New species for EU aquaculture

Technical Leaflets – Pikeperch (*Sander lucioperca*)

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DIVERSIFY 2018

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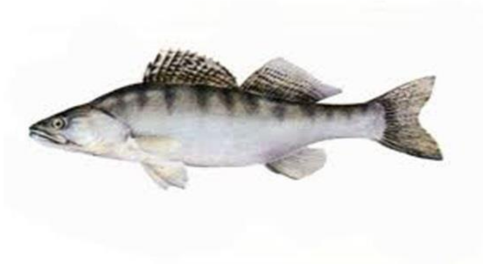
Introduction



This freshwater fish is considered to have the highest potential for inland aquaculture diversification in Europe (Wang et al., 2008; Kestemont et al., 2015). Based on EU projects LUCIOPERCA and LUCIOPERCIMPROVE and national projects, reproductive control (Kucharczyk et al., 2007) and bio-economic feasibility of pikeperch intensive rearing (Steenfeldt and Lund, 2008; Dalsgaard et al., 2013) have been demonstrated. Pikeperch demand has been strengthened by the strong decline of wild catches from 50.000 t in 1950 to 17.000 t in 2014 (FAO, 2015). Over the last decade, new farms have been built in Europe to produce pikeperch using (RAS), producing an estimated 1000 t. Numerous commercial operations have been designed and/or are under construction in Belgium, Czech Republic, Denmark, France, Germany, Hungary, Italy, Poland, Portugal, the Netherlands and Switzerland. Year-round production of pikeperch requires constant high temperatures (24-26°C), which is only feasible in RAS to ensure relatively high growth rates (*i.e.*, **production of 1.2 kg fish in 15 -18 months** from non-selected strains). These RAS also allow high densities of 80-100 kg m^{-3} (Dalsgaard et al., 2013). Pikeperch flesh quality has a neutral taste, thus lending itself to different forms of preparation, and the filets are without bones --unlike carp, which competes on the same market segment. At present, pikeperch is sold either as whole fish at a weight of 600-3000 g or as filets of 100-800 g to markets in Europe (mainly Western, Eastern and Northern areas) and North-America, showing strong demand. The market value is high at 8-11 € kg^{-1} at farm gate, whole fish.

Identified by a survey addressed to fish farmers in preparation for DIVERSIFY, the **major bottlenecks for further expansion of pikeperch** culture today include (a) **high sensitivity to stressors, handling and husbandry practices** that result in high and sudden mortalities, (b) **low larval survival** (typical 5-10%) and **high incidence of deformities**, (c) **lack of knowledge of the genetic variability of the used broodstocks**. Identification of genetic relationships among different broodstocks, inbreeding phenomena and loss of heterozygosity is important in aquaculture, since it may result in subsequent reproductive and productive failure (reduced progeny survival, growth, food conversion efficiency and increased frequency of deformities). It is also important to know how the domesticated stocks differ from their wild counterparts, which could potentially be a future source of fish to implement in effective breeding programs. Overcoming the above bottlenecks is very important to reduce production costs and, therefore, expand the aquaculture production of this species in the EU, and will be the objective of DIVERSIFY.

Reproduction and Genetics

Genetic variation in domesticated and wild populations of pikeperch

The objective was to use genetic markers to evaluate population genetic parameters (allelic richness, heterozygosity indices, inbreeding coefficients) in captive broodstocks from commercial farms and compare them to those estimated in wild pikeperch populations **(Table 1)**. We analyzed 13 cultured and 8 wild populations (950 fish) for a final set of 10 microsatellite genetic markers.

Table 1. Basic population genetics parameters for **wild** and **domesticated** populations: mean number of alleles per locus, allelic richness (A_R), observed (H_O) and expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}). Asterisks indicate significance at p=0.05.

	Population	Sample Size	Origin	Mean Nb of alleles	A_R	H_E	H_O	F_{IS}
1	Hungary-1	53		6.2	4.9	0.6826	0.7472	-0.08424
2	Hungary-2	49	Hungary	7.8	5.4	0.7182	0.6759	**0.06962***
3	Denmark-1	54	Netherlands	2.6	2.4	0.4675	0.6796	-0.44607
4	Denmark-2	38	Czech Rep.	3.3	2.7	0.4616	0.4882	**-0.04401***
5	Denmark-3	14	Netherlands	2.8	2.6	0.3408	0.4100	-0.16229
6	Denmark-4	73	Hungary	8.2	5.5	0.7194	0.7165	**0.01110***
7	Denmark-5	19	Denmark	3.1	2.7	0.4169	0.3985	**0.07185***
8	Germany	46	Germany	5.7	3.9	0.5567	0.5502	**0.02343***
9	Finland-1	31	Finland	3.7	3.1	0.5257	0.5819	-0.09055
10	Finland-2	20	Finland	2.8	2.6	0.4743	0.6032	-0.24757
11	France-1	63	France	5.4	3.9	0.5940	0.5913	0.01261
12	Belgium-1	100	Germany	7.2	5.1	0.7224	0.8099	**-0.11621***
13	Belgium-2	100	Netherlands	4.7	3.6	0.6156	0.6465	-0.04510
14	Tunisia	59		3.7	2.7	0.4013	0.3585	**0.11512***
15	Sweden	30	Sweden	4.4	3.6	0.5250	0.5817	-0.08989
16	France-2	51		4.6	4.0	0.5923	0.6706	-0.12237
17	Czech Rep.	70		3.8	2.9	0.4692	0.4382	**0.07357***
18	Poland-1	14		4.6	4.2	0.5763	0.5643	**0.05780***
19	Poland-2	11		4.2	4.1	0.6149	0.6764	**-0.05217***
20	Finland-3	32		4.8	3.6	0.5946	0.5995	**0.00787***
21	Finland-4	31		4.7	3.9	0.6034	0.5340	**0.13148***

In aquaculture, breeding populations are vulnerable to inbreeding depression because a loss of genetic variability is expected due to genetic drift and selection. Surprisingly, the mean allelic richness and the unbiased expected heterozygosity estimates in the 13 domesticated broodstocks are only slightly lower from the wild ones, and not significantly different. 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 (**Table 1**). Additionally, 95 samples in total (4-5 fish per population) were partially sequenced for a portion (571bp) of the mitochondrial Cytochrome b gene and a median-joining network of haplotypes was constructed to infer phylogeographic relationships (**Fig. 1**).

