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Microalgae in fish farming

Advances in pikeperch research

DIVERSIFY

Improving technology uptake and market impact of genetic research

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Advances in pikeperch (*Sander lucioperca*) research during the first 18 months of the project

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Pikeperch (*Sander lucioperca*) is a promising emerging fish species for intensive freshwater aquaculture, based on Recirculation Aquaculture Systems (RAS). During the first 18 months of the DIVERSIFY project, a number of studies have been initiated (a) to obtain knowledge in support of future breeding programs and (b) to solve the major bottlenecks identified previously by fish farmers (*e.g.*, cannibalism during larval rearing and stress sensitivity). This article presents a summary of the main results obtained so far.

GENETICS

The first genetic work was organized under the responsibility of Dr C. Tsigenopoulos (Hellenic Center for Marine Research, Greece). The primary objective was to use genetic markers (microsatellite loci) to evaluate the genetic variability of some wild pikeperch populations in comparison to the variability of captive broodstocks in commercial RAS farms around Europe. Thirteen cultured and eight wild populations with more than 950 fish in total were analyzed for a final set of 10 microsatellite genetic markers. On average, and contrary to what could be theoretically expected, the thirteen domesticated populations exhibited a slightly higher number of alleles compared to the wild ones (2.634 *versus* 2.580, not significantly different with an F-test). Likewise, unbiased expected heterozygosity estimates were slightly higher in wild population (0.573 *versus* 0.553, but again not significantly different with an F-test). Inbreeding coefficient (F_{IS}) values showed that the domesticated populations are in general not inbred and that some wild populations may also suffer from kin mating, too. In general, the mean heterozygosity estimates and the count of the number of alleles per population indicate that domesticated samples do not suffer from inbreeding. There are few domesticated populations that have some level of inbreeding, either due to their small sample size or their use as 'selected' fish.

Our studies also provide evidence that pikeperch populations in Europe are part of at least two genetically differentiated groups (Figs. 1 & 2). The first group is found in northern Europe from the Netherlands/Denmark to the West, Poland (at least) to the East, and to Finland to the North (Fig. 2). The second group comprises all remaining populations in Central Europe to as south as Tunisia (and probably Spain, Italy and northern Greece). In the second stock, the Hungarian

