SIXTH FRAMEWORK PROGRAMME HORIZONTAL ACTIVITIES INVOLVING SMES CO-OPERATIVE RESEARCH

Improving pikeperch larval quality and production by broodstock management and nutrition, husbandry and sex control

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PROJECT OBJECTIVES

Diversification has been suggested as a potential tool to develop the freshwater aquaculture production in Europe. Recent research has demonstrated the feasibility of cultivating intensively pikeperch *Sander lucioperca*, a valuable fish under increasing demand for both the consumption (large size fish, 2-4 kg each) and the restocking (0+ and 1-year old fish) markets in Europe. However, the supply of eggs and larvae is still largely dependent on the spawning of wild breeders or captive fish held in ponds during the maturation process. Production of pikeperch eggs and larvae is thus seasonally restricted and extremely variable in quality and quantity. In order to support the development and sustainability of pikeperch farms recently established in several European countries, the project will develop reliable methods securing the supply of high quality eggs and larvae of pikeperch.

Therefore, the general objective of this two-year proposal was to improve and control egg and larval supply and quality of pikeperch by improving broodstock management and nutrition. This comprises several specific objectives:

1. The set up of broodstock management programme, including the comparison of broodstock rearing conditions as well as the development of all-season spawning technology (year 1) and reliable artificial propagation methods.
2. The comparison of pikeperch eggs and larvae from broodstock held in ponds (extensive culture), cages (semi-intensive culture) and tanks in recirculating systems (intensive culture).
3. The improvement of broodstock feeding and nutrition, aiming to produce, from captive broodstock, eggs and larvae of comparable quality than those from wild broodstock.
4. The production of all-female population, divided into two phases: production of sex-reversed males and production of all-female juveniles from the breeding of normal females and neo-males.

The research programme is divided into 7 workpackages (WP), which are listed below :

WP 2 – The first aspect of the project is to secure year round production of pikeperch eggs and larvae by improving broodstock management and developing all season spawning reproduction and artificial fertilisation.

WP 3 – Improving egg and larval quality by improving broodstock diets.

WP 4 – Optimization of broodstock management using a multifactorial approach.

WP 5 – Setting a procedure for all-female pikeperch.

WP 6 – Influence of breeders stocking conditions on gamete and larval production and quality.

The **WP1** and **WP7** correspond respectively to the co-ordination action and the dissemination and use of knowledge transfer of technology respectively, both involving all partners and supervised by partner 1.

WORK PERFORMED AND END RESULTS

I – Project coordination and management (WP1)

I.1 – Workpackage objectives

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

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 different workpackages within the overall objectives of the project and communication with the EC,
6. Organization of management meetings (5 meetings during the 2-year period)

I.2 – Progress towards objectives

Overall management and co-ordination of all project objectives and financial matters were achieved. A website (www.luciopercimprove.be) was created and updated throughout the two years of the project. A first meeting (kick-off meeting) occurred in Namur, Belgium (09-10/11/05), a second one (first progress meeting) in Olsztyn, Poland (20-21/06/06) and a third one (first annual meeting) in Valkenswaard, The Netherlands (09-10/10/06). The first meeting 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 and the third meetings mainly focused on the presentation, analysis of the results obtained during the first 6 months and the first year of the project, respectively.
The first task of the consortium management for year 2 has consisted on the organization of the second periodic meeting in Nancy (Partner 11) on the 6th and 7th of March 2007. Results of year 1, methodologies and running experiments protocols were intensively discussed. Especially, SMEs gave useful advice to RTD partners for broodstock management during the reproductive season in April. The final meeting was held in Liege (Partner) on the 25th and 26th of September 2007. There, the results of both years were discussed, as well as the methodologies for the last experiments still running. SMEs were very enthusiastic regarding the obtained results. They proposed some last modifications of different running protocols, according to their needs. These modifications were taken into account and totally followed by the RTD performers.
All five meetings occurred as originally scheduled within the two year of the project. Each meeting allowed, besides discussing about the advances of the project, to share productive exchanges between all 11 partners, especially between SMEs and between SMES and RTDs. Such meetings also enabled each partner to show his own facilities and talked more deeply about his own experience (difficulties encountered, market targeted, other species reared…). It is worthwhile to notice that during all the project duration, all partners have always exchanged reliable information to others, and have sent all reports on time.

I.3 – Extent to which objectives were met

All objectives of this workpackage were met.

II – Improving egg and larval availability by developing out-of-season spawning and artificial fertilization techniques (WP2)

II.1 – Workpackage objectives

In current pikeperch farming, wild breeders are collected just before the spawning season and induced to spawn naturally. Production of pikeperch eggs and larvae is thus seasonally restricted and extremely variable in quality and quantity. For commercial pikeperch aquaculture to develop and expand, controlled maturation and spawning pikeperch in captivity is needed to achieve reliable production of juveniles on a year-round basis. Therefore the first objective of this workpackage was to contribute to the development of out-of-season spawning techniques by investigating different aspects of pikeperch reproduction. In total, three subtasks were developed. The aim of the first one was to gain understanding of the endocrine and physiological changes during a reproductive cycle. For this purpose an experiment was conducted to gain knowledge on the reproductive cycle and reproduction performance in pikeperch. In this study both wild and cultured (F1) breeders were assessed because in view of safe and controlled reproduction and breeding, it is of major importance to be able to use own produced offspring as broodstock. Independency of natural populations makes safe, predictable and controlled production possible and therefore can increase commercial opportunities in several ways. At this moment, cultured broodstocks are still less successful in reproduction compared to wild breeders (pers. comm. partner 2). In related species such as perch and walleye it has been shown that control of reproduction cycle is mainly determined by temperature and photoperiod variations (Migaud *et al*., 2002, 2003, 2004a,b, 2006). Therefore a thermal and photoperiod manipulated out-of-season protocol was established in order to obtain spawning on a year-round basis. This protocol was applied at a commercial farm, where both wild and F1 breeders were used, and reproduction performance was monitored (subtask 2). During the second year an additional experiment was conducted on optimizing out-of-season spawning techniques by varying thermal regimes and duration of the cold period (subtask 3).
For synchronised spawning and developing breeding programs, stripping eggs, artificial fertilization and incubation of eggs in small containers is essential. Current commercial pikeperch farming is reliable on semi-controlled nest-spawning while artificial fertilization is rarely used. The second objective of this workpackage was to establish reliable artificial fertilization techniques. Therefore a protocol for artificial spawning was developed and different spawning agents were tested (subtask 4). The third objective was to develop a suitable method for storing pikeperch semen under cryopreservation. In aquaculture, semen cryopreservation is applied for synchronization of artificial reproduction. An on-farm protocol was developed by testing different extenders, cryoprotectants and sperm-motility activators, and different freezing and thawing rates (subtask 5).
The fourth objective was to make a first step towards a breeding program. The importance of establishing a controlled breeding program is high in order to improve for example growth rates and feed conversion rates. It is, however, out of the scope of this project to establish a fully controlled breeding programme. A first step was made by investigating available family marking methods and using one method to do some preliminary tests on inter- and intra-family growth performance (subtask 6).

II.2 – Progress towards objectives

II.2.1 – Subtask 1: Reproduction biology in pikeperch breeders

The elucidation of mechanisms that govern reproduction in pikeperch requires a basic understanding of the gonadal and hormonal changes which occur during the reproductive cycle. The annual cycle of sex steroids has been established for related species such as Eurasian perch (Sulistyo et al. 1998; Sulistyo et al. 2000) and walleye (Malison et al., 1994) but not pikeperch. In the current study changes in the reproductive cycle were monitored for F1 pikeperch reared in intensive (tank) as well

as semi-natural (pond) conditions. Wild breeders were sampled as control and provide reference data. Therefore wild breeders stocked in a pond were used and also wild breeders from fisheries were sampled (table 1.1). Samples (n=10 for each group) were realized each month in the period August-April for F1 breeders and January-April for wild breeders. To determine the reproductive cycle we measured sex steroids profiles (T, 11kT/E_2), gonad development (histology) and morpho-anatomical parameters. At the end of the reproductive cycle, reproduction performance was indicated by fecundity, sperm quality (using CASA measurements; Kime et al., 2001; Rurangwa et al., 2004) and spawning success.

Table 1.1 Origin and holding condition of the different broodstock groups

BS Group	Origin	Holding condition	Number	Length (cm)	Weight (gram)
Group A	F1	Recirculation tank	110	46 ± 5	922 ± 195
Group B	F1	Outdoor pond	114	47 ± 3	948 ± 187
Group C	Wild	Outdoor pond	90	49 ± 9	1585 ± 595
Group D	Wild	Natural environment (fishery)	30	53 ± 6	1324 ± 439

Male breeders

Gonadosomatic indices (figure 1.1, left panel) in both F1 breeders reared in a tank and reared in a pond rose during the first months (August-November). This peak in autumn was a result from an active spermatogonia transformation to form spermatocytes (spermiogenesis) as evidenced by histological data. GSI levels of male pikeperch breeders slightly decreased during winter and rose again prior to spawning. Maximum GSI levels of wild breeders (0.6-0.7%) were significantly higher compared to F1 breeders (0.25-0.3%). GSI dynamics of related percid male breeders is similar to patterns observed in the present study. Hepatosomatic values rose gradually towards the spawning season and dropped just prior to spawning. The liver was probably increasingly active in the elaboration of lipoproteins and other constituents deposited in the gonads. Maximum VSI values in F1 pikeperch breeders were reported in August. This is in coincidence to the suggestion that broodstock deplete visceral energy stores due to gonadal recrudescence.

Two periods of increasing plasma testosterone (T) levels were observed in F1 pikeperch breeders (figure 1.1). The first occurred between August and December and seems correlated to spermiogenesis and subsequently an increase of GSI levels. The second peak was observed from January towards spawning. Testosterone levels were highest in wild pikeperch reared in a pond but lowest in wild caught breeders. The functional significance of such a pre-spawning rise in T may be to stimulate secondary sexual behaviors, increase pituitary GTH levels in preparation for the preovulatory GTH surge, or serve as a precursor for the production of other steroids such as 11kT. 11kT levels in male pikeperch breeders were low during the entire period and peaked just prior to spawning. Since 11kT is released prior to spawning, it is thought that this steroid is involved in the initiation of spermiation in pikeperch. No statistical difference was found for 11kT levels between the pikeperch broodstock groups of different origin and reared under different holding conditions. Results of the current study on pikeperch confirm the suggestion by Kime & Manning (1982) that T may be involved in spermiogenesis, and 11kT in the initiation of spermiation.

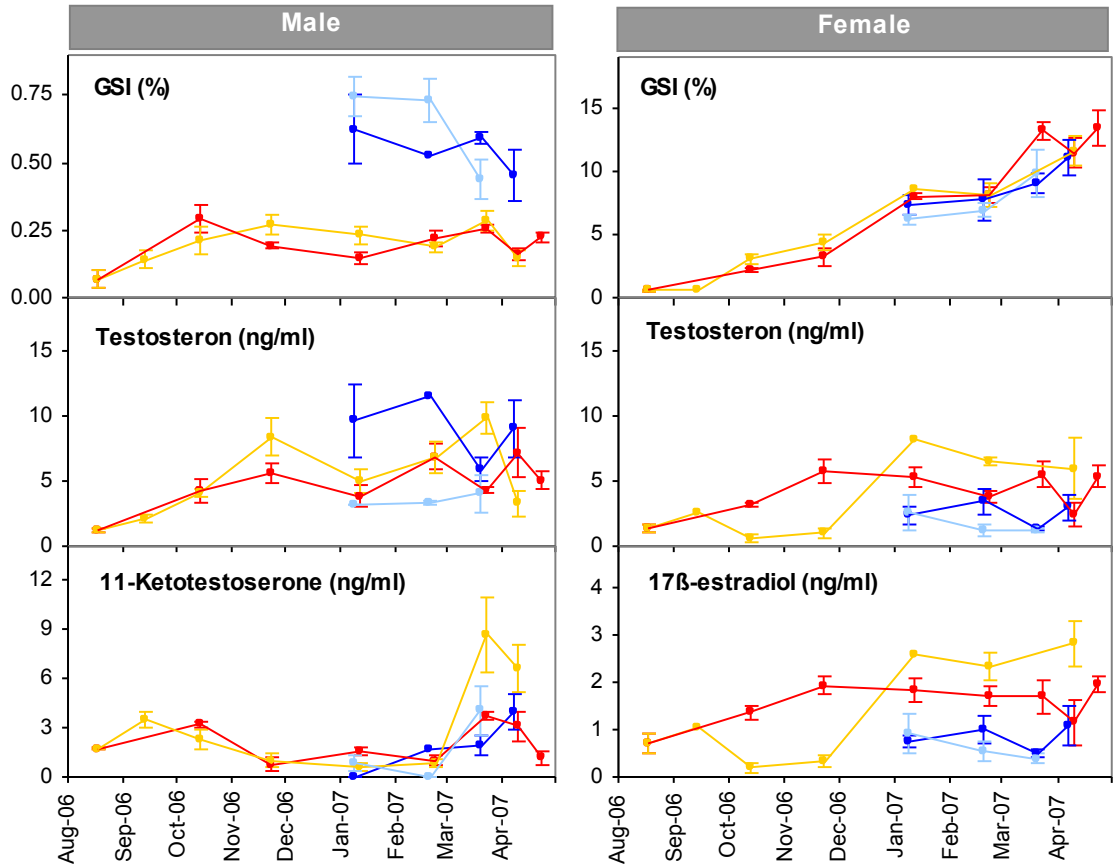


Figure 1.1 Gonadosomatic indices and sex steroid concentrations (T, 11kT and E_2) for the four broodstockgroups during one reproductive cycle. Data are expressed as means ± standard error. Left for males and right for females.
— F1 in tank (A) ; — F1 in pond (B); — wild in pond (C) ; — wild from fishery (D)

Reproduction performance (sperm quality) showed best results for F1 breeders reared in a tank, and lowest results for wild breeders reared in a pond (figure 1.2). Highest sperm concentrations were found for F1 breeders in a tank and lowest values were found for wild breeders. However, since highly concentrated sperm does not always give highest motility or the highest fertilization rates (Geffen & Evans, 2000; Williot et al., 2000), we also measured motility parameters. The current study has shown that sperm concentration and motility are positively correlated in pikeperch breeders. All motility measurements (mot, VCL, VSL) were highest for F1 breeders reared in a tank.

Female breeders

GSI values of female breeders of the four broodstock groups were not statistically different and the maximum measured value was 13% (figure 1.1, right panel). Changes in female GSI paralleled changes in average oocyte diameters. Highest oocyte diameters were observed in pikeperch breeders prior to spawning (912 and 988 µm). Like in male breeders, highest HSI values were observed in winter and a depletion of HSI was measured prior to spawning. Variations in condition factor (K_1, K_2 and K_3) indicate that during the reproductive cycle energy reserves were allocated to oocyte development.

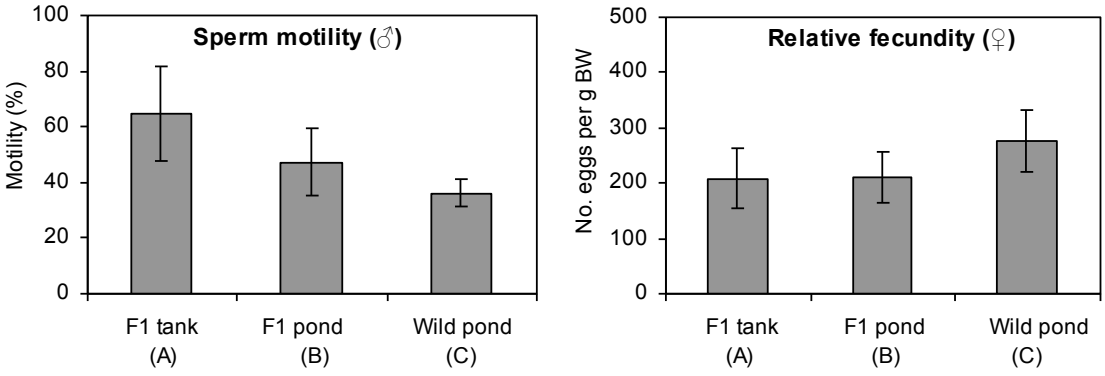


Figure 1.2 Reproduction performances of different pikeperch broodstock groups. Right: sperm quality measurements for male breeders. Left: Relative fecundity estimates for female breeders. Data are expressed as means ± standard deviation.

Testosterone (T) is thought to be the precursor of E_2 which acts on hepatic vitellogenesis. Patterns in female steroid concentrations testosterone (T) and 17β-estradiol (E_2) were closely linked for all broodstock groups in the present study (figure 1.1). An increase of T and E_2 levels was observed during August-January, followed by a high but stable pattern up to spawning. The increase of T and E_2 levels in female pikeperch breeders coincident with the periods of most rapid GSI rises. Maximum T (>9 ng/ml) and E_2 (2.8 ng/ml) levels values were measured prior to spawning.

Absolute and relative fecundity of wild pikeperch breeders was higher compared to F1 breeders (figure 1.2). There was no statistical difference between F1 breeders reared in an intensive broodstock tank or F1 breeders reared in an extensive pond system. In the present study relative fecundity varied between 209 eggs/gram for F1 breeders and 276 eggs/gram for wild breeders, and are in the ranges as reported in literature.

From this experiment the following can be concluded:

- The current study provides insight in the reproductive cycle of male and female pikeperch and distinguishes between wild and F1 breeders reared under intensive respectively extensive culture conditions. The greater understanding of the endocrine and physiological changes during the reproductive cycle provided by this study is essential for further studies on out-of-season spawning.
- Based on this study it can be concluded that neither fish origin nor production environment negatively affects the reproductive cycle; no differences between morpho-anatomical, sex steroid or histological performance of male and female pikeperch breeders was found for the different broodstock groups (except for GSI levels of male breeders).
- No lack of reproduction performance was observed for intensively reared F1 breeders (male & female). This indicates that intensive reproduction of pikeperch seems feasible, without loss of reproduction performance and thus provides a strong perspective for intensive aquaculture of this species.
- Furthermore, the essential gonadal maturation of male breeders appears to be completed several months before spawning (spermiogenesis), suggesting that it might be possible to induce male pikeperch (wild and F1) to spawn several months out-of-season. In female breeders, vitellogenesis has occurred during early winter. However, much GSI growth and final maturation occurs during the last months of the reproduction cycle. It is unknown to what extent this process can be accelerated and advanced spawning can be induced.

II.2.2 – Subtask 2: Out-of-season spawning on-farm

In this study broodstock facilities (two 25m^3 circular tanks) with temperature and photoperiod control have been established at a commercial farm. Breeders have been collected from wild stocks as well as from the production facilities (F1). During the two years of the project in total five broodstock groups were used; two groups in 2005/2006 and three groups in 2006/2007 (see table 2.1). As photoperiod and temperature are generally considered as the most important cues in gametogenesis and spawning (Migaud et al., 2002) a photo-thermal protocol was applied in order to induce out-of-season reproduction in pikeperch. The protocol consisted of four successive periods; ongrowing stage, cooling down, cold period and reproduction phase (figure 2.1). As wild fish are caught in winter they will only be exposed to the cold and the reproduction period. At the start of the reproduction period, spawners were selected at regular intervals and stocked in pairs in spawning tanks where temperature gradually increased. Spawning was then induced by a single hormonal injection. Spawning tanks were equipped with an artificial nest, and the fish were allowed to spawn naturally on the nest. After spawning the nests were transferred from the spawning tank to an incubator for hatching. Reproduction performance was determined by qualitative nest assessments.