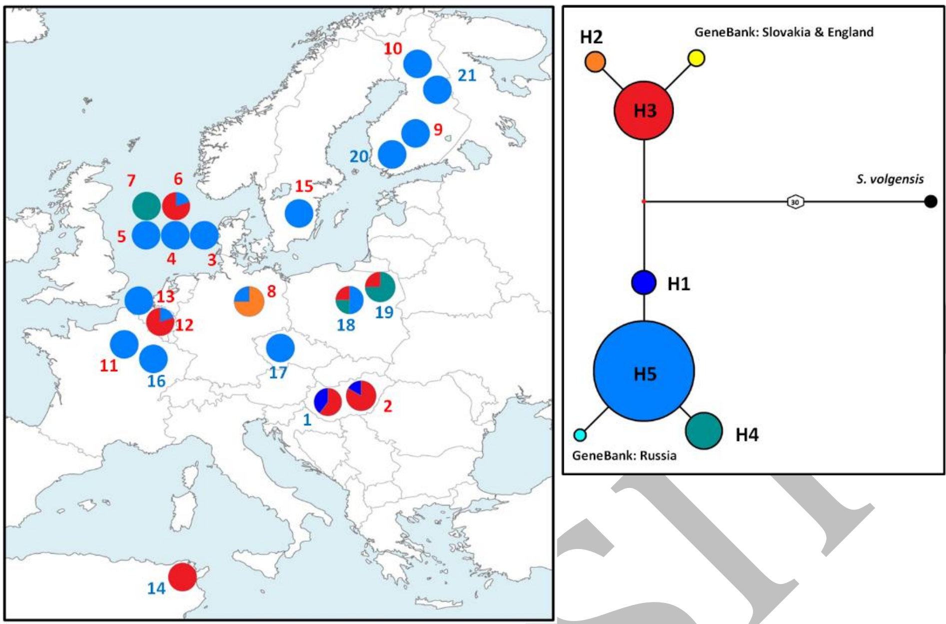


Figure 1. Geographic distribution of pikeperch *cyt-b* haplotypes and network of haplotypes. Each line connecting the haplotypes represents one mutation unless a different number is given.

Main conclusions from the genetic analyses are:

- ✓ The majority of the populations shows medium to low levels of genetic diversity and some of them may suffer from inbreeding,
- ✓ Differentiation between domesticated broodstocks was high in most cases,
- ✓ Average values of heterozygosity and allelic richness are not significantly different between wild and domesticated populations,
- ✓ Microsatellites analysis shows at least two genetically differentiated groups:
    - ➢ The first is found in **Northern Europe** from Netherlands/Denmark in the West to Poland (at least) in the East and up to the North of Finland.
    - ➢ The second comprises all remaining populations in **Central Europe** to as south as Tunisia (and probably Spain, Italy and Northern Greece).
- ✓ In the Central Europe group, **Hungarian populations** (1, 2, 6) seem to have a key-position being differentiated from adjacent populations, e.g., from Czech Republic and Germany.

Nutrition

Effect of selected nutrients on pikeperch larval development and performance

Importance of dietary phospholipids and essential fatty acids for larval development and skeleton alterations in pikeperch

No pelleted commercial feeds have been formulated specifically for pikeperch larvae and there is a need to develop feed types with the right composition to ensure survival, growth, welfare and decrease stress. The origin and level of dietary lipids and their fatty acid composition have proven very important for the development of pikeperch larvae. Thus, pikeperch larvae have a dietary requirement of essential fatty acids (EFA, Ω-3 fatty acid) and for a type of lipids called phospholipids which is found mainly in some fish oils or in oils of marine origin and not in vegetable oils. This requirement is unusual for freshwater fish larvae and is more commonly observed in marine species. Phospholipid level is generally low in dietary oils used in fish feeds, but some fish oils can have high concentrations. Phospholipids may be particularly important in fish larvae as these oils have an important function during larval development and are particularly present in larval brain and cellular membranes. Phospholipids may improve digestion and lipid feed utilization and have positive benefits in larval development. It was thus important to determine optimal phospholipid levels and levels of EFA in dry feeds for pike perch larvae on the performance and development.

Three dietary levels of phospholipids were tested in larval dry feed diets (PL1-PL3) and effect on larval growth and development. Additionally, supplementation of EFA in three other diets (PL1H1-PL3H3) was tested (**Table 2**). Fish meal, soluble fish protein concentrate and krill meal were the main ingredients and olive oil was used as main fat source. Soy lecithin was included at different levels to regulate phospholipid levels, and for diets PL1H1-PL3H3 a source of essential FA was included to regulate EFA level. Larvae were fed the dry diets from 10 days until 30 days after hatching. Diet composition is shown in **Table 2**.

Table 2. Main analytical content of the 6 experimental diets.

Analysed content (% ww)	PL1	PL2	PL3	PL1H1	PL2H2	PL3H3
Crude proteins	54.1	54.7	55.6	54.1	55.8	55.3
Crude lipids	26.8	25.9	24.6	26.6	25.6	24.8
Total phospholipids (TPL)	**3.73**	**8.19**	**14.38**	**3.70**	**8.32**	**14.51**
Essential Ω-3 fatty acid (inclusion %)	**0.0**	**0.0**	**0.0**	**0.55**	**2.0**	**3.4**

Results showed either a specific effect of the EFA, Ω-3 fatty acids or a combined effect of phospholipids and fatty acids. Combined supplementation of up to 14.5% phospholipids with EFA, Ω-3 fatty acids lead to the highest growth (**Fig. 2**) and lowest anomalies (**Table 3**). Survival was much lower for larval groups reared on the lowest phospholipid level PL1 and PLH1. The highest phospholipid EFA level improved enzymatic activity in the larval digestive tract, which was likely due to a higher maturation of the gut followed by growth improvement. Several of the proteins expressed in the liver (which is the main metabolic organ in the body) such as FAS (fatty acid synthase) showed a marked increase, when larvae were fed low levels of EFA in the diets, suggesting a higher energy demand of these smallest larvae. An increase in dietary phospholipids from 3.7 up to 8.2% did not lower the incidence of skeletal deformities, but inclusion of 14.5 % phospholipids significantly reduced the incidence of severe skeletal anomalies, and was lowest in larvae fed 14.5 % phospholipids + EFA. Most of the anomalies were found in the rays or in the cranium (**Table 3**).

