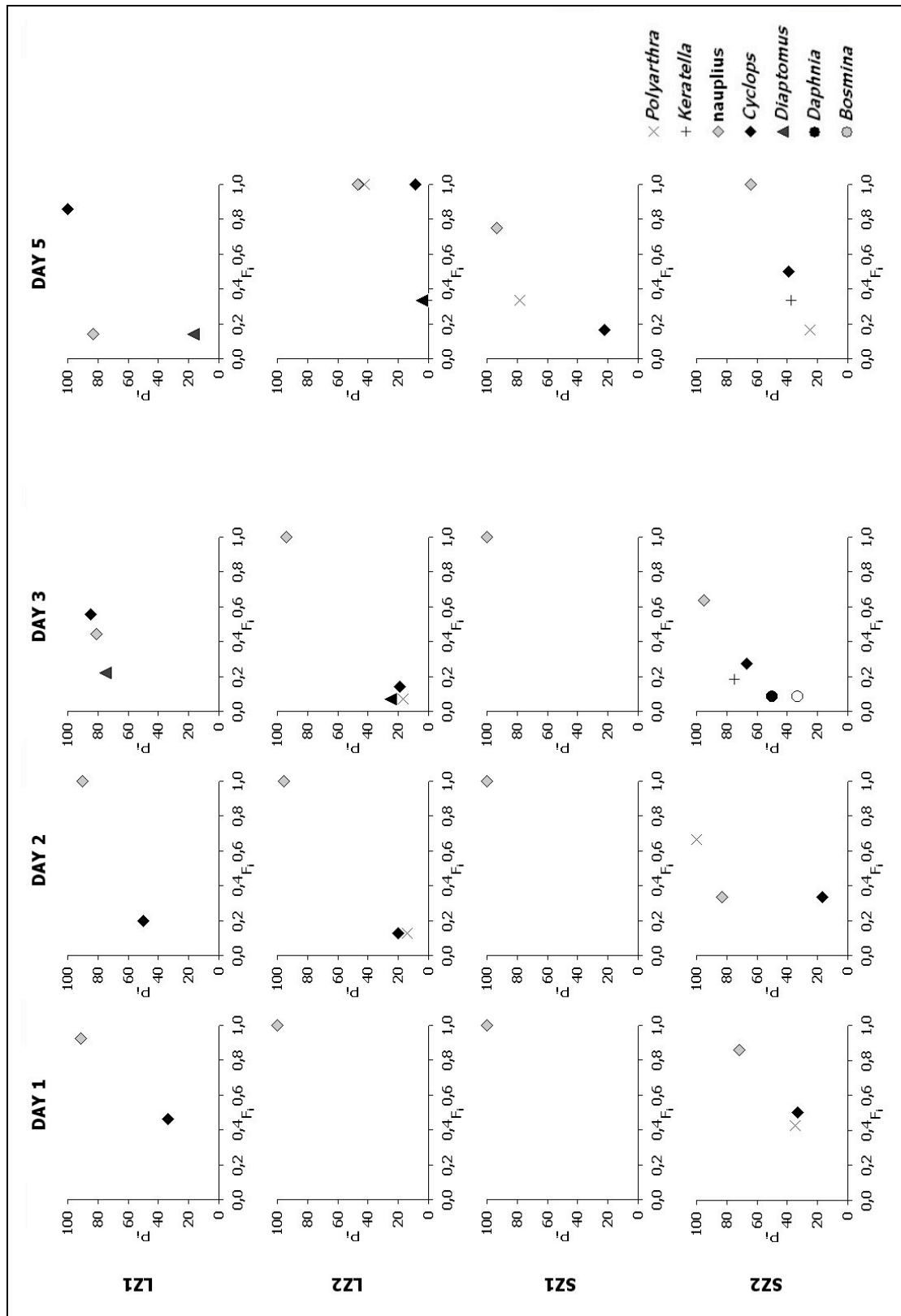