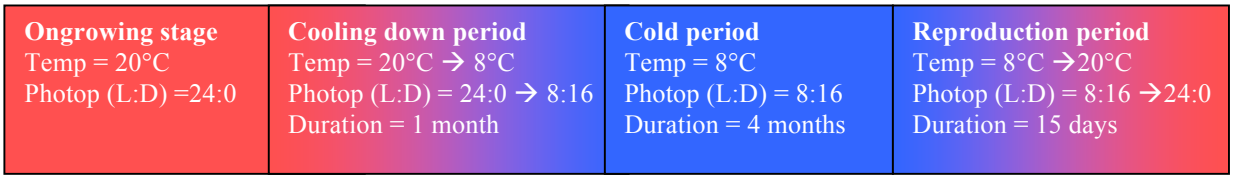


Figure 2.1 Temperature and photoperiod manipulated out-of-season spawning protocol

During the first year in total 25 nests were successfully produced from both F1 and wild breeders (table 2.1). However, less than 40% of the initial stocked breeders showed dilated abdomen or were spermiating males and thus were used for reproduction. This is similar to other years (pers. com. partner 2 and partner 6). On average 90% to 100% of the selected breeders did spawn after hormonal injection. Low reproduction numbers and bad nest quality of delayed wild breeders in July 2006 suggest that spawning is limited to a range of storage time under low water temperatures. Due to unexpected and unknown reasons, out-of-season spawning of F1 breeders completely failed in 2007. Both groups showed very few developing male and female breeders at the end of the cycle and only two successful reproduction occasions were observed. It was therefore not possible to determine the effect of age of the cultured breeders. In total 11 nests were obtained from an group of 50 wild breeders (III), resulting in an efficiency of more than 40%.

Spawning of both wild and F1 breeders in and out-of-season occurred 3 to 7 days after hormonal stimulation, but most often after 4 to 5 days. Hatching occurred 3 to 5 days after spawning, and hatching rates varied between 60-80%. The adhesiveness of the eggs to the nest material was good for all nests. Division of the eggs on the nest, however, showed higher variation between the nests but no pattern between groups or within time could be observed. One nest was severely covered with fungus.

Table 2.1 Information on broodstock groups during the spawning season 2005/2006 (A and B) and spawning season 2006/2007 (I to V)

Group characteristics	Group number	Total number of fish	Reproduction date	Number of nests
F1	A	80	January-March 2006	12
Wild	B	75	April-June 2006	13
F1 (age 1)	I	75	November-December 2006	2
F1 (age 2)	II	75	February- March 2007	0
Wild	III	50	January-March 2007	11

From this study it can be concluded that out-of-season spawning in wild and F1 breeders was successfully introduced during the first year of the project. Based on the established managements plan it seemed possible to produce eggs and larvae on a year round basis when using both F1 and wild breeders during the first year. During the second year F1 reproduction in winter completely failed due to unknown reasons. Those results indicate that out-of-season reproduction of pikeperch breeders should be further studied to improve its security.

II.2.3 – Subtask 3: Optimizing out-of-season spawning protocol

In order to optimize the out-of-season spawning protocol (see also subtask 2), the effects of different temperatures and different durations of the cold period on reproduction performance in cultivated pikeperch were tested. In total three photothermal regulated tanks were established. The same out-of-season protocol as previously described in subtask 2 was applied to induce out-of-season spawning and three different temperature levels for the cold period were tested; 8°C (tank 1), 10°C (tank 2) and 13 °C (tank 3). For each thermal regime, three different durations of the cold period were tested: 100, 135 and 164 days. Approximately 90 F1 breeders, which had never spawned before the onset of the experiment, were stocked in each tank. After each time interval batches of breeders (n=10 to 20) were transferred from the broodstock tanks to the reproduction tanks and induced to spawn by increasing the photothermal regime and a hCG injection. Reproduction performance was determined in two ways. 1) General reproduction performance, such as number of maturing breeders and number of successful produced nests, was determined for all batches. 2). In order to test the effect of temperature on sperm quality, semen samples were taken from all temperature profiles at day 100. To test the effect of duration of the cold period on sperm quality, semen samples of the 8°C-group were taken at day 100 and day 135. Sperm quality was determined by CASA analysis (Kime *et al.,* 2001; Rurangwa et al., 2004).

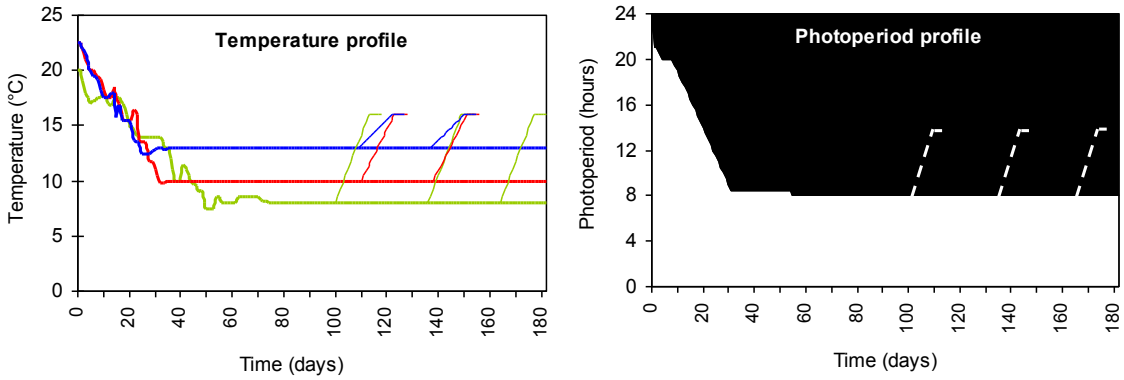


Figure 3.1 Out-of-season spawning protocol based on photothermal regimes**.** Right panel: temperature of tank 1 (— 8 °C), tank 2 (— 10 °C) and tank 3 (— 13 °C) and during the warming up phases. Let panel: photoperiod profile applied to all tanks, dashed line represents values during warming up stage in the reproduction tanks

For all treatments gametogenesis was observed in at least a fraction of breeders. The fraction of maturing fish was lower for the 8°C treatment compared to the 10 and 13°C treatments (figure 3.2). All breeders of the higher temperature treatments (10, 13°C) were mature (spermiating or egg release) while only 60-83% of the breeders of the 8°C treatment were mature. All female breeders originating from treatments 10 and 13°C released eggs, but nest quality was most of the time low and only a few fertilized and/or hatched nests were observed. We observed no difference between the different durations of the cold period (100, 135 or 164 days). This different from Eurasian perch for which it was shown that gonad development and reproductive success depended on the chilling duration with better results for a long cold period (Migaud et al., 2002). Spawning took place 4 to 12 days after injection but in general some days later than during the natural spawning season and

later than observed by Zakes (2007) for second and third year out-of-season spawners. Finally, we observed a lower condition for the breeders after rearing them by 8°C and probably weight loss has occurred.

No strong effects of temperature nor of duration of the cold period was found by sperm quality measurements (figure 3.2, right panel). Motility, curvilinear and straight line velocity were lowest for the 8°C group. These results confirm the observations made by the general reproduction performance measurements that performance of the 8°C group is slightly lower compared to the two higher temperature groups.

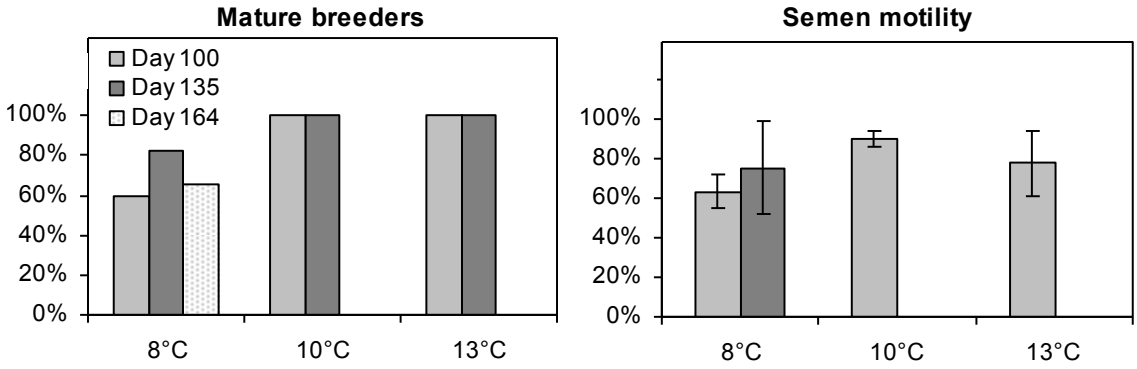


Figure 3.2: Left: fraction of mature breeders. Right: Semen motility as measured by CASA

From this study the following can be concluded :

- The applied photo-thermal regimes have successfully led to maturation of both male and female breeders. However, fertilization and/or hatching rates are still low.
- Thus, out-of-season spawning performed with cultivated specimens held exclusively (from larvae to spawners) in re-circulating systems with artificial temperature and light regimes and fed with formulated feed is possible and further studies should improve the productivity.
- All three temperature treatments resulted in maturing and spawning pikeperch breeders. The number of mature breeders was slightly lower for the 8°C temperature treatment compared to the two higher temperature treatments. No difference between different durations of the cold period was observed.
- A weight loss in breeders reared by 8°C was observed. This confirms earlier observations that rearing pikeperch under cold temperatures reduces the condition of the fish (unpublished). Bad health status might be caused by low temperature conditions over a prolonged time interval. Results of this experiment might suggest that bad health status negatively may affect the reproduction performance of pikeperch.
- For many fish species it has been shown that the effects of reproduction of younger specimens, especially those spawning for the first time, are significantly lower (Kamler, 2005). Because first spawning breeders were used in this experiment, it was decided to retain them after the end of the experiment and again apply a full photo-thermal regime in order to induce a second spawning next year. Spawning success is expected to be higher for those breeders which spawn for the second time. Those results will, however, be gained several months after the end of this project.

II.2.4 – Subtask 4: Artificial reproduction

Since a manual on artificial reproduction techniques was developed and a workshop on the same topic was organised, knowledge was efficiently transferred to all partners involved in the project. The manual is also available on the Luciopercimprove website (www.luciopercimprove.be) for people outside the project consortium and interested in artificial pikeperch propagation.

II.2.5 – Subtask 5: Cryopreservation

Four experiments were conducted in order to develop a suitable protocol for storing pikeperch semen under cryopreservation. Different extenders, cryoprotectants and sperm-motility activators were evaluated (exp. I & II), and the effectiveness of a simple and practical freezer (on-farm method) was compared to a computer-freezer (exp III & IV).

Experiment I
Within the first experiment different extenders, cryoprotectants and sperm-motility activators were evaluated. This experiment was designed in a factorial 3 extenders (NaCl 200 mM, glucose 277 mM and Ginsburg fish Ringer) x 2 cryoprotectants (methanol CH_3OH and dimethyl sulphoxide – DMSO $(CH_3)_2SO$) x 3 activating solutions ($NaHCO_3$ 119 mM, tank water and NaCl 50 mM). Initial sperm motility was analyzed directly after semen collection. Thereafter, semen was diluted in each of the three semen extenders, the solutions were divided into two portions and 10% of cryoprotectants was added. Cryovials with diluted semen were frozen by a controlled computer-freezer. After few days, cryovials were thawed and sperm motility was scored after activation with three solutions. Motility was scored in two different ways: post-thaw motility (% of moving cells relative to the whole population of cells) and relative motility (% of moving cells relative to pre-freezing motility). Results from this study indicate that NaCl 50 mM and tank water are better sperm-motility activators compared to $NaHCO_3$. Highest sperm motilities were obtained in samples frozen using the extender NaCl 200 mM and methanol as cryoprotectants (figure 5.1).

Experiment II
In the second experiment some additional extenders and cryoprotectants were evaluated. This experiment was designed in a factorial of 2 extenders (NaCl and glucose) x 2 egg yolk concentrations (0 and 5%) x 3 cryoprotectants (methanol, DMSO and methyl glycol) x 3 activating solutions ($NaHCO_3$, tap water and NaCl 50 mM). The methods used were similar to the previous experiment. Results of the second experiment show that the addition of egg yolk to the extender was beneficial only when combined with glucose (extender) and DMSO (cryoprotectants), for the other combinations post-thaw sperm motility decreased when compared to the same freezing media without egg yolk. Best results were found for NaCl (extender) without egg yolk; relative sperm motility was above 50% in samples frozen in NaCl combined with all cryoprotectants. NaCl 50 mM and tap water were better activating solutions compared to $NaHCO_3$ (figure 5.1).

Experiment III
The effectiveness of a shipping dewar (dry-shipper) as freezer was tested in the third experiment. A shipping dewar is a simple and practical freezer and allows freezing semen samples on the farm. Furthermore, one additional extender (Rathbun) was tested. In this experiment, semen was diluted in 6 freezing media as a combination of 3 extenders (NaCl 200 mM, Rathbun and Ginsburg fish Ringer) and 2 cryoprotectants (methanol and DMSO). Because the freezing rate used during experiment I and II was slow and the freezing rate in the dry-shipper is fast, 0.25-mL straws were used rather than cryovials. Diluted semen was loaded into the straws and frozen under nitrogen vapour inside the dry-shipper. The following day, straws were transferred to liquid nitrogen for storage until sperm analysis. After thawing semen was activated with NaCl 50 mM and sperm motility was scored. Results from this study show that pikeperch semen was successfully frozen using the dry-shipper as a freezer (figure 5.1). All combinations showed more or less equal motilities (above 40% relative motility), except for the combination DMSO and Ginsburg fish Ringer which was much lower (~10%).

Experiment IV
As promising results using the dry-shipper were obtained, in the final experiment, all extenders and cryoprotectants tested during experiments I and II were evaluated using the dry-shipper. Additionally, Hanks balanced salt solution (HBSS) was included as extra extender. Semen was

diluted in 15 freezing media as a combination of 5 extenders (NaCl 200 mM, Rathbun, Ginsburg fish Ringer, glucose 277 mM and HBSS) and 3 cryoprotectants (methanol, methyl glycol and DMSO). Further methods were similar as described for experiment III. Results of this study showed high relative post-thaw motilities (above 60%) in samples frozen in methanol as cryoprotectant combined with NaCl, Rathbun and HBSS, as well as in methyl glycol as cryoprotectant combined with NaCl and HBSS (figure 5.1).

From those experiments the following can be concluded:

- Initial semen quality of pikeperch is a problem. Sperm auto-activation was observed in most of the samples, which can be a strong cause of motility reduction. It is therefore suggested to use an immobilizing solution during the stripping process.
- The use of a nitrogen vapor vessel, dry-shipper, was effective for pikeperch semen. This is a very simple and cheap method that can be manipulated by farmers without the need of expensive equipments or technicians.
- Different extenders were tested but NaCl 200 mM always produced high post-thaw sperm motility. As this is a very simple solution compared to Rathbun and HBSS (that also produced high post-thaw motility), it is recommended to use this solution as semen extender.
- The addition of egg yolk to the freezing media was beneficial only when combined with glucose (extender) and DMSO (cryoprotectant).
- Both methanol and methyl glycol are effective cryoprotectants in a controlled-rate freezer as well as in a dry-shipper. DMSO is only effective when slower freezing rates (controlled-rate freezer) are used.
- The post-thaw motilities obtained at the end of this project suggest that good fertilization rates may be obtained, but this is yet to be tested. As semen volume in pikeperch is low, it would also be interesting to use pooled semen of 4-5 males, combined with larger straws (4 mL) in order to optimize the fertilization process.

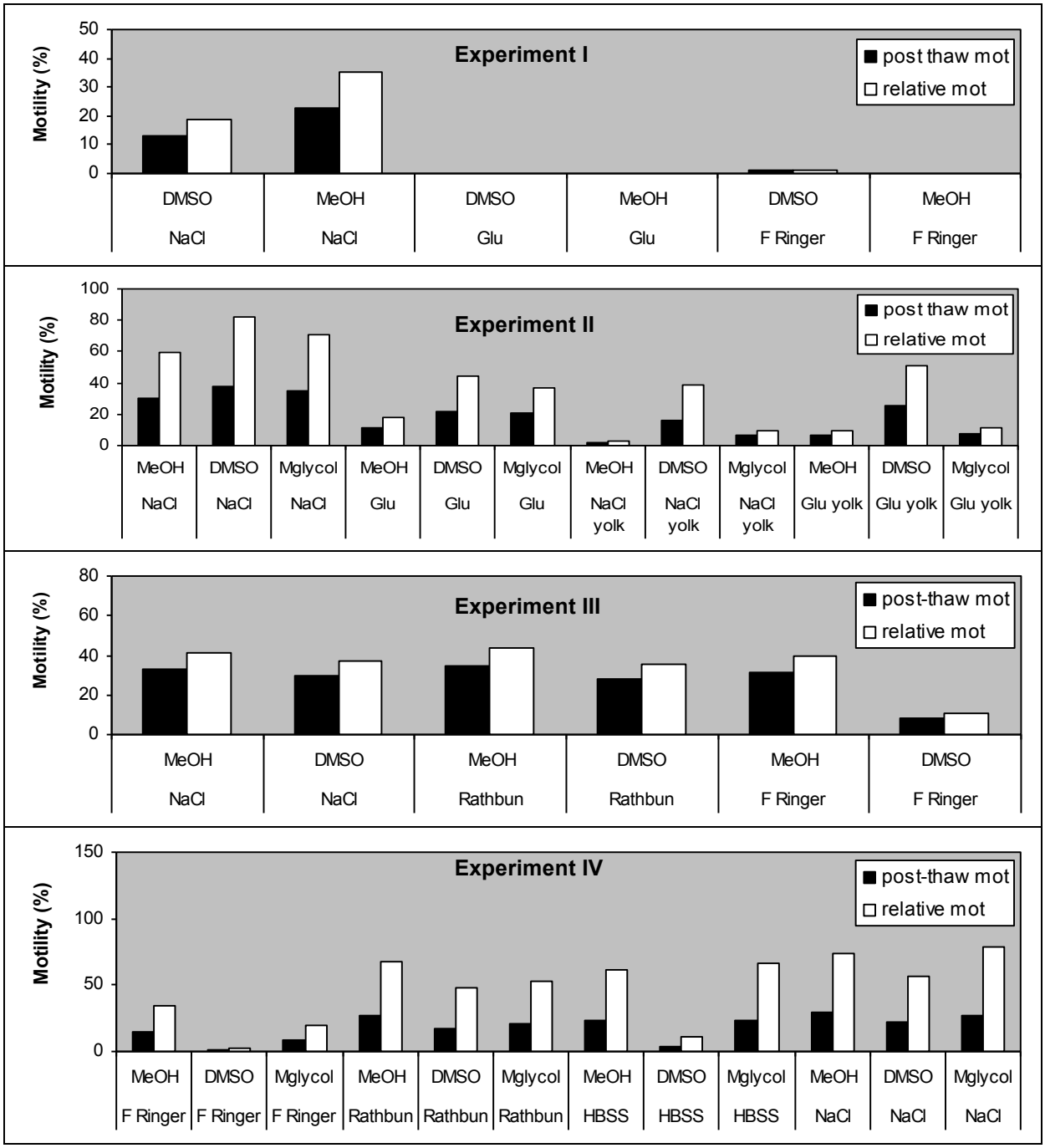


Figure 5.1: Sperm motility of pikeperch semen frozen in different freezing media (as displayed on the x-axes) and activated with NaCl 50 mM. Post-thaw motility is expressed as % of moving cells relative to the whole cell population. Relative motility is expressed as % of moving cell relative to pre-freezing sperm motility.

II.2.6 – Subtask 6: Marking of families

A first step towards a breeding program for pikeperch culture was made by investigating available family marking methods and using one method to do some preliminary tests on inter- and intrafamily growth performance. Based on literature research, three marking methods seemed feasible for marking of pikeperch fry: microsatellites, fluorescent marking and external marking. In general, CRAFT projects aim towards high benefits in terms of knowledge and development of techniques for fish farmers. It is therefore very important that the chosen method can and will be continued after finishing this project (*Luciopercimprove*). In that sense, the use of microsatellites for distinguishing between families was judged to be too expensive and unpractical for fish farmers in the long term. Fluorescent marking of consumption fish is not allowed by legislation in the Netherlands. It is also questionable if one should apply such techniques in consumption fish in terms of consumer perception. Altogether, this made us decide to work on external marking (Floy tag ©).

Inter-family growth performance

In commercial pikeperch farming sorting of pikeperch fry starts as early as 10-15mm to reduce the effects of cannibalism. Individuals of the same length originating from several families are then stocked together in one tank and monitoring family growth performance is therefore impossible in such systems. In an experiment where growth rates of different families were evaluated, we managed to culture four families (3x F1 and 1x F2) in separate tanks. By removing the extremely large/small specimens is was shown that cannibalism could be suppressed. The individual families were cultured as one groups from hatching until they reached 40-60 gram at which they could easily be externally marked.