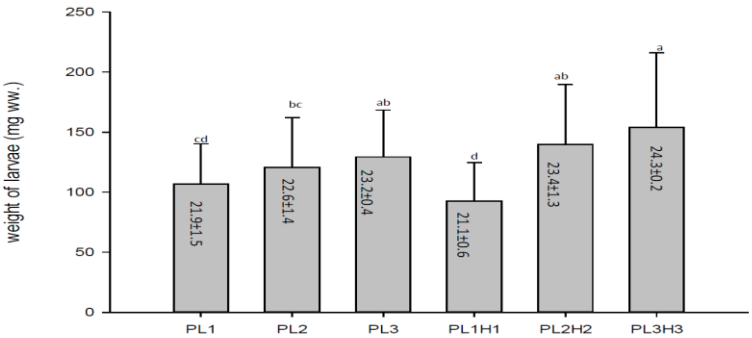


Figure 2. Weight of larvae 30 days after hatching according to feeding regimes.

Table 3. Main rate (±SD) of skeletal deformities observed in 30 DPH pikeperch larvae after 20 days feeding experimental diets (n= 3).

Diet	PL1		PL2		PL3		PL1H1		PL2H3		PL3H3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Prevalence of Anomaly Type (%)												
Severe	86.7^{a}	18.9	81.5^{a}	13.9	54.2^{ab}	13.7	96.7^{a}	4.7	75.1^{ab}	11.4	35.2^{b}	10.9
Dentary	26.5^{ab}	11.6	59.1^{a}	15.8	19.1^{b}	7.1	35.2^{ab}	7.3	25.3^{ab}	9.1	18.8^{b}	14.9
Branchiostegal rays	76.3^{b}	8.2	17.0^{a}	11.9	3.3^{a}	3.3	90.0^{b}	5.8	18.6^{ab}	7.4	0.0^{a}	0.0
Cranium anomalies	86.7^{a}	18.9	66.5^{ab}	17.6	25.5^{b}	11.2	96.7^{a}	4.7	61.0^{ab}	19.8	18.8^{b}	14.9

Values in a row followed by a different superscript are significantly different (P<0.05). PL1, PL2, PL3 (soybean lecithin (SBL): 3%, 10%, 19 %). PL1H1, PL2H2, PL1H3 (SBL: 3%, 10%, 19 %) + Algatrium DHA 70: 0.55%, 2.05, 3.4%).

In conclusion, we observed that **pikeperch requires** a **high level of PL** in micro diets to sustain optimal growth as inclusion of **14.5 % PL** supplemented as SBL gave the best growth. Moreover, a possible additional beneficiary effect by supplementation of EFA, Ω-3 fatty acids (DHA+ EPA) is suggested. A high dietary PL content reduced skeletal deformities indicating some additionally positive effects of LC PUFA inclusion. Overall the results indicate that essential fatty acids may not need to be incorporated in the PL fraction of the diet and **therefore EFA could be supplemented as triglycerides** (in normal oil) to have a **beneficial effect** in pikeperch larvae development.

Optimal levels of essential nutrients: LC-PUFAs, vitamins (A, E, C, D) and minerals (Ca/P)

The requirements for essential nutrients (vitamins A, E, C, D) and fatty acids have not been determined in pikeperch larvae during early weaning to dry feeds. Commercial diets used for freshwater percid fishes have been developed for marine fishes, therefore additionally CA/P levels may not be optimal, and little is known about the implication in bone mineralization. This study examined larval development and performance, digestive capacity and skeleton malformation using 16 diets with two levels of 8 variables: Ca/P, EPA+DHA, ARA, Vit. E, D, C, A and Se, according to **Table 4**. Diets were similar in terms of proteins and lipids.

Table 4. Dietary variables and their 2 levels applied in experimental diets.

Dietary variables	Low level	High level
Ca /P	0.6	1.2
DHA+EPA (%)	1.25	3.5
ARA (%)	0.8	1.6
Vit. E (mg/kg)	1000	3000
Vit. C (mg/kg)	2000	3600
Vit. A (mg/kg)	8000	30000
Vit. D (mg/kg)	2800	28000
Se mg/kg	3	12

The highest survival was recorded in larvae fed high Ca/P (**Fig. 3**), but final weight and specific growth rate (SGR) were lower in larvae fed high Ca/P, and these larvae had higher incidence of kyphosis (curvature of the spine) and fin deformities (**Fig. 3**).

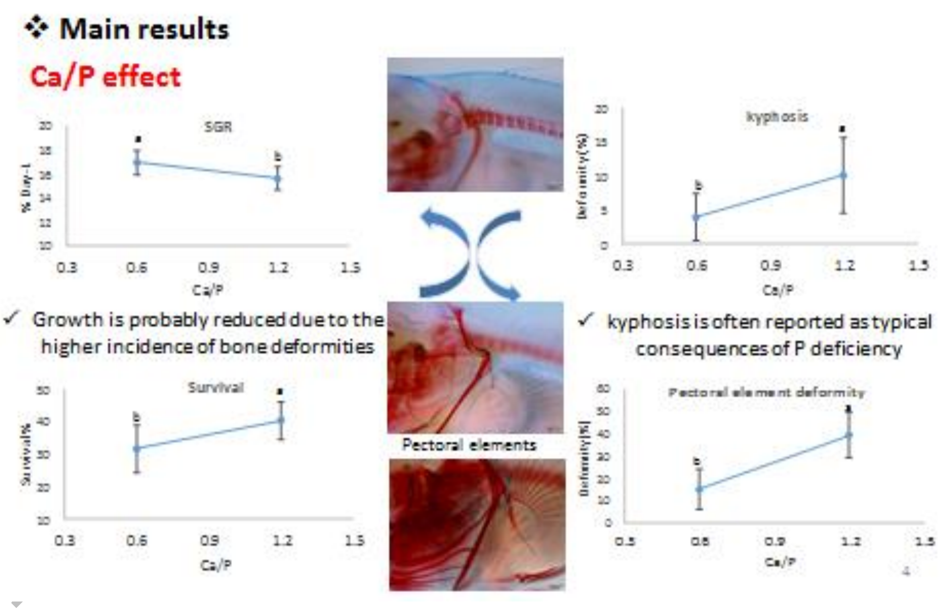


Figure 3. Ca/P effects on larval weight, SGR, survival and kyphosis.

