New species for EU aquaculture

Deliverable Report

Deliverable No:	D16.6	**Delivery Month:**	60
Deliverable Title	Evaluation of selected rearing combinations for pikeperch on farm condition		
WP No:	16	**WP Lead beneficiary:**	P9. UL
WP Title:	Larval husbandry – pikeperch		
Task No:	16.2	**Task Lead beneficiary:**	P39. F2B
Task Title:	Proposition of an industrial protocol for pikeperch rearing		
Other beneficiaries:	P9. UL	P22. DTU	
Status:	Delivered/delayed	**Expected month:**	57
……..			

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Other Scientists participating: Fontaine, P. (UL), Lund, I. (DTU)

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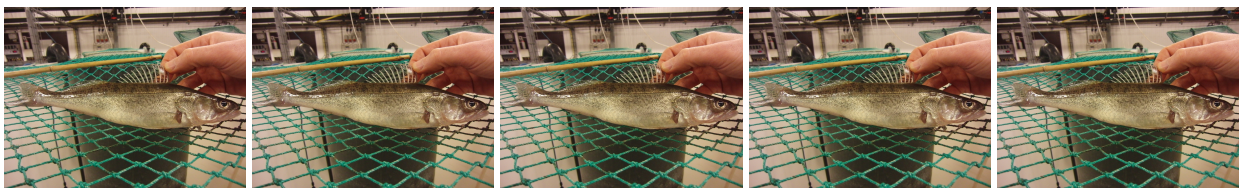

Objective:

The objective was to develop an industrial protocol to improve larval performance (growth, survival) during rearing of pikeperch.

Updated bibliographical analysis:

Pikeperch is considered to have the highest potential for inland aquaculture diversification in Europe (Wang et al., 2008). 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). To promote pikeperch intensive culture, researches have concerned its biology and culture over the last decade mainly (Kestemont et al., 2015). Bio-economic feasibility of pikeperch intensive rearing using RAS has been demonstrated (Steenfeldt and Lund, 2008; Steenfeldt et al., 2010a; Dalsgaard et al., 2013), currently ~ twenty farms have been built in Europe to produce pikeperch using RAS. A major bottleneck for further expansion of pikeperch culture today corresponds to the weak performances at the nursery level (low survival, high cannibalism and deformity rates). Cannibalistic behaviour becomes very severe from 18 to 39 dph representing up to 80% of all mortality at weaning time (19 to 23 dph) in pikeperch (Kestemont *et al*., 2007; Szkudlarek and Zakęś, 2011). The provision of a reliable protocol for pikeperch larval rearing is necessary to secure the development of its culture.

However, the development of such reliable protocol in the short term is not easy because larval rearing is a complex system, and numerous factors influence larval development as well as behavior and survival (Kestemont et al., 2003). The influential factors can be classified into three categories: environmental factors (a.o. temperature, light intensity, photoperiod, water quality), nutritional factors (a.o. food composition, feeding frequency and ratio, meal distribution timing and population parameters (a.o. fish density, strain, domestication level). Like any complex systems, the effects of the interactions between factors are often more important than the simple effects of the corresponding factors and the determination of the performances is multifactorial. In that context, the use of factorial design experiments is particularly adapted to determine the combinations of factors, that would be required to improve the rearing system. However, in the field of aquaculture, only few studies have been designed using such a multifactorial approach (Hamre et al., 2004; Gardeur et al., 2007; Teletchea et al., 2009; Trabelsi et al., 2011).

Previous works related to pikeperch larval rearing have already been published based mainly on monofactorial approaches, but the integration of corresponding knowledge and their application on farm conditions are very difficult for several reasons. Firstly most data have not been specified, especially concerning the origin (broodstock management) and the pre-experimental history of the biological material used for experimental trials (eggs incubation, larvae and post-larvae rearing). This lack of data concerns also the experiment itself. For example, that may concern: Initial weight or size of larvae (Lund et al., 2012, 2014; Tielmann et al., 2017), the type and volume of the rearing tanks (Lund et al., 2012, 2014), the tank wall color (rarely indicated), the tank cleaning or not (Mamcarz et al., 1997; Hamza et al., 2007), the use or not of a protocol or technique against the water surface soiling, the light spectrum or the direction of the water flow (almost never indicated). Secondly, rearing practices vary a lot and may not be detailed So to be sure that food availability is not a limiting factor, fish are fed *ad libitum* with very few details (Mamcarz et al., 1997; Molnar et al., 2004; Król and Zakęś, 2016; Policar et al., 2016) or in excess (Szkudlarek and Zakęś, 2007; Szczepkowski et al., 2011). Thirdly, according to the specific objective and local constraints (equipment, fish), the experimental designs are very heterogeneous. Some trials have been carried out with larvae at different ages and stages: at early stages (1-4 day post-hatching (dph), before the transition from an endogenous to the exogenous feeding) (Szkudlarek and Zakęś, 2007; Lund et al., 2012; Policar et al., 2016), at later stages (9-15 dph, after the feeding transition (Hamza et al., 2007; Kestemont et al., 2007; Ljubobratović et al., 2015; Król and Zakęś, 2016) or more later (42-48 dph after the weaning as second feeding transition) (Molnar et al., 2004; Szczepkowski et al., 2011). In the same way rearing practices are highly variable, i.e.initial fish density (from 2.5 to 100 ind.L^{-1}), rearing systems (glass aquarium, plastic rectangular bowls, circular or resin cylindro-conical tanks; green, grey or blue tanks wall color), water renewal rate (from 0.15 to 2.5 $L.min^{-1}$ for larvae), tank cleaning practice

