Wageningen IMARES
Institute for Marine Resources & Ecosystem Studies

Location IJmuiden
P.O. Box 68
1970 AB IJmuiden
The Netherlands
Tel.: +31 255 564646
Fax: +31 255 564644

Location Yerseke
P.O. Box 77
4400 AB Yerseke
The Netherlands
Tel.: +31 113 672300
Fax: +31 113 573477

Location Texel
P.O. Box 167
1790 AD Den Burg Texel
The Netherlands
Tel.: +31 222 369700
Fax: +31 222-319235

Internet: www.wageningenimares.wur.nl
E-mail: imares@wur.nl

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Task 2: Broodstock management
Sub-task 2.1: Extension of the natural reproduction period – early or delayed spawning (WP2)

H.M. Jansen and J. Kals

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DG IV Fisheries
Rue de la Loi 200
B-1049 Brussels
Belgium

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Summary

The overall objective of task 2 is to extend the spawning period of 'wild' common perch from some weeks (generally 3 or 4) to 6 months. In order to reach this objective three experiments were performed. Experiment 1 focused on the effect of extending the reproduction period of 'wild' breeders – early or delayed spawning - on their ability to spawn and reproduce. Experiment 2 focussed on optimization of the holding conditions for harvested "wild" breeders. The effects of catch and captivity were evaluated in experiment 3.

During all experiments high mortalities (mostly due to fungal infections) were observed. In previous studies also high mortality rates of wild breeders were observed after capture and in the first phase of captivity conditions. The present results indicate that these mortalities were related to a weakness in immune defences, such as the decrease in temperature (winter syndrome).

Early and delayed reproduction of 'wild' breeders (experiment 1)

During the period of December-April, mature wild perch were collected and stored in a broodstock holding system. Monthly, randomly selected individuals were transferred to the reproduction facilities, where the fish were induced to spawn by using HCG-injections followed by a natural spawning in the tank. After the spawning, the available egg strings were collected from the tanks and approximately 300 eggs from each string were incubated. Fertilization and hatching rates were used as indicators for egg quality.

Six different spawning experiments were executed. Several unexpected problems were experienced, as for example difficulties with the collection of broodstock, disease problems, mortality and behavioural differences of the breeders during the successive spawning induction periods. Therefore multiple batches of broodstock needed to be collected and the protocol to induce spawning needed to be adapted almost every spawning trail. This makes comparison between groups difficult and no hard conclusions could be drawn. Although fertilization rate of the eggs throughout the period was not that bad, the hatching of the larvae was dramatically low. We have observed early and spontaneous release of eggs during storage. This suggests that spawning is limited to a range of approximately 10 weeks of storage, regardless of temperature or photoperiodicity.

As a result of high mortalities and problems with collection of wild breeders in the first batch, it was impossible to evaluate the possibility to use early spawning as a tool to extend the reproduction period (out of season spawning) of wild breeders.
Holding wild breeders under winter conditions for prolonged time for the purpose to delay spawning appears to have less potential than originally thought due to diseases, mortality, lack of appetite and spontaneous release of eggs during storage.

Optimization of holding conditions (experiment 2)

Fish originating from an extensive culture pond were caught, acclimatized and stored at three different holding temperatures (6°C, 12°C and 18°C). Differences between the different holding conditions were evaluated by monitoring the effects on (chronic) stress parameters. Monthly, randomly five fish were selected for analysis. Serum and plasma samples were taken to evaluate respectively the effects on stress physiology (cortisol and glucose) and immunological status (lysozyme activity, immonoglobulin level and haemolysis titre). It was difficult to get sufficient blood of the smaller fish and of fish stored at 6 °C, which resulted in bad quality of those samples.

During the process of recovery and acclimatization 25 percent of the fish died. After the recovery period, the overall mortality during this experiment was 37%, mostly due to fungal infections. Mortality rates were not significantly different between temperature treatments.

There was a large difference in the feeding behaviour of the fish reared under different circumstances. During the last sampling point it appeared that most of the female gonads were almost completely degenerated again.

High cortisol levels were observed on day 0, most likely caused by the effects of transport. Cortisolemia markedly decreased on Day 15, indicating an acclimatization of fish to experimental conditions. The decrease was not influenced by rearing temperature. Low temperature regime induced an increase in cortisol and glucose level after day 29 confirming an enhancement of stress physiology by a prolonged holding at low temperature. Apart from the reproduction process, lowering temperature is one of the major stressors which trigger the mechanisms controlling stress response. Total immunoglobulin level for fish submitted to low temperature conditions (6°C) was comparable to values known to induce stress. No differences in total immunoglobulin level were observed between 12°C and 18°C. Lysozyme activity was not affected by temperature regimes. Results from the present study indicate that low temperature only affected specific immune system not non-specific immune status.

Low temperature regimes should be avoided for a prolonged time since it enhances stress physiology. We used an absolute temperature protocol to bring the broodstock into the physiological state necessary to start gonadal development. It is unknown if a Δ T can be used instead. If possible, this would result in the possibility for keeping broodstock at higher "winter" temperatures, which will increase their metabolism and feeding behaviour giving them a better scope to fight diseases and increase survival rates.

Effects of catch and confinement (experiment 3)

Wild breeders were caught in the River Meuse (Limburg) in April 2006. After transport the fish were immediately stored in a temperature controlled recirculation. The majority of the females were not sexually mature yet or appeared to be already in post-spawning stage. At regular time intervals fish were sampled. High mortalities caused an early finish of the experiment (10 days instead of the expected 21 days) and sample points were taken relatively close at each other. Blood samples were taken for both plasma and serum to evaluate respectively the effects on stress physiology (cortisol and glucose) and immunological status (lysozyme activity, immonoglobulin level and hemonysis). It was difficult to get sufficient blood of the smaller fish. This resulted in bad quality of the samples of the smaller fish.

The overall mortality during this experiment was 38%, mostly due to fungal infections. Average cortisol and glucose levels were lower compared to previous studies on Eurasian perch, and did not indicate an increase in stress physiology during the ten days in captivity conditions. Levels for both lyzozyme activity and haemolysis titre were low at the time of capture and were significantly affected by captivity conditions with an increase over the time.

Introduction

The overall objective of task 2 is to extend the spawning period of 'wild' common perch from some weeks (generally 3 or 4) to 6 months. Specific objectives are:

- to define the optimal period for harvesting of 'wild' breeders,
- to optimise the photo thermal program of spawning induction in an extended season and
- to optimise stocking conditions for harvested 'wild' breeders.

Experiments regarding objectives 1 and 2 were finalized in experiment 1 during the first year of the project. The research of the first experiment was focused on the effect of extending the reproduction period of 'wild' breeders – early or delayed spawning - on their ability to spawn and reproduce. During the period of December-April, mature wild perch was collected and stored under an artificial natural photoperiod and low water temperature in a temperature controlled closed recirculation system. Males and females were stored separately. Monthly, during a period from March until July, randomly selected individuals, males and females, were transferred to the reproduction facilities, where the fish was induced to spawn by slowly increasing water temperature over a period of 2 weeks from 'natural' to 12 °C. At first the fish was induced to spawn by HCG-injection and stripped according to the protocol developed by Kucharczyk *et al.* (1996), but as stripping of the wild breeders appeared to be very difficult it was decided to let the wild breeders spawn "naturally" in the tank after the spawning induction period and HCG injections. This proved to work very well in the beginning, but as the holding period of the broodstock prolonged the perch did already spawn during the spawning induction period, sometimes even before the HCG injections could be given. From each string of eggs, which could be collected, approximately 300 eggs were incubated in duplo using a Petri dish. Fertilization and hatching rates were determined and used as indicators for egg quality.

In the second year the focus was on the optimization of the holding conditions for harvested "wild" breeders. Wild breeders were stored under different holding conditions (temperature) for evaluation of effects on stress physiology and immunological status (experiment 2). However due to disappointing results during experiment 2 due to mortality and difficulties to obtain sufficient amounts of blood we decided to perform a follow-up experiment (experiment 3). The initial objective of experiment 3 was to characterize the stress physiology and immunological status of wild broodstock directly after capture, before, during and after spawning. However this turned out not to be possible (see section about Experiment 3), therefore the new objective of experiment 3 is: to determine the effects of catch and confinement on stress physiology and immunological status of wild broodstock in indoor water recirculated holding facilities.

The experimental work of experiment 2 and 3 is part of work package 2 and analysis and interpretation of the data are part of work package 5. Nevertheless, all information (materials and methods as well as data, results and discussion) will be reported in this section of the report.

Overview of important findings

During all experiments high mortalities (mostly due to fungal infections) were observed. In previous studies also high mortality rates of wild breeders were observed after capture and in the first phase of captivity conditions. The present results may indicate that these mortalities were related to a previous weakness in immune defences, due to the decrease in temperature (winter syndrome) and low food intake. We also have the impression that quality of breeders originating from fisheries is highly important for successful production in tanks.

Experiment 1 - Out of season reproduction of perch, early or delayed spawning; the effect of extending the reproduction period of 'wild' breeders on their ability to spawn and reproduce

Due to several unexpected problems, as for example difficulties with the collection of broodstock, disease problems, mortality and behavioural differences of the breeders during the successive spawning induction periods as spawning was delayed further into the season, multiple batches of broodstock needed to be collected and the protocol to induce spawning needed to be adapted almost every spawning trail. This makes comparison between groups difficult and no hard conclusion could be drawn. Some of the most important findings of this experiment are stated below:

- Collection of wild breeders appeared to be more difficult than expected
- Under the experimental conditions encountered stripping of wild breeders appeared to be impossible
- Holding wild breeders under winter conditions for prolonged time for the purpose to delay spawning appears to have less potential than originally thought. This due to problems with:
    - Diseases, especially of fungal origin
    - High mortality, throughout the holding period as well as during the spawning induction period, does besides directly affecting reproduction also makes it more difficult to hold broodstock for selection purposes
    - Lack of appetite during storage at winter conditions
    - Early and spontaneous release of eggs during storage suggest that spawning is limited to a range of approximately 10 weeks of storage, regardless of temperature or photoperiodicity. But although the suggestion seems obvious, care should be taken, as it is important to realize that this particular batch of broodstock was collected as late as April. The gonadal development might have been already to far to be able to put the fish back in winter conditions for an extended period of time.
    - Egg quality is most likely to deteriorate the more spawning is delayed as indicated by the fact that although the fertilization rate of the eggs throughout the period was not that bad, the hatching of the larvae was dramatically low

As a result of high mortalities and problems with collection of wild breeders in the first batch, it was impossible to evaluate the possibility to use early spawning as a tool to extend the reproduction period (out of season spawning) of wild breeders.

Experiment 2 - Optimization of holding conditions

Due to high mortalities less samplings were performed then originally planned. It turned out to be difficult to obtain sufficient amounts of blood to perform all analysis. Some of the most important findings of this experiment are stated below:

- High mortality was observed through out the experiment. During the recovery period 25% died. After the recovery period, the total mortality within this experiment was 37% (42% at treatment 6˚C; 33% at treatment 12˚C; 43% at treatment 18˚C, not significant different)

- Mortality was mostly caused by fungal infections
- There was a large difference by the feeding behaviour of the fish reared under different circumstances; fish reared at 6˚C reacted very apathetic and hardly ate, fish reared at 12˚C ate carefully, and fish reared at 18˚C were very eager to eat and did so.
- During the last sampling point it appeared that most of the female gonads were almost completely degenerated again.