No difference was observed in growth rates between pikeperch juveniles originating from the three F1 families (figure 6.1). On the other hand, lower growth rates were observed for F2 offspring originating from the fourth family. Those results are in contrast to results found in many other studies, where higher growth rates are normally found for more domesticated families (among others Hershberger et al., 1990; Tymchuk and Devlin, 2005; Tymchuk *et al.*, 2006). It should, however, be taken in to account that this experiment comprised only three F1 families and one F2 family which is too low to draw hard conclusions on whether differences in growth performance be attributed to the genetic background. The negative results on domestication are nonetheless surprising. Another important result from this study was that it seems possible to culture one family as one group from hatching until a size at which can be marked. This allows distinguishing between individual families. Doing so at a commercial farm, desires a good management plan since larvae and juvenile tanks will be used less efficient.

Intra-family growth performance

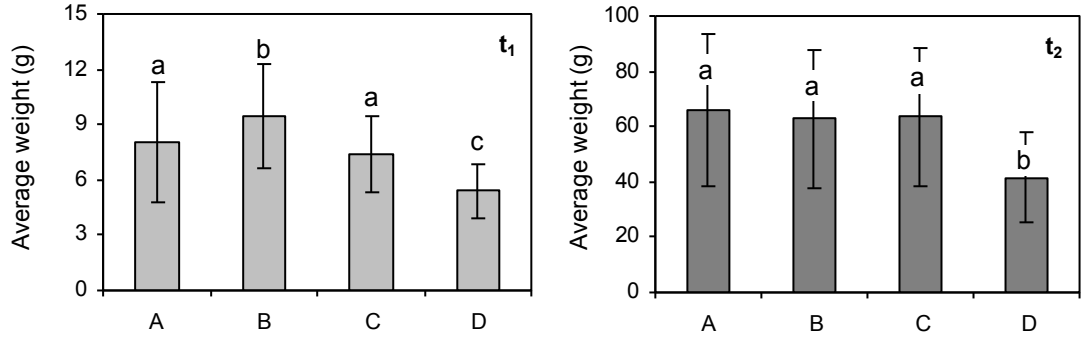


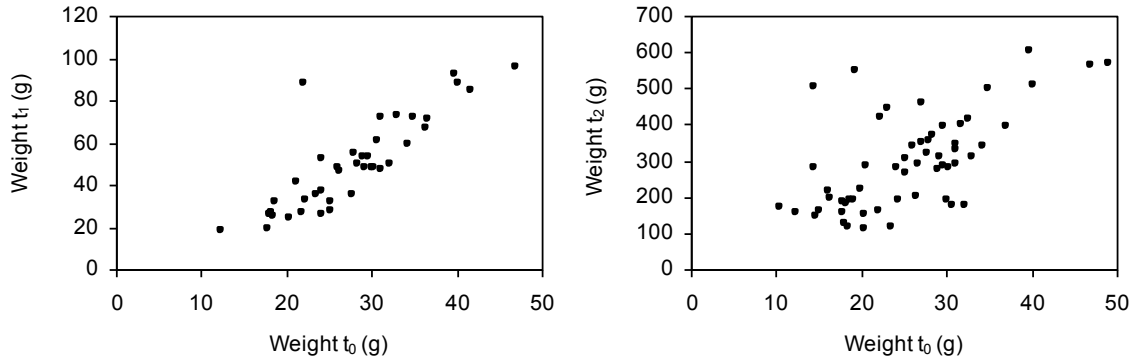
Figure 6.1: Average weight of the four families at t_1 (left panel) and t_2 (right panel). N at t_1 was 200 individuals for each family. Error bars represent the standard deviation. Values with different letters are statistically different (α=0.01)

In a second experiment intra-family growth performance was determined. Therefore 362 fish from one family were externally marked (floy-tags). Because relatively small fish were used (10-50

gram), the smallest T-bar floy-tags were used (FF-94, Floy tag ©). All marked fish were transferred back into the production tank and were cultured under 'normal' culture circumstances. Individual length and weight were measured before the floy-tag was attached (t_0) and twice during the following six months (day 33= t_1 and day 176= t_2).

During both samplings (t_1 and t_2) a strong positive correlation between initial weight and weight during sampling was found (figure 6.2). Similar correlations are also found for weight at different time intervals in sea bass (Grobler et al., 1992; Gorshkov, 2004). This correlation indicates that larger fingerlings will remain larger during the entire production cycle and so reach the marketable weight at a younger age than relatively small fingerlings. This can both be used for within-family selection programs as well as selection within the fingerling stock to achieve weight at market size with a shorter production cycle. It is shown that a within-family selection strategy is effective in increasing growth rate in several fish species (the freshwater yabby, Jerry et al., 2005; Chevassus et al., 2004; sea bream, Murata et al., 1996).

Figure 6.2 Individual growth. Relation between initial weight (t_0) and weight during sampling. Left for the sampling at day 33 (t_1) and right for the sampling at day 176 (t_2)



From those experiments the following can be concluded:

- External marking with floy-tags seems a good on-farm marking method for family and individual marking purposes.
- By removing the extremely large and extremely small individuals, cannibalisms can be suppressed. It is therefore possible to culture individual families until they can be externally marked (25-50 gram).
- Preliminary results showed lower growth rates for one F2 family compared to three other F1 families. This is surprising since other studies have shown higher growth rates for more domesticated families. However, it should be noted that in this study only one F2 family could be taken into account. The experiment will be continued after the end of the project to gain more information on growth differences between families during a whole production cycle. More families should be monitored to gain a representative view on domestication processes.
- The intra-family growth performance study showed that weight of individual pikeperch was correlated during different time intervals, which indicates that large fingerlings will remain larger during the entire reproduction cycle and reach marketable size at an earlier age. This may be useful for the choice of selection criteria, raising the possibility that the response to selection for weight at market time may be achieved by conducting selection on young fish.
- Results from this study provide a good perspective for implementation of breeding programs in pikeperch farming, using external marking to distinguish between families and individuals.
- Broodstock domestication and selective breeding in pikeperch have yet to be exploited on experimental as well as production scale. Since fecundity of pikeperch is high, inbreeding might be a problem. A combined individual and family selection is therefore recommended (Gjedrem, 1998). In any case, it is advisable to start the domestication process from a population with a large

genetic basis, and to manage it in order to maintain suitable levels of genetic variability across generations (Vandeputte & Launey, 2004).

II.3 – Degree to which the objectives were met

Objective 1: To develop methods for year-round spawning of pikeperch
Greater understanding on the annual cycle of sex steroids, GSI and gonad development in pikeperch broodstock has been gained during this project. Evidence was provided that reproduction performance of F1 breeders reared in intensive rearing systems has not decreased compared to wild breeders. This is important from an aquaculture point of view since F1 breeders are most important for commercial farming.

A temperature and photoperiod manipulated protocol for out-of-season spawning was successfully introduced. Good results were obtained but the unpredictability of the results remains. The protocol needs to be optimized during further studies. Results of the study focussing on optimization of the out-of-season spawning protocol showed that slightly higher temperatures during the cold stage might increase the reproduction performance of pikeperch breeders.

The above indicates that objective 1 has been reached: for the first time in pikeperch culture out-of-season spawning using F1 breeders was successfully introduced. Further studies are required to optimise the protocol.

Objective 2: To develop methods for artificial fertilization in pikeperch
Objective 2 has been reached since a successful method for artificial stripping and fertilization has been established and optimized during this project. The method focuses on techniques which can easily applied at farm level. This has resulted in the extensive and detailed document "Manual on artificial pikeperch reproduction" and is available for everyone interested in pikeperch farming. Furthermore a workshop was held in the University of Nancy where experts on artificial fertilization showed how to perform artificial fertilization. A movie was done and given to all partners. This manual was distributed to all participants attending the Workshop on Percid Culture held in the University of Namur in January 2008.

Objective 3: To develop cryopreservation of pikeperch sperm
A protocol for storing pikeperch semen under cryogenic circumstances was developed and the post-thaw motilities obtained at the end of the project suggest that good fertilization rates may be obtained. The use of a dry-shipper (a simple and cheap method that can easily be used by farmers) was found to work effective for pikeperch semen. Although the protocol needs to be fine-tuned in future studies, the objective has been reached since a practical on-farm technique for cryopreservation of pikeperch semen was developed during this project. Such protocol can be immediately transferred to SMEs partners.

Objective 4: To develop marking methods for family marking of pikeperch fry/juveniles
An external method for individual and family marking was tested and found to work efficiently for marking pikeperch juveniles at a commercial scale. By using external marking methods (floy-tag) inter- and intra- family growth performance can be monitored and the results can be used in a selective breeding program. It can be concluded that this study has provided a good perspective for implementation of breeding programs in pikeperch farming by using external marking methods and so objective 4 was reached.

III – Improving egg and larval quality of farmed pikeperch through improvement of broodstock diet (WP3)

III.1 – Workpackage objectives

The overall objective of WP3 was to improve the egg and larval quality of farmed pikeperch through improvement of broodstock diet. In order to reach this objective, three subtasks have been undertaken.

Feeding strategy (live vs co-feeding vs dry food)
The objective of this subtask was to improve broodstock management during the reproductive cycle (10 months). The results of this study conducted from September 2006 to June 2007 showed that it is possible to mix dry feed to live feed in co-feeding and obtain good quality spawning. Furthermore, it showed that live feed can be easily reared intensively in indoor facilities, avoiding contamination occurring when live feed is harvested in the wild (what is usually done). Therefore, it can reduce the occurrence of pathologies in pikeperch and reduce the cost of feeding (live feed is expensive).

Broodstock diet improvement
The objective of this subtask was to improve an existing commercial diet, specifically formulated for fish reproduction, with arachidonic acid which is known to enhance quality of reproduction in fish. Nutreco has changed the composition of the commercial Vitalis in 2007. The results suggest that it is better adapted to pikeperch and enrichment with arachidonic acid could improve the quality of reproduction.

Efficiency of phospholipids addition in larval diets
The effect of supplementation of the pikeperch larval diet with phospholipid was tested. It was shown that the increase of phospholipids in the diet could improve larval growth.

III.2 – Progress towards objectives

III.2.1 – Subtask 3.1: Feeding strategy (live vs co-feeding vs dry food)

An experiment on pikeperch broodstock nutrition was conducted over one year, from September 2006 to May 2007. Three broodstock diets were tested. The first one was a dry feed (DD group, Vitalis, Nutreco). The second diet, considered as a control, consisted in forage fish (*Cyprinus carpio*), (FF group). The third one was a mix of Vitalis and forage fish alternatively given to broodstock over a week (FD group). The reproduction occurred in April 2007 (hormonal injection) and the larval quality was assessed.
During the spawning season, many injected females of the DD group (57%) failed to spawn (Table 1). It was also observed that less male were spermiating than in the two other treatments and that breeder mortality was higher. This indicates that the Vitalis feed composition is less adequate for pikeperch health and reproduction than forage fish. As expected, feeds containing forage fish gave the best reproductive performances. Indeed, both FF and FD groups displayed higher similar proportions of running males and hatching rates than the DD group. All these data suggest that disruptions of physiological processes may have occurred in fish fed only with Vitalis, perhaps because of lacks and/or too high levels of some nutrients in this diet compared to the two others.

Table 1: Broodstock reproductive performances at the end of the experiment. Numbers under brackets represent numbers of spawning.

	Diets		
	FF	**FD**	**DD**
Injected couples	8	8	7
Running male (%)	76	89	54
Spawning rate (%)	100	88	57
No hatch (%)	37,5 (3)	14 (1)	50 (2)
Few hatch (%) (<1000 larvae)	25 (2)	29 (2)	25 (1)
High hatch (%) (>2000 larvae)	37,5 (3)	57 (4)	25 (1)
Broodstock growth (%)	31,5	27,5	26,5
Broodstock mortality (%)	14	25	43

Thus, fatty acid profiles in the broodstock diets have often been shown to strongly influence reproductive performances and progeny survival in fish (Izquierdo *et al*., 2001). Too low or high levels of n-3 FA levels in the diets of sea bream (*Sparus aurata*) and Japanese flounder (*Paralychthys olivaceus*) have already been shown to alter larval quality (Fernandez-Palacios *et al*., 1995 ; Furuita *et al*., 2002). In our experiment, the FA profiles of Vitalis and forage fish were very similar except for the EPA level which was slightly higher in Vitalis. Thus, there was a difference between the two diets in term of EPA/AA ratio. It is known that EPA and AA compete in the same enzymatic system for production of 3-series and 2-series prostaglandins respectively (Sargent, 1995). Since these prostaglandins do not share the same functionalities, the EPA/AA ratio in the broodstock diet is important for the fish to be able to maintain homeostasis. It was shown *in vitro* that PGE_2 induce oocyte maturation in sea bass while PGE_3 does not (Sorbera *et al*., 2001). An unbalanced EPA/AA ratio in the diet in favour of EPA could lead to a predominant biosynthesis of 3-series prostaglandins, leading to a lesser ability to undergo final maturation. Thus, the high level of EPA in the Vitalis diet could partly explain why only a low proportion of injected females spawn in this treatment. A lack of PGE_2 synthesis may have led to a lower receptivity to HCG in these fish.

Eicosaenoids derived from AA and EPA have also been implicated in immune system regulation and sensitivity to stress. Thus, too high or low levels of AA in the diet have been shown to lead to severe physiological disorders in post-smolt salmon, *Salmo salar*, (Bell *et al*., 1990, 1992). From fish farmer experience, pikeperch is very sensitive to stress and especially to factors caused by captivity (human presence, handling, high light intensity…). This has already been observed in Eurasian perch (Acerete *et al*., 2004 ; Wang *et al*., 2006 ; Strand *et al*., 2007), but never studied in pikeperch. Nevertheless, the high mortality observed in the DD group during the whole experiment could be due to a combine effect of stress factors and a disrupted physiological state of the broodstock due to inappropriate feeding regime.

Throughout the experiment, growth did not differ among treatments, indicating that all groups of fish were eating correctly (Table 1). Broodstock survival was lower when fed with commercial food. In addition, fewer males were spermiating and spawning rate was lower than in the two other treatments. FD gave the highest amount of larvae whereas FF and DD gave only one spawning with a high hatching rate (>2000 larvae).

At hatching, larval weight, length, body lipid composition, dry matter and results of challenge stress tests did not differ among treatments (Table 2). This indicates that larval quality was similar or did not differ significantly among treatments.

Table 2: Larval morphology, gross composition and resistance to stress tests, (mean ± S.E.). No significant difference ($P<0.05$).

	Diet		
	FF	**FD**	**DD**
Larval weight (mg)	0.40 ± 0.01	0.40 ± 0.05	0.34 ± 0.22
Larval length (mm)	5.17 ± 0.52	5.03 ± 0.31	5.14 ± 0.08
Total lipid (%)	20.5 ± 0.7	21.0 ± 0.5	23.7 ± 0.6
Dry matter (%)	19.2 ± 3.3	15.5 ± 3.7	16.1 ± 0.7
Starvation (h)	106 ± 75	197 ± 90	292 ± 28
Osmotic shock (%)	45 ± 46	55 ± 37	42 ± 12

Larval growth performances did not differ between treatments, indicating that potentiality of larvae was globally the same (Table 3). Survival is in the range of what has been found in other studies. In fish farm, larval survival is generally 7-10%. Kestemont *et al*., (2007) observed slightly higher survival (13-17%) and similar mortality rates (48-69%) in their experiment on pikeperch larval rearing. Pikeperch larvae are very small at hatching (0.3-0.4 mg). For example, Eurasian perch larvae weight is 3-4 mg at this period. Therefore, it is not surprising that larval survival is low in this species. Its reproductive strategy is to rely on the number of eggs produced (small amount of yolk but high fecundity), rather than on the ability of the larvae to survive.

Table 3: Results of growth trials according to the tested treatment (mean ± S.D.)

	Diet		
	FF	**FD**	**DD**
Mortality (%)	48 ± 1	45 ± 9	61 ± 16
Cannibalism (%)	45 ± 6	49 ± 11	36 ± 20
Survival (%)	8 ± 6	6 ± 6	3 ± 3
Malformation (%)	31 ± 8	24 ± 20	34 ± 22

Table 4: Fatty acid profile of larvae (in % of total lipids), (mean ± S.E.). No significant difference ($P<0.05$).

	Diets		
	FF	**FD**	**DD**
C14:0	4.7 ± 0.6	5.3 ± 0.5	5.2 ± 0.7
C16:0	5.6 ± 0.6	5.7 ± 0.5	6.7 ± 0.8
C18:0	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.3
C20:0	1.4 ± 0.3	1.6 ± 0.2	2.0 ± 0.3
Total SFA	13.0 ± 1.4	13.9 ± 1.3	15.2 ± 1.8
C16:1n-7	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
C18:1n-7	2.0 ± 0.3	1.9 ± 0.2	1.9 ± 0.4
C18:1n-9	8.7 ± 1.0	9.9 ± 0.9	9.1 ± 1.2
C20:1n-9	5.2 ± 0.7	5.9 ± 0.6	6.6 ± 0.9
Total MUFA	16.7 ± 1.9	18.3 ± 1.6	18.4 ± 2.3
C16:4n-1	30.8 ± 7.1	28.4 ± 6.1	20.2 ± 8.7
C18:4n-1	0.7 ± 0.1	0.7 ± 0.1	1.2 ± 0.2
C16:2n-4	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
C16:3n-4	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
C18:3n-4	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
C18:2n-6	5.3 ± 0.8	4.8 ± 0.7	7.5 ± 1.0
C20:4n-6	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Total n-6	6.1 ± 0.8	5.9 ± 0.7	8.4 ± 1.0
C18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C18:4n-3	5.3 ± 1.6	5.8 ± 1.4	3.5 ± 1.9
C20:4n-3	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.2
C20:5n-3	6.0 ± 1.1	5.4 ± 1.0	7.8 ± 1.4
C22:5n-3	2.5 ± 0.4	2.3 ± 0.4	3.2 ± 0.5
C22:6n-3	17.2 ± 3.0	18.0 ± 2.6	20.4 ± 3.7
Total n-3	31.7 ± 3.1	32.0 ± 2.7	35.4 ± 3.8
Total PUFA	70.3 ± 3.4	67.9 ± 2.9	66.4 ± 4.1
n-3/n-6	5.4 ± 0.3	5.5 ± 0.3	4.2 ± 0.4
DHA/EPA	3.0 ± 0.2	3.4 ± 0.2	2.7 ± 0.2
EPA/ARA	7.1 ± 1.1	5.4 ± 0.9	8.1 ± 1.3

Larval quality and body lipid composition did not differ between treatments (Table 4). As reported in other species, larval body fatty acid profiles correlated well with those of feeds, except for C14:0 and C16:4n-1. The latter was accumulated in huge amount (20-30% according to treatment), perhaps to be used as an energy source by the larvae. These profiles quite differed from those measured in just hatched Eurasian perch and pikeperch larvae from spawning of breeders harvested in the wild (Abi-Ayad et al., 2001, 2004). However, they correlated well to those found in eggs of Eurasian perch reared under the exact same conditions of temperature, photoperiod and tested feeding (Vitalis or forage fish) in the same type of tank and during the same year as in the present experiment (unpublished data). Linear correlation calculations of the fatty acid profiles of our pikeperch larvae against Eurasian perch eggs for each treatment (Vitalis or forage fish) gave R^2 coefficients of 0.74 and 0.77 respectively. Therefore, first, it shows that Percids apparently share a same pattern of lipid deposition in the eggs. Second, it suggests that this pattern strongly depends on environmental and nutritional breeder rearing/living conditions. Third, as hypothesised by Abi-Ayad et al. (2000, 2004), Percids may poorly use lipids as an energy source during embryognesis.

To conclude, spawning qualities of FF and FD pikeperch broodstock were similar and higher than fish fed with Vitalis alone. On a scientific point of view, this indicates that feeding breeders with Vitalis only is not sufficient to obtain acceptable reproductive performances. However, according to the producers, these results should be acceptable on a large scale. Indeed, the reproductive strategy of pikeperch relies on the high number of eggs and larvae produced. Larvae are small and survival in the wild is very low. Nevertheless, to enhance the quality of the diet, one key would be to add arachidonic acid, which is quite low and lacking in commercial diets in general.