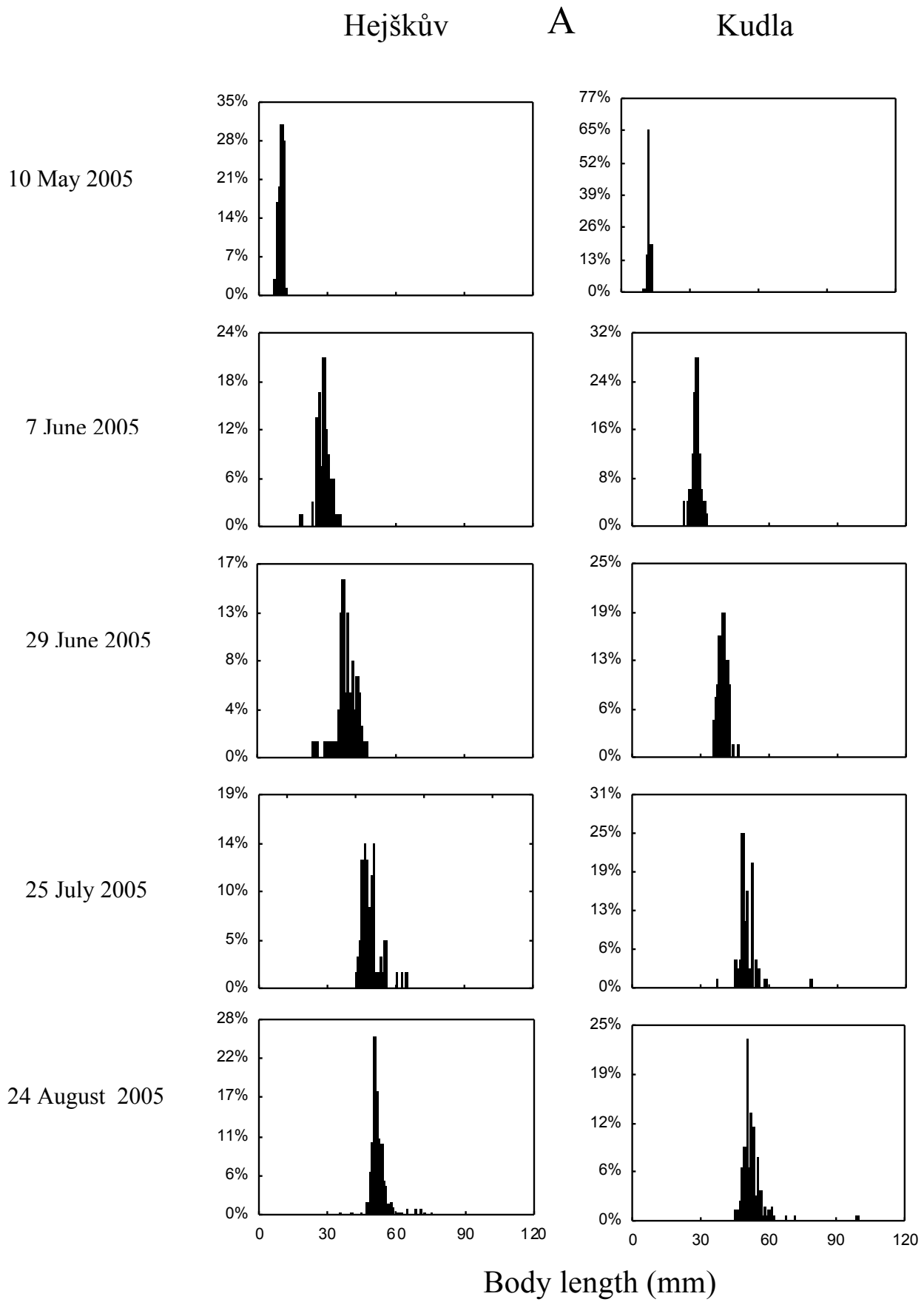