In terms of stress response the following could be concluded:

- High cortisol levels were observed on day 0, most likely caused by the effects of transport. Cortisolemia markedly decreased on Day 15, indicating an acclimatization of fish to experimental conditions. The decrease was not influenced by rearing temperature.
- Low temperature regime induced an increase in cortisol and glucose level after day 29 confirming an enhancement of stress physiology by a long time holding at low temperature.
- Results from the present study indicate that low temperature only affected specific immune system not non-specific immune status:
    - Total immunoglobulin level for fish submitted to low temperature conditions (6°C) was comparable to values known to induce stress. No differences in total immunoglobulin level were observed between 12°C and 18°C.
    - In contrast, lysozyme activity was not affected by temperature regimes

Experiment 3 – Effects of catch and captivity

Again high mortalities caused by fungal infections were observed within this experiment (38%). Both cortisol and glucose levels were lower as compared to values in previous studies on Eurasian perch, and did not indicate an increase in stress physiology during the twelve days in captivity conditions. Levels for both lyzozyme activity and haemolysis titre were low at the time of capture and were significantly affected by captivity conditions with an increase over the time.

Suggestions for further research

It would be interesting to test if an absolute temperature protocol using winter conditions or low temperatures is a necessity to bring the broodstock into the physiological state necessary to start gonadal development. It might for example be possible that it is not the absolute temperature that triggers gonadal development or spawning, but a certain change in temperature. It is unknown if a Δ T can be used instead. If possible, this would result in the possibility for keeping broodstock at higher “winter” temperatures, which will increase their metabolism and feeding behaviour giving them a better scope to fight diseases and increase survival rates.

Experiment 1: Out of season reproduction of perch – early or delayed spawning; the effect of extending the reproduction period of 'wild' breeders on their ability to spawn and reproduce

Introduction

The research of this experiment was focused on the effect of extending the reproduction period of 'wild' breeders – early or delayed spawning - on their ability to spawn and reproduce, the main objective of this work package. During the period of December-April, mature wild perch was collected and stored under an artificial natural photoperiod (15h D: 9h L), and low water temperature of 6°C in a temperature controlled closed recirculation system (the so called "broodstock holding system"). Males and females were stored separately. Monthly, during a period from March until July, randomly selected individuals, males and females, were transferred to the reproduction facilities, where the fish was induced to spawn by slowly increasing water temperature over a period of 2 weeks from 'natural' to 12 °C (table 1). At first the fish were induced to spawn by using HCG-injections (table 2) and stripped according to the protocol developed by Kucharczyk *et al.* (1996). But as stripping of the wild breeders appeared to be very difficult it was decided to let the wild breeders spawn "naturally" in the tank after the spawning induction period and HCG injections. This proved to work well in the beginning, but as the holding period of the broodstock prolonged the perch did already spawn during the spawning induction period, sometimes even before the HCG injections could be given. After the breeders did spawn, the available egg strings were collected from the tanks and approximately 300 eggs from each string were incubated in duplo using a Petri dish. Fertilization and hatching rates were used as indicators for egg quality.

Materials and methods

Broodstock holding facilities

The broodstock holding tanks had a diameter of 160 cm, and a water depth of 100-cm. The total water volume of the tanks used was 2 m^3. The tanks were made of plastic and had a black colour. The tanks contained pieces of PVC piping and plastic flaps to provide cover material for the fish. The tanks were situated in a cold room with a constant air temperature. The water in the tanks was kept at a constant low temperature of 6°C by circulating cold air through the tanks. Water is pumped over a filter and water exchange was set at 15% per day. The photoperiod in the area is (15 h D: 9 h L), which corresponded to the natural photoperiod in December in the Netherlands. Males and females were kept in separate tanks. The broodstock holding facilities are shown in figure 1 and 2.

Figure 1 and 2 Some images of the temperature-controlled broodstock system. PVC pipes were used to provide shelter.

Reproduction facilities and experimental protocol

Monthly, randomly selected individuals, ♀ and ♂, were transferred to the reproduction facilities in another climate control room. The size of these tanks was 100 x 70 x 80 cm (length x height x width). The tanks are shown in figure 3 and 4. In these tanks water temperature was increased over a period of 15 days from 6 to12 °C, while the photoperiod gradually changes to the photoperiod of April/May (9 h D: 15 h L) as shown in table 1.

Figure 3 and 4 Some views of the temperature controlled spawning induction system. Artificial "weeds" and PVC pipes were added to provide shelter and a possible spawning substrate.

At first the fish was induced to spawn by using three HCG-injections, a priming injection, stimulatory injection and a resolving injection respectively as shown in table 2 and stripped according to the protocol developed by Kucharczyk *et al.* (1996), but as stripping of the wild breeders appeared to be very difficult it was decided to let the wild breeders spawn "naturally" in the tank after the spawning induction period and three successive HCG injections. This proved to work very well in the beginning, but as the holding period of the broodstock prolonged the perch did already spawn during the spawning induction period sometimes even before the HCG injections could be given. From that moment on it was decided to put females and males together during the spawning induction period, as it was more important to get

fertilized eggs than unfertilised strings of eggs. From each string of eggs, which could be collected, approximately 300 eggs were incubated in a Petri dish (diameter 200 mm) in duplo. As a control an unknown number of eggs were incubated in flow through incubation system. Both incubation systems are shown in figure 5 and 6. Fertilization rate (number of fertilized eggs/total number of eggs) and hatching rates (number of hatched larvae/number of fertilized eggs), were used as indicators for egg quality. Incubation was conducted at 15 ^{o}C and O_2 >6 ppm applying a photoperiod of April/May (9 h D: 15 h L).


Figure 5 and 6 Incubation systems.

Table 1: The timetable of the spawning induction period, incubations of eggs and the estimation of the fertilisation and hatching rate.

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

* Hatching was originally expected 14-21 days after incubation (15 ± 0.5 Cº) in our experiments hatching started 7 days after incubation (17.5 ± 0.5 Cº).

** The use of hCG was not always possible as can be read in the specific description of the different spawning trails executed.

Table 2: The three successive HCG injections given with a maximum injection volume of 1.0 ml/kg per injection

Type of injection, time of injection and amount of hCG in IU/kg	♀	♂
Priming injection, time (0 h)	200	100
Stimulatory injection, time (24 h)	500	250
Resolving injection, time (48 h)	5000	2500

Results & Discussion

The results of the first experiment are described below. Due to several unexpected problems, as for example difficulties with the collection of broodstock, disease problems, mortality and behavioural differences of the breeders during the successive spawning induction periods as spawning was delayed further into the season, multiple batches of broodstock needed to be collected and the protocol to induce spawning needed to be adapted almost every spawning trail. Therefore it is decided to describe the results and activities of the first experiment chronologically in time starting with the collection broodstock:

1) Collection of broodstock	(December	04)
2) Second batch of wild breeders	(March	05)
3) First spawning trail	(March	05)
4) Third batch of wild breeders	(April	05)
5) Second spawning trail	(April	05 batch 1)
6) Third spawning trail	(April	05 batch 2)
7) Fourth batch of wild breeders	(April	05)
8) Fourth spawning trail	(May	05 batch 1 and 2)
9) Unexpected eggs	(June	05)
10) Fifth spawning trail	(June/July	05)
11) Sixth spawning trail	(July	05)

Chronological description of activities (including results)

Broodstock

The catch of broodstock appeared to be more difficult than expected. Even though four fishermen and the OVB (Organisation for improvement of inland fisheries) were willing to look out for wild breeders. During cold periods of the year, perch stayed in deeper waters, from where it was difficult to catch the fish and keep them alive. The rapid change in water pressure, which does occur when collecting the fish from deeper waters, proved to demand a serious toll on the breeders. The spines of the common perch also caused problems during the collection of wild breeders. Many individuals suffered severe damage during the process of fishing, handling and transport, which often affected the chance of survival during storage, especially at lower temperatures. The first batch of wild breeders became available as late as December the 31st of 2004. The fishing company "Klop" caught these fish in the river Merwede. The wild caught broodstock were sexed and stocked separately in the Broodstock Holding System at RIVO in Ijmuiden. The broodstock holding facility is shown in figure 1. The batch characteristics are summarized in table 3.

Table 3: Batch characteristics:

♂ n=44	L (cm)	Weight (g)	♀ n=52	L (cm)	Weight (g)
Average	31.3	476	**Average**	33.3	626
Std	3.3	176	**Std**	5.2	329

The water temperature in the broodstock tanks was brought down from an average of 11 ºC to 5.5 ºC in a period of 20 days. Water quality in the holding facilities remained at suitable levels during the whole trial. Oxygen level fluctuated from 8.5 to 14.6 mg/l, temperature remained at 5.5 ºC, pH remained constant at 7.6, and water replacement was 15% per day as shown in table 4. During this period of decreasing the water temperature all the fish died, even though the fish didn't show disease related external symptoms and looked quite healthy. As the wild breeders in this batch died within two weeks, the broodstock didn't survive long enough to bring the fish into winter conditions, which is a necessary phase before starting the spawning induction period. The lack of a cold period makes spawning impossible. As a result it was not

possible to bring a batch of wild breeders in a physiological condition ready to spawn as early as January-February, which was necessary for our early spawning trails. Therefore it was impossible to evaluate the possibility to use early spawning as a tool to extend the reproduction period (out of season spawning) of wild breeders.

Table 4: Water and other parameters:

Parameter	Measured
Oxygen (mg/l)	8.5-14.6
Temperature (ºC)	11 going down to 5.5
PH	7.63-7.68
Light/Dark period	9/15
Suppletion	200 ml/minute = 15% daily

Second batch of wild breeders

The second batch of wild breeders became available from a lake in the south of the Netherlands, near Tholen, as late as the 15th of March and were provided by Mr. P. Kooistra, a local fisherman. It was only a small batch of wild breeders containing 10 males with an average weight 85 g and 13 females with an average weight 108 g. It was decided to start a spawning trail immediately.

The first spawning trail (March 05)

At the 15th of March the first spawning trail was started by the transfer of wild breeders (5 ♀, average 755 g, std 237 and 5 ♂, average 203 g, std 142) to the spawning induction system necessary to follow the spawning induction protocol as shown in table 1. During this period of two weeks the water temperature increased from 6 to 12°C and the photoperiod was adapted from winter conditions (15D/9L) to the conditions of April/May (15L: 9D) as occurring in the Netherlands (table 1). ♀ and ♂ were stocked separately. Figure 9 and 10 show wild breeders swimming in the spawning induction system. At the end of the "spawning induction period" the wild breeders were induced to spawn by using three successive HCG-injections (table 2) and stripped according to the protocol as developed by Kucharczyk et al. (1996). As stripping of the wild breeders appeared to be very difficult, even though it was tried several times with different females between 12 and 24 hours after giving them the resolving injection, it was decided to let the wild breeders spawn "naturally" in the tank after the spawning induction period and HCG injections during the future spawning trails. During this trail some eggs were obtained by stripping females and mixed with sperm, but all of the eggs obtained died within 12 hours. "Natural" spawning took place between 72 and 84 hours after giving the resolving injection. The total weight of eggs collected and weight of the fish involved are written in table 5. Eggs were immediately incubated in Petri dishes and incubation trays as a control (figure 5 and 6), at a temperature of 17.5 ± 0.5 Cº and oxygen levels around 9.5 mg/l.

Table 5: Weight of fish involved and eggs collected during the first spawning trail. The ration between the eggs divided by the weight of the females is shown in figure 15.

	Weight of eggs (g)	Weight of ♂ (g)	Weight of ♀ (g)
Tank 1	905	218, 85, 77	595, 699
Tank 2	853	164, 430	403, 885

Fertilization of the first spawning trail, were the fish have been treated exactly as the protocol, except for the fact that they were allowed to "naturally" spawn in the tank instead of being stripping was quit good with an average of 78% as shown in figure 13. Hatching was originally expected to start 14-21 days after incubation (15 ± 0.5 Cº), but in our experiments hatching did start already as soon as 7 days after incubation (17.5 ± 0.5 Cº). The hatching percentage

of the fertilized eggs was 44% as shown in figure 14. The quality of the larvae that do hatch is good. They were very active, growing well as can be seen in figure 7 and 8.