This preliminary study suggests that mixing dry feed and forage fish in the broodstock diet could be an effective mid-term method to obtain spawning of good quality. Furthermore, after presentation of the results, the productivity of breeders fed with a dry diet alone seemed acceptable to fish farmers.

III.2.2 – Subtask 3.2: Broodstock diet improvement

Following the results of the first experiment, it was decided to test the effect of arachidonic acid supplementation in the diet on the quality of pikeperch reproduction. Indeed, it is known that commercial diets often lack this fatty acid since it is quite expensive to add. Therefore, it was decided to test different levels of ARA enrichment of Vitalis and to compare the results to broodstock fed with wild forage fish (considered as a reference diet). We assessed the reproductive status of fish in mid-January. This period was chosen because it is the lower limit period where the first out-of-season spawning can be obtained by fish farmers. Therefore, precocious information on the reproductive status of the broodstock at this date would be useful in order to evaluate the effect of food composition on pikeperch reproduction.

The experiment was conducted in the facilities of Partner 1. To this respect, a thermal group was installed in August 2007 in the University in order to control water temperature. A batch of 102 breeders (1 kg, 1^{+}) were provided by partner 2 and equally distributed into 6 tanks (17 breeders per tank). A photothermal program mimicking the natural variations of temperature and photoperiod was applied (**Figure 1**).

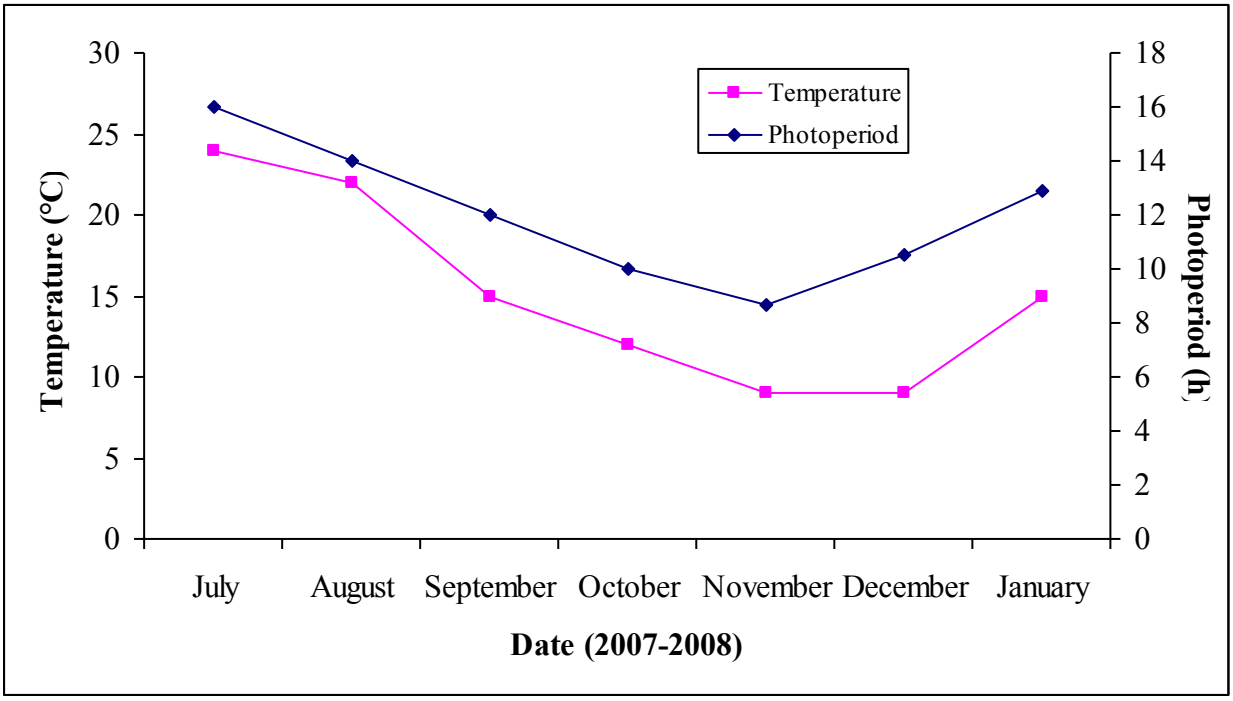


Figure 1: Variations of temperature and photoperiod applied to broodstock

Fish were fed to satiation every day with 3 different diets (2 tanks per feed). First feed (DD) was a commercial one (Vitalis, produced by Partner 5). Second feed (FF) consisted in small roach (*Rutilus rutilis*) caught in the wild. The last one (**DD+ARA**) was the dry diet enriched with 6‰ of arachidonic acid per kg of food.

In January, male and female GSI were about 0.3 and 3.2-4.5% respectively (**Tables 5 and 6**). This is quite similar to what was observed in other pikeperch broodstock at the same date (see WP2). FI were quite low (0.2-0.6), indicating that male and female pikeperch consume the energy content of their fat storage as energy during gametogenesis, as Eurasian perch does (Wang *et al*., 2006).

Sexual steroids concentrations in the plasma of males and females were also similar to those observed in WP2 and in Wang (2006) in Eurasian perch during the same period. The histological analysis indicated that oocytes were in exogenous vitellogenesis in all females and oocyte diameters were between 700 and 771 µm, which is quite homogenous and in the range of those measured at the

same period during the experiment on the characterisation of the annual reproductive cycle of pikeperch (see results of WP2).
These observations indicate that the reproductive status of all fish in all treatments were globally similar to pikeperch caught in the wild. At this level, the different commercial feeds did not appear to alter fish reproduction.
Regarding the fatty acid profile of the female gonads, no differences were found between treatments (**Table 7**). High levels of DHA were found in the eggs, as it is one of the most important fatty acid for the development of the brain and the retina (Sargent, 1995). A higher level of ARA (+30%) was found in the eggs of fish fed with Vitalis+ARA than in the eggs of fish fed with Vitalis only. ARA is essential for the development of the embryo and the larva. This suggest that Vitalis+ARA could be an enhanced diet compared to Vitalis alone. However, ARA content was higher in the eggs of fish fed with forage fish, probably because this fatty acid representing 7 to 10% of the total fatty acids of forage fish.
It has to be noted that the composition of Vitalis has changed in 2007 to better fulfill the requirements of breeders during the reproductive cycle. In particular, it has been enriched in protein (from 44 to 54%) and to a lesser extent, also in lipids (from 16 to 18%). It is explained by the fact that pikeperch would require more dietary protein than Eurasian perch (Wang *et al*., 2008).

Table 5: Morphometric indexes and plasma sexual steroid concentrations in male pikeperch at the end of the experiment.

Diet	GSI (%)	HSI (%)	FI (%)	T (ng/mL)	11K T (ng/mL)	E2 (ng/mL)
DD	0.28 ± 0.3	0.64 ± 0.1	0.38 ± 0.1	10.8 ± 1.0	1.3 ± 0.2	1.2 ± 0.4
FF	0.25 ± 0.3	0.84 ± 0.1	0.68 ± 0.1	7.4 ± 1.0	1.8 ± 0.2	1.1 ± 0.4
DD+ARA	0.27 ± 0.3	0.72 ± 0.1	0.62 ± 0.1	8.7 ± 1.0	1.6 ± 0.2	0.9 ± 0.4

Table 6: Morphometric indexes, oocyte diameter (OD) and plasma sexual steroid concentrations in female pikeperch at the end of the experiment.

Diet	GSI (%)	HSI (%)	FI (%)	OD (µm)	Lip (%)	T (ng/mL)	11K T (ng/mL)	E2 (ng/mL)
DD	4.2 ± 0.7	0.94 ± 0.1	0.59 ± 0.1	731 ± 22	7.7 ± 0.3	2.9 ± 0.1	0.46 ± 0.1	2.3 ± 0.2
FF	4.5 ± 0.7	0.90 ± 0.1	0.16 ± 0.1	771 ± 22	7.6 ± 0.3	2.9 ± 0.1	0.48 ± 0.1	2.2 ± 0.2
DD+ARA	3.2 ± 0.7	0.96 ± 0.1	0.34 ± 0.1	694 ± 22	7.1 ± 0.3	3.2 ± 0.1	0.50 ± 0.1	2.7 ± 0.2

Table 7: Fatty acid composition of female pikeperch gonad at the end of the experiment.

	Diets		
	DD	**FF**	**DD+ARA**
C14:0	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3
C16:0	4.0 ± 0.5	5.1 ± 0.5	3.3 ± 0.5
C17:0	14.9 ± 0.9	13.7 ± 0.9	13.6 ± 0.9
C20:0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Total SFA	23.7 ± 0.7	23.6 ± 0.7	21.6 ± 0.7
C16:1n-7	15.5 ± 1.6	16.4 ± 1.6	18.5 ± 1.6
C18:1n-9	9.7 ± 0.4	9.1 ± 0.4	8.4 ± 0.4
C20:1n-9	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Total MUFA	25.5 ± 1.5	25.7 ± 1.5	27.0 ± 1.5
C16:4n-1	5.7 ± 0.9	7.2 ± 0.9	5.2 ± 0.9
C16:3n-4	2.2 ± 0.2	2.5 ± 0.2	1.7 ± 0.2
C18:3n-4	1.6 ± 0.5	2.4 ± 0.5	1.3 ± 0.5
C18:2n-6	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
C20:4n-6	0.9 ± 0.3	1.7 ± 0.3	1.2 ± 0.3
Total n-6	1.1 ± 0.3	2.0 ± 0.3	1.2 ± 0.3
C18:3n-3	1.8 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
C18:4n-3	2.9 ± 0.6	3.7 ± 0.6	3.5 ± 0.6
C20:4n-3	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
C20:5n-3	7.1 ± 0.7	5.9 ± 0.7	5.9 ± 0.7
C22:5n-3	2.0 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
C22:6n-3	26.2 ± 2.1	21.9 ± 2.1	27.9 ± 2.1
Total n-3	40.1 ± 2.4	36.3 ± 2.4	42.0 ± 2.4
Total PUFA	50.9 ± 1.1	50.8 ± 1.1	51.5 ± 1.1
n-3/n-6	37 ± 5.8	20 ± 5.8	36 ± 5.8
DHA/EPA	3.8 ± 0.5	3.9 ± 0.5	4.8 ± 0.5
DHA/ARA	29 ± 4.4	14 ± 4.4	26 ± 4.4

In conclusion, there was no major difference between the reproductive status of the breeders fed different diets. The “new” Vitalis composition, with higher protein and lipid contents may not alter the quality of reproduction of pikeperch anymore, compared to when breeders are fed with wild forage fish. An enrichment of Vitalis with arachidonic acid slightly allows enriching the eggs with this fatty acid. However, it is still in higher proportions in the eggs of breeders fed with wild forage

fish. We recommend further investigations on this feed that seems better than the old one and to test it on a small scale in fish farm. This practice avoids introducing any disease from wild forage fish into a recirculating system, what would be catastrophic for any fish farm.

III.2.3 – Subtask 3.3: Effect of different level of phospholipids content in the diet on larval quality

Even though the improvement of broodstock diet, through a well-balanced ratio of essential fatty acids and vitamins should improve the quality of eggs and larvae, the supplementation of diet with lecithin as phospholipids sources, should be considered as an alternative way (Coutteau, 1997). Indeed, larval diets enriched with phospholipids were reported as allowing a better quality of larvae. The objective was to test whether the quality of the larvae fed with such an enriched diet could be improved. If phospholipids contents have effective effects on the larval quality, the inclusion of phospholipids directly into the broodstock diet could be study during the second year of the project.
Three experimental isoproteic (56%) and isolipidic (20%) larval diets containing different phospholipid contents (1, 5 and 8%) were tested (PL1, PL5, PL8, respectively).
No effect of the diet was found on survival rate (**Figure 8**), cannibalism rate (**Figure 9**) and malformation rate (**Figure 10**). These results differed from those reported for others species as beneficial effect of dietary phospholipids on survival rate was reported for sea bass larvae, *Dicentrarchus labrax,* (Zambonino Infante et al., 1999) or for carp larvae, *Cyprinus carpio* (Geurden et al., 1995). Moreover, for seabream, Cahu et al. (2003) showed a reduction of malformation (jaw deformities and spinal malformations) with the increase of diet phospholipids contents. Indeed, 35% of the larvae fed the diet with the low phospholipids level exhibited malformation whereas only 2% of the larvae fed the diet with 11.7% phospholipids exhibited malformations.

Figure 8: Survival rate at the end of the experiment (34-days posthtach).

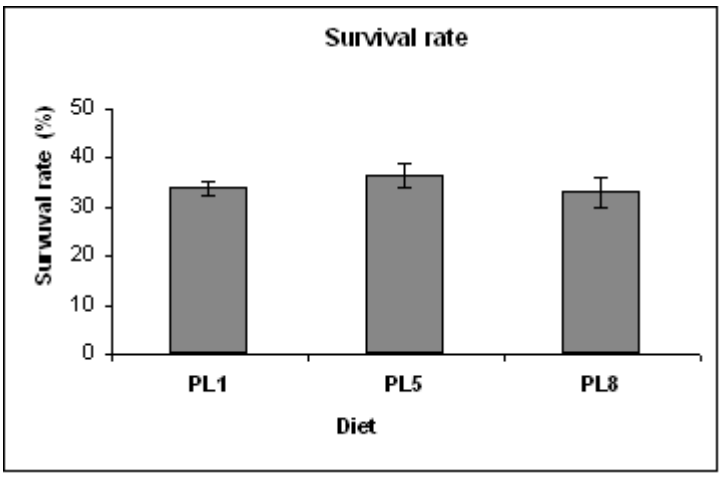


Figure 9: Cannibalism rate at the end of the experiment (34-days posthtach).

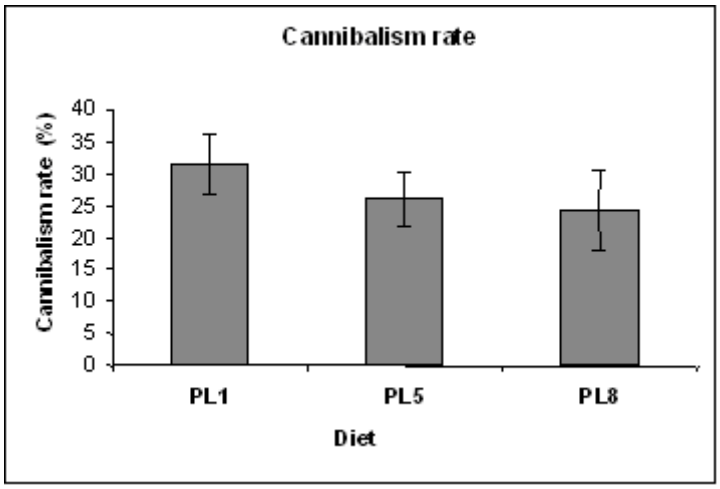
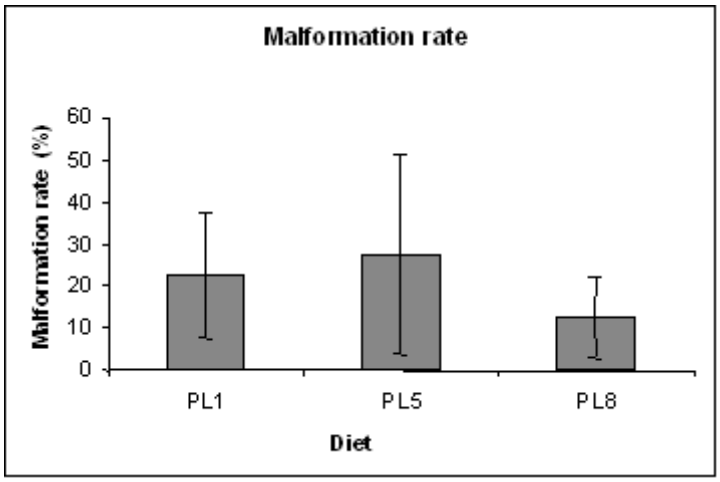
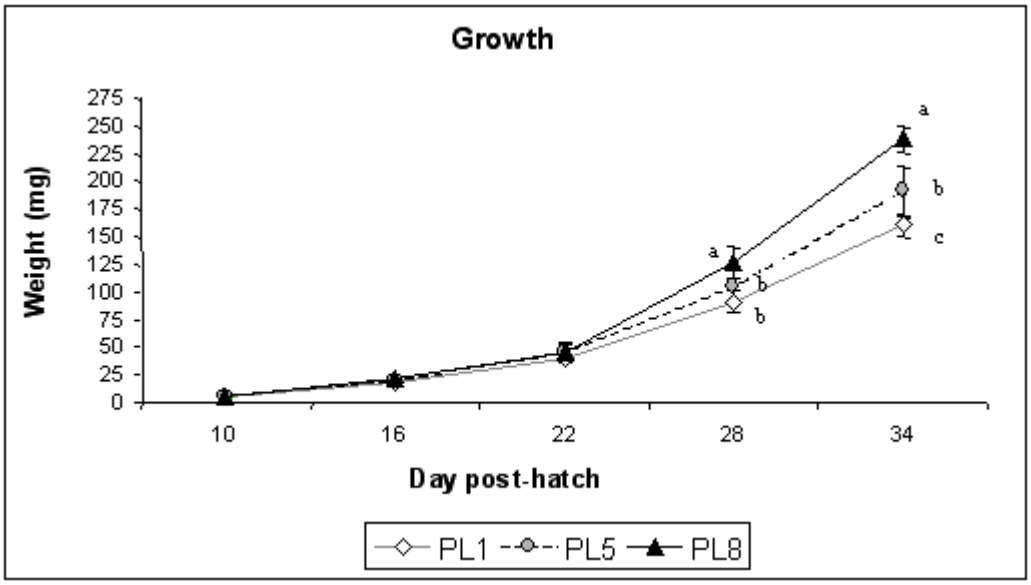


Figure 10: Malformation rate at the end of the experiment (34-days posthtach).



Larval growth was significantly improved in PL8 (**Figure 11**). The supplementation of the diet with phospholipids increased the growth performances of pikeperch larvae. The positive relationship between dietary phospholipids content and larval growth was previously reported in several studied and reviewed by Coutteau et al. (1997). Regarding to the transfer of lipids from breeders towards eggs that occurred during the maturation process (Sargent, 1989), the inclusion of phospholipids into broodstock diet could be interesting for the improvement of the larval quality, as suggested by Izquierdo et al. (2001).

Figure 11: Larval growth during the experiment.



In the present experiment, it was shown that higher phospholipids content in larval diets could improve the growth without affecting survival, malformations and cannibalism rates.

III.3 – Degree to which the objectives were met

Objective 1: To reduce the use of wild forage fish in the broodstock diet
The first experiment allowed to obtain spawning and larvae with fish fed with Vitalis only. Furthermore, the quality of reproduction of pikeperch fed with a mix of forage fish reared under intensive conditions (parasites and pathogen free) was similar to what can be obtained with pikeperch caught in the wild. In the second experiment, the results suggest that Vitalis has been improved by the producer and now fits better to the nutrient requirements of pikeperch. This should enhance the productivity of pikeperch breeder in the future.

Objective I1: To improve the quality of dry feed
The aim was to improve the fatty acid composition of a commercial dry diet given to pikeperch broodstock during the reproductive cycle. The results of the second experiment suggest that an enrichment of the diet with arachidonic acid leads to an increase of this fatty acid in the lipids stored into the egg. Knowing that this fatty acid is essential for the larvae to develop, we can assume that this supplementation should be effective in pikeperch.

Objective I1I: To improve the quality of larval diet
To complete the enhancement of breeder diet, the aim of this study was to enhance the quality of the diet given to pikeperch larvae. The supplementation of this diet in phospholipids allowed clear growth improvement. A supplementation of larval diet with 8% phospholipids at least is therefore advised.

IV – Optimization of broodstock management using a multifactorial approach (WP4)

IV.1 – Workpackage objectives

The main objective of this workpackage is the definition of optimal conditions of broodstock management during an entire reproductive cycle, aiming to improve the reproductive performances as well as the egg and larval qualities. This study is based on the use of a multifactorial designed experiment that enables taking into account several influential factors and their possible interactions (environmental and nutritional factors).

Based on fruitful discussions with both RTDs and SMEs partners during the first two meetings, four factors among the 12 were originally planned to test, were selected for the multifactorial approach. These four factors, which could be tested within our outdoor rearing facilities, were considered as the most influential by all partners, especially by SMEs. Consequently we decided, in accordance with all partners, to realize one big experiment during the project testing these four factors.