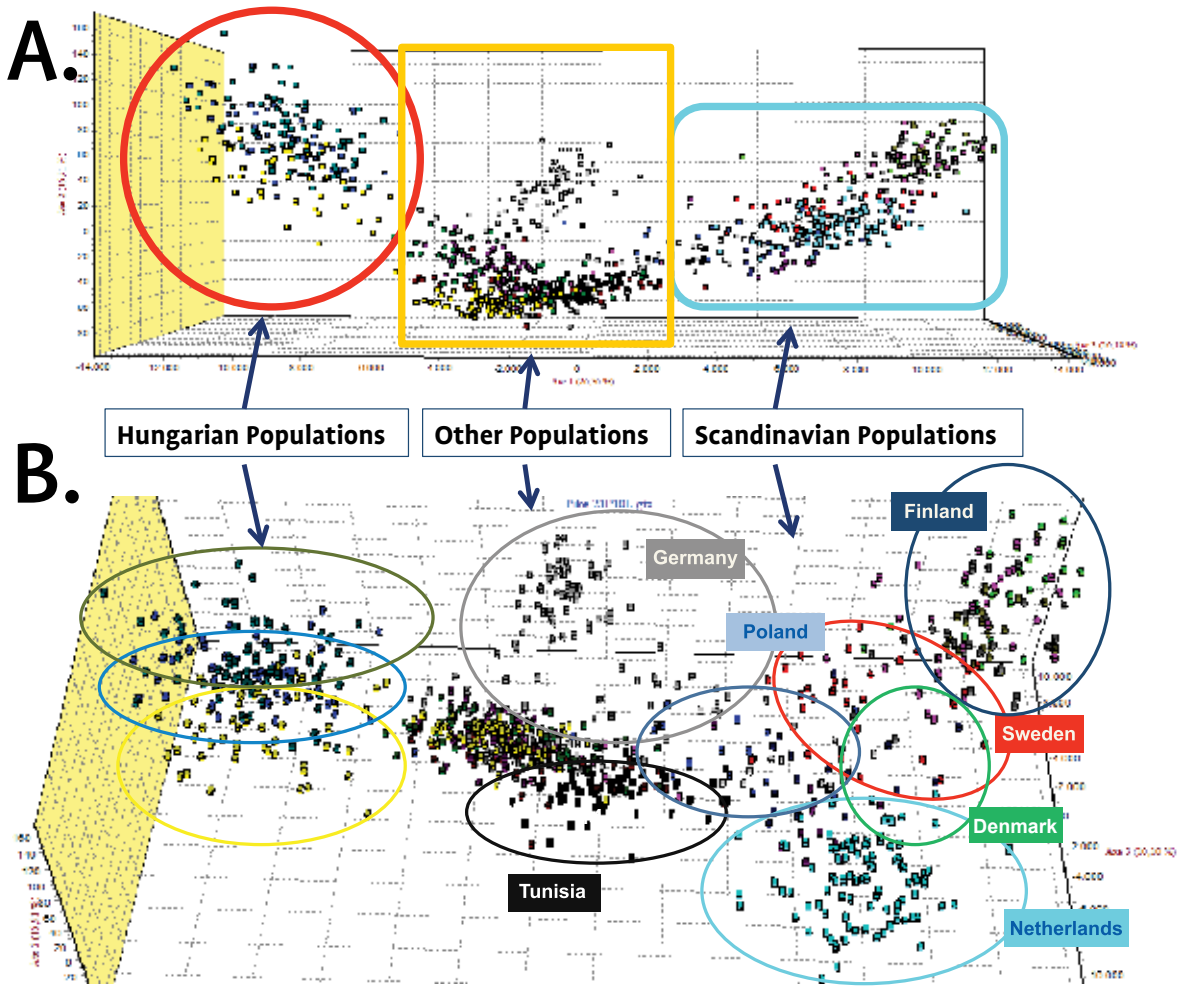

Figure 1. Factorial Correspondence Analysis (FCA) for all twenty-one populations and ten loci using the GENETIX v. 4.05 software.

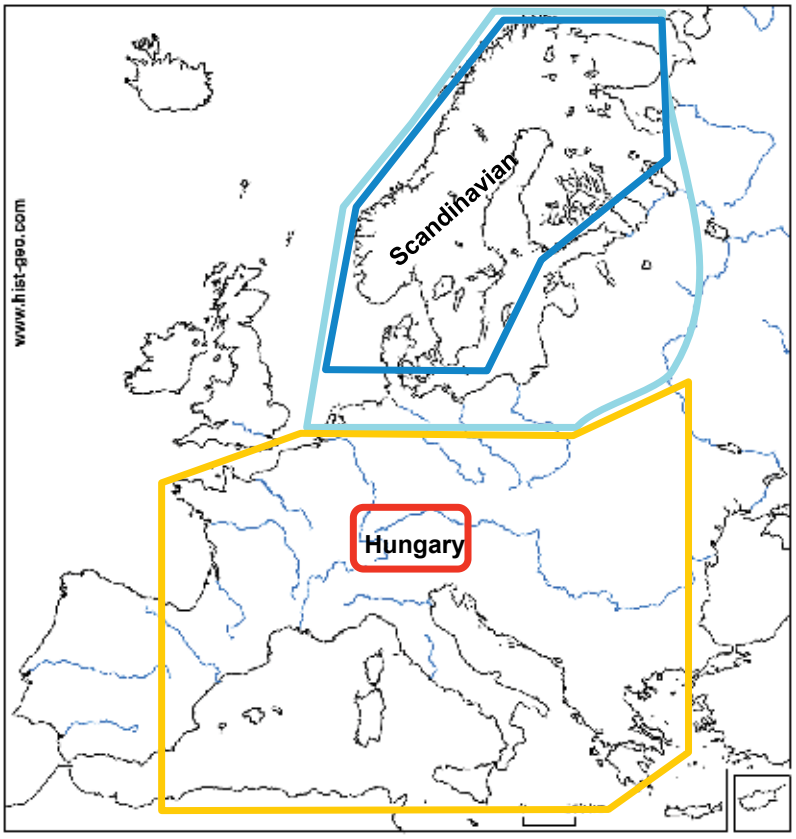

Figure 2. Map of Europe showing the major pikeperch genetic groups included in the study.

Figure 3. Pilot scale larval rearing system used for pikeperch research at the University of Lorraine (France).

populations are having a key-position being different from those found geographically close, *e.g.*, from Czech Republic and Germany. It might be another stock associated with Hungarian lakes, as opposed to all other populations that probably has dispersed through the Danube River west and southwards. Based on this grouping, it can be stated that most analyzed populations seemed to contain fish of a single origin; nevertheless, in few domesticated populations this ratio varied from 5-19%, possibly due to the mixing of fish from multiple sources.