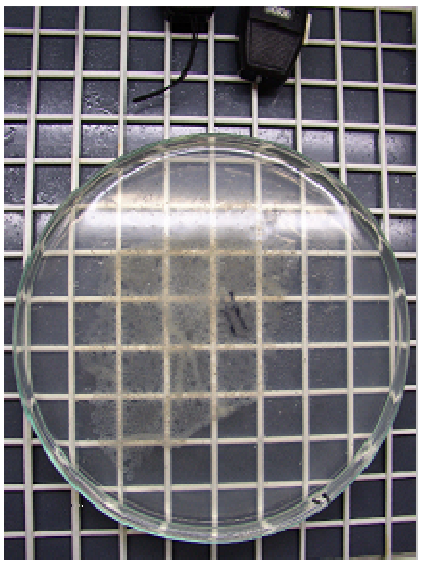
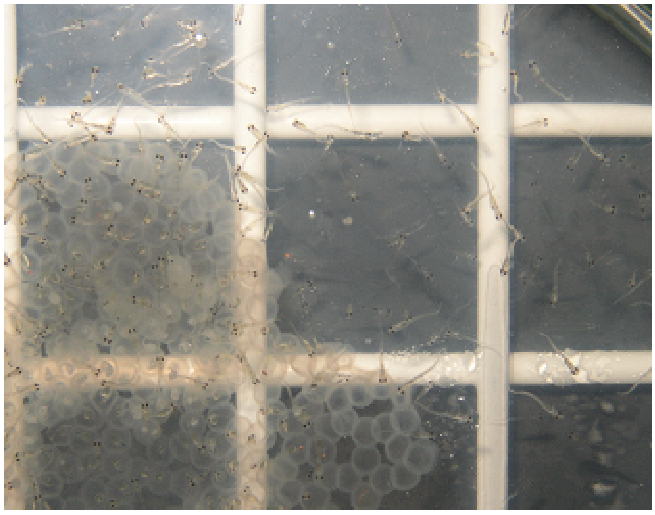

Figure 7 and 8: Petri- dishes with both eggs and hatched larvae.


Figure 9 and 10 Wild breeders swimming in the spawning induction system

Third batch of wild breeders

At 01-04-05 a third batch of wild breeders, caught by the fishing company of Klop in the River Merwede became available. The temperature of the water from the river did reach already 13.5°C, the preferred spawning temperature for European perch. The wild breeders were physiologically seen ready to spawn, but had to be brought back into winter conditions. The fish was stored in the broodstock holding system for the delayed spawning trails. It appeared that the temperature of the broodstock holding system was to low for the fish, as they didn't eat and behaved very aphetic. It was therefore decided to increase the water temperature of the broodstock holding system up to around 6.8 °C.

Second spawning trail (April 05 batch 1)

At 22-04-05, 2 ♂ and 2 ♀ were directly transferred from the broodstock holding system (6,6-7 °C) to the spawning induction system (11-12°C). After 2 days severe fungi developed on all the fish and at the third day one fish died. The fourth day all the fish were almost completely covered with fungi, but surprisingly did spawn. Weight of eggs collected was 343 g. Eggs were incubated immediately. Fertilization of the second spawning trail was low with only 18% of the eggs fertilized (figure 13). Hatching percentage of the fertilized eggs was even worse with only 0.82% of the fertilized eggs being able to hatch as shown in figure 14. The fact that the

breeders in this batch were not able to pass through the spawning induction protocol and therefore didn't receive any hormonal treatment due to fungal problems could very well be the cause of these results. It surprising to see that the fish even did spawn.

Third spawning trail (April 05 batch 2)
At 04-04-05, 6 ♂ (average 202 g std 37) and 6 ♀ (average 352 g std 31) were transferred from the broodstock holding system to the spawning induction system. At the 8th day one female died. All fish were having problems with fungi. Fish were treated with salt (from 5 to 7.5 and eventually 10 ppt). On the 18th of April the fish got their first HCG injection. They all had severe fungi problems and it was decided to treat them with Malagite green (50 minutes at 5 mg/l, which is suppose to be a low concentration), but unfortunately the next morning all fish were dead, except for one. This ♀ (352 g) was put together with 3 ♂ coming form the warm holding tank and 1 ♂ from the broodstock tank (figure April Batch 2). The ♀ did run through the spawning induction period but didn't receive hormonal treatment. Three days later 206 g of eggs could be collected without the use of HCG and were incubated. The ration between the eggs divided by the weight of the females is shown in figure 15. Fertilization was not all that bad with 56% of the eggs fertilized (figure 13), but the hatching percentage of the fertilized eggs was bad with only 1.64% of the fertilized eggs being able to hatch as shown in figure 14.

Fourth batch of wild breeders (April 05)
At 8-4-05 a fourth batch of wild breeders became available. Mr. Van Manshanden caught these fish in Lake Ijssel. It was decided to add these freshly caught wild breeders to our broodstock population in the broodstock holding tanks. A strong selection took place to get rid of less conditioned and damaged fish.

Fourth spawning trail (May 05 batch 1 and 2)
At 03-05-05, 7 ♂ and 7 ♀ were transferred from the broodstock holding tank to the spawning induction tank. Salt (7.5 ppt) was added to prevent development of fungi. The fish did well until day 10 when 2 males died and one string of eggs was found. At day 14 a second string of eggs was found. At this point 5 "full" females were still available. Regarding the experience from earlier trails it was decided to put half (3 ♂ + 2 ♀) of the still living ♂ and ♀ together directly after the spawning induction period **(Batch May 2)** and kept the other half (2 ♂ (average 227 g std 30) + 3 ♀ (average 326 g std 110) separately so these fish were still able to receive their HCG injections, before the fish were also put together **(Batch May 1).**

Batch May 1:
The fish from this batch did run through the whole spawning induction protocol including their hormonal treatment. ♂ and ♀ were put together on 19-05 after they received their final HCG injection. After 26 hours the first ♀ did spawn and released 192 gram of eggs, after 48 hours the second ♀ did spawn and released 340 grams of eggs and after 72 hours the last ♀ did spawn and released 133.5 gram of eggs. The ration between the eggs divided by the weight of the females is shown in figure 15. Fertilization was not all that bad with 51% of the eggs fertilized (figure 13), but the hatching percentage of the fertilized eggs was really bad with only 0.13% of the fertilized eggs being able to hatch as shown in figure 14.
.

Batch May 2:
Fish run through the spawning induction period, but didn't get hormonal treatment. ♂ + ♀ were put together after the induction period while batch May 1* went through hormonal treatment. Collected eggs are from one ♀ only, the other one unfortunately died before releasing her eggs. The fish (2 ♀ Average 330 g std 8) ran through the spawning induction period, but didn't receive hormonal treatment. Only one fish did spawn and released 194 grams of eggs. The ration between the eggs divided by the weight of the females is shown in figure 15. The other one unfortunately died before releasing her eggs. None of these eggs were fertilized as is shown in figure 13.

Unexpected eggs; Batch June 1

Surprisingly one string of eggs was found in the **male** broodstock tank at the 13th of June! Therefore these eggs were released under "winter conditions" (L/D 9/15, T 6.6 ºC). Assuming the eggs were fertilized they were incubated just as the other batches. Off course the weight of the responsible female is unknown. Fertilization was not all that bad with 33% of the eggs fertilized (figure 13), but hatching of the fertilized eggs was less than 1% as shown in figure 14.

The fifth spawning trail (June/July 05)

At 13-06-05, 8 ♂ + 8 ♀ were transferred from the broodstock holding tank to the spawning induction tank. Salt (7.5 ppt) was added to prevent development of fungi. After 7 days one ♀ started to release her eggs, at day 10 three other ♀ did release their eggs and at day 11, 13 and 14 three other ♀ released their eggs without any hormonal treatment. These eggs were not fertilized because the ♀ were hold separately from the ♂. At this time only 8 ♂ (average 82 g std 14) and 3 ♀ (average 622 g std 645) were left to start the hormonal treatment. Unfortunately one female (230 g) released her eggs (193 g) already after the first HCG injection (priming injection) and it was decided to put one of each of the two ♀ left together with 4 ♂ in separate tanks, while continuing their hormonal treatment. Approximately 14 hours after the last (resolving) injection the biggest ♀ (1047 g) released 399 g of eggs, which were incubated directly. The small ♀ (260 g) released her eggs (147 g) approximately 36 hours after her final resolving injection. The ration between the eggs divided by the weight of the females is shown in figure 15. The breeders who survived the spawning protocol were transferred to the warm perch holding tank were they died within 24 hours. Fertilization rates did differ greatly between the eggs from the large female (3%) and the eggs from the smaller female 77%. Average fertilization was not all that bad with 40% of the eggs fertilized (figure 13), but none of the fertilized eggs did hatch as shown in figure 14.

The sixth spawning trail (July 05)

At 05-07 there were only 4 females left with eggs, all the other ♀ in the broodstock holding tank were “empty” (10 fish) and released their eggs spontaneously in the broodstock system during the holding period regardless of the low temperature. These “empty” ♀ and the rest of the ♂ were transferred to the warm holding tank and treated with Flumquine (25 g/m3). These fish except for one died within three days. The ♀ with eggs, as also the 10 best ♂ selected out of the male broodstock tank, were transferred to the spawning induction system and put in separate tanks and were treated with salt 7.5 ppt and Oxytetracycline (20g/m3). The treatment appeared to be working because all fish survived except one ♀, which died at day 5. At day 12, just before the first HCG injection, two ♀ released their eggs (230 g). Because no males were present in this tank none of these eggs were fertilized. At this time only one ♀ with eggs (537 g) was left together with 10 males (average 151 g std 90), but she was severely infected. It was decided to put the ♀ together with all the ♂. The remaining fish finished their hormonal treatment, but unfortunately the ♀ died approximately 12 hours after her final resolving injection, leaving no eggs.

Figure 11 On the right side; ex-breeders swimming in the holding tank. Figure 12 on the left; after the transferring the fish from brackish water (7.5 ppt) to fresh water the ex-breeders developed spots as shown within a day.