Figure 8. Prey occurrence (F_i) and prey-specific abundance (P_i) in the digestive tracts of perch larvae in nursing ponds on 1st, 2nd, 3rd and 5th day of exogenous feeding.



Kamenný

B

Dvorčák

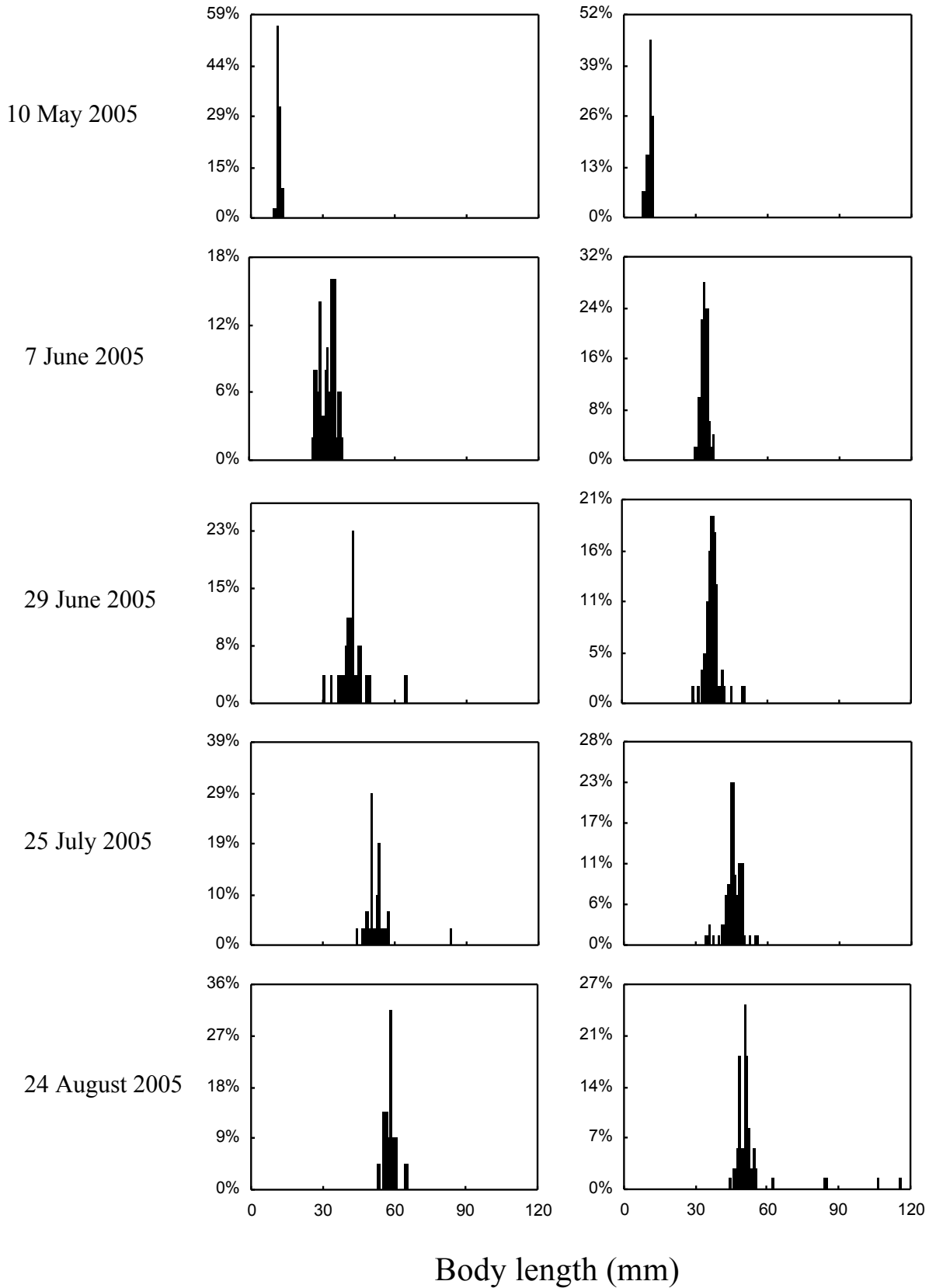


Figure 9. Length-frequency distribution of 0+ perch caught by seine nets. A - indicate ponds where prey fish were stocked in, B - no presence of prey fish respectively.

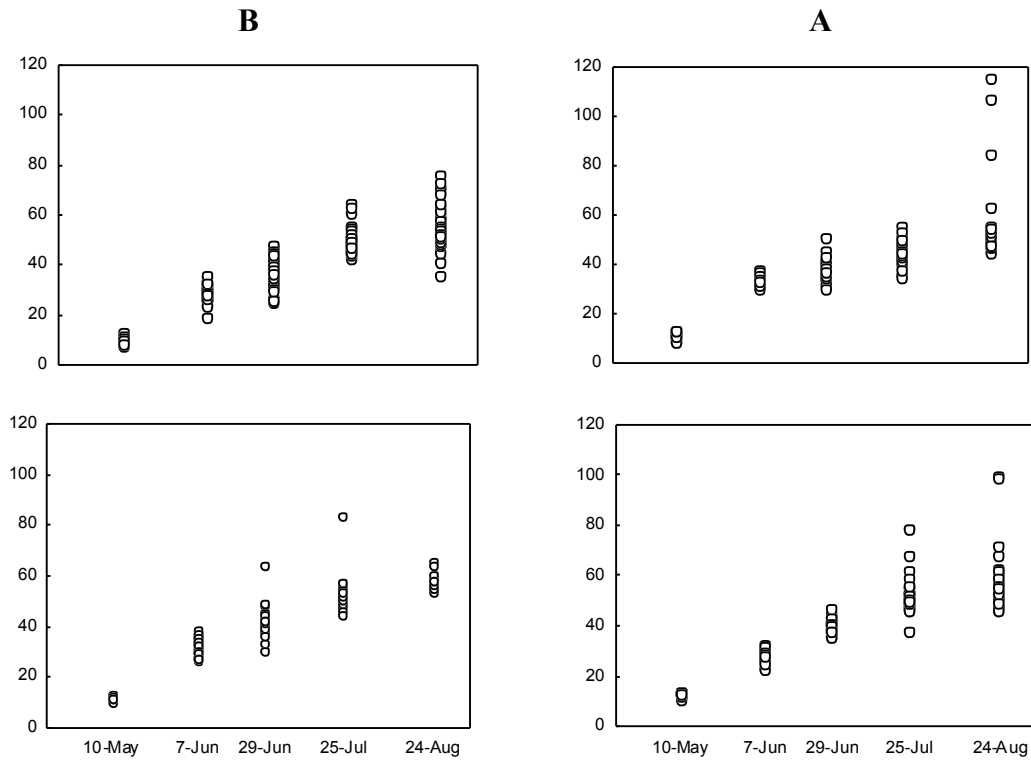


Figure 10. Length-frequency distribution of 0+ perch caught by seine nets. A - indicate ponds where prey fish were stocked in, B - no presence of prey fish respectively.