LARVAL REARING

Three main bottlenecks have been identified as preventing the success of larval rearing: a high rate of mortality due mainly to cannibalism, a high rate of deformities and a strong growth heterogeneity characterized by important differences in size between larvae of the same age but at various developmental stages. Several trials have been completed using a pilot scale larval rearing system (RAS, eight 700 L tanks, Fig. 3). The main goal was the identification of optimal combinations of major culture factors (environment, population and nutrition) to increase larval survival and growth. This task has been managed by Pr P. Fontaine (University of Lorraine, France). In a first experiment, the effects of four environmental factors with two modalities were tested (light intensity: 5 *vs* 50 lx, water renewal rate: 50 *vs* 100% per hour, water current direction: up-flow *vs* down-flow, tank cleaning time: at morning just after the first feeding vs at the evening after the last meal). A fractional factorial experimental design (24-1) of resolution IV was used to study simultaneously the effect of these four factors and their possible interactions. Every week, from four days post hatching (dph) and after the first feeding, 60 larvae were sampled in each tank. Individual weight, morphological measurements (total length, mouth size, myotome height and eye diameter), occurrence of deformities, inflation rate of swim bladder and histological analyses (retina, intestine and musculature of jaws, in collaboration with Dr E. Gisbert, IRTA, Spain) were made at 25 and 40 dph. Results showed that light intensity, water renewal rate and cleaning period have a direct impact on growth, deformities and swim bladder inflation success. For example, larval total length at 40 dph was influenced by the interactions between (i) light intensity and water renewal rate (Fig. 4) and (ii) light intensity and cleaning period. The water current direction had no impact on these developmental parameters.

Then, in a second experiment using similar methodology, we studied the impact of four feeding factors on the growth of pikeperch larvae during the first weeks after fertilization. The four tested factors were: quantity of live preys (2,100 or 10,500 *Artemia* nauplii/larvae/day), weaning duration (three or nine days), frequency of food distribution (continuous or a meal each 1.5 hour from 8:30 to 17:30) and use of co-feeding (Prowean Larviva, BioMar) or not. Significant effects were observed in pikeperch larval growth and growth heterogeneity (CV, %) at 11 and 18 dph. At both sampling dates, the mean body weight of larvae was significantly higher when a higher quantity of *Artemia* nauplii were distributed (4.79 mg *vs* 3.07 mg at 11 dph and 20.10 mg *vs* 9.39 mg at 18 dph). Likewise, similar effects were observed on larval length (1.35 cm *vs* 1.08 cm at 11 dph). Moreover, significant effects on growth heterogeneity were caused by the quantity of live preys, the frequency of food distribution and co-feeding. A higher coefficient of variation for body weight at 11 dph was observed in response to discontinuous *vs* continuous food distribution (29.8% *vs* 28.3%) and to a lower *vs* higher quantity of live preys (37.8% *vs* 30.3%). Finally, at 18 dph, a significant interaction between co-feeding and duration of the weaning period was observed. When co-feeding was applied, the duration of the weaning period had no effect on larval size heterogeneity, whereas without co-feeding a significant increase of size heterogeneity appeared when the weaning period was longer *vs* shorter (13.9% *vs* 10.5%). In conclusion, the amount of distributed food was the main factor affecting the development of pikeperch larvae, but some effects of food distribution, co-feeding and weaning duration were also observed.

NUTRITION

Some nutritional bottlenecks remain to be solved to sustain a successful commercial production of pikeperch. Among these is the development of larval diets for optimal growth and performance. Several studies have indicated that low dietary levels of long-chain highly unsaturated fatty acids (LC HUFAs) may cause dysfunctions, such as increased stress sensitivity

and mortality and may have long term consequences on brain size, behavior and neuromuscular escape responses. The research undertaken so far in the project (managed by Dr I. Lund, Technical University of Denmark, DTU Aqua) involves a study on larval requirement of phospholipids (PL) and LC HUFAs and a pilot study on tolerance to salinities in order to observe how performance, essential fatty acid (FA) requirements and FA metabolism may be altered by changes in environmental salinity. To study the effects of PL and LC HUFAs, six different diets with increasing content of PL, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were tested (Table 1). The diets were fabricated by SPAROS (Portugal). At 30 dph, larval performance, biochemical composition as well as digestive enzymatic activity, liver proteomics, gene expression and skeleton morphogenesis were evaluated. Larvae were obtained by AquaPri A/S (Denmark) and reared on *Artemia* nauplii until 10 dph and then gradually switched to compound feeds within 5 days. The trial was performed in 18 tanks of 50 L (6x3) in which ~800 larvae were stocked and fed in surplus (approximately 25% of estimated total average wet weight) until 30 dph. Results are still being analysed, but the growth result (Fig. 5) showed that increasing levels of PL had a significant positive impact on larval size and that LC HUFAs such as DHA and EPA may increase those values.