Meanwhile, a strong request also emerged during the first two meetings from the SMEs to test the quality of reproduction between wild and domestic breeders. For this reason, and in accordance with all partners, we decided, besides our main experiment, to run a second experiment within four tanks in our outdoor facilities using two new batches of wild breeders coming from two different sources (issued from partner 3 and 6), and to compare the results obtained between the three different batches.

At last, according to the revised schedule sent in April 2006 to the European Commission, we proposed to video-tape all our experiment to provide to all partners a clear illustration of the main steps of the artificial reproduction in pikeperch.

IV.2 – Progress towards objectives

Description of the experiment

Domestic breeders

Based on fruitful discussions with both RTDs and SMEs partners during the first two meetings, four factors among the 12 were originally planned to test, were selected for the multifactorial approach. These four factors (Table 1), which could be tested within our outdoor rearing facilities, were considered as the most influential by all partners.

Table 1. List of the factors and levels tested during the experiment.

Factors	Levels
1.Initial nutritional status	Feeding forage fish *ad libitum* (from July 27th to October 20th)
	Feeding pellets *ad libitum* and starving alternately every two weeks (from July 27th to October 20th)
2. Photoperiodic treatment	Natural
	Natural Simulated
3. Food type	Pellets (Nutreco - Skretting, Europa 5)
	Forage fish (carp, roach, 5-10 cm)
4. Feeding rate	Feeding *ad libitum*
	Feeding *ad libitum* and starving alternately every two weeks

Domestic breeders (F1 generation) were purchased from the fishfarm Excellence Fish (Partner 2) on June 28th, 2006. Fish were transferred by truck over a long distance (5:30 hours) with no mortality during either the transfer (only 2 fish) or just after the transfer (no delayed disease). Salt was introduced into the tanks used for the transport.

Upon their arrival, the gonado-somatic index of breeders was below 1% (n=27), indicating that no fish displayed any gonadal development. Breeders were evenly distributed into eight 3 m^3 outdoor tanks (19-20 fish) in an isolated 40 m^3 recirculating system (Domaine de la Bouzule, Meurthe et Moselle, France). Temperature and photoperiod were natural. Fish were fed *ad libitum* pellets from Skretting as in the Excellence Fish farm, from their arrival until July 27th 2006.

Two different feeding programs (Table 1) were then applied during three months, each on four tanks, to obtain two batches of fish displaying significant differences in term of initial nutritional status (perivisceral fat level). This first phase succeeded very well, and statically differences were found for the fat index: 1.11 ± 0.36 *vs* 0.49 ± 0.52 (p-value of 0.015), between the batches fed forage fish (n=8) and pellets (n=8), respectively. As expected, these two batches of fish also displayed widely divergent final total weight: 1.40 ± 0.22 kg *vs* 1.11 ± 0.16 kg (p-value of 0.010) between the batches fed forage fish (n=8) and pellets (n=8), respectively

On October 9-10th, because pikeperch has neither morphological nor anatomical differences between sexes, we decided to (i) perform a blood sampling for the 11 keto-testosterone assay (a typically male hormone) and (ii) mark all fish (1 or 2 marks per fish were inserted below the skin in the opercula region), to balance the sex-ratio between the eight tanks. The 11 keto-testosterone assay revealed two main groups of individuals: the putative "males" (325.05 ± 221.60 $pg.ml^{-1}$, n=96) and the putative "females" (38. 25 ± 20.81 $pg.ml^{-1}$; n=40), which thus enabled us to equilibrate the sex-ratio between tanks, i.e., 4-5 "females" and 12-13 "males", for a total of 16-18 individuals per tank.

The multifactorial experiment started on October, 23rd 2006, and ended in April 2007, i.e., corresponding at the beginning of the spawning season this year. We applied on four tanks the natural simulated photoperiod (factor 2, Table 1) by using artificial lights (2 x 58 W daylight). The duration of the simulated photoperiod was changed every week with clock, based on the natural duration of the day between sunrise and sunset. Fish were fed by hand every morning according to the treatment applied (factors 3 and 4, table 1).

During the entire experiment, along with temperature, oxygen (>7 mg/l), pH (>7.4), ammonium (< 1mg/l) and nitrite (< 1mg/l) levels were checked three times a week (data not shown). In parenthesis are indicated the targeted values for each parameters.

All fish were checked on April 4th in order to:

(i) determinate the actual composition of tanks, in terms of number of fish and sex-ratio and to compare it with the results obtained with the 11 keto-testosterone assay,

(ii) evaluate the development stage of females and the spermiation of males,

(iii) weigh and measure the length of all fish.

All fish were sampled a second time on April 16th, and then everyday until the end of the experiment, on April 24th. The analysis of gametogenesis of females was realized in collaboration with two Polish colleagues (partner 10: Andrzej Szczerbowski and Maciej Szkudlarek) that came to help us during four days (April 17-19th). The analysis of sperm quality was realized in collaboration with two Czech colleagues, namely *Martin Psenicka*, *Vojtich Kaspar* who came between 27th April and May 2nd (not involved in the Luciopercimprove project).

The comparison between the actual sex-ratio *vs* the sex-ratio determinate with the 11 keto-testosterone assay appeared to be 95% correct, only 7 out of the 136 fish tested were attributed to the wrong sex. Taking into account the four fish that died during the winter period, the number of fish remaining in all eight tanks before the spawning season (on April 4th) was 132 (15-18 fish per tank) including 99 males (10-14 per tank) and 33 females (2-6 females per tank. The detailed composition of each tank is provided on figure 1.

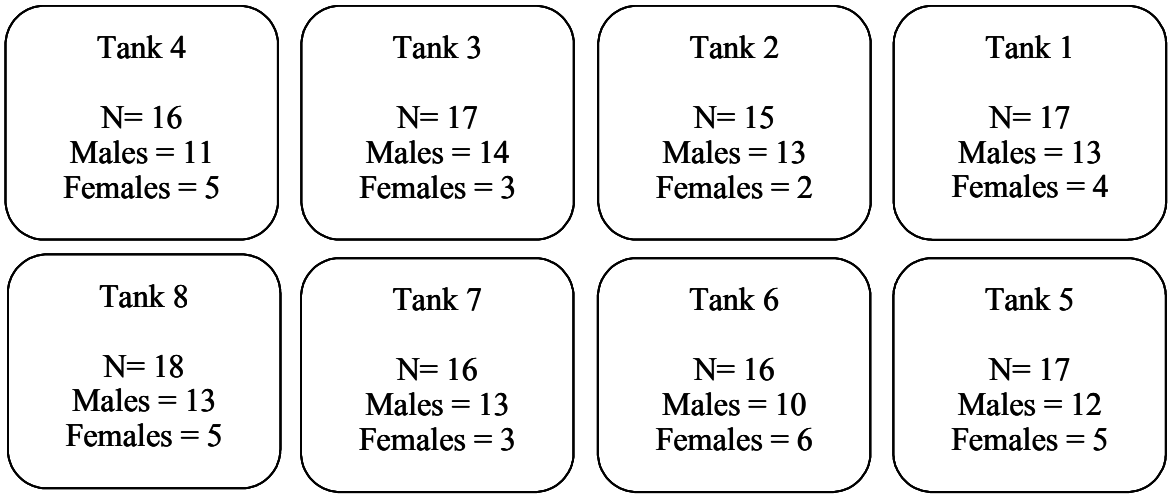


Figure 1. Detailed composition of each eight tanks determinate on April 4th, i.e., just before the spawning season.

During the beginning of April, we observed completely unexpected and unusual warm temperatures i.e., the temperature doubled in 10 days from April 6th to April 16th, from 8 to 17°C respectively. This tremendous increase led to an unusual early spawning season (advanced by nearly one month), and the first larvae were observed in the tank 5 by April 16th. Just after this peak of temperature during April (the water was as high as 20°C), it then slightly decreased and never attained such high values (only in summer).

Wild breeders

The first batch of wild breeders came from the Netherlands (Viskweekcentrum Valkenswaard, partner 6), and it arrived within our facilities on January 23rd. This batch was originally constituted of 44 pikeperch (47 kg), yet chiefly because of sanitary problems (*Flavobacterium*, *Saprolenia*), eight fish died in the first days upon arrival. Thus, we decided to treat all these fish. These treatments succeeded partly, yet eight more fish died prior to April 4th. Consequently, 15 fish have died among the 44: seven females and eight males. Eight additional fish died during the spawning season: six females and two males. This means that during the entire experiment, more than half (23/44) of the wild pikeperch died.

The second batch coming from France (Piscival, partner 3) arrived on February, 22nd. This batch, constituted of 36 fish, was in good health. No fish died prior to April 4th, and then four females died mostly due to repeated manipulations (anaesthesia bath, stripping).

Quantitative analysis of males

Domestic breeders

We confirmed in the present study that pikeperch males produce a little quantity of sperm (ca. 0.5 ml or less). As we pressed the abdomen, urine or shit could also come out in the meantime. Thus, in order to get the maximal amount of good milt, we took a tiny sample, then cleaned the surface and pressed again.

Among the 16 variables analyzed for five males per tank, eight appeared significantly influenced by the four tested factors: Δ weight (final-initial weight), Δ condition factor (final –initial weight), frequency (%) of non spermiating males on April 4th, frequency (%) of slightly spermiating males on April 4th, frequency (%) of spermiating males on April 4th, concentration of spermatozoa, motility of spermatozoa after 15s at 45s (data not shown).

The variation of weight ranged from a decrease of 330 g (in tank 1) to a gain of 267 g (in tank 8). A significant interaction was found between the initial nutritional status (factor 1) and the feeding rate (factor 4). The variation of the condition factor (k) varied from a minimum of -0.01 (in tank 5) to a maximum of + 0.08 (in tank 3); even tough the variation was low in most tanks. Two factors significantly influenced the evolution of the condition factor during the experiment, namely the interaction between the initial nutritional status (factor 1) and the lighting conditions (factor 2) (Figure 2b).

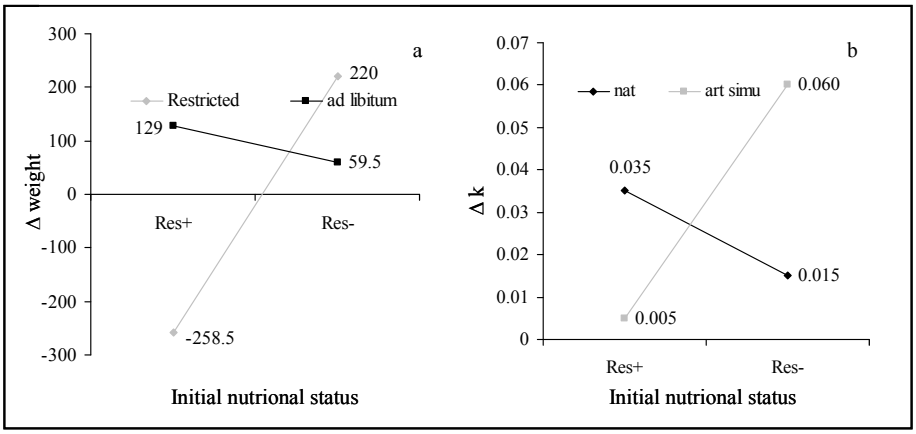


Figure 2. Significant effects of tested factors on (a) the evolution of weight (Δ weight) and (b) the evolution of the condition factor (Δ k).

Males were checked for spermiation three times during the spawning season, on April 4th, 16th, and 24th. Significant effects of the four tested factors were found for the first sampling date only. The initial nutritional status (factor 1) significantly influenced the percentage of non spermiating males (Figure 3a). Likewise, there was a significant interaction between the initial nutritional status (factor 1) and the type of food (factor 3) on the percentage of slightly spermiating males (Figure 3b). At last, two interactions significantly influenced the percentage of spermiating males: the first involved the initial nutritional status (factor 1) and the type of food (factor 3) (Figure 3c), the second involved the lighting conditions (factor 2) and type of food (factor 3) (Figure 3d).

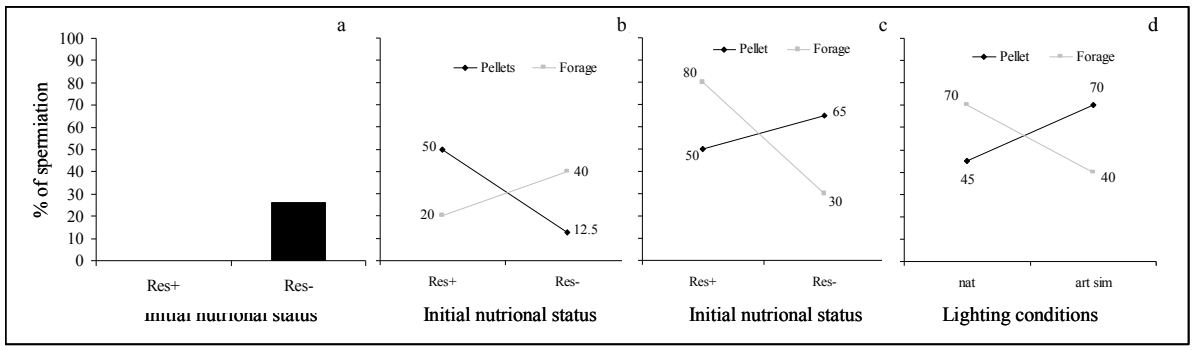


Figure 3. Significant effects of the tested factors on the percentage of (a) non spermiating males, (b) slightly spermiating males and (c) and (d) spermiating males.

The final concentration of spermatozoa ranged from a minimum of 8.13 (tank 5) to a maximum of 21.14 10^9 ml^{-1} of sperm (in tank 1). This concentration was significantly higher for males with restricted food (factor 3) than those fed *ad libitum* (factor 3) (Figure 4). Likewise, the initial nutritional status (factor 1) had a significant effect on the final concentration of spermatozoa (Figure 4). At last an interaction between initial reserves (factor 1) and lighting conditions (factor 2) was found (Figure 4).

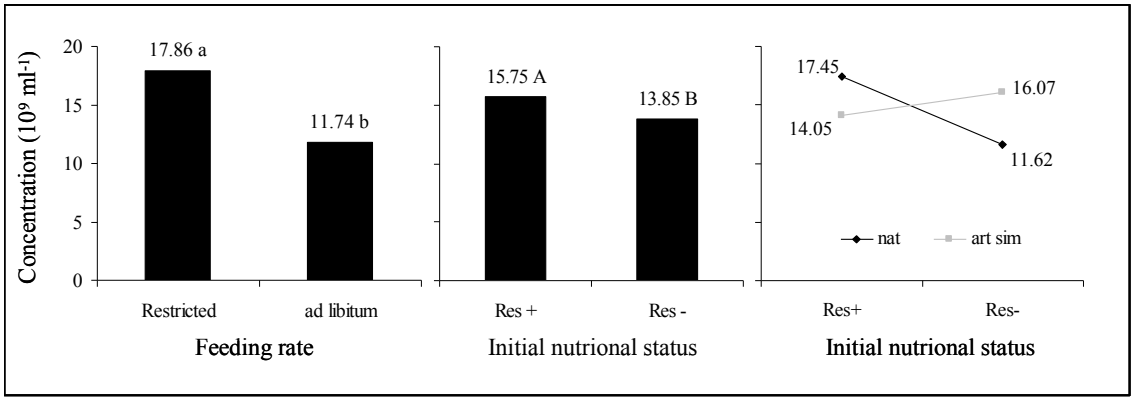


Figure 4. Significant effects of factors on the final concentration of spermatozoa. From left to the right: simple effect of the feeding rate, simple effect of the initial nutritional status, and interaction between initial nutritional status and lighting conditions.

The percentage of motile spermatozoa at 15 seconds, which ranged from a minimum of 60.93% (in tank 1) to a maximum of 84.97% (in tank 4), was significantly influenced by the lighting conditions (figure 2) (Figure 5). There was also a significant interaction between this factor and the initial nutritional status (factor 1) (Figure 5).

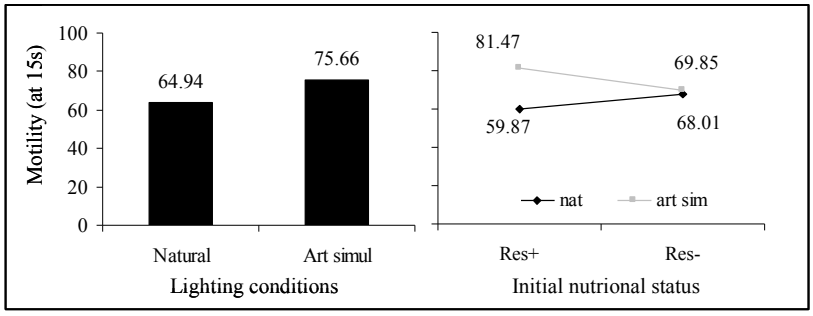


Figure 5. Significant effects of the factors on motility at 15s. From the left to the right: simple effect of the lighting conditions, and interaction between initial nutritional status and lighting conditions.

The present study showed that the initial nutritional status (factor 1) and the food availability (factor 4) chiefly explained the growth variation recorded over the "wintering" period (October – April). It seemed that if pikeperch males possess enough reserves to go through all its gametogenesis cycle, it will almost not feed during the winter, whereas if it has not enough reserves, males will

continue to feed during the whole year. Interestingly, neither the quality of food provided (factor 3) during the experiment nor the lighting conditions (factor 2) led to significant differences of weight.

The initial nutritional status (factor 1) also influenced the percentage of spermiating males observed in early April (4th). Similarly, an interaction between the lighting conditions (factor 2) and the type of food (factor 3) was observed on this variable. These results clearly demonstrated that the conditions applied for the broodstock management act on the gonad development, and more specifically on the timing of spermiation. Concerning the quality of sperm, the spermatozoa concentration was significantly increased when the food was restricted (factor 4) and the initial perivisceral reserves were high (factor 1). Data on sperm motility recorded after 15s traduced a very good sperm quality (compared to wild individuals, see below), and was significantly influenced by the initial nutritional status (factor 1) and lighting conditions (factor 2).

To conclude, this study demonstrated clearly that the initial nutritional status (factor 1) highly influenced the male status during the spawning season (and its feeding behaviour during the wintering period) and the sperm quality (concentration and motility), whereas the type of food (factor 3) given during the wintering period appeared less important.

The use of pikeperch male with a good initial nutritional status (high level of perivisceral reserves) is thus strongly recommended.

Domestic *vs* wild breeders

Five wild males coming either from France (Partner 3) and the Netherlands (Partner 6) were also analyzed for the analysis of the quality of sperm. Yet, due to the poor quality of some videotapes, three males per origin were successfully analyzed. The comparison between domestic and wild breeders coming from the two different areas (Table 5) revealed that among the five parameters analyzed, four differ significantly between the three batches (for motility at 45 s, the coefficient of variation of the standard residual error was too high to decisively conclude).

Table 5. Comparison of the quality of sperm between domestic and wild breeders coming from France and the Netherlands.

	Domestic	The Netherlands	France	*P*	CvSre (%)
Concentration	14.8 a	24.2 ab	25.8 b	0.019	29
Osmolarity	257 a	292.2 b	284.8 ab	0.015	6
Velocity 15 s	148.1 a	154.4 a	121.3 b	0.017	9
Motility 15 s	69.8 a	66 a	30.2 b	0.001	18
Motility 45 s	21.5 a	23.2 a	10.1 a	0.37	64

The concentration of spermatozoa was significantly inferior for the domestic breeders than for the wild breeders coming from France (14.8 *vs* 25.8 10^9 ml^{-1}). For osmolarity, the difference was significant between the domestic and the wild breeders from The Netherlands. Besides, the wild breeders from France displayed clearly a velocity of spermatozoa (at 15 s) and motility at 15 s much inferior than either wild breeders from the Netherlands or domestic breeders.

In conclusion, significant differences were observed between the three different batches. Domestic breeders appeared to have a much lower concentration of spermatozoa than wild breeders, yet displayed a quality of spermatozoa (in term of motility and velocity at 15s) similar to the wild breeders of the Netherlands and much superior to those coming from France. Yet, the interpretation of these results must be taken with high precautions because numerous parameters differ between these three broodstocks (i.e., history, age, size…).