Summarized results of the different spawning trails

As described in detail six different spawning experiments were executed on respectively 15/3/2005, 4/4/2005, 22/4/2005, 3/5/2005, 13/6/2005, 5/7/2005. The results of the different trails are summarized and presented in figures 13 – 15. The different labels March, April1, April 2, May 1, May 2, June 1 and June/July represent the different trails and their specific circumstances, which are briefly explained below:

- **March:** These fish have been treated exactly as the protocol in table 1 with the only difference that the fish were allowed to "naturally" spawn in the tank instead of being stripping.
- **April 1*:** 2 ♂ + 2 ♀ were directly transferred from the broodstock holding tank (T 6.6ºC, L/D:9/15) to the spawning tank T 11.5ºC , in which the temperature was immediately increased to (12ºC, L/D 16/8).
- **April 2*:** Females did run through the spawning induction period but didn't receive hormonal treatment. From the ♂ used 1 came directly out of the broodstock tank (L/D 9/15, 6.6 ºC) and the others came out the warm holding tank (L/D 16/8, 16ºC). The latter were also used during the spawning trail in March.
- **May 1*** The fish of this batch did run through the whole protocol and the hormonal treatment. ♂ and ♀ were put together after the last HCG injection.
- **May 2*:** The fish did run through the spawning induction period, but didn't get hormonal treatment. ♂ + ♀ were put together after the induction period. Collected eggs are from one ♀ only, the other one unfortunately died before releasing her eggs.
- **June 1*:** These eggs were collected from the ♂ broodstock tank and released under "winter conditions" (L/D 9/15, T 6.6 ºC).
- **June/July**: These fish were treated as written in table 1 with the only difference that they were allowed to "naturally" spawn in the tank instead of stripping.
- **July**: Due to high mortality no females remained

Spawning trail	Label	Hormone injection	Followed protocol while in holding system	Followed protocol while in induction system
1	March	Yes	Yes	Yes
2	April – 1	Yes	Yes	No
3	April – 2	No	♀ Yes ♂ No	Yes
4a	May – 1	Yes	Yes	Yes
4b	May – 2	No	Yes	
	June	No	No	No
5	June/July	Yes	Yes	Yes
6	July	-	-	-

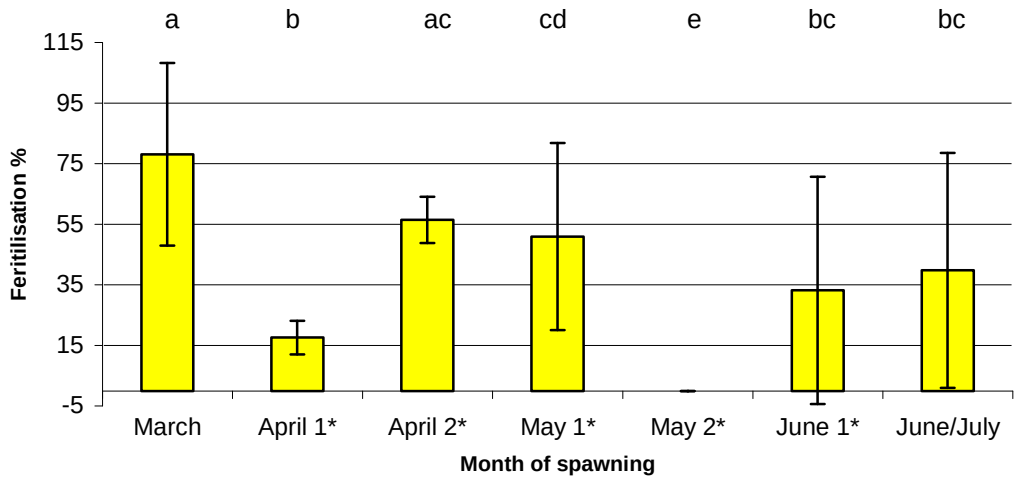

Figure 13: The average fertilization % of the wild breeders of the successive spawning trails as estimated during the delayed spawning trails. Groups with different letters are significantly different (t-test assuming equal variances)

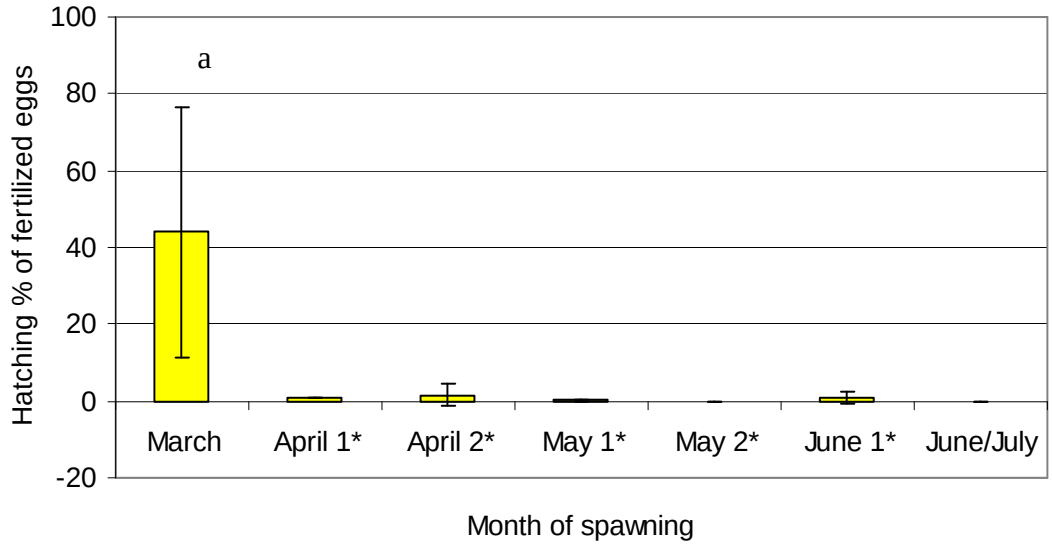

Figure 14 The hatching % of the fertilized eggs from the wild breeders as estimated during the delayed spawning trails.

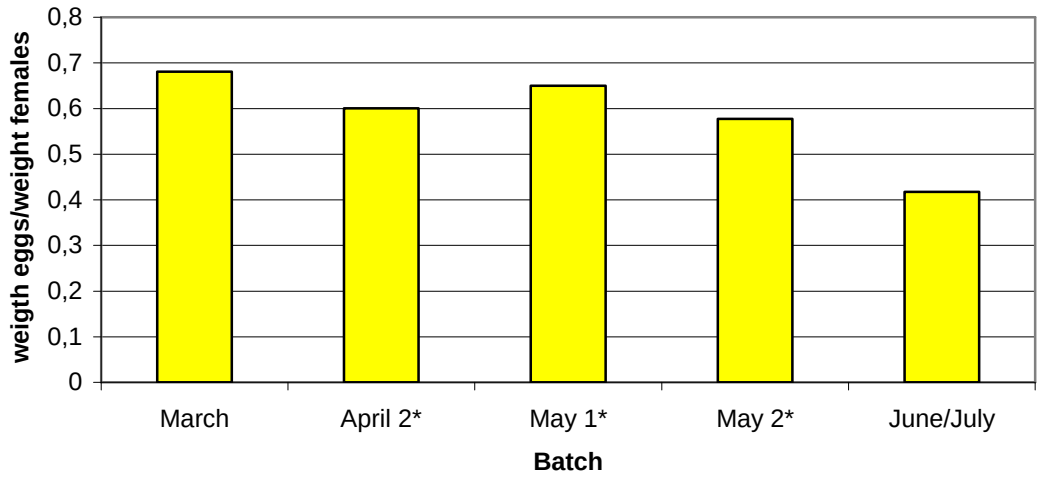

*Figure 15 The average "fecundity" (weight eggs/weight fish) of the different batches). It is Important to stress; that we didn't strip the fish but let them "naturally" spawn in the tanks. Therefore the weight of the eggs is the weight after collection from the spawning induction tank instead of the weight of the eggs after stripping. The weight of the eggs is therefore higher than normal due to absorption of water. From batch April 1 * and June 1 * the weight of the responsible females is unknown.*

Storage of breeders

Mortality and diseases

The first batch of breeders died within one month after arrival. No reproduction trial could be conducted with these fish. An obvious reason for this mortality could not be observed. Although some fish did show signs of damaged tissue around the mouth area and sunken eyes none of them did show serious damage or any signs of infection. But their behaviour was very striking; all fish were very apathetic and did show major difficulties in their gravitational orientation.

Of the other batches of breeders 28% of the males and 49% of the females died during their stay in the brood stock holding facilities, especially due to fungal infections. The mortality as percentage of death males in the male broodstock tank and the percentage of females in the female brood stock tank per week within the experimental period from April until July are shown in figure 16 and 17.

But most of the mortality during the experiment did occur after the transfer of the fish from the broodstock holding facilities towards the reproduction facilities, with fungal infection as the main problem. As soon as the temperatures increased the fungi appeared massively on the breeders, resulting in mortality of several fish, and very poor conditions of the others. Because of these severe fungal problems it was necessary to treat the fish. Treatments using salt up to 10ppt, Malagite green (5mg/l for 50 minutes) and H_2O_2 (250 ug/l for one hour were tried. Malachite green, as used, appeared to be deadly for European perch and H_2O_2 didn't show the results necessary. Salt gave the best results; it appeared that in salt (10 ppt) the fungi couldn't grow out. However, fungi reappeared on the fish within one day after the fish returned to fresh water. Fungal problems are also known to occur with largemouth bass hold at winter conditions. A local pikeperch farmer experienced the same kind of difficulties with his breeders kept for the out of season reproduction of pikeperch.

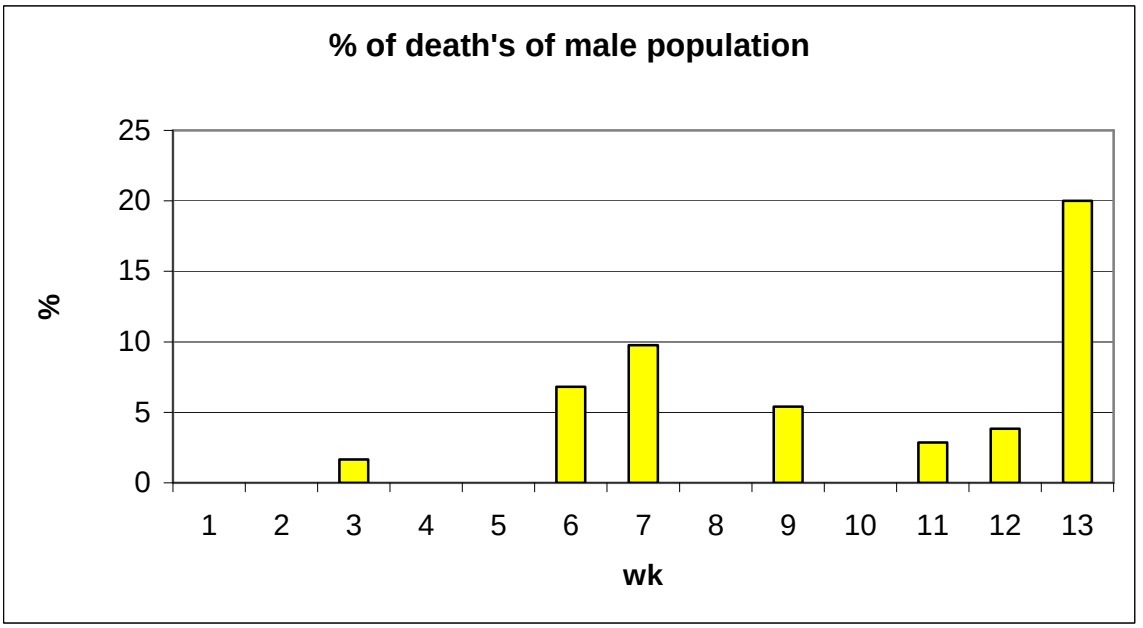

Figure 16: The % of death males in the male broodstock tank per week within the experimental period from April until July.

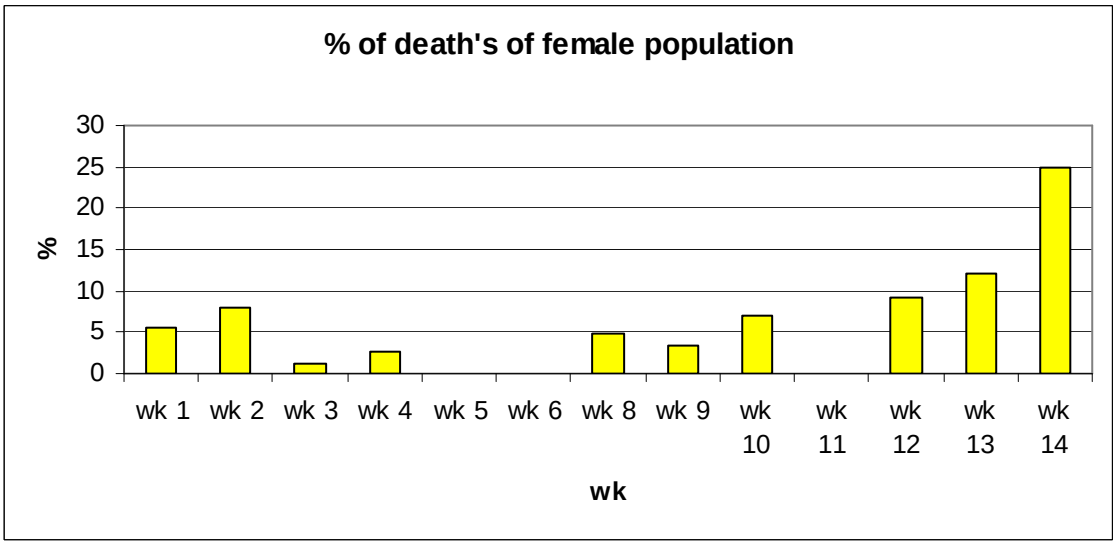

Figure 17: The % of death females in the female broodstock tank per week within the experimental period from April until July.