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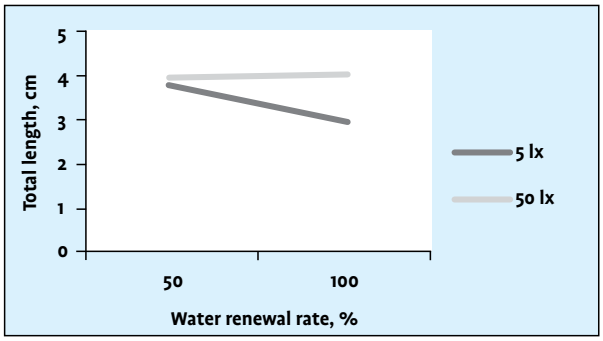

Figure 4. Influence of light intensity and water renewal rate on the total length of pikeperch larvae at 40 days post hatching (dph).

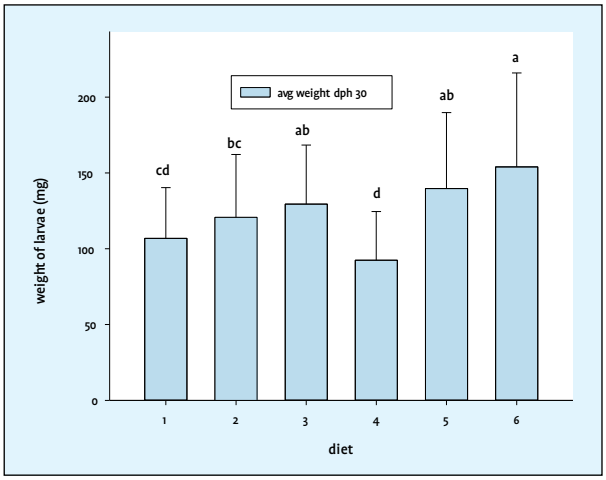

Figure 5. Size of pikeperch (*Sander lucioperca*) larvae at 30 days post hatching (dph) in response to six experimental diets. Means with statistically significant differences (ANOVA, P<0.05) are indicated by different letter superscripts.

As fed basis (% wet weight)	1	2	3	4	5	6
Crude protein	52,74	52,72	52,69	52,74	52,72	52,69
Crude fat	27,01	26,99	27,01	27,01	26,99	27,01
Fiber	0,14	0,14	0,13	0,14	0,14	0,13
Starch	4,02	3,90	3,72	4,02	3,90	3,72
Ash	8,12	8,12	8,11	8,12	8,12	8,11
Gross Energy	24,02	23,34	22,48	24,02	23,34	22,48
Fatty acids (% total fat)						
Eicosapentaenoic acid (EPA)	0,41	0,41	0,41	0,47	0,61	0,75
Docosahexaenoic acid (DHA)	0,66	0,66	0,66	1,04	2,06	3,04
Phospholipid classes (% total fat)						
Phosphatidylcholine (PC)	1,51	2,88	4,64	1,51	2,88	4,64
Phosphatidylethanol-amine (PE)	0,62	1,58	2,81	0,62	1,58	2,81
Phosphatidylinositol (PI)	0,69	2,10	3,90	0,69	2,10	3,90
Total phospholipids (TPL)	3,16	7,45	12,96	3,16	7,45	12,96

Table 1. - Dietary content of six formulated weaning diets.

Another pilot study showed that pikeperch larvae can survive abrupt salinity changes of 8-10 ppt directly after hatching without noticeable mortality, but 12 ppt might be the upper tolerance level. A subsequent study involving three salinity levels was initiated (0, 5 and 10 ppt) in a triplicate set-up on larvae fed either *Artemia* enriched with high levels of linolenic acid (ALA, 18:3n-3) or linoleic acid (LA, 18:2n-6). Larvae seemed to grow well, but the study needs to be repeated later this year, as the initial stocked numbers of larvae were too low. The capability of larvae to synthesize LC-HUFAs, as well as the fatty acid esterification pattern into different lipid classes will be studied by *in vivo* radio-tracing of 14C fatty acids and lipid classes (phosphatidylcholine-phosphatidylethanolamine, PC-PE).