Qualitative analysis of females

Domestic breeders

On April 4th, most of the females were on stage I and II, indicating that these females have completed ovogenesis but were not ready for either injection or spawning. From the 6th up to the 16th of April, among the 33 females present in all tanks prior to the spawning season: six females were still undeveloped, four died, six spawned naturally (without any nest, and with no obvious aggressive behaviour of males), ten already displayed overripening eggs, and seven females were injected, and placed in tanks with females only.

The seven females that were in stage II and III were injected, and placed in tanks with only females. The time latency between injection and either further stripping or spawning is a critical point. In our conditions (the temperature was warm, 16-20°C everyday), the time of latency was short, mostly 24-48 hours. Therefore, even though we checked females every morning, few released eggs in the tanks before we could obtain their oocytes (pikeperch may spawn early in the morning). Some injected females, could thus spawn alone with no males around them.

We stripped all injected females to get oocytes for further artificial reproduction trials. When the oocytes were ready to be released they came out easily, and good oocytes were very small and clear yellowish. In contrast, when it was either too soon or too late, oocytes seemed destroyed, more whitish, and some liquid and sometimes blood also went out.

Unfortunately, because of both the low number of females per tanks (Figure 1) and the fact that few females have spawned prior to the planned sampling it was not possible to quantitatively analysis the effect of the four tested factors on the quality of the ovogenesis and oocytes, even though differences appeared between the different treatments.

Wild breeders

On April 4th, all the 29 fish remaining from The Netherlands (Partner 6) were checked, they were nine females (most in stage 1), 14 males (not or slightly spermiating) and six fish undeveloped, thus indeterminate. The gonado-somatic index was widely different between the six females euthanatized during the spawning season (5.60 to 21.29%) indicating that only part of females have undergone a full reproductive cycle. Seven females were injected on April 23rd at stage II and III, and some eggs were found three days later in the tanks. One female gave oocytes from stripping that were successfully fertilized with sperm of three males.

On April 4th, 32 fish were sampled, they were 14 females (all stage I), 16 males (most not or slightly spermiating) and two were indeterminate. Most fish were quite small and immature. Seven females were injected on April 19th. Eggs were artificially obtained from one female, and larvae hatched (4.66 ± 0.34 mm upon hatching).

Artificial reproduction trials

Overall, we were able to obtain seven batches of eggs for domestic females. Two spawning were fertilized with high rates (79-88%). Despite the treatment against stickiness, the eggs remained adhesive and clustered in few masses, preventing them of getting sufficient oxygen to develop. Besides, numerous microorganisms destroyed the eggs. We tried to kill such microorganisms with formol (1ml per 10 litres of waters during ten minutes). This treatment appeared efficient for a period of time, but afterwards, the microorganisms came back. This resulted that we were able to obtain larvae from one female only.

Seven wild females coming from The Netherlands were injected on April 23rd at stage II and III, and some eggs were found three days later in the tanks. One female gave oocytes from stripping that were successfully fertilized with sperm of three males. Those eggs took 84 degree-days to incubate at 12.7°C, which is quite lower than in other studies (Schlumberger and Proteau, 1993). Larvae were obtained from this female, which were 4.35 ± 0.18 mm in total length, similar to what is usually described for pikeperch (Lappaleinen *et al*., 2003).

Seven wild females coming from France were injected on April 19th. Eggs were artificially obtained from one female, and larvae hatched (4.66 ± 0.34 mm upon hatching).

In conclusion, it seemed clear that the treatment against stickiness was not enough sufficient and that our incubating facilities were not well adapted for pikeperch (we usually used them for perch and it worked really well), and thus an incubation in Zoug bottles is clearly recommended.

Conclusions

To our knowledge this study is the first experiment conducted on pikeperch reproduction in order to better understand the multifactorial determinism of the quality of the reproduction, and more specifically the effect of the broodstock management (initial nutritional status, food availability and quality over the reproductive cycle, photoperiod conditions). Our study clearly showed that the initial nutritional status highly influenced the male status during the spawning season (and its feeding behaviour during the wintering period) and the sperm quality, whereas the type of food given during the wintering period appears less important. The use of pikeperch male with a good initial nutritional status (high level of perivisceral reserves) is thus strongly recommended. Interestingly, whereas all SMEs and some RTD performers considered pikeperch as a very sensitive species to handling and storage conditions (high levels of mortality frequently observed without explanations), a very low level of mortality has been recorded in our experience, even if fish were often handled (sometimes everyday during the spawning season). That indicates that in the environment of the Bouzule station, conditions (tank wall colour?) were optimal for pikeperch breeder management.

Another original point concerned the use of an artificial propagation method, poorly used in pikeperch culture (Schlumberger and Proteau, 1996). This last choice has been decided to support the development of this method for some SME partners. Our study showed that (i) the sperm collection from males is very difficult (low production of sperm per male, mixture of urine and sperm), (ii) pikeperch females can spontaneously spawn in tanks without nests (with or without males, and no obvious aggressive behaviour of males), and (iii) the latency between hormonal injection and ova collection is not evident to determine (1-3 days depending on temperature and stages of females). Consequently, we highly suggest to fish farmer to use the traditional method for pikeperch reproduction (hormonal injection of HCG + introduction of couple of breeders + reproduction on nest). Such way appears more reliable and less labour consuming. However, based chiefly on the successful spawning trials (2 females with high fertilization rates: 80%), a short videotape was realized and will be soon supplied to all partners (end of January) to show the different steps of the artificial propagation method.

In conclusion, even though this study showed some limits (initial unbalanced sex ratio and a very low number of females, surprising temperature variations just before the expected spawning period…), this long study (10 months) and the corresponding results carried out important information for the progress of pikeperch culture, especially for the SME partners of the Luciopercimprove project.

To summarize, the four main milestones of the present study are that:

- It is possible to accurately sex pikeperch using the 11 keto-testosterone assay (95% of success in our study).
- Pikeperch appeared much more resistant to manipulations than originally stated.
- The favoured way of propagation of pikeperch is clearly by isolating couples into a tank and let them spawn freely on nests (until artificial reproduction is improved significantly)
- The use of males with high nutritional reserves are highly recommended before the starting of a reproductive cycle

IV.3 – Degree to which the objectives were reached

The revised objectives of this workpackage were reached for the most part, particularly concerning the effects of the four tested factors on both the male condition during the spawning season and the quality of sperm.

V – Production of all-female populations and effect on productivity in intensive culture (WP5)

V.1 – Workpackage objectives

The overall objective of this workpackage was to produce all-females families with hormonally sex-reversed male breeders, and to compare the growth performances of all-females families to mixed-sex families. In order to attain these objectives, this WP was divided into 3 sub-tasks :

- The determination of the optimal protocol to produce hormonally sex-reversed XX males (sub-task 5.1).
- The comparison of growth performances of all-females and mixed-sex families (sub-task 5.2).

V.2 – Progress towards objectives

V.2.1 – Subtask 5.1: Production of hormonally sex-reversed males breeders

In order to determine an optimal protocol to produce hormonally sex-reversed male breeders, different hormonal doses (40 or 80mg kg^{-1} food), treatment duration (30 and 60 days) and initial mean body weight (70 and 900mg) were tested. After larval rearing in mesocosm system (10m^2 / 5m^3 tanks at 20°C and O_2 > 6 ppm), weaned larvae were fed during 30 days with 17α-methyltestosterone (17MT - masculinizing steroid) incorporated into the food. Sex ratio was determined on 100 fish per batch (or all fish if the survival is lower), when individuals will reach a mean body weight > 20g. After sacrifice with a lethal dose of anaesthetic (2-phenoxyethanol), a piece of the gonad will be removed, coloured with acetocarmine (Guerrero and Shelton, 1974) and observed with an optical microscope (photo 1).

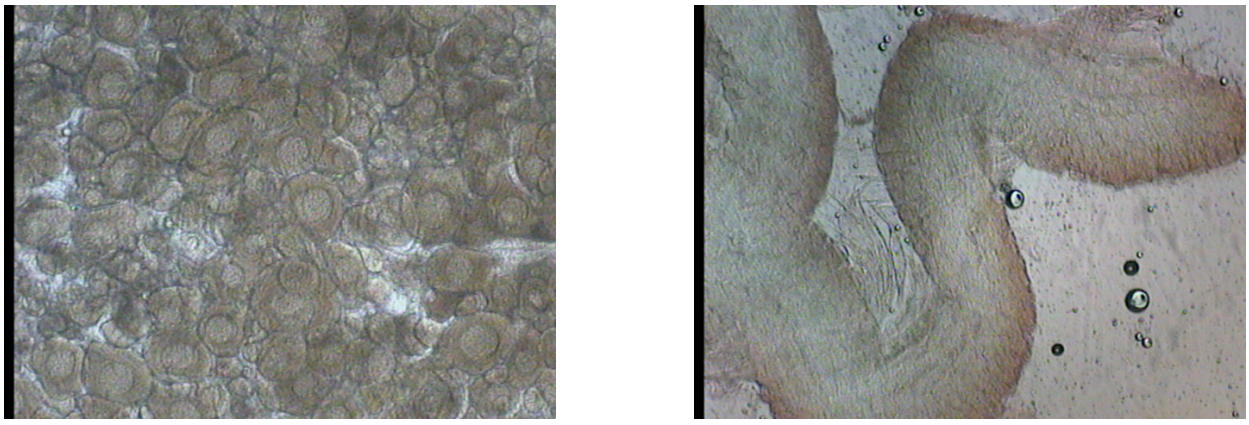


Photo 1 : Microscopic observation of a female (left) gonad with ovocytes and males gonad (right) of sander.

Hormonal treatment applied on fish with an initial mean body weight of 74.3 mg resulted up to 97.0% of males in the progenies, whatever the MT doses (table 1). On contrary, hormonal treatment applied on fish with an initial mean body weight of 900mg do not produce sex reversed progenies, whatever the doses or duration tested (sex ratio ranged from 41.1 to 65.0 of males). At 900mg, sander gonads were probably differentiated into males or females and it was no more possible to modify the phenotypic sex (Baroiller et al., 1999; Devlin and Nagahama, 2002). Based upon these results and because huge mortalities (>99%) due to the transfer from mesocosm to the recirculating system (2006), the production of hormonally sex-reversed males breeders were directly done in the mesocosm system. Sex reversal treatments were applied on fish with an initial mean body weight of 70 mg at a hormonal dose of 40 mg kg^{-1} food. This treatment gave a minimum of 97.0% of males in

the treated families (table 2). At present (MBW = 25g) it was not possible to discriminate between normal and hormonally sex-reversed males and no gonads abnormalities were observed in hormonally treated fish.

Table 1 : Sex ratio of the progenies fed with two different doses of MT during 30 or 60 days at an initial mean body weight of 70mg (30 days post-hatching) or 900mg (75 dph).

		IMBW : 70mg		IMBW : 900mg	
MT doses ($mg.kg^{-1}$)	Duration (days)	% males	% females	% males	% females
0 (control)	-	50.0	50.0	52.0	48.0
40	30	100	0.0	41.1	58.9
	60	-	-	65.0	35.0
80	30	97.4	2.6	59.2	40.8
	60	-	-	50.0	50.0

Table 2: Sex ratio of the progenies fed with 40mg MT kg^{-1} food during 30 days at 30 days post-hatching (n = 50).

Origin	**MT doses (mg)**	**% males**	**% females**
CEFRA	Control	52.0	48.0
	40	100	0
VV	Control	50.0	50.0
	40	97.4	2.6

V.2.2 – Subtask 5.2: Comparative growth of male and female pikeperch

The comparative individual growth performance trial was performed on a mixed-sex families (generation 2006) reared in $1.6m^3$ tank in a recirculating system at 23°C, O_2 > 6ppm. Every two months, 50 fish were sacrificed with a lethal dose of anaesthetize, weighted (±0.1g), measured (±1mm) and sex determined based upon the gonad morphology (photo 2). Gonads were removed and weighted (±0.01g) in order to calculate the gonado-somatic index (GSI = [Weight gonads (g) /body weight (g)]*100). The condition coefficient was calculated as [body weight (g) /body length ^{3}(cm)]*100.

Under our experimental rearing conditions, until 450 days post-hatching, sander reached a mean body weight of 180g which was 25% lower than mean body weight obtained in a previous project (Lucioperca – CRAFT Q5R-2001-70594) for which sander reached 235g at the same age in the same rearing conditions. No sexual growth dimorphism was observed between males and females sander until 450 days post-hatching (P>0.05, table 3).

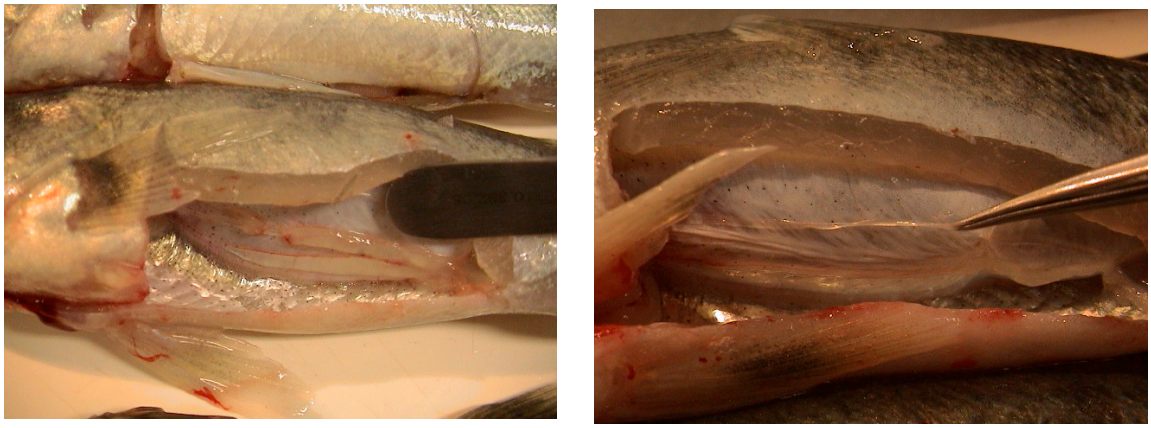


Photo 2: gonads morphology of female (left) and males (right) sander (MBW > 15g).

Table 3: Mean body weight (g), gonado-somatic index (GSI) and condition coefficient (K) of males and females juveniles sander reared in recirculating system at 23°C (n = 50). Values were means ± SE.

	MBW (g)		GSI (%)		K	
Age (dph)	M	F	M	F	M	F
325	49.8±4.5	62.0±5.7	0.01±0.00	0.50±0.08	0.71±0.09	0.73±0.10
385	96.2±6.1	100.5±6.3	0.02±0.00	0.48±0.16	0.86±0.11	0.86±0.06
445	178.2±12.0	178.0±7.2	0.04±0.00	0.81±0.13	0.86±0.15	0.86±0.13

V. 3 – Degree to which the objectives were met

The main objective of this workpackage "determination of a protocol to produce hormonally sex-reversed males breeders" was attained as we obtained 100% males when feeding undifferentiated juveniles at an initial mean body weight of 70 mg (30 days post-hatching) with 17α-methyltestosterone at 40 mg kg^{-1} food during 30 days. Until 180 g (450 days post-hatching), there is no sexual growth dimorphism between males and females juveniles sanders.

VI – Influence of breeder stocking conditions on gamete and larval production and quality (WP6)

VI.1 – Workpackage objectives

The objective of this task was to reproduce pikeperch from different environmental conditions: (ponds, cages, tanks) for evaluation of the breeder stocking conditions on spawning quality.

VI.2 – Progress towards objectives

During the first year of the project, the comparison of the reproduction pikeperch from different stocking conditions was evaluated.

Breeders – year 1

Breeders (Fig. 1) were obtained from FFP (SME partner). In July 2005 fish were divided into three groups:

- Pond conditions (42 specimens) – Fig. 2.
- Cage conditions (41 specimens) – Fig. 3.
- Tanks conditions (32 specimens) – Fig. 4.

Fish whole season were kept at the present above conditions and were spawn it them.

Breeders – year 2

Breeders (Fig. 1) are obtained from FFP (SME partner). In August 2006 fish were divided into three groups:

- Pond conditions (45 specimens) – Fig. 2.
- Cage conditions (43 specimens) – Fig. 3.
- Tanks conditions (39 specimens) – Fig. 4.

Fish whole season were kept at the present above conditions but were spawn artificially under hatchery conditions.

Fig. 1. Pikeperch breeders in anesthesia.

Fig. 2. Ponds used for breeding pikeperch.

Fig. 3. Cages used for pikeperch breeders.

2006
In pond and cage (located in the ponds) environmental conditions are natural. In tanks, the water temperature and photoperiod was artificially stimulated as in natural conditions. Fish were fed with live prey fish: small roach, carp, tench or perch. During winter, some cages were broken by ice cover, but in the early spring, fish were moved back to the new cages.

In May 2006, when the water temperature in ponds warmed to 14 - 15 °C (Lappalainen at al. 2003), the fish were selected and obtained hormonal stimulation (hCG at 750 IU kg^{-1}) (Fig. 5). The same dose of hCG was applied for fish from both sexes. Fish were spawning at the same conditions in which they were keeping. The nests from artificial grass were put down as a substrate for eggs. In all conditions, 10 pair of pikeperch were kept.

2007
In pond and cage (located in the ponds) environmental conditions are natural. In tanks, the water temperature and photoperiod was artificially stimulated as in natural conditions. Fish were fed with live prey fish: small roach, carp, tench or perch. In April 2007, when the water temperature in ponds warmed to 13 - 14 °C (Lappalainen at al. 2003), fish from ponds and cages were moved to the hatchery, when they kept in 1000 dm^3 tanks worked at closed system. Then the fish were selected and obtained hormonal stimulation (hCG at 450 IU kg^{-1}) (Fig. 5). The same dose of hCG was applied for fish from both sexes. Fish were spawning artificially.

Fig. 4. Tank used for pikeperch in FFP.

Fig. 5. Female injected using hCG.

2006

The nests were checked after 3rd, 6th and 9th after hormonal stimulation. The number of nests (Fig. 6) with eggs was noted. Samples of nests with eggs (Fig. 7 and 8) were transported to the UWM laboratory for further incubation. All taken samples were in duplicate.

Fig. 6. The nest with pikeperch eggs.

Fig. 7. Sample of pikeperch eggs.

Pikeperch eggs were incubated at the hatchery conditions in closed water system. The egg survival was estimated after three days of spawning and shortly before hatching. There were some problems with counting live/dead embryos and the final estimating was done under the microscope. During incubation the eggs were bath in formalin solution to protect before diseases.

After spawning time, the spawners were moved from their conditions for evaluating their survival and spawning success.

Fig. 8. Pikeperch egg sample ready to transport to the hatchery.

2007

Maturation of the oocytes is a long process that involves complex physiological and biochemical changes. One important step, vitellogenesis, is a process in which yolk protein are produced in the liver, transported to the ovary, and stored in the egg, resulting in tremendous egg enlargement. Also critical are germinal vesicle (GV) migration and germinal vesicle breakdown (GVBD). In present experiment the maturity stage of females was checked. For further treatment in all groups were taken females in 2-3 oocytes maturity stages. There were no differences in maturity stages in females from different groups.

There are many different methods of sampling oocytes from fish females. One of them is taking sample using a catheter (Fig. 11).

Before taking egg sample, fish should be treated in anaesthesia solution. It was very important, because pikeperch is very sensitive fish. On the other way, taken sample sometimes taking few minutes, and during this time the fish should do not moving. The next problem is: when the catheter should be bringing in? In which pore?

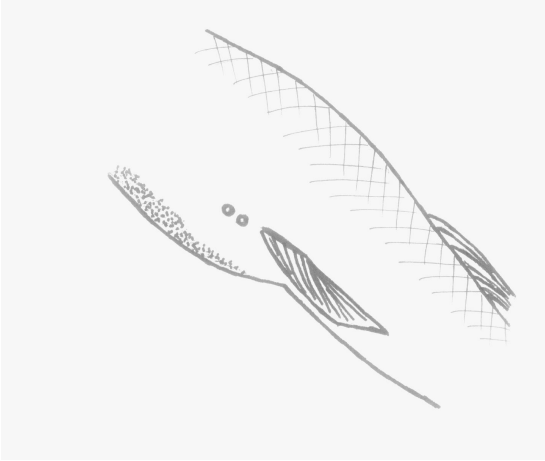


Fig. 9. The belly of male.