Spontaneous release of eggs

The storage of wild breeders under winter conditions for prolonged time did appear to cause several problems such as disease, bad eating, deterioration of egg quality etc of which some are worse than others. But one problem in particular, the spontaneous release of eggs during the storage of the breeders under winter conditions, did immediately affect the reproductive capacity of individuals. In the beginning the spontaneous release of eggs was incidental, but going further into season the frequency of the fish spontaneously releasing their eggs increased regardless of the low temperature they were stored as is shown in figure 18. The data does suggest that the breeders will release their eggs after approximately 3 months of storage, regardless of temperature or photoperiodicity. This observation might be of great importance as it suggest that the storage of breeders for delayed spawning is limited to a range of approximately 10 weeks regardless of the results found with fertilization and hatching. But although the suggestion seems obvious, care should be taken, as it is important to realize that this particular batch of broodstock was collected as late as April. The gonadal

development might have been already to far to be able to put the fish back in winter conditions for an extended period of time.

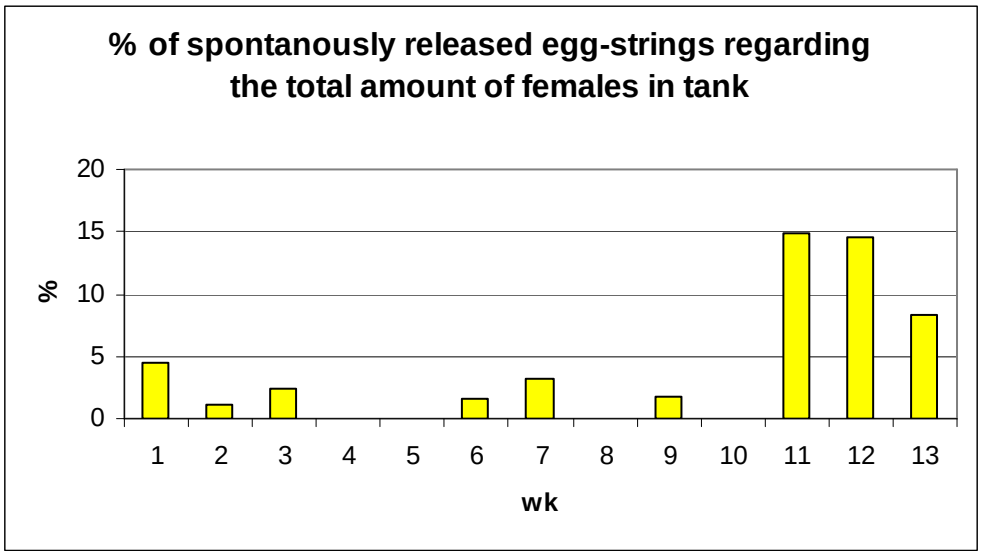

Figure 18: The % of spontaneously released egg strings per week regarding the population in the broodstock tank in the period between April and July.

Feeding the broodstock

We experienced great difficulties with feeing the wild breeders. In the beginning pieces of fish were tried, but the fish didn't eat it. Also life goldfish (3 cm) has been tried, but the fish didn't eat them. Maggots were also not taken, as weren't commercial pellets from Trouvit. After one month the broodstock started to take some earthworms, but was very cautious. The males were more aggressive in taking earthworms than the females. The broodstock in de broodstock holding system was fed two to three times a week depending on how the fish reacted on the food given. Summarizing it can be stated that the breeders didn't eat much during their holding period and even completely stopped eating in the end of June.

Conclusions

Due to several unexpected problems, as for example difficulties with the collection of broodstock, disease problems, mortality and behavioural differences of the breeders during the successive spawning induction periods as spawning was delayed further into the season, multiple batches of broodstock needed to be collected and the protocol to induce spawning needed to be adapted almost every spawning trail. This makes comparison between groups difficult and no hard conclusion could be drawn. Some of the most important findings of this experiment are stated below:

- Collection of wild breeders appeared to be more difficult than expected
- Under the experimental conditions encountered stripping of wild breeders appeared to be impossible
- Holding wild breeders under winter conditions for prolonged time for the purpose to delay spawning appears to have less potential than originally thought. This due to problems with:
    - Diseases, especially of fungal origin
    - High mortality, throughout the holding period as well as during the spawning induction period, does besides directly affecting reproduction also makes it more difficult to hold broodstock for selection purposes
    - Lack of appetite during storage at winter conditions

- Early and spontaneous release of eggs during storage suggest that spawning is limited to a range of approximately 10 weeks of storage, regardless of temperature or photoperiodicity. But although the suggestion seems obvious, care should be taken, as it is important to realize that this particular batch of broodstock was collected as late as April. The gonadal development might have been already to far to be able to put the fish back in winter conditions for an extended period of time.
- Egg quality is most likely to deteriorate the more spawning is delayed as indicated by the fact that although the fertilization rate of the eggs throughout the period was not that bad, the hatching of the larvae was dramatically low

As a result of high mortalities and problems with collection of wild breeders in the first batch, it was impossible to evaluate the possibility to use early spawning as a tool to extend the reproduction period (out of season spawning) of wild breeders.

Experiment 2: Optimization of holding conditions

Objective

Commercial aquaculture requires minimizing stressors to maintain healthy growing fish. One of the objectives of sub-task 2.1 was to optimize the holding conditions of perch by reducing stressors. Therefore wild breeders were caught, acclimatized and stored at three different holding temperatures (6°C, 12°C and 18°C). Differences between the different holding conditions were evaluated by monitoring the effects on (chronic) stress parameters. Recent experiments have shown the importance of distinguishing between acute and chronic responses to stress (Davis, 2006). Acute responses to stressors may be beneficial to the fish and extend their normal adaptive ability, whereas chronic exposure to stressful conditions may result in decreased performance or survival. In our study stress was characterised by physiological changes such as plasma cortisol, glucose and the immunological status of the fish.

Materials and methods

Experimental set-up

Catching of "wild" breeders during winter season appeared to be difficult just as last year (see section experiment 1) and fish from an extensive culture pond were used as an alternative. The fish originated from Belgium and were collected in December 2005. The average length of fish was 20.4 cm and the average weight 118.1 gr (table 6).

Table 6: Batch characteristics

	Number of fish	Average length (cm)	Average weight (gr)
Male ♂	14	19.1	90.4
Female ♀	54	20.8	125.3
Total	68	20.4	118.1

The breeders were transported in oxygenated tanks and aloud to recover in an outside recovery pond enriched with both submerged and floating substrate. After two weeks the breeders were transferred to different temperature controlled holding systems. In total there were three controlled recirculation systems (A,B,C) consisting of circular and square tanks with PVC pipes providing shelter (figure 1). Each system had an UV filtration unit (8W), oxygen levels were between 9.8-12.7 mg/l and water exchange was 15% per day. The systems had a fixed Light/Day regime of 12/12. The fish were acclimatized from 10°C, the outside water temperature, to the experimental temperatures of 6°C (system A), 12°C (system B) and 18°C (system C), which took respectively 8, 4 and 10 days. Fish were fed earthworms and clamworms (*Nereis diversicolor)*. The experimental set-up is shown in table 2. There was a large difference by the feeding behaviour of the fish reared under different circumstances; fish reared at 6°C reacted very apathetic and hardly ate, fish reared at 12°C ate carefully, and fish reared at 18°C were very eager to eat and did so.

Table 7: The experimental set-up for the optimization of holding conditions for captive wild breeders.

Temperature		**6˚C.**	**12˚C**	**18˚C**
Sampling period	*Running time exp. In weeks*			
.t(0) sampling	*0*	n=5		
1) December	*2*	n=5	5	5
2) January period 1	*4*	"	"	"
3) January period 2	*6*	"	"	"
4) February	*8*	"	"	"
5) March	*12*	"	"	"
6) April	*16*	"	"	"
Total samples (90+5)		**30**	**30**	**30**

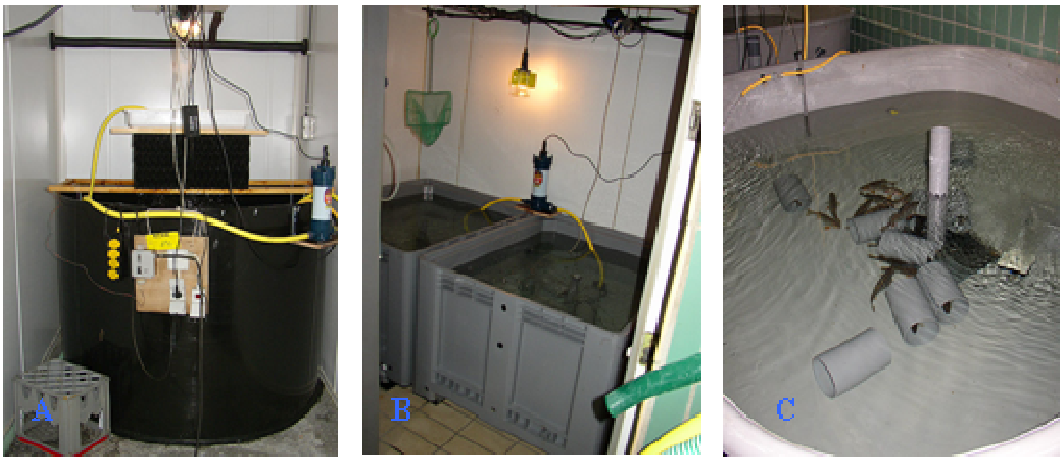

Figure 19 Controlled recirculation systems (A=6°C B=12°C C=18°C)

Blood sampling

Blood needed to be sampled for both plasma and serum necessary to evaluate the effects on stress physiology and immunological status. Five fish per sampling point were sedated by using phenoxy-ethanol (1:1000). A maximum allowable time window of 5 minutes to catch and sedate the fish is taken into account for the analysis of the stress parameters. Blood for plasma is sampled using a syringe with heparin and blood for serum is sampled using a syringe without heparin. After the blood sampling, the sedated fish were weighted, length was measured and sex was determined.