GROWTH - STRESS

A first experiment was carried out in order to study the effects of husbandry practices and environmental factors on pikeperch growth, immune and physiological status (task led by Pr P. Kestemont, University of Namur, Belgium). The aim was to optimize the conditions for pikeperch grow out. This study is a screening approach of the main stressful factors identified for pikeperch juveniles reared in intensive culture conditions such as RAS. Eight factors with two modalities have been selected according to the bibliography and fish farmer observations (Table 2). This experiment (June – August 2015) is based on a multifactorial experimental design and new experimental facilities consisting of 16 independent RAS of 3 m^3 each (Fig. 6), located at the University of Lorraine (France). Pikeperch juveniles (70 g) were supplied by the company SARL Asialor, also a partner of the DIVERSIFY project. Different parameters of stress response will be analyzed, including cortisol, glucose or cerebral serotonin. Immune markers will be also investigated, including lysozyme and complement activities, concentration of immunoglobulin (Ig) and the expression of immune genes (*e.g.*, lysozyme, C3-1, TNF-α, IL-1β). Following this multifactorial experiment, a validation experiment will be done at the University of Namur facilities for further investigation, focusing on the interaction between stress intensity and resistance against pathogens. The results will enable the determination of the major aquaculture stressors in pikeperch in order (a) to reduce stress exposure during rearing by applying the optimal rearing conditions, (b) to increase disease resistance and (c) to improve growth performance.

Before the actual start of this multifactorial experiment, two preliminary experiments were planned in order to (i) standardize the analytical protocols for physiological and immune markers using pikeperch submitted to stressors and (ii) define the lethal dose of *Aeromonas hydrophila* or *A. salmonicida* that will be used for the challenge tests after stress experiments. Compared to salmonids, the first observations showed high levels of cortisolemia in pikeperch (88-122 ng/ml), confirming its high sensitivity to captive environmental conditions, and that emersion stress induced a significant increase in plasma glucose.

Factor	Modality
1 - Grading	- 2 times per month - No
2 - Initial rearing density	- 30 kg/m^3 - 15 kg/m^3
3 - Light intensity	- 100 Lx - 10 Lx
4 - Light spectrum	- White - Red
5 - Photoperiod	- L:D 24 : 0 - L:D 10 : 14
6 - Hypoxia	- 50-60 % of O_2 saturation - 90-100 % of O_2 saturation
7 - Temperature	- 21-22°C - 26-27°C
8 – Type of feed	- Semi floating - Sinking

Table 2. - Experimental conditions and modalities

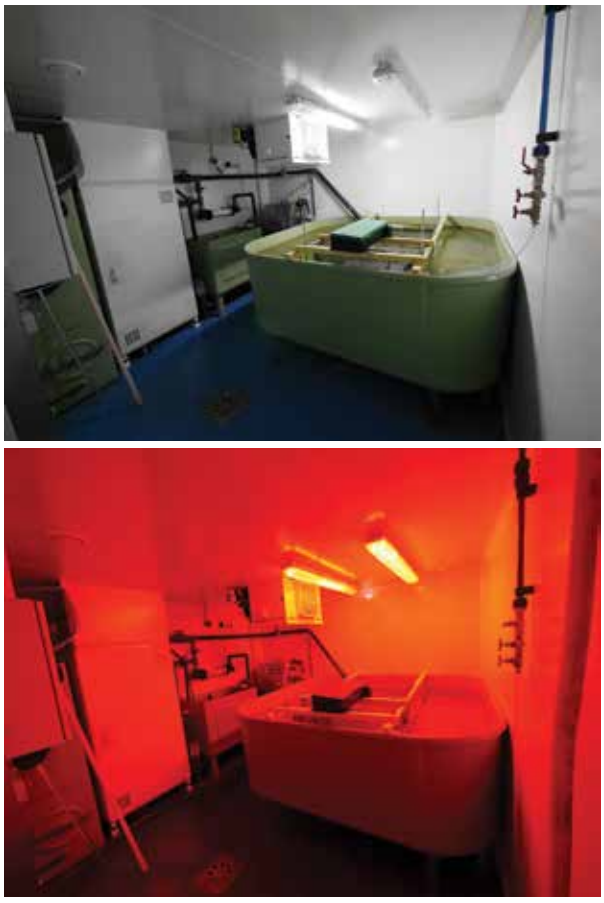

Figure 6. Examples of two environmental conditions applied on pikeperch juveniles to study. Upper photo: white spectrum, 100 Lx, lower photo: red spectrum, 100 Lx

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