Growth was probably reduced due to the higher incidence of bone deformities. **Ca/P, fatty acids and their interaction** seem to be **key nutritional factors** influencing pikeperch larval development.

Effect of early fatty acid nutrition on short and long-term stress sensitivity, behavior and metabolism

During early pikeperch larval development, diets low in LC-PUFAs, especially DHA, may provoke increased mortality and shock syndromes. Information is lacking about the influence of dietary lipid composition early in ontogeny and the robustness of produced fish. We examined if dietary fatty acid composition in larval feed of pikeperch affected larvae and fry behavioral responses to challenges, and if it could affect fry learning and stress response. This was carried out by studying behavioral responses to visually simulated predator attacks and fast escape responses to abrupt sensory stimuli during the larval stage. During the fry stage the fast escape response test was repeated, learning ability was studied by a maze test and effects on the stress response were quantified by post stress plasma cortisol levels.

Four dietary emulsions were made by the substitution of olive oil with either EFA, Ω-3 fatty acids, so called DHA oil consisting of 500 mg DHA/g; ≤100 mg EPA/g (EPA; eicosapentaenoic acid) or a fish oil rich in phospholipids. From 7–27 dph, larval groups were fed *Artemia* (INVE-*Artemia* Systems) enriched by one of 4 emulsions (0.6 g emulsion L^{-1} for 24 h). Larvae fed low levels of essential fatty acids, i.e. EPA + DHA Ω-3 fatty acid displayed a tendency towards delayed escape responses towards a simulated predator and had slower peak acceleration rates during escape responses following a sensory stimulus. This effect was consistent up to 90 days after the dietary treatment was terminated, demonstrating long-term effects of early nutritional history in fish. This indicates a more anxious behavioral profile of the fry fed diets low in EPA+ DHA and suggests changes in brain developmental pattern, being the cause of these behavioral effects. Inclusion of essential fatty acids in early diets for pike perch larvae, seems therefore important for optimal behavior and - development of pikeperch larvae and juveniles.

Essential fatty acid metabolism

Combinations of nutritional requirements and husbandry rearing conditions during early ontogeny are poorly studied in pikeperch. The substitution of marine oils with vegetable oils has reduced stress tolerance and caused neurophysiological changes in pike perch larvae, but effects of environmental cues are limited. Saline water influences on a range of physiological functions during early fish larval ontogeny and may affect FA metabolism, so that larvae are better able to convert non-essential fatty acids to essential fatty acids and thus have less requirement for essential fatty acids provided by the food. First feeding larvae were fed two *Artemia* experimental diets differing in levels of non-essential fatty acids, 18:2n-6 (LA) and 18:3n-3 (ALA) contents by enrichment with sunflower oil (SFO) or linseed oil (LO) and held under environmental salinities (0, 5, 10 ppt) until 30 days post hatching. Consequences for larval performance, skeletal anomalies and stress sensitivity were examined. Salinity had no effect on the growth performance of the larvae. Larvae possessed a marked specificity to incorporate and esterify essential Ω-3 fatty acids especially ARA, EPA and DHA into lipids. Salinity had no effect on the ability of larvae to esterify and incorporate unsaturated PUFA precursors and thus to biosynthesize lipid classes containing essential FA. A confinement stress test caused high acute mortality in all groups (50-70%), however significantly lowest for a control group given high levels of essential Ω-

3 fatty acids. The prevalence of severe skeletal anomalies was generally high, affecting over 75% of the larval population with negative effects by increase in salinity.

It is recommended that essential Ω-3 fatty (EPA + DHA) must be supplied in diets of pikeperch larvae for normal development and to reduce stress sensitivity. The results pointed out a high occurrence of deformities in endochondral bones and increased incidence at higher salinities.

Larval husbandry

Optimal combination factors to improve larval rearing

Until now several bottlenecks have prevented the success of the rearing of the pikeperch larvae. Three major bottlenecks have been identified: (1) high mortality due mainly to cannibalism, (2) high rate of deformities and (3) a large size heterogeneity between larvae cohorts at various ontogenic development stages. Using a pilot scale larval rearing system (RAS, ten 700 L tanks, **Fig. 4**) and based on existing protocols used by the SMEs, successive experiments were conducted using factorial designs (4 factors tested with 8 experimental units) which are efficient methods to successfully optimize larval protocols. Such methodology allows (i) to integrate the effects of each simple factor tested and interactions between them, (ii) to rank and evaluate the effects induced by factors or interactions, (iii) to identify rapidly an optimal combination of factors that increase larval survival, and (iv) to establish a first modeling of the complex multifactorial determinism of output variables. This method has been already applied successfully in fish larviculture (Trabelsi et al., 2011). Our objective was to study successively the effects of environmental, nutritional and population variables. For each experiment, the choice of these factors was a trade-off between data available in the literature and the constraints of our system (*i.e.* the impossibility for varying the temperature in each tank). From each experiment, according to results obtained, the most influent factors and modalities were conserved and integrated in the following experiment in order to optimize the protocol.

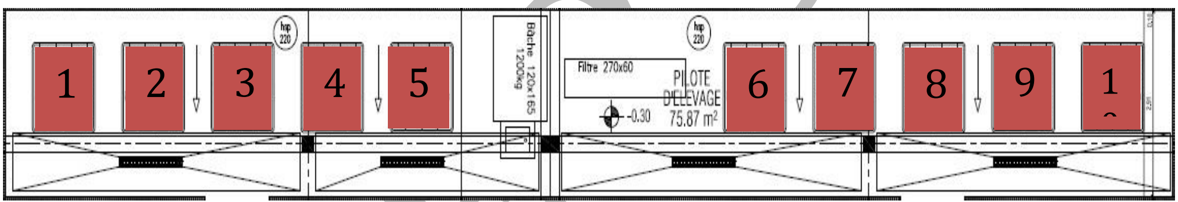


Figure 4. Diagram of the experimental facility (UR AFPA, Vandœuvre-lès-Nancy, France): a ten (two rows of 5 tanks) 700-L indoor recirculating aquaculture system (RAS) using mechanical and biological filters, as well as a UV sterilization unit (same water quality in all tanks). Tanks 2-9 were used during the experiments, while tank 1 served as an additional moving bed filter.