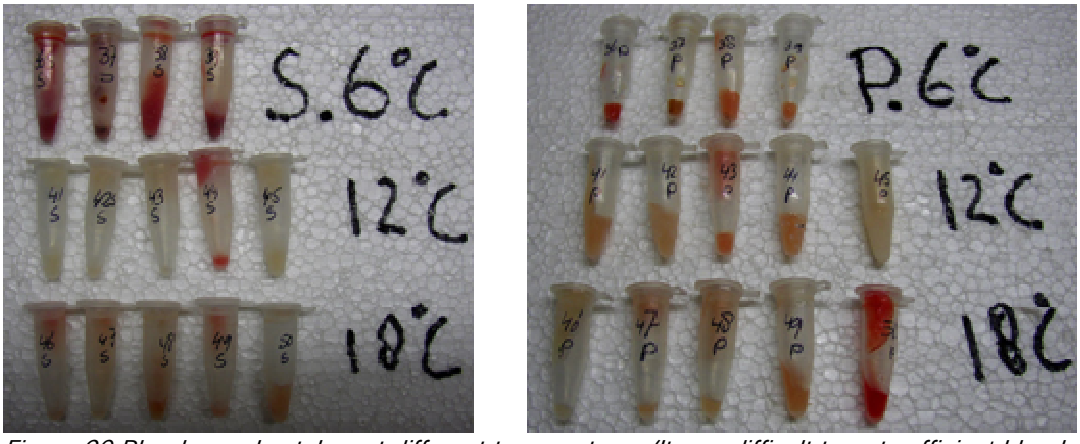

Figure 20 Blood samples taken at different temperatures (It was difficult to get sufficient blood from the fish)

It appeared to be difficult (almost impossible) to get sufficient blood of the smaller fish, especially fish stored at 6 ˚C. Holding temperature seems to have a negative effect on the ease to obtain blood. These effects are noticeable in the quality of the samples. Samples from small fish at 6 ˚C do show increasing problems with haemolysis. There appears to be more fluid in the abdominal cavity of fish kept at 18˚C compared to the other two groups. The fish used appears to be too small to get sufficient blood to sample both plasma and serum

Table 8: Number of samples taken at each sample point for the three rearing methods

Days after stocking	**Serum Samples**				**Plasma samples**			
	6˚C	12˚C	18˚C	**Total**	6˚C	12˚C	18˚C	**Total**
0								**5**
15	4	5	5	**14**	4	5	5	**14**
29	4	5	5	**14**	6	5	5	**16**
43	1	5	5	**11**	4	5	5	**14**
62		7	6	**13**	2	7	4	**13**
Total	9	22	21	**52**	16	22	19	**62**

Analysis

Plasma serum was separated from the blood cells by centrifuging using 5000 rpm for 10 minutes. After centrifuging both plasma and serum are separated from blood cells and kept in frozen storage (-80 °C) until assayed. Immunological status will be based on analysis of serum samples on haemolysis titre, lysozyme activity, and Ig-level. Plasma samples will be used to analyze stress physiology by evaluation of cortisol and blood glucose

Statistical analysis

All quantitative data are expressed as means ± SD (standard deviation). Statistical analysis of this data was performed using the SAS system (version 9.1) with general linear model (GLM) procedures for unbalanced one-way analysis of variance. The minimal level of significance was set at P≤0.05. Before statistical analysis values of cortical, glucose and lysozyme activity were ln-transformed, to satisfy tests for normality and homogeneity of variances.

Results & Discussion

Mortality and diseases

During the process of recovery and acclimatization 25 percent of the fish died. The total mortality within this experiment was 37%, mostly due to fungal infections:

- System A (6˚C) 42% (13/31)
- System B (12˚C) 33% (10/30)
- System C (18˚C) 43% (16/37)

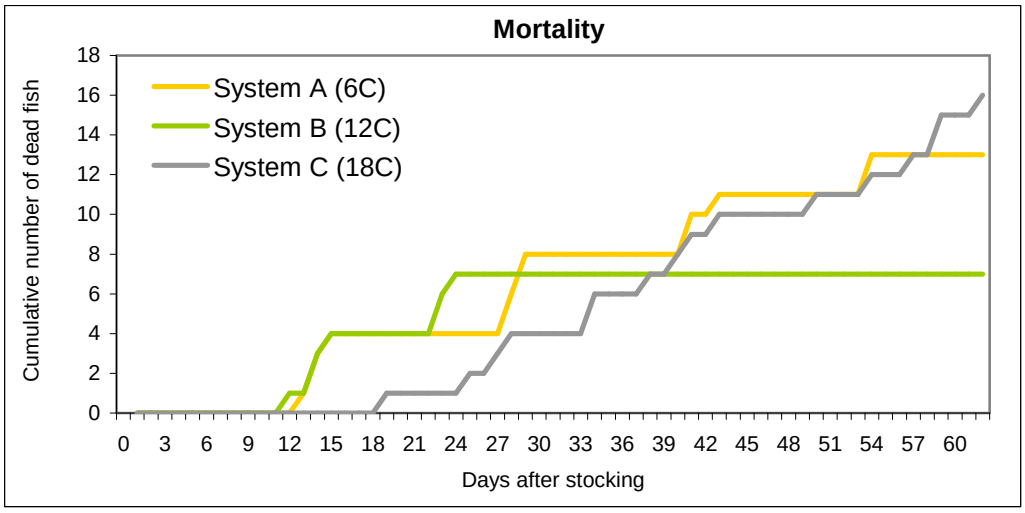

Figure 21 Cumulative mortality in the three holding systems during the trail

Water quality in the holding facilities remained at suitable levels during the whole trial. The high mortalities have caused an early finish of the experiment. The final running time of the experiment came to 8 weeks giving us 5 sample points instead of the 7 points as originally planned. The plasma and serum samples from the sample period of March and April are therefore missing.

Figure 22 Perch severely suffering from fungal infections

Stress physiology and immunological status

In terms of cortisol level (Figure 24), a high stress response to transfer of fish from Belgium was observed on Day 0 as compared to previous reports on Eurasian perch (Acerete et al., 2004; Jentoft et al., 2005) and other fish species (Rotlant et al., 2001). Cortisolemia markedly decreased on Day 15 whatever the temperature conditions of rearing, indicating an acclimatization of fish to experimental conditions. However, low temperature regime induced an increase in cortisol level until Day 29 to the end of experiment (Day 62). The same profile was observed for glucose level (Figure 23) confirming an enhancement of stress physiology by a long time holding at low temperature.

This finding strengthen the results from a previous experiment conducted by partner 8 in the frame work of Percatech project that spawning period is associated to an increase in stress physiology in Eurasian perch. Apart from reproduction process, lowering temperature by winter conditions is one of the major stressors, which trigger the mechanisms controlling stress response.

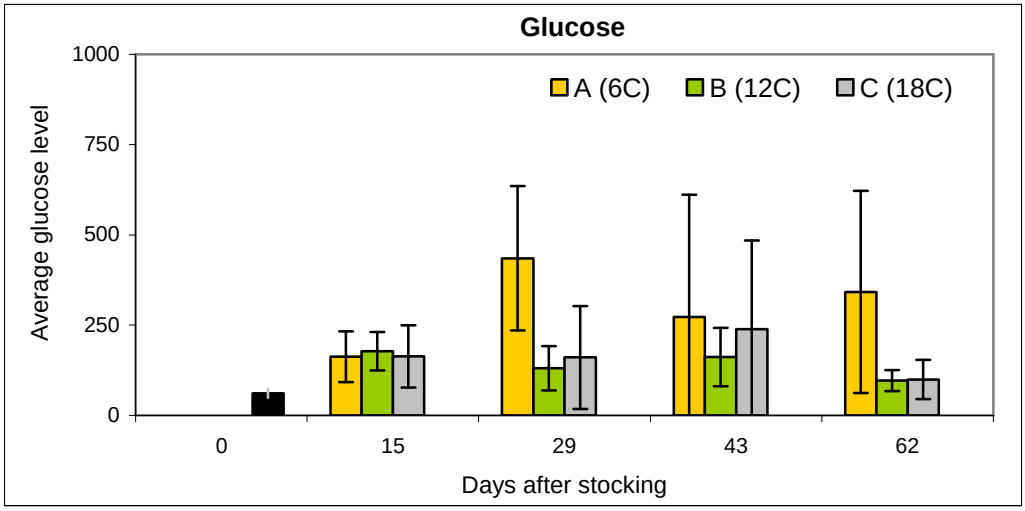

Figure 23 Changes in plasma glucose level (µg/ml) of Eurasian perch breeders submitted to 6, 12 and 18°C conditions of rearing during two months.

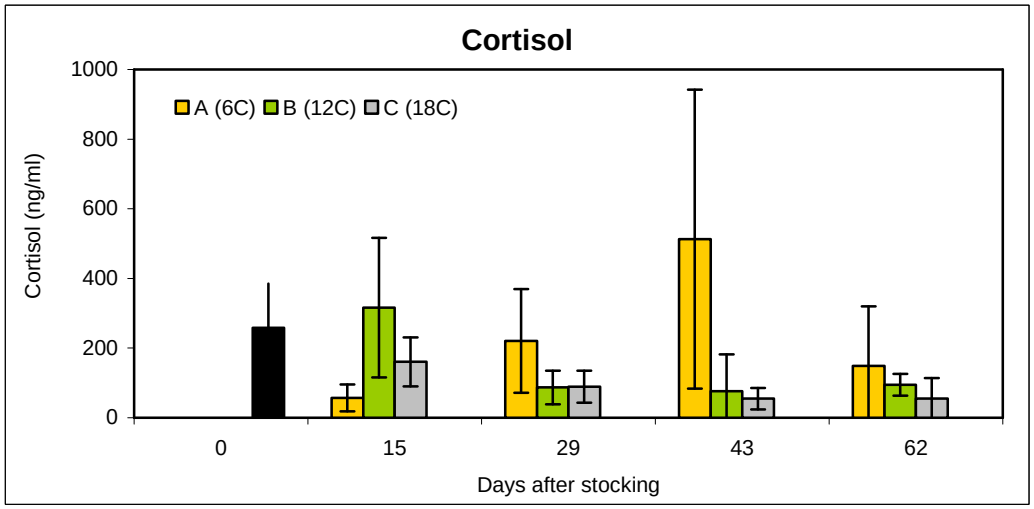

Figure 24 Changes in plasma cortisol level (ng/ml) of Eurasian perch breeders submitted to 6, 12 and 18°C conditions of rearing during two months.

Increase in stress physiology during the spawning period has been associated to a depression in immunological status in some fish species such as *Rutilus rutilus* (Kortet et al., 2003). Results from the present study indicate that low temperature only affected specific immune system not non-specific immune status. Indeed, elevated levels of total serum immunoglobulin in fish submitted to low temperature conditions (6°C, Figure 25) were comparable to those previously observed in other fish species (*Dicentrarchus labrax* and *Cyprinus carpio*) exposed to chronic stress-induced ammonia, copper or parasite infections (Coeurdacier and Dutto, 1999; Dautremepuits et al., 2004), while no differences were observed between 12 and 18°C. In contrast to the alteration in specific immune defence, non-specific immune parameters, such as lysozyme activity and haemolysis titre (Figures 26 and 27) were not affected by temperature regimes. This finding do not corroborate previous reports that lysozyme activity was temperature related during gonad maturation in Eurasian perch (partner 8, final report, 2005) and that a significant increase occurs during the spawning period, as an increased disease susceptibility (Felvoden et al., 1994; Wang et al., 2003, Fatima et al., 2006). The present result may indicate that the increase in lysozyme activity observed at the time of spawning for Eurasian perch may be simply related to changes in environmental conditions at the beginning of spring season.

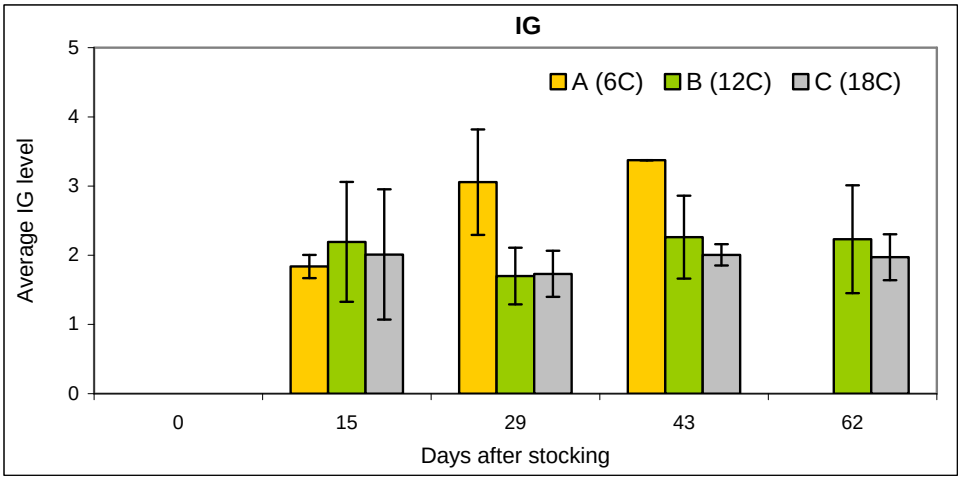

Figure 25 Changes in total immunoglobulin level of Eurasian perch breeders submitted to 6, 12 and 18°C conditions of rearing during two months. Samples taken at sample point 0 (start) were of bad quality and could not be analysed for immunological status.