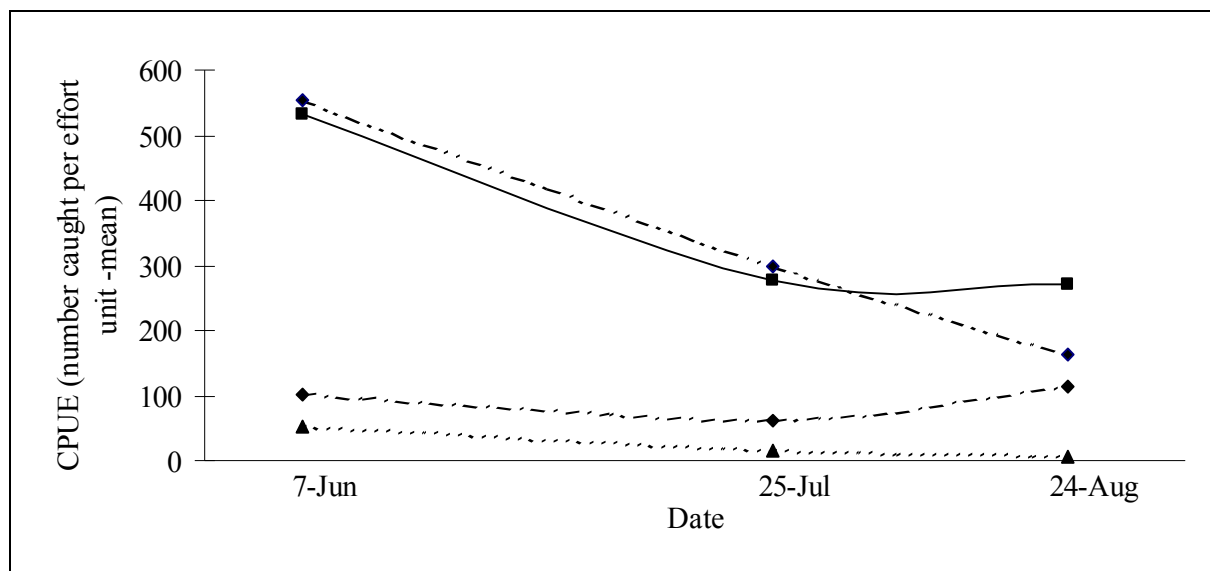


Figure 11. Numbers of captured perch per sampling effort unit – expressed as mean numbers respectively.

- *Deviations from the project workprogramme*

In Ireland, field trials have been conducted to compare the cost of production of fry under intensive and extensive management. The intensive system used cylindro-conical tanks, the extensive system used a mesocosm system with cages in ponds. Both systems had problems during the first year. The intensive system experienced a sudden increase in pH resulting in

high mortality, and consequently in very low production. The extensive system experienced poor hatching results and high mortality. Both systems will be continued during the next season.

F - Task 6: Transfer of technology (WP8)

Responsible: LSA - UHP (Partner 7)

Associated partners

- SMEs: all partners

- RTDs: all partners

- *Workpackage objectives and starting point of work*

The objectives of this task are:

To elaborate a strategy for dissemination of the results and transfer of the technology to other organisations

To promote the knowledge and the know-how of the consortium

- *Progress towards objectives*

Firstly, at the beginning of the first year (November 2004), a consortium agreement has been signed by all partners. It defines the property of the results and their way of diffusion. Secondly, a PERCATECH website (www.percatech.org) has been developed and put online in September 2004 to promote the activities conducted by the Percatech consortium within the framework of this programme. The reference of our website has been transmitted to both the European Aquaculture Society (EAS) and the Federation of European Aquaculture Producers (FEAP) for dissemination to their members and to each national organisation of professionals. Thirdly, a recent contact (September 2005) was established with the General Secretary (Mr C. Hough) of the FEAP to organize a further workshop on progress in Percids culture during the year 2007 or 2008. Besides, a contact has also been established with the coordinator of the Luciopercimprove project, dealing with pikeperch culture, a closed species of perch belonging to the same family. He agreed with the opportunity to organize together such a meeting.

- *Deviations from the workprogramme*

For the moment, all scheduled experiments have been carried out in the corresponding delay.