Effects of environmental factors

The effects of the light intensity (5 or 50 lx), water renewal rate (50 or 100% per hour), water current direction (at the bottom or the surface of the tank) and time of tank cleaning (morning or afternoon) have been studied. The multifactorial experimental design was based on the application of 8 combinations of factors. From the spawn of a domesticated broodstock 500,000 newly hatched larvae (<1 dph) were obtained from the SME Asialor (Pierrevillers, France). Then larvae were distributed into 8 tanks (62 500 per tank, 90 larvae L^{-1}), where water temperature was initially kept at 15-16°C. Photoperiod was fixed at 12 h of light and 12 h of darkness (Hamza et al. 2007) with a progressive increase of light intensity (from 0 to 5 or 50 lx) from 07:30 to 08:00 and a decrease of light intensity (from 50 or 5 to 0 lx) from 19:30 to 20:00. Temperature was incrementally increasing by 1°C per day to 20°C (Hamza et al., 2007; Kestemont et al., 2007; Szkudlarek and Zakes, 2007). The frequency of feeding was a meal every 1.5 hours during the light period (Hamza et al., 2007; Kestemont et al., 2007). Dissolved oxygen was maintained above 6 mg L^{-1}.

In this experiment (39 days), it was demonstrated that weaned juveniles of 0.50±0.06 g mean body weight can be produced in 5 weeks, but survival rates (0.3-2.6%) were very low. Finally, it appears that a water inlet at the bottom of the tank is better to reduce size heterogeneity. Considering all the results, **we recommend to apply a light intensity of 50 lx, a water renewal rate of 100%, a cleaning of the tank during the afternoon and an inlet of the water at the bottom level**. According to behavior, this first experiment allowed us to know that it is possible to determine the personality on pikeperch juveniles and maybe highlight in a future experiment the link between personality and cannibalism.

Effects of nutritional factors

A second experiment (53 days) was done in order to evaluate the effects of four feeding factors: the timing of the beginning of weaning (at 10 or 16 dph), the method of food distribution (continuous or discontinuous during the lighting period), the implementing or not of a co-feeding approach (6 day before the weaning period) and the weaning duration (3 or 9 days). Larvae (240,000, 30,000 larvae per tank ca. 43 larvae L^{-1}) were obtained from the SME Asialor (Pierrevillers, France). The results suggest that **a later onset and longer duration of weaning followed by discontinuous feeding** will improve larval survival, growth and reduce deformities in pikeperch populations.

Effects of population factors

For the third experiment (52 days), we have tested the effects of the initial larvae density (50 or 100 larvae L^{-1}), sorting out fish jumpers (yes or not), stocking sibling or not sibling larval group (larvae from one or two females) and female weight (< 2.8 kg or > 3.3 kg). Larvae (420,000) were obtained from the SARL Asialor (Pierrevillers, France) and transferred to the UL experimental platform (UR AFPA, Vandœuvre-lès-Nancy, France). **High final biomass seemed correlated to a higher initial larvae density (100 larvae L^{-1}) and the use of larvae supplied by bigger females**, but independent of jumper sorting and the use of sibling population.

Identification of optimal combinations of factors

According to the best results obtained in the previous experiments, an optimal combination of factors (**Table 5**) was proposed to improve pikeperch larval rearing and tested in the same rearing system using 7 replicates (52 days).

Table 5. Applied modality for each factor. This combination of factors was repeated in 7 experimental tanks (n = 7).

Factor	**Modality**
Density	100 larvae L^{-1}
Sorting of fish jumper	No
Sibling or not sibling	Not sibling

Female weight	Large (> 3.3 kg)
Feeding schedule	Discontinuous
Light regime	12:12
Light intensity	50 lx
Weaning start (dph)	16
Weaning duration (days)	9
Water renewal rate (tank vol./h)	1
Tank cleaning period	Morning
Tank current direction	Bottom to top

Table 6. Summary of performance parameter recorded in all tanks.

Tanks	Swim bladder inflation rate (%)	Initial biomass (g)	Final biomass (g)	Mean initial body weight (mg)	Mean final body weight (mg)	Survival rate (%)	SGR (%/day)	FCR
2	90.8	34.6	9526	0.49±0.02	710.0 ±161.7	19.2	14.8	0.66
3	96.9	34.6	9722	0.55±0.06	938.3 ±177.4	14.8	15.2	0.65
5	88.1	34.6	9754	0.57±0.03	945.4 ±311.9	14.0	15.1	0.65
6	94.7	34.6	9638	0.52±0.01	740.6 ±258.0	13.7	14.8	0.65
7	90.4	34.6	9658	0.47±0.03	806.8 ±259.0	14.0	15.2	0.65
8	95.5	34.6	9483	0.34±0.24	827.8 ±273.6	14.7	15.9	0.66
9	91.8	34.6	9075	0.52±0.03	740.6 ±163.4	13.7	14.8	0.69
Average	92.6	34.6	9550.9	0.49±0.13	816.0 ±248.8	14.9	15.1	0.66

The optimal combination of factors allowed the production of 0.8 g juveniles (mean specific growth rate of 15% per day) in 52 days with a swim bladder inflation rate above 90%, a survival rate between 13.7 and 19.2%, a final biomass of 9.5 kg per tank and a food conversion rate of 0.66 (**Table 6**). The production costs were calculated (**Fig. 5**), the production of a juvenile costs 0.2 euro.

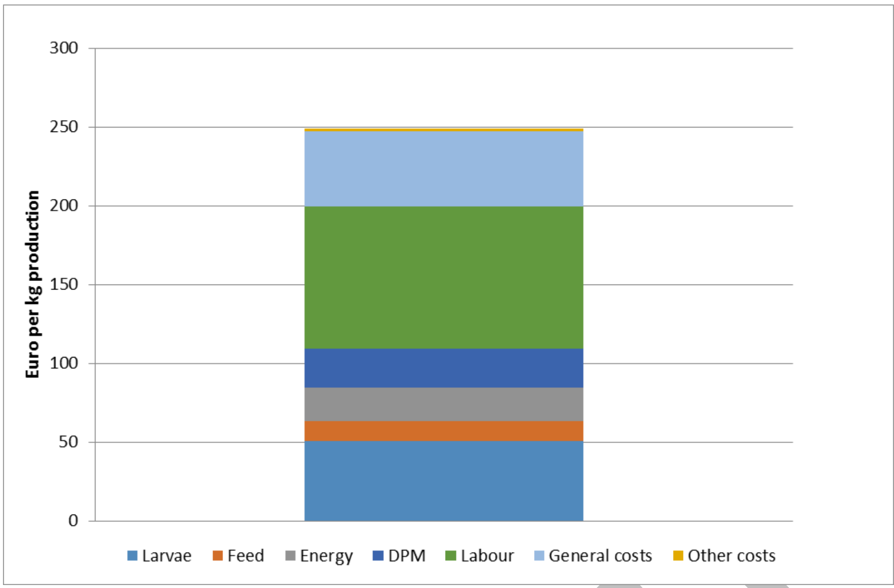


Figure 5. Production costs per kilogram of 0.8 g pikeperch juvenile in RAS.