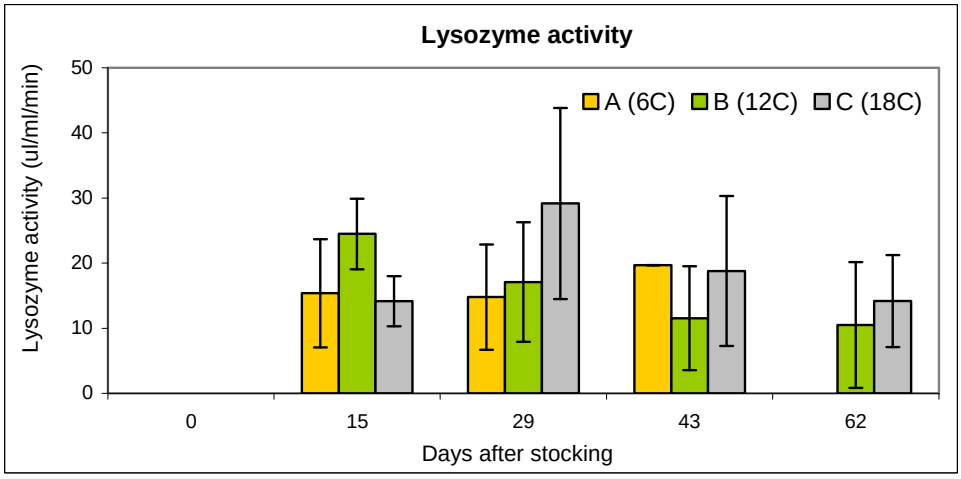

Figure 26 Changes in lysozyme activity (Ul/ml/min) of Eurasian perch breeders submitted to 6, 12 and 18°C conditions of rearing during two months. Samples taken at sample point 0 (start) were of bad quality and could not be analysed for immunological status.

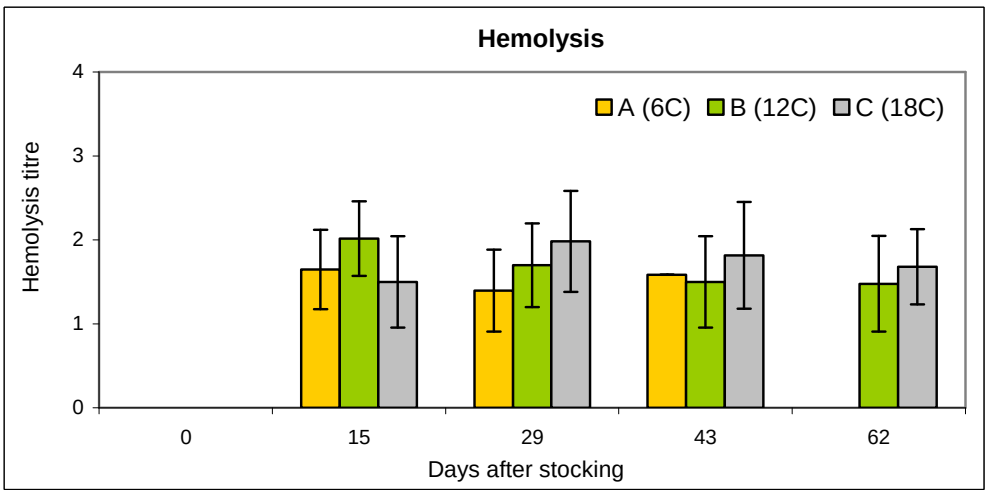

Figure 27 Changes haemolysis titre of Eurasian perch breeders submitted to 6, 12 and 18°C conditions of rearing during two months. Samples taken at sample point 0 (start) were of bad quality and could not be analysed for immunological status.

Experiment 3: Effects of catch and captivity

Objective

However due to disappointing results during experiment 2 due to mortality and difficulties to obtain sufficient amounts of blood we decided to perform a follow-up experiment.

The initial objective of this experiment was to characterize the stress physiology and immunological status of wild broodstock directly after capture, before, during and after spawning. However, the females appeared to be already in post-spawning stage or not sexually mature yet, pre-spawning and during spawning levels could therefore not be defined. During this experiment again high disease levels and mortality occurred. Therefore the new objective of experiment 3 is: to determine the effects of catch and captivity on stress physiology and immunological status of wild broodstock in indoor water recirculated holding facilities.

Materials and methods

Experimental set-up

Wild breeders were caught in the River Meuse (Limburg) in April 2006. The average length of the experimental fish was 19.4 cm and the average weight was 89.4 gr. Females were significantly larger and had a higher weight compared to the males.

Table 9: Batch characteristics; Average length and weight of the experimental fish

	Average length (cm)	Average weight (gr)
Male ♂	18.5	73.8
Female ♀	20.8	114.1
Total	19.4	89.4

After transport the fish were immediately stored in a temperature controlled recirculation system at IMARES. The holding facilities consisted of two temperature and light controlled circular and square tanks (respectively system A and B also used in experiment 1; figure 26) with PVC pipes as shelter (duplicates).

Figure 26 Perch seeking shelter in PVC pipes

The initial set-up was that breeders will be induced to spawn by using the temperature and light protocol (spawning induction protocol (table 11), and that sample points would be taken respectively at the period immediately after capture, the period before, during and after spawning. However, during the first sample points it was observed that the majority of the females were not sexually mature yet or appeared to be already in post-spawning stage (table 10). Most of the males were in their spawning period since they released sperm.

Table 10: Maturation stage of the experimental fish

	Not sexually mature	Spawning	Post-spawning
Male ♂	4%	96%	0%
Female ♀	81%	6%	13%
Total	36%	59%	5%

The same protocol was used in order to determine the effects of catch and captivity (Table 11). In total 47 perch were stocked in system A and 47 in system B.

Table 11: Experimental protocol

Day	**T (°C)**	**Photo period**	**Planned protocol**	**Actual protocol**
0	± 6°C	15D:9L	Sample point A (Directly after capture)	Sample point A (Directly after capture)
1	6.0	15D:9L		
2	6.0	15D:9L		
3	6.5	14D:10L	Sample point B (Before spawning)	Sample point B
4	7.0	14D:10L		
5	7.5	13D:11L		
6	8.0	13D:11L		
7	8.5	12D:12L		
8	9.0	12D:12L		Sample point C
9	9.5	11D:13L		
10	10.0	11D:13L		
11	10.5	10D:14L		Sample point D
12	11.0	10D:14L		
13	11.5	9D:15L		
14	12.0	9D:15L	Sample point C (During spawning)	
15	12.0	9D:15L		
16	12.0	9D:15L		
17	12.0	9D:15L		
18	12.0	9D:15L		
19	12.0	9D:15L		
20	12.0	9D:15L		
21	12.0	9D:15L		
??	12.0	9D:15L	Sample point D (After spawning)	

Table 12: System characteristics

	System A	**System B**
Volume tanks (liter)	2000	450x2
Initial number of fish	47	47
Oxygen (ppm)	10.9 (98% saturation)	11.0 (99% saturation)
PH	8.08	7.69
Water exchange (l/min)	0.388	0.350

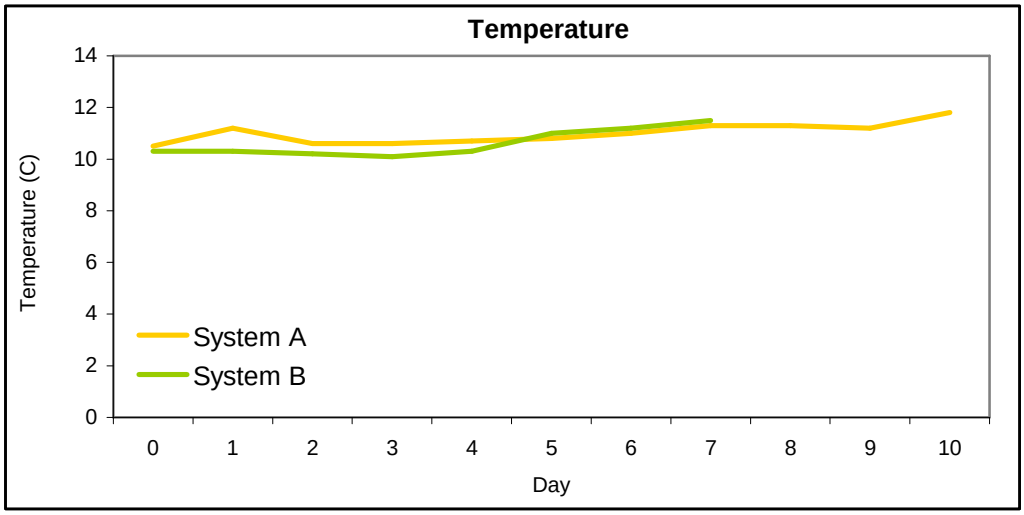

Figure 27 Actual temperature profile of the two systems used within this experiment

Blood sampling

Blood samples obtained immediately after capture (sample point A) were only analyzed for immunological parameters (serum). Blood samples taken at regular time-intervals (sample point B to D) were sampled for both plasma and serum to evaluate the effects of on stress physiology and immunological status. Blood for plasma is sampled using a syringe with heparin and blood for serum is sampled using a syringe without heparin. Ten fish per sampling point were sedated by using phenoxy-ethanol (1:1000). A maximum allowable time window of 5 minutes to catch and sedate the fish were taken into account for the analysis of the stress parameters. After the blood sampling, the sedated fish were weighted, length was measured and sex was determined. Plasma and serum were separated by centrifuging using 5000 rpm for 10 minutes. After centrifuging both plasma and serum are separated from blood cells and kept in frozen storage (-80 °C) until assayed. Immunological status will be based on analysis of serum samples on haemolysis titre, lysozyme activity, and Ig-level. Plasma samples will be used to analyze stress physiology by evaluation of cortisol and blood glucose.

It appeared to be difficult (almost impossible) to get sufficient blood of the smaller fish. These effects are noticeable in the quality of the samples. High mortalities causing an early finish of the experiment. The final running time of the experiment came to 10 days instead of the expected 21 days. This means that sample points are taken relatively close to each other. Because of the relatively high mortality rates and bad quality of the samples, the samples can not be considered as duplicates but the samples are analysed as one group.