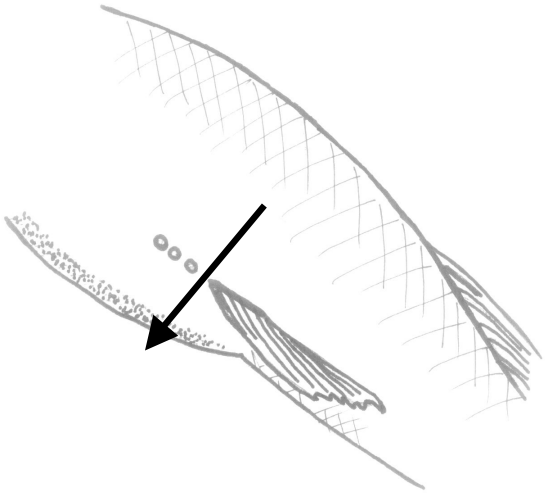


Fig. 10. The belly of the female. Arrow indicates the accurate pore for application catheter.

In pikeperch females on the belly there are three pores and the catheter should be bring into middle one (Fig. 10). The details are presented in Fig. 11 and 12.

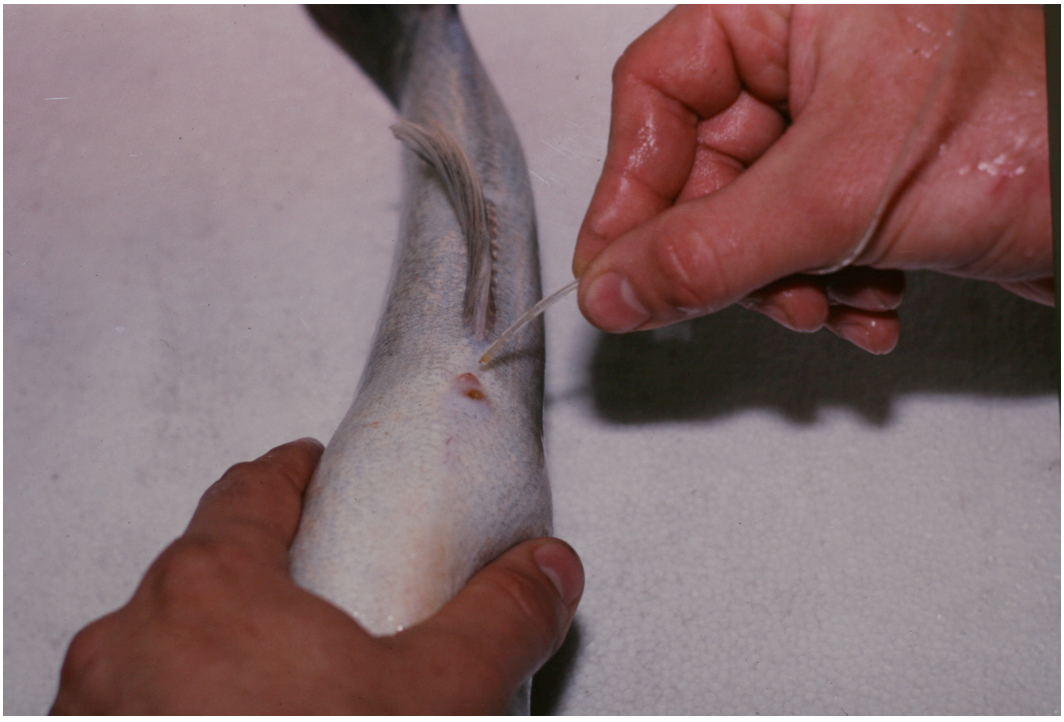


Fig. 11. Shortly before bring in a catheter to pikeperch female.

Fig.12. Sampling oocytes from pikeperch female.

The maturity of oocytes can be determined by using biopsy techniques. In this technique eggs (oocytes) are removed from the ovary with the use of catheter, cleared with a prepared solution (Serra's solution), and viewed under a microscope.

The main evaluation criteria of pikeperch oocytes maturity stages like other Teleostei fishes are the location of germinal vesicle (GV) and additionally coalescence of the oil droplets.

Oocytes classified according above-mentioned criteria are divided into four stages:

I. Oocytes in I maturity stage have GV in a central position and many small oil droplets. In this stage freshly sampled oocytes from ovary have a yellow-white coloration and they are relatively opaque. The clearing time in Serra's fluid to obtain fully transparency varies from 120 to 170 s.

II. Oocytes classified as II maturity stage have shifted GV less than a half radius, in addition the oil droplets are less numerous and have bigger dimensions. In this stage oocytes freshly sampled from ovary are straw-colored and less opaque then in previous stage. The clearing time into Serra's fluid to obtain fully transparency amounted c. 110 s.

III. Oocytes classified as III maturity stage have positioned GV on the periphery, near the oocyte membrane, and oil coalesced in one big droplet. In this stage oocytes freshly sampled from ovary are more transparency than in previous stage. The clearing time into Serra's fluid to obtain fully transparency is shorter than in previous stage and amounted c. 60 s.

IV. Oocytes without visible GV, i.e. in which the process of GV breakdown (GVBD) had begun or GV is present near the zone, should be classified as IV maturity stage. In this

stage oocytes freshly sampled from ovary have light yellow coloration and clear transparency. The clearing time in Serra's fluid to obtain fully transparency is shorter than in previous stage and amounted c. 20 s.

If the oocytes placed in Serra's solution are damages it might means that oocyte are during ovulation or resorption process. When the oocytes are in stage I – IV, the shape of oocytes in Serra's solution is still constant – they are round.

Pikeperch breeders must be handled very gently. They are sensitive to shocks or temperature changes and may die during handling. In this situation fish before handling should be anaesthetized with "propiscin" at a concentration of 0.5 cm^3*dm^{-3} (or other solution applied in aquaculture as anesthetics). When handling the fish, gentle firmness should be the rule. Covering the fish with wet towel or cloth, will help to keep them calm. When the pikeperch female is ready to spawn, the eggs are usually start do drop spontaneously from the genital pore. It is very well visible, because the color of eggs is yellow. Before stripping, both the male and female should be cleaned and dried using a soft towel. Water should not be allowed to mix with the gametes as they are stripped.

To strip the fish, the female should be held around the caudal fin with one hand, while applying slight pressure to the abdomen with the other hand. If ovulation has occurred, a stream of eggs will emerge. If there is a stream of eggs, the abdomen should be messaged from front to back to strip out all the eggs. After egg collection the skin in females belly are going inside and looks empty. Eggs are usually collected to the small plastic basin. The artificially stripped eggs are usually waiting for adding milt.

The best way of obtaining milt is collecting it using plastic syringes. Milt from individual males should be collected separately and later add to the eggs. Milt should be white without any pollution, especially from blood addition.

The females in present experiment were checked twice daily from second day after hormonal stimulation. The number of ovulated females were noted. Eggs were mixed with the semen obtained from the males from the same group (Fig. 13) and fertilized. After anty-stick bath they were incubated on Wiess jars at constant temperature 15 °C. The number of live embryos were noted. All taken samples were in duplicate.

Fig. 13. The artificially obtained pikeperch eggs mixed with milt.

Pikeperch eggs were incubated at the hatchery conditions in closed water system at Weiss jars. The eggs survival was estimated after three days of spawning and shortly before hatching. There were no problems with counting live/dead embryos and the final estimating was done under the microscope. During incubation the eggs were bath in formalin solution to protect before diseases.

After spawning time, the spawners were kept additionally for next two weeks for evaluating their survival.

2006

There are find a differences in percentage of spawning pikeperch pairs in different conditions (Fig. 14). The highest results were obtained in tanks conditions, where 90% of females spawn.

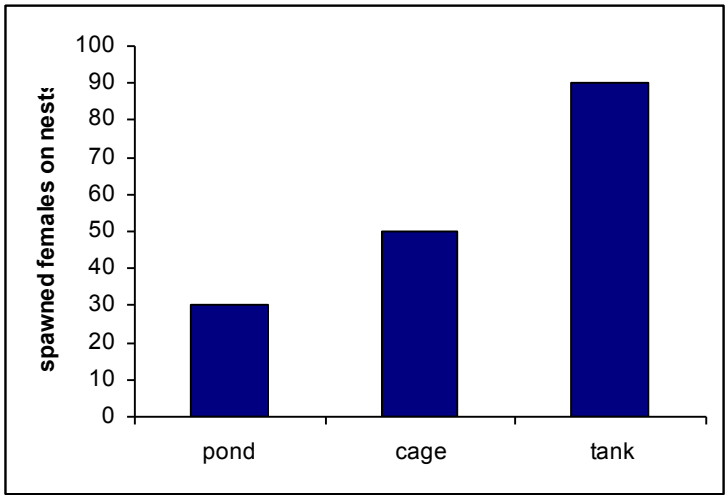


Fig. 14. The percentage of nests with eggs obtained from different conditions.

In pond, probably two females spawn on grounds or dropped eggs. There are significant differences in embryo survival obtained from breeders kept in different conditions. The highest survival rate after three days of incubation was noted in case of fish kept whole period in tanks (Fig. 15). The same situation was noted, after longer eggs incubation (Fig. 16). Quite low survival in groups “pond” and “cage” might be involved after protozoa and copepods (Fig. 17), which damaged the eggs. These results are similar to obtained by Kaszubowski (2005) for fish from other part of Poland than those used in present experiment.

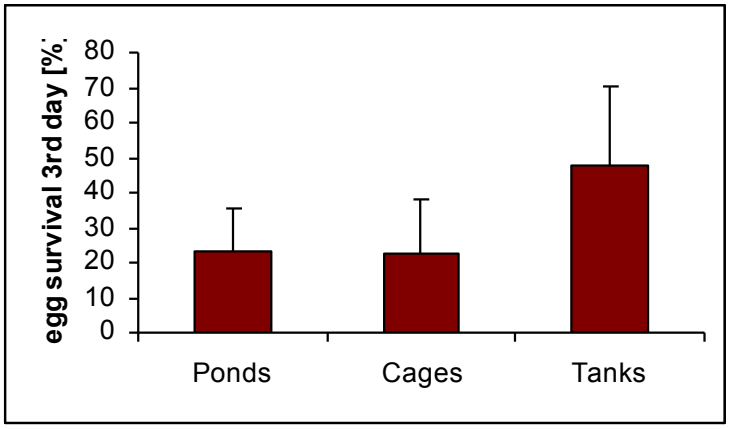


Fig. 15. 3-days-old embryos survival. There are a significant difference in survival between "tanks" group and other groups (Duncan's multiple range test, $P < 0.05$).

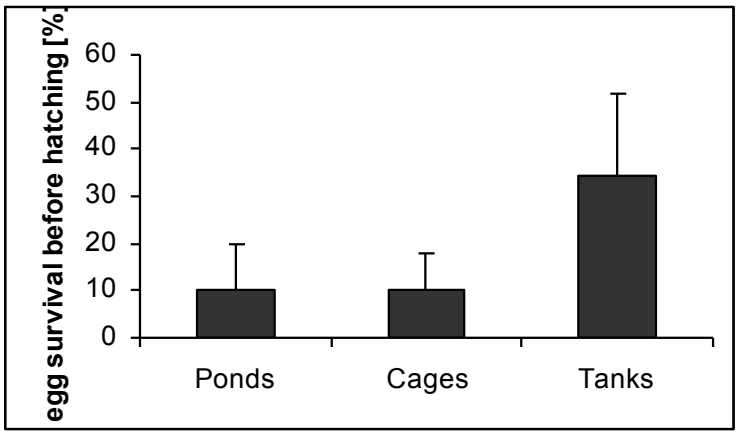


Fig. 16. Shortly before hatching embryos survival. There are a significant difference in survival between "tanks" group and other groups (Duncan's multiple range test, $P < 0.05$).

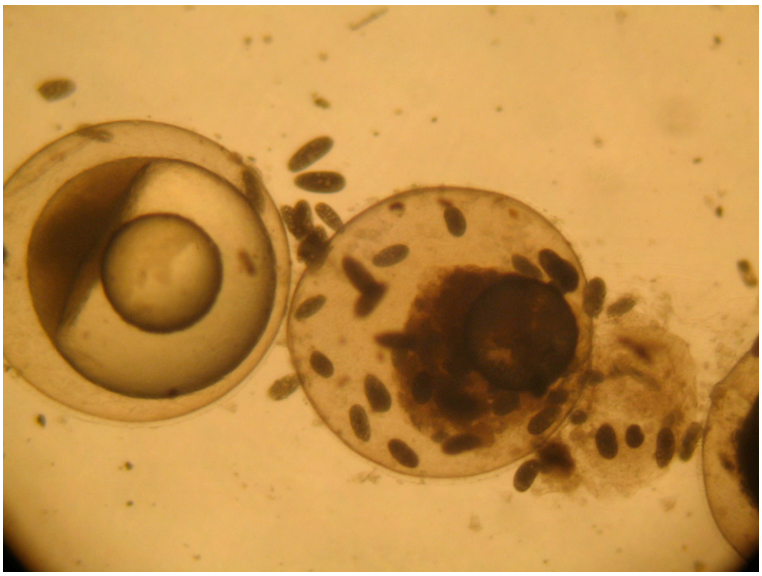


Fig. 17. The eggs collected from pond conditions with protozoan infection.

The spawner's survival was not excellent. The highest mortality was noted in group "cage"- over 30%. In other groups the mortality rate was similar and was over 20%.

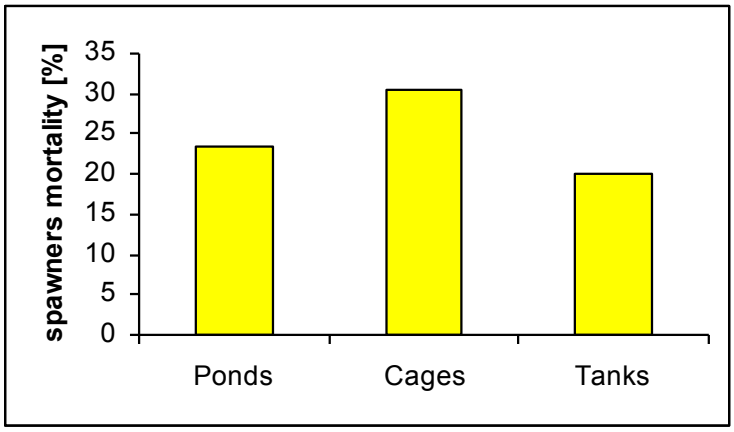


Fig. 18. Spawner's mortality of fish holds in different stocking conditions.

2007

There are find a differences in percentage of spawning pikeperch pairs in different conditions (Fig. 19). The highest results were obtained in tanks conditions, where 80% of females spawn.

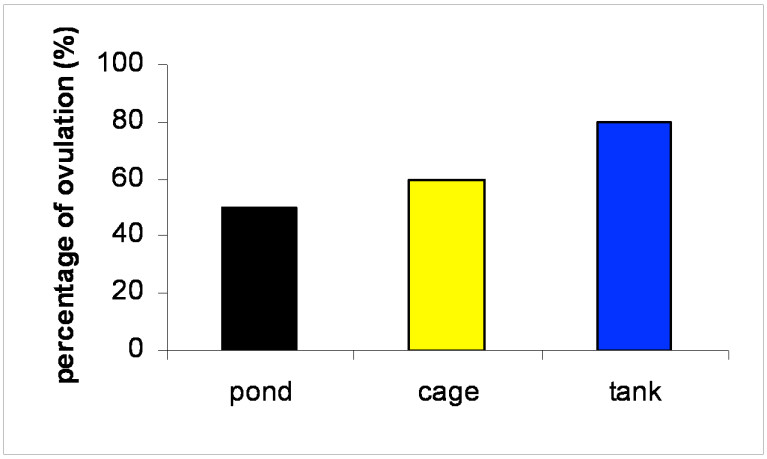


Fig. 19. The percentage of ovulated females from different conditions.

The same situation was noted in the case of males (Fig. 20), where highest values were noted in fish kept whole season at tanks. Spermatozoa motility was different in different groups, but without statistical differences. Data obtained on 5th day after hormonal stimulation were much higher than these collected on 1st day. The spermatozoa motility was correlated with semen osmolality (Fig. 21). Higher osmolality influenced higher spermatozoa motility. Such situation was observed in all groups.

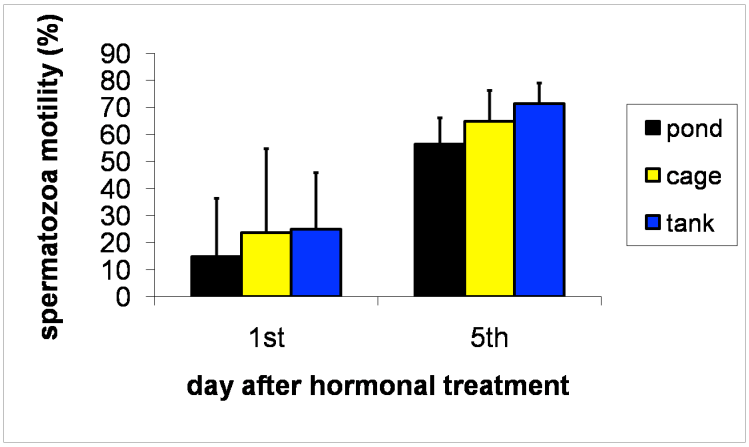


Fig. 20. The motility of pikeperch semen from different groups.

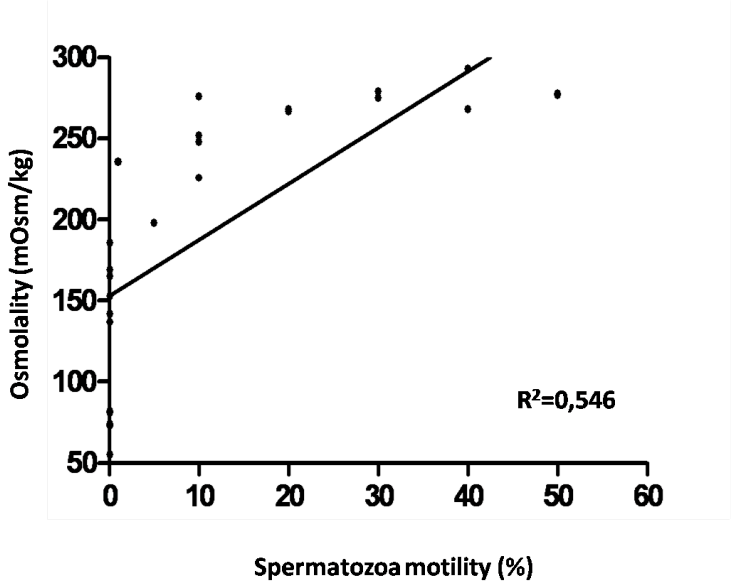


Fig. 21. The relationship between spermatozoa motility and osmolality.

There were significant differences in embryo (Fig. 22) survival obtained from breeders kept before spawning in different conditions, and the highest survival rate after three days of incubation was noted in case of fish kept whole period in tanks (Fig. 23). These results are similar to obtained by Kaszubowski (2005) for fish from other part of Poland than those used in present experiment and very close to data obtained one year earlier.

Fig. 22. Pikeperch eggs.

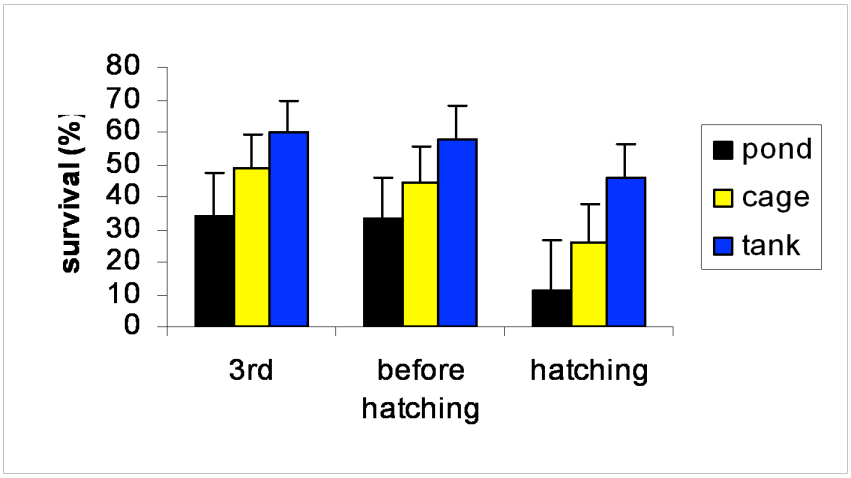


Fig. 23. Pikeperch embryos survival. There were significant difference in survival between tank and other groups (Duncan's multiple range test, $P < 0.05$).