Proposed industrial protocol for pikeperch production

As a last step, an industrial protocol (larval rearing + weaning) will be proposed and tested by an SME (Partner 39. FISH2BE) in 2018 to improve pikeperch larval growth and to reduce significantly cannibalism and larval mortality. This final protocol will integrate SME constraints.

Grow out husbandry

Effect of husbandry practices and environmental factors on growth, immune and physiological status

High (and sometimes sudden) mortalities and unpredictable growth rate at different developmental stages are major bottlenecks for the development of pikeperch aquaculture. These failures may be related to high stress responsiveness since they are often observed after fish handling. A previous study demonstrated that juveniles of Eurasian perch *Perca fluviatilis*, a percid species close to pikeperch, are more sensitive to aquaculture management than species with a long domestication history, such as rainbow trout. Moreover, several studies have demonstrated that high physiological stress may suppress the immune competence in fish and decrease their disease resistance. Therefore, the objectives of this study were: (1) to characterize the effects of major husbandry and environmental factors on growth related parameters as well as on physiological and immune responses of cultured pikeperch and (2) to identify the optimal husbandry and environmental conditions for improving the growth and the survival rates as well as the welfare of pikeperch in intensive culture.

These objectives were achieved through an experimental setting considering a multifactorial protocol. This experimental design considered 16 combinations (8 factors in two modalities, **Table 7**) of husbandry and environmental modalities in order to identify the best culture conditions.

Table 7. Selected modalities for the 8 environmental factors.

Factor	**Levels**	
Light intensity (lx)	100	10
Light spectra **(Fig.6)**	Industrial white	Red (610 nm)
Photoperiod (L:D)	24:00	10:14
Density (kg.m^{-3})	30	15
Temperature (°C)	26	21
Oxygen saturation (%)	90	60
Feed type	Mid-floating	Sinking
Fish grading	Yes	No

The growth performance results (**Table 8**) demonstrated significant and positive effects of low light intensity on survival. Positive interactions were also calculated when light intensity was associated with other factors such as temperature and rearing density. Thus, high mortality rates were observed in experimental conditions composed of high light intensity (100 lx) and low temperature level (21°C), confirming that pikeperch prefers low light intensity. Negative interactions between light characteristics and high density were observed even if the density levels tested did not exceed the known limits for pikeperch juveniles.

Figure 6. Experimental tanks (University of Lorraine, France) with different light conditions.

Growth related results were mainly affected by the feed type with positive effects of sinking feed. Positive interactions were calculated with the feed type and red spectrum, low light intensity and low temperature or high oxygen saturation. The impact of other factors-modalities, such as photoperiod, varied greatly between treatments without a conclusive trend. Information about the effect of feed type on husbandry performances is limited in fish, and it is not yet clear whether pikeperch prefers sinking or floating feeds. Some pikeperch farmers are using floating feeds to facilitate the control of food intake, but it seems that it is necessary to habituate juveniles at an early age to avoid rejection of this type of food later. So, it is possible that the feeding strategy interfered slightly with the positive effect obtained in the current experiment since fish were fed with sinking feed before the experiment.

As far as the sinking feed was considered, the highest growth performances were mainly observed in experimental conditions composed of "red light-10 lux-24h-21°C", indicating the importance of light characteristics for good performances of pikeperch in RAS conditions. Higher growth rates of fish reared under long photoperiod conditions are usually reported in relation to high food intake. Our results demonstrated the same trend for pikeperch if the rearing system includes sinking feed, red spectrum and low light intensity. They also indicate that pikeperch needs specific requirements for light characteristics, perhaps due to the presence of the *tapetum lucidum*, a specific reflective layer of the retina which may amplify the light level, explaining why this species may be highly sensitive to high light intensity.

The results of the present study did not support the hypothesis that high temperature promotes growth rate in pikeperch juveniles but corroborate the report that energy is spent for increased metabolic rates over 25°C. Indeed, temperature was not found as the main directive factor, and the positive interactions with sinking feed were observed with the treatments which included low temperature of 21°C.

Table 8. Combinations (c1 - c16) of the tested factors and results. Final weight heterogeneity (CV); Light spectrum: W = White, R = Red; Feed: S = Sinking, F = mid-Floating; Grading: Y = with

manipulations mimicking grading, N = without grading. The grey lines correspond to the five best combinations according to the global score of interest.

	Variables tested								Variables studied			
Combination of the factors	Light intensity (lux)	Density (kg.m^{-3})	Light spectra	Photoperiod (h)	Temperature (°C)	Feed	Grading	Oxygen saturation (%)	Final individual weight (g)	Mortality Rate (%)	CV (%)	Specific growth rate (%d-1)
c1	10	30	W	24	21	S	Y	90	168	4	37	0.9
c2	100	15	R	10	26	F	N	60	146	3	50	0.7
c3	100	15	W	24	21	S	N	60	172	13	40	1.0
c4	100	30	R	10	21	S	N	90	143	31	29	0.7
c5	10	15	R	10	21	S	Y	60	131	7	53	0.5
c6	10	15	W	10	21	F	N	90	88	7	67	0
c7	100	15	R	24	21	F	Y	90	113	24	61	0.3
c8	10	15	W	24	26	F	Y	60	146	10	53	0.7
c9	100	15	W	10	26	S	Y	90	158	13	39	1.1
c10	100	30	W	10	21	F	Y	60	122	41	52	0.7
c11	100	30	W	24	26	F	N	90	148	18	61	0.8
c12	10	30	R	10	26	F	Y	90	114	24	57	0.3
c13	100	30	R	24	26	S	Y	60	151	32	37	0.8
c14	10	30	R	24	21	F	N	60	117	4	72	0.4
c15	10	30	W	10	26	S	N	60	167	3	36	0.9
c16	10	15	R	24	26	S	N	90	169	7	40	0.9
Mean									140	15	49	0.7
SD									24	12	13	0.3

Regarding fish manipulation and size heterogeneity, our results did not clearly demonstrate that grading was a directive impact factor; and good growth performances were obtained in experimental conditions including or not grading manipulations. Perhaps the frequency of grading every two weeks, and the rather low manipulations were not so detrimental at the developmental stage used in the present experiment.