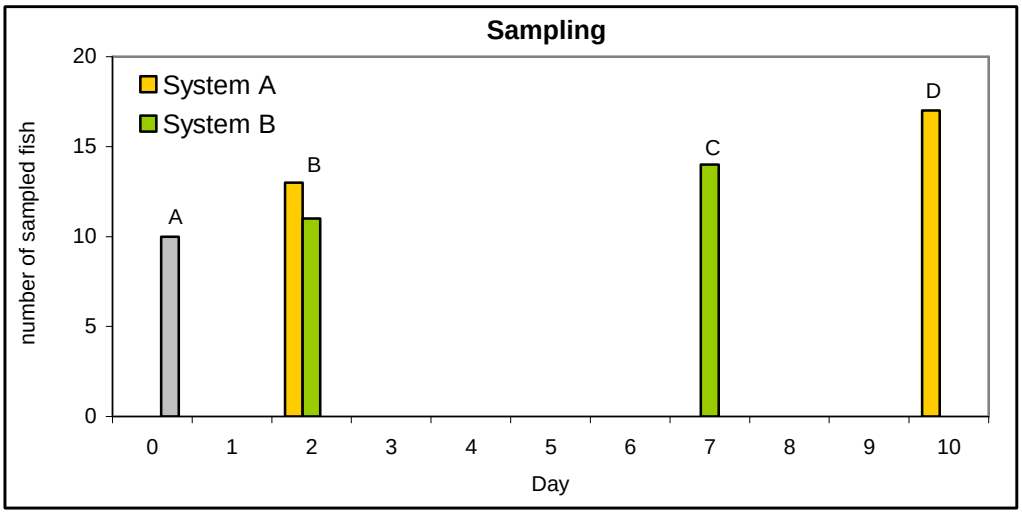

Figure 28 Actual sampling protocol

Table 13: Number of samples at each sample point for the three rearing methods

Days after stocking	Number of fish sampled	Number of serum samples	Number of plasma samples
0	10	8	-
2	24	13	8
7	14	14	8
10	17	1	11
Total	**65**	**36**	**27**

Statistical analysis

All quantitative data are expressed as means ± SD (standard deviation). Statistical analysis of this data was performed using the SAS system (version 9.1) with general linear model (GLM) procedures for unbalanced one-way analysis of variance. The minimal level of significance was set at P≤0.05. Before statistical analysis values of cortical and glucose were ln-transformed, to satisfy tests for normality and homogeneity of variances.

Results & Discussion

Mortality and diseases

The total mortality within this experiment was 38%, mostly due to fungal infections (respectively 36 (17/47) and 40% (19/47) in system A and B). Because of the severe fungi problems it was necessary to treat the fish. At day 4 both systems were treated with 7 ppt salt to reduce the mortality. In system A the treatment seemed to work, and relatively low mortality was found the days following the treatment. The first day after treatment no mortality was found in system B, however, severe fungal infections were observed, resulting in high mortalities the second day after treatment. We decided to sample all remaining fish in system a at day 7 (3 days after treatment).

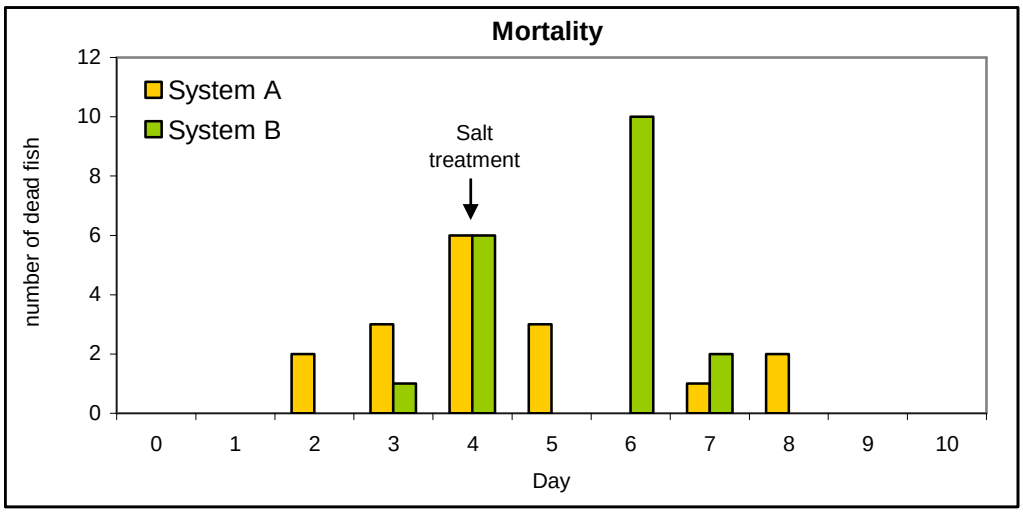

Figure 29 Mortality of fish during the experiment

Stress physiology and immunological status

Average cortisol and glucose levels were lower compared to previous studies on Eurasian perch, (Acerete et al., 2004; Percatech annual report, 2005), and did not indicate an increase in stress physiology during the twelve days in captivity conditions.

As observed for stress parameters, the first phase of captivity did not affect specific immune status as evidenced by the profile of total serum immunoglobulin levels (Figure 32). It is worthy noting that levels for both lyzozyme activity and haemolysis titre were low at the time of capture and were significantly ($P < 0.001$) affected by captivity conditions with an increase over the time, as a result of the gradual elevation in temperature. The low haemolysis titre account for problems encountered in blood sampling, and may indicate a weakness in blood characteristics in relation to winter conditions. Moreover, the fact that captivity conditions did not impact specific immune status in the present study may be related to the non-stressful spawning protocol, as reported above. In previous studies (Wang et al., 2003, Percatech annual report, 2005) a high mortality rate of wild breeders was observed after capture and in the first phase of captivity conditions. The present results may indicate that these mortalities were related to a previous weakness in immune defences related to the decrease in temperature (winter syndrome) and in food intake, as already reported for sea beam *Sparus auratus* by Tort et al. (2004).

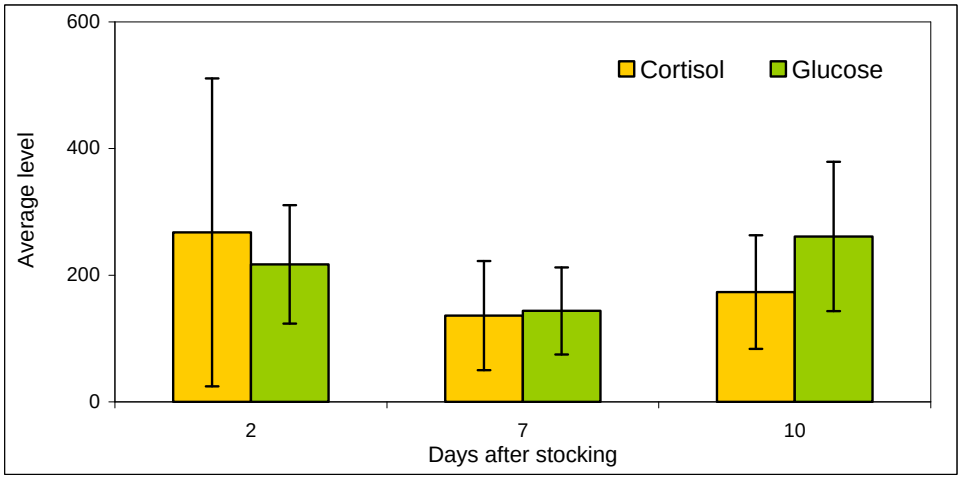

Figure 30 Changes in cortisol level (ng/ml) and glucose level (µg/ml) of wild Eurasian perch breeders submitted captivity conditions of rearing during ten days.

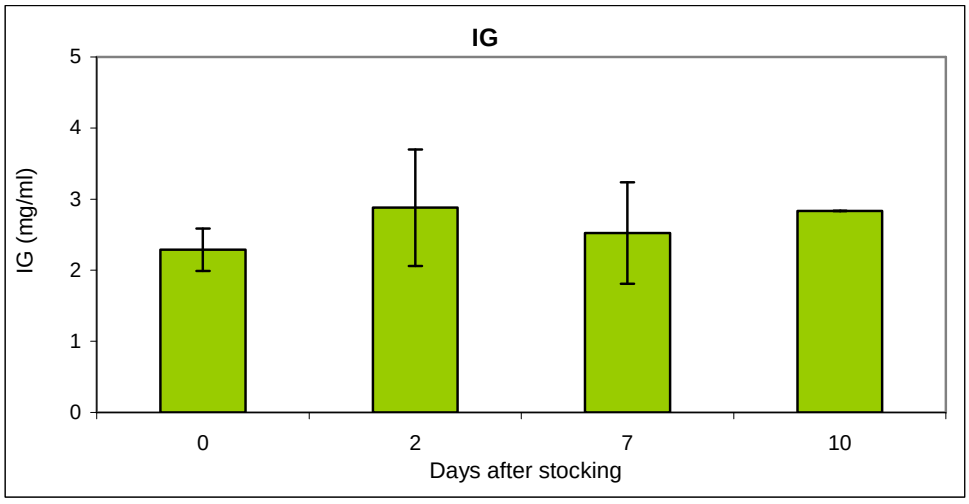

Figure 31 Changes in total immunoglobulin level (mg/ml) of wild Eurasian perch breeders submitted captivity conditions of rearing during ten days.

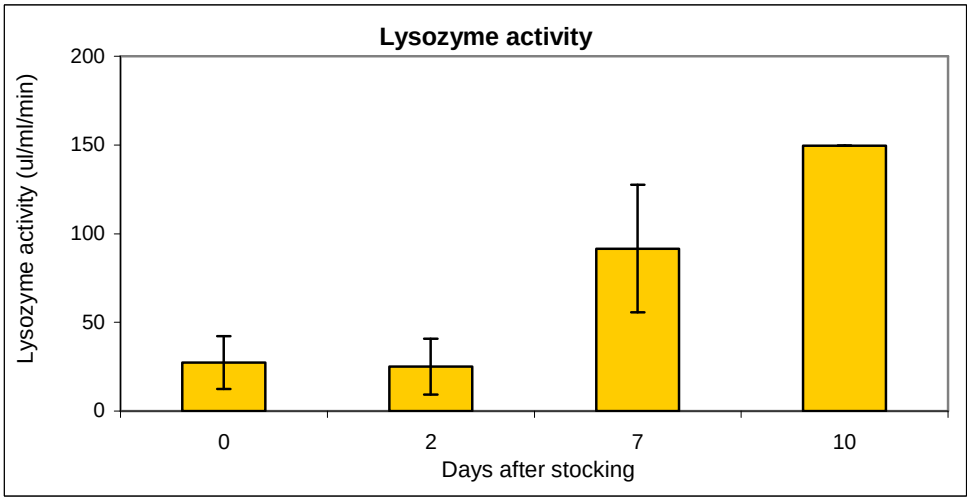

Figure 32 Changes in lysozyme activity (Ul/ml/min) of wild Eurasian perch breeders submitted captivity conditions of rearing during ten days.

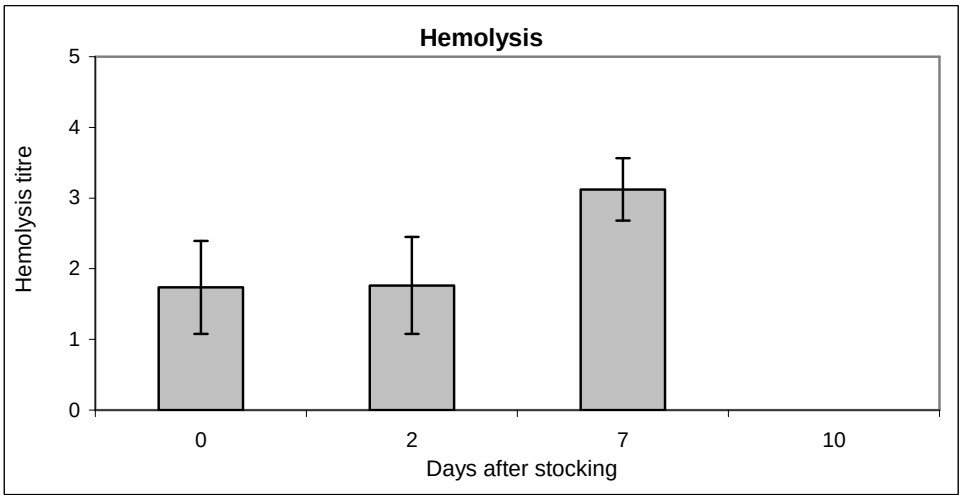

Figure 33: Changes in haemolysis titre of wild Eurasian perch breeders submitted captivity conditions of rearing during ten days.

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Drs. E. Jagtman

Signature: _____

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