The spawner's survival was not excellence (Fig. 24). The highest mortality was noted in group "cage"- 30%. In other groups the mortality rate was similar and was 20% in cage group and 15 in tank group. In this experiment the **spawners mortality was lower than in previous experiment carried out one year earlier.**

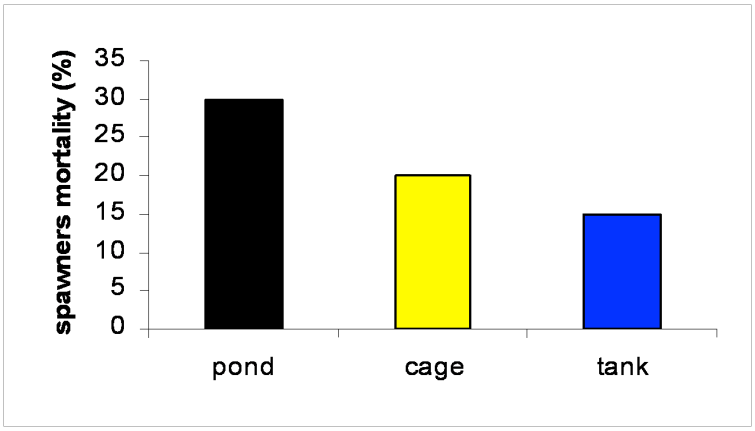


Fig. 24. Spawner's mortality of fish holds in different stocking conditions.

The reasons of this mortality were probably stress. Fish kept at captivity whole season probably showed lower influence on manipulations. There were some differences between larvae length obtained from different groups. This was surprise because female size was very similar. Embryos from cage and tank groups hatched later than from pond group, so it may be the influence of incubation period.

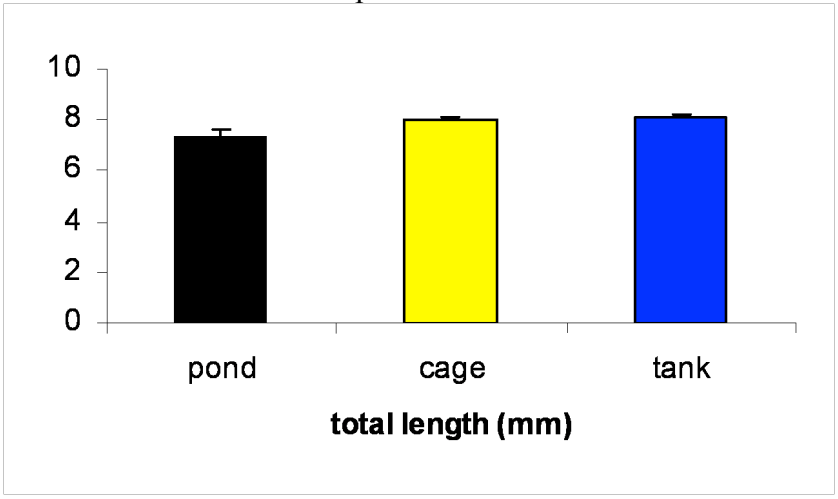


Fig. 25. The larval length. Samples were taken during mass hatching.

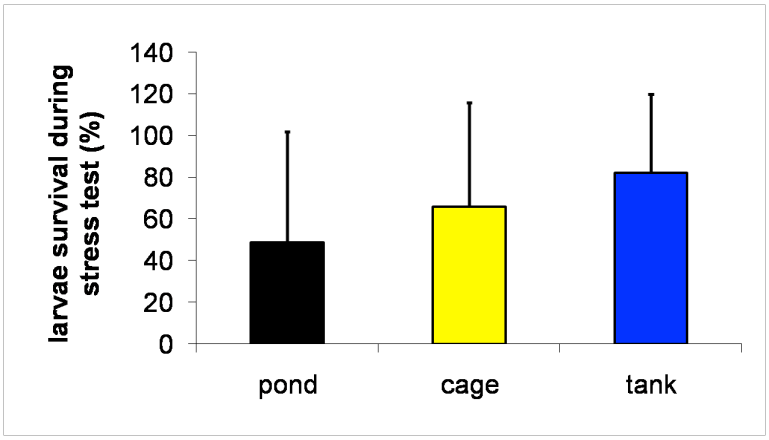


Fig. 26. The results of the stress test.

The stress test of the larvae did not shown the differences between groups. The highest survival was noted at tank group, but very high variation (also SD) did not gave possibility to find statistical differences. The larvae (Fig. 27) usually shown excellent or very poor survival.

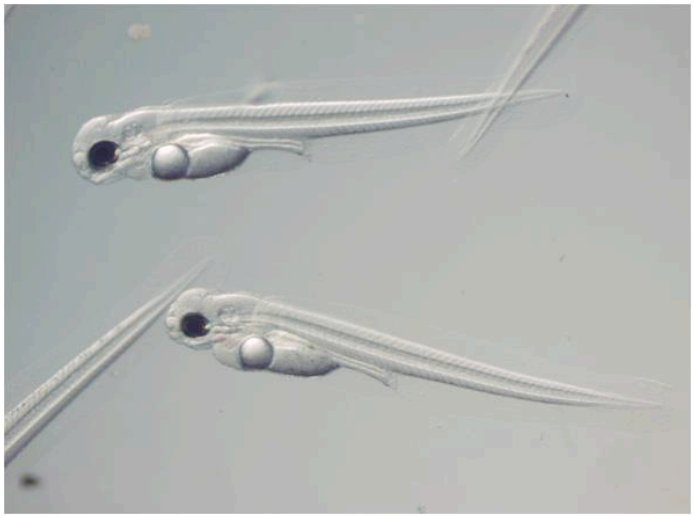


Fig. 27. Pikeperch larvae.

The biochemical analysis including fatty acid of eggs and larvae body composition did not shown differences.

VI.3 – Degree to which the objectives were met

All objectives of this workpackage were met. It was shown that:

1. Pikeperch can be spawn easily in captivity.
2. There is an influence of stocking conditions to spawning effects.
3. The best results (highest ovulation/spawning rate, highest embryo survival/ highest spawners survival, highest quality of milt, ect ...) was noted in group “tanks”.
4. There were no differences in chemical body composition and results of stress test between groups.

VII – Use of knowledge and transfer of technology (WP7)

VII.1 – Workpackage objectives

The work package includes different activities:

- Weekly updating of Luciopercimprove website for the dissemination of information within the consortium (intranet with restricted access to the project partners only) and for the presentation of general information on partners, project progress, main activities, etc. to the public (www.luciopercimprove.be).
- Organisation of a workshop on Percid Fish Culture (in collaboration with Percatech coordinator) (www.percid.be).
- Finalization of a technical manual on artificial reproduction method towards partners of the project and fish farmers in general.
- Publication of technical papers in professional magazine.
- Publication of scientific papers in peer-review journals.
- Redaction and dissemination of progress and final reports (restricted use within the consortium and European Commission Services).

VII.2 – Progress toward objectives

The specific website (www.luciopercimprove.be) presenting the LUCIOPERCIMPROVE project has been created and updated throughout the two years of the project to inform all publics interested by the pikeperch culture and to promote the main results obtained in this project. It was also used to disseminate the results and for communication aspects within the consortium.
A specific workshop on Percid culture will be organised on the 23-24-25 of January 2008, in association with coordinator of Percatech project. Sponsors have been found and a website containing all required information has been created and updated weekly (www.percid.be). Provisional programme was discussed during the meetings. This event will ensure the dissemination of the results to the European fish farmers, scientists and other people interested by Percid fish culture.
A manual based on the Polish expertise (Partner 10) on pikeperch artificial reproduction has been finalized, printed and distributed to all partners of the consortium. It was also put within the restricted area of the Project website. The goal of this book is first of all for all partners to gain more experience with this technique. It will also be distributed during the workshop on Percid Fish Culture to all participants.
Firstly, at the beginning of the first year (November 2005), a consortium agreement has been signed by all partners. It defines the property of the results and their way of diffusion.

Website of Luciopercimprove
A specific website (www.luciopercimprove.be) has been developed and put online in November 2005 to promote the communications between partners related to the activities conducted by the Luciopercimprove consortium within the framework of this program. A restricted area is accessible to each partner with a personal password (http://www.luciopercimprove.be/protege/index.htm). This area contains all the documents dealing with the project such as: technical annex and consortium agreement, provisional dates of the meetings, meeting programs and minutes, methodologies for the different workpackages and main results. The website is regularly updated and the partners can download all

documents electronically. The management of this website is done by the coordinator. It is updated weekly.

Workshop on Percid Culture: From Research to Production

A specific workshop on Percid culture was organised on the 23rd, 24th and 25th of January 2008 in the University of Namur (Partner 1), in association with coordinator of Percatech project from the University of Nancy (Partner 11). This event was sponsorised by Bord Iascaigh Mhara (BIM, Irelande), DanaFeed (Denmark), Filière Lorraine d'Aquaculture Continentale (FLAC, France), Nutreco (Partner 5 of Luciopercimprove), Dil Import-Export (The Netherlands) and the Wallone Region (Belgium). This workshop aimed at ensuring the dissemination of the results of both project to the European fish farmers, scientists and other people interested by Percid fish culture in general. Furthermore, knowledge dissemination was not restricted to these two projects. Scientists presented wide synthesis of the work performed on the 15 past years in the field of Percid research and professionals implied in Percid Aquaculture also shared their own experience, presenting their activities and facilities. In addition, other producers and scientists attending to the workshop completed the conferences by poster communications.

Concerning the preparation of this event, a website containing all required information for the participants was created and updated weekly since May 2007 (www.percid.be). At this period, workshop official announcement was made and a call for communications was launched. Its provisional programme was discussed during the Luciopercimprove meetings and also during two specific meetings between chairmen (Partner 1 and 11) and Partner 8 in May and September 2007.

In January, a total of 115 people attended to the workshop, of which 60 professionals of the aquaculture industry (fish farmers, feed producers, advisor or network organisation in aquaculture, people from the European Commission…) and 48 researchers (**Figure 1**). A total of 20 countries were represented, of which 17 from Europe (**Table 1**). The most represented countries were Belgium, Denmark, France, Ireland, Poland, Romania and the Netherlands (**Figure 2**). This denoted the high intensity of research and Percid fish farming development in these countries in particular. However, the multiplicity of the countries represented – from Ireland to Romania – clearly demonstrated that the development of the Aquaculture industry of Percid fish culture and the research activity in this field is definitely pan-European.

A total of 53 communications were presented. On arrival of participants, an abstract book of these communications (150 pages) and the handbook on artificial reproduction of pikeperch elaborated by partner 10 (University of Warmia and Mazury) within the framework of the Luciopercimprove project were distributed. Regarding the program of the workshop (**see annex**), the two first days were dedicated to 23 conferences presented by specialists of Percid Aquaculture (scientists or fish farmers according to the topic) on all aspects of Percid Fish Culture (e.g. markets, husbandry management, reproduction, growth, production systems, fish farmers experience…). 13 conferences were presented by professionals while 10 conferences concerned synthesis of applied research of the 15 past years. Interestingly, this ratio 13/10 perfectly reflected the ratio of professional/researchers present at the meeting. Since the workshop was directly made to the attention of producers, the results of research presented only concerned knowledge susceptible to be transferred and applied in fish farms at short, mid or long term. This knowledge was completed by the presentation of about 30 posters on recent studies or experience gained in the field of Percid culture.

The third day consisted in a visit of the facilities of a laboratory of Percid aquaculture (University of Liège, CEFRA, Partner 8) and a pikeperch fish farm (Excellence Fish, Partner

2). This excursion had a great success since about half of the total participants attended to it of which mainly were producers.
In conclusion, it appears that the participants were very pleased to participate to this workshop and found what they learned very interesting. Some of them were about to launch their own fish farm (e.g. in Italy). The representant of the European commission (S. Varsamos) was also very enthusiastic and advise the "Percid community" to build a network of producers and scientists in the aim of allowing a harmonized development of the Percid fish culture in Europe. For example, it was identified as a main conclusion that the major bottleneck to the development of Percid culture is the supply of juveniles all year round. It is no doubt that the results of Percatech for Eurasian perch and Luciopercimprove for pikeperch will help to the development of this industry. From the results obtained, Excellence Fish (partner 2) has already launched an activity of hatchery-nursery for pikeperch and two participants from Ireland are also building their own hatchery for Eurasian perch. S. Varsamos appeared confident for the future of this industry and also advised to propose more projects of applied research within the area of Percid fish culture to the European commission.

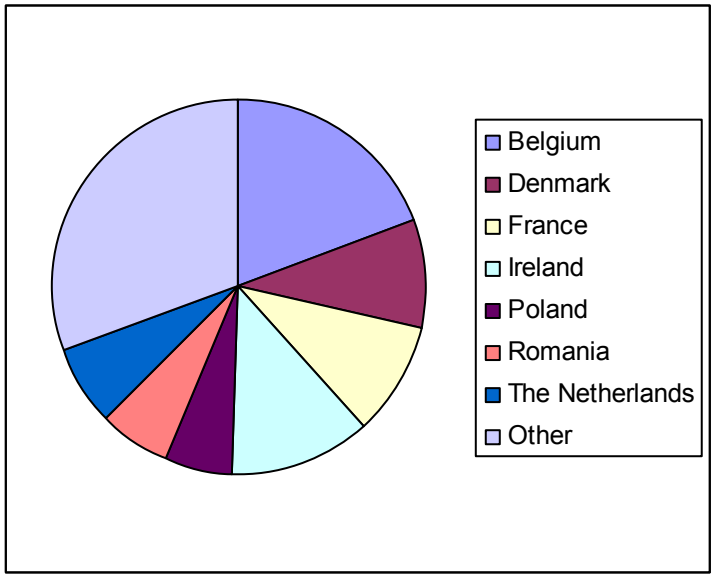


Figure 1: Diagram of the most represented countries at the workshop

Table 1: Total participants of each country represented at the workshop

Country	Number of participants
Austria	1
Belgium	22
Czech Republic	2
Denmark	11
Finland	2
France	11
Germany	5

Hungary	5
Ireland	14
Italy	3
Poland	7
Romania	7
Slovenia	2
Sweden	4
Switzerland	4
The Netherlands	8
Canada	2
New Zealand	2
United Kingdom	2
USA	1

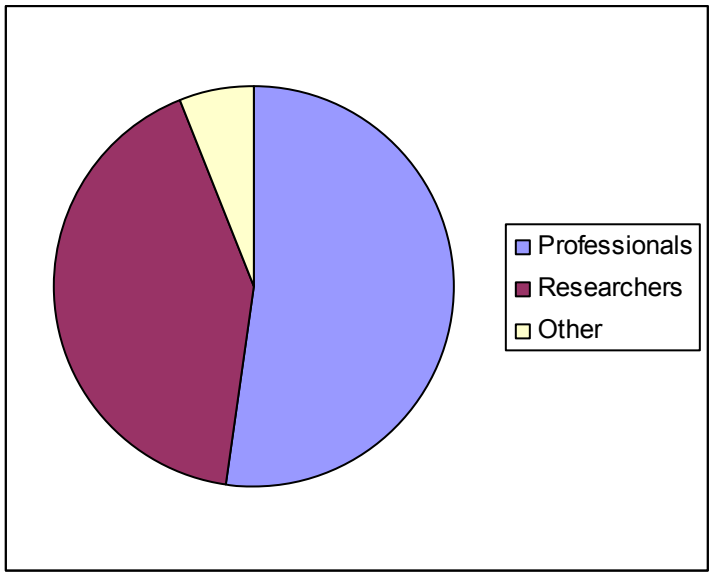


Figure 2: Diagram of the professionals/researchers representation at the workshop

Videotape and manual on artificial reproduction

A manual based on the Polish expertise (Partner 10) on pikeperch artificial reproduction has been finalized, printed and distributed to all partners of the consortium. It was also put within the restricted area of the Project website. The goal of this book is first of all for all partners to gain more experience with this technique that is particularly delicate with pikeperch (sensitivity of fish to handling, small eggs and egg stickiness). It will also be distributed during the workshop on Percid Fish Culture to all participants.

PERCID FISH CULTURE - FROM RESEARCH TO PRODUCTION

Namur (Belgium) 23 - 25 January 2008

DAY 1

8:00 Registration and poster installation

9:00 Welcome address by the organizers

9:15 Address by FEAP (C. Hough)

9:30 Support to aquaculture research in FP6 and new opportunities in FP7 (S. Varsamos, European Commission)

9:45 Session 1 : The European market and its prospects (Chair person: D. Toner, IRL)

• 9:45 The European market for perch Perca fluviatilis (L. Watson, IRL)
• 10:15 Poster Session and Coffee break
• 10:45 The European market of the pikeperch for consumption (H. Dil, NL; F. Teletchea, F)
• 11:05 The restocking market for percids (L. Tamazouzt, F)
• 11:25 Discussion (D. Toner, IRL)

12:00 Lunch

13:30 Session 2 : The hatchery-nursery activity: juvenile production and gamete quality (Chair person: C. Melard, B)

• 13:30 Recent improvement in the control of the percid reproductive cycle (P. Fontaine F; H. Jansen, NL)
• 14:00 Sperm quality and cryopreservation in Perca fluviatilis (O. Linhart, CZ)
• 14:30 Feeding and nutrition of European percid broodstock and early life stages (P. Kestemont et al., B)
• 15:00 Genetic improvement of growth in perch production: domestication, sex control, hybridization and strain selection (C. Rougeot, B)

• 15:30 Poster Session and Coffee break

16:00 Session 3 : The producer experience (Chair person: H. Paulsen, DK)

• 16:00 Bornholms Hatchery: Control of out-of-season spawning of perch (J. Overton, DK)
• 16:20 Perch juvenile production in Ireland – Grasping the potential (D. Toner, IRL)
• 16:40 Viskweekcentrum Valkenswaard: extensive vs intensive production of pikeperch juveniles (J. Van Mechelen, NL)

• 17:00 Discussion (H. Paulsen, DK)

• 19:30 Banquet (Grenier, Arsenal, University of Namur)

DAY 2

8:30 Session 4 : The ongrowing phase (Chair person: R. Mandiki, B)

• 8:30 Recent progress in feed and nutrition in percids (P. Kestemont, B)
• 9:00 Growth and husbandry effects in percids (C. Mélard, B)
• 9:30 Rearing system and flesh quality in Eurasian perch (M. Thomas, F)
• 10:00 Percid culture and pathology control (E. Ariel, DK)

• 10:30 Poster Session (Coffee break)

11:00 Session 5 : The producer experience (Chair person: J. Van Mechelen, NL)

• 11:00 Lucas Perches : production of Eurasian perch in recirculating system (D. Vandeworde, F)
• 11:20 Excellence Fish : production of pikeperch in recirculating system (E. Philipsen, NL)

• 11:40 Discussion (J. Van Mechelen, NL)

12:15 Lunch

13:50 Session 6 : Techniques and economics of percid culture (Chair Person: L. Watson, IRL)

• 13:50 Presentation of the German experience (H. Wedekind, D)
• 14:10 Presentation of the Swedish experience (O. Oberg, S)
• 14:30 Economical feasibility of pikeperch rearing and presentation of an economical model for percid culture (E. Schram, NL)

• 14:50 Coffee break

• 15:20 Percid culture in Canada (L. Therrien, CAN)
• 15:50 Percid culture in North Central region of USA (J. Malison, USA)

16:20 Discussion: future and prospects of Percid culture in Europe (P. Kestemont, B; P. Fontaine, F)

DAY 3

8:00 – 16:30 Visit of the CEFRA facilities (Research Laboratory in Aquaculture, ULG, Tihange) in Belgium, followed by a visit of pikeperch farm (Excellence Fish, Horst) in The Netherlands.