When considering stress markers in the presented study, red light spectrum at low intensity or white light spectrum at high intensity induced both a higher stress status. More research is, however, needed on this interaction between light characteristics and stress level in pikeperch. Concerning the immune status, humoral immune activities were only slightly impacted by some tested factors during the 2-month period and no conclusion can be drawn without further investigations.

Characterization of pikeperch growth, immune and physiological status in farm conditions

Based on the results from the multifactorial experiment, growth and physio-immunological status of pikeperch at different developmental stages (from 10 g to 100 g) were compared, in farm conditions, between standard husbandry conditions usually applied in routine by the SME. Because light characteristics may be an important factor in pikeperch culture, it was decided to maintain fish under the two experimental modalities defined as “optimal” but testing only red vs. white light spectrum, since other factors modalities induced less variability.

The experiment (periods 1 and 2) was run by Fish2Be farm (Belgium) and good growth was observed in all conditions (about 3.0 and 2.5 %.day^{-1} for periods 1 and 2 respectively) (**Fig. 7**). Grading manipulations were applied on days 49 (15th May) and 83 (19 Jun.) due to increased size heterogeneity. However, only few fish were discarded and cumulated loss of biomass did not exceed 10 %.

Figure 7: Experimental tank in Fish2Be farm.

Environmental colors affect the vision of fishes, influencing for example food intake, signals for hierarchical status, reproduction, growth and even survival. In pikeperch, it was shown that the use of red light improves specific growth rate and feed efficiency without any clear influence on stress status. In the present experiment, the light spectrum (red or white) did not significantly affect the growth parameters or the stress level (**Fig. 8**). However, the red light improved lysozyme activity while the industrial white light increased peroxidase activity, suggesting that environmental colors may influence immune status and susceptibility to diseases. However, to our knowledge, no study has focused so far on the potential link between the environmental colors and immunity in fish.

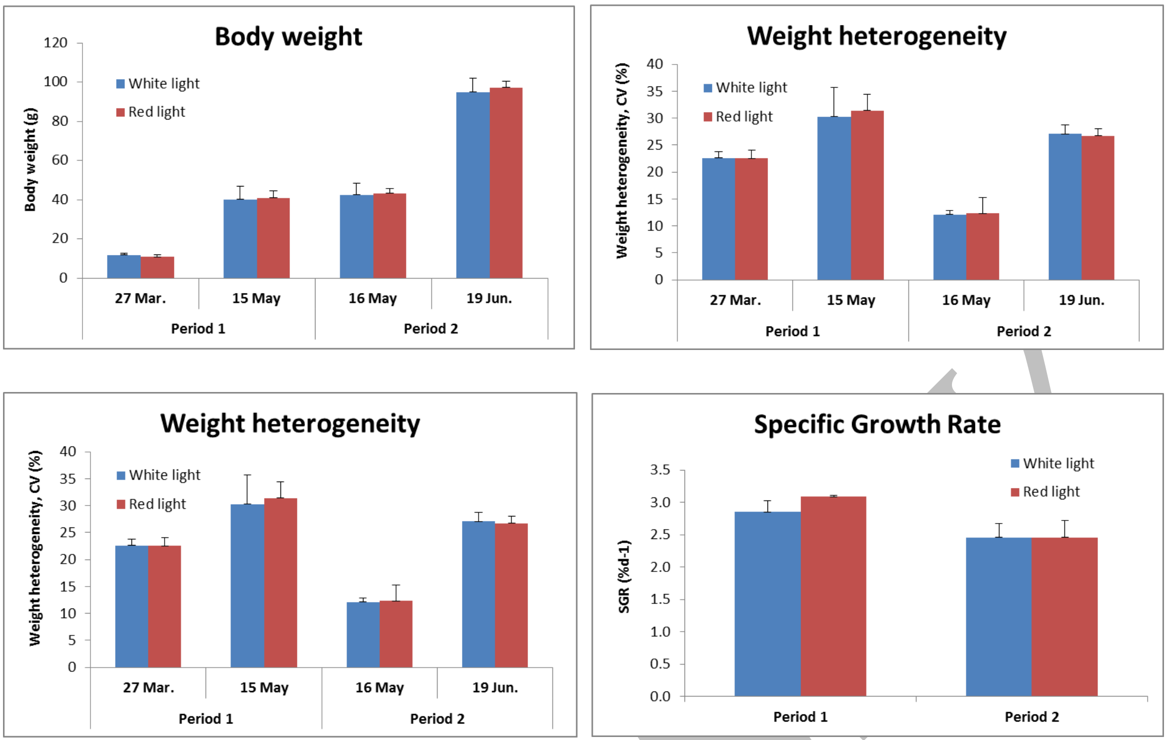


Figure 8: Growth performances (final body weight, weight heterogeneity, biomass and SGR) of pikeperch reared under a white (W) or a red (R) spectrum for periods 1 (27 Mar. to 15 May) and 2 (16 May to 19 Jun.). Each light condition was applied on 3 tanks. Lowercase letters indicate a significant difference between light spectra at $p < 0.05$.

Since light appears to be an important factor to consider for pikeperch rearing, it was decided to conduct another experiment to better understand the effects of the light intensity (10 *vs* 120 lx) and the light spectra (white *vs* red) on stress status and immune status of pikeperch juveniles. Briefly, fish were initially maintained under a white light spectrum at low intensity. At day 1, tanks were exposed to new light conditions, including two light intensities (10 or 120 lx) and two light spectra (industrial white or red at 610 nm). Specimens were reared under these conditions with a 12 L:12 D daily cycle for a month. Results indicated a strong effect of the light on physio-immunological status of pikeperch. The change of light environment was perceived as a short-term stress, characterized by increases in stress and immune markers. However, 30 days of high light intensity exposure led to a significant immune suppression. Combined with previous results, this experiment suggests that light-induced stress is of particular concern in pikeperch and low light intensity should be preferred for its culture.

Effect of pikeperch domestication level and geographical origin on growth and stress sensitivity

Due to recent intensive culture, pikeperch populations displaying various domestication levels are reared so far. Domestication highly affects the stress and immune status of fish in farm conditions. The effects of domestication process (wild vs domesticated strains) and geographical origin were investigated. Three batches including a wild French F0 strain (Lindre pond, France) and two Czech strains with a wild (F0) and a domesticated (F1, 4th reproduction cycle) batches were used. Fish were randomly distributed (5 kg.m^{-3}) into 9 indoor 800 L-tanks (3 tanks per batch) for acclimation and on growing until they reached 20 g body weight. During acclimation and experiment, fish were maintained in constant conditions (temperature: 21 °C; light intensity: 15 lx; photoperiod: 12D:12L). Moreover, to characterize the stress response of the different batches, we exposed pikeperch to a 30s-chasing. Samples were collected both before and after the application of this stress event.

While no effects of the geographical origin were detected, the stress response was stronger among the fish of the Czech strain with a high domestication level (F1) than in the wild ones. Although it is suggested that stress can impact negatively the immune defense in fish, stress response is a beneficial physiological adjustment to maintain homeostasis. So, the trade-off between stress and immune functions depends on the stress intensity, and may be species related in fish. As an example, it has been reported that reduction of stress responsiveness may be an important part of domestication, because of the positive selection of stress-resistant fish with an improvement of fitness along generations. In salmonids, this improvement was associated with low cortisol response, which was shown to be highly heritable through generations. However, the decrease in stress responsiveness with selection may be species related.

The domesticated Czech strain was also characterized by increased immune markers suggesting that domestication promotes immunocompetence and thereby limits pathogen outbreaks. However, this experiment was viewed as a preliminary one and further investigations on stress, immunophysiology and domestication process are needed to better understand the underlying mechanisms.

Market, consumer perception, new products and business model

The socio-economic research in DIVERSIFY includes applied market development approach clarifications on perception of aquaculture products, market demand evaluation, consumer preferences, new product development (**Fig. 9**), value adding and market development. The studies have been performed across five largest European fish markets: France, Germany, Italy, Spain and the United Kingdom.

Market analysis

- The market analysis demonstrated that important buyers (*i.e.* retailers) in the five countries find it very difficult to position the 6 new species (*e.g.* pikeperch) in relation to the current species in the market.
- Species such as pikeperch are known as wild catch but less as aquaculture products. Still industrial buyers position this fish not easily in relation to other fish species. White fish is easily seen as equal, so that *Pangasius* might become a direct competitor.
- Buyers are open to welcome new species under the following conditions:
    - The product must be cultured in a sustainable way,
    - The product should be available as a fresh product (especially southern-Europe)
    - The product must be easy to prepare and/or ready to eat (Germany and United Kingdom) and
    - The product must be priced competitively.

New Product Development

- Co-creation with consumers identified very promising product ideas for new fish products per investigated country.
- The most important drivers and barriers for the choice of the new product ideas have also been identified and recommendations for new product development of selected fish species.

Sensory characterization of new fish species and consumer acceptance of new product development

- New fish species need to be properly introduced to create a diversification in the current market.
- Sensory, compositional, instrumental texture parameters and somatic properties of DIVERSIFY five emerging fish species, namely wreckfish, greater amberjack, grey mullet, meagre, and pikeperch, were examined for characterization purposes.
- Regarding the compositional parameters, fat content was among the most relevant discriminating aspect between species, while hardness was among the most differentiating ones when dealing with texture.

Figure 9. Examples of sample presentation to the participants in the test.

- Greater amberjack was described with sour flavor, pikeperch was described as a crumbly, pasty white fish and grey mullet was characterized by bitter flavor. Sensory firmness was clearly distinctive for wreckfish, while meagre related to juicy texture.
- The species in this study exhibited a wide range of physicochemical and sensory characteristics that show their potential for being further exploited when designing new products.
- In a **consumer acceptance** test, it was demonstrated the influence of having the product information in advance on the consumer acceptance degree **(Fig.10**).
- In the case of pikeperch, it is presented as a pate, and it was best rated in France and Spain and low rated in the UK, Italy and Germany. This implicates that next to the fillet, other products might be interesting.

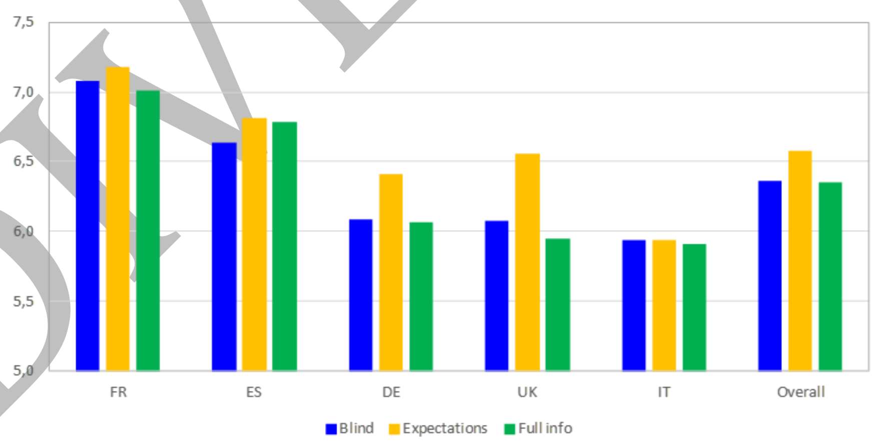


Figure 10. Results of the consumers' acceptance tests for new developed products performed in 5 European countries. Consumers were not informed about the product (blue bar), then knowing the product to be tested, they were asked about their expectation once informed about the products (orange bar) and finally they had the full information before tasting the product (green bar).

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Co-funded by the Seventh Framework Programme of the European Union

This 5-year-long project (2013-2018) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY). The consortium includes 38 partners from 12 European countries –including 9 SMEs, 2 Large Enterprises, 5 professional associations and 1 Consumer NGO- and is coordinated by the Hellenic Center for Marine Research, Greece. Further information may be obtained from the project site at "www.diversifyfish.eu".