(once or twice a day), water temperature (from 16-17 to 22-23°C), photoperiod (from L:D 10:14 to L:D 24:0), feeding frequency and period (from a meal each 0.5 hour to a meal 3 hours over the lighting period) and weaning duration (from 2 to 11 days) (Hamza et al., 2007; Kestemont et al., 2007; Szkudlarek and Zakęś, 2007; Lund et al., 2014; Ljubobratović et al., 2015; Policar et al., 2016; Tielmann et al., 2017; Yanes-Roca et al., 2018). Furthermore, specific practices are sometimes used like sorting of jumpers (Molnar et al., 2004), salt adding (2‰ of salinity, Schaefer et al., 2017) or a co-feeding before weaning (Hamza et al., 2007, 2008). Finally, it must be noted that most of studies have been realized at a small scale with 2-60 L tank volume and over very short and targeted periods, mainly 2-4 weeks. The lack of data and the extreme heterogeneity in experimental designs, mainly conducted in laboratory conditions, limit the possibility to aggregate and integrate knowledge for the development of a reliable protocol relevant in commercial nursery conditions.

In consequence, in the framework of the European Project DIVERSIFY, a multifactorial approach was developed to identify an optimal combination of factors to improve pikeperch larval rearing. Four experiments (See D16.1 ***Determination of the effect of environmental factors on pikeperch larval rearing***, D16.2 ***Determination of the effect of nutritional factors on pikeperch larval rearing*** and D16.3 ***Determination of the effect of population factors on pikeperch larval rearing and*** D16.4 ***Identification of optimal combinations of factors for pikeperch larval rearing),*** were conducted in laboratory conditions using an experimental recirculation aquaculture system (RAS) with ten 700 l tanks. This rearing system was very similar to those used under commercial conditions in percid hatchery – nursery (ex: Lucas Perches and Asialor). An optimal combination of factors was proposed (**Table 1)**. However, during these successive experiments, we have tested a limited number of parameters (12 parameters with two modalities per factor) and consequently some potential influential factors have not been considered and studied. Also, considering the experience of the SME in pikeperch larval rearing, including results presented in the deliverable D16.5, and the results obtained in WP10 (pikeperch larvae nutrition), this last objective of WP16 is to propose an industrial protocol for pikeperch larval rearing integrating also new results published recently.

Optimal combination of factors:

The table 1 summarizes the optimal combination of environmental, nutritional (feeding) and population factors selected from experiences made in the task 16.1.

Table 1: Optimal combination of factors validated in pilot-scale RAS (see D16.4).

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

Considering the factors and associated modalities presented in **table 1** and the results obtained (growth and survival rates, biomass gain, swim bladder inflation), it must be indicated that the most important ones are higher light intensity (50 lx *vs* 5 lx) and water renewal rate (100% per hour *vs* 50% per hour). Other factors that have an influence: are cleaning the tanks during the morning routine, rather than the afternoon and a bottom to top water current for environmental factors (Colchen et al., 2015), a later onset (16 dph *vs* 10 dph), a longer duration (9 days *vs* 3 days) of weaning and a discontinuous food distribution (*vs* continuous) for the feeding factors and a higher initial larvae density (100 larvae.l^{-1} vs 50 larvae.l^{-1}) and the use of larvae from bigger females (> 3.3 kg) for population factors (Fontaine et al., 2017). The practice of a co-feeding period before weaning (*vs* no co-feeding), the use of sibling populations (*vs* mixed populations) and sorting fish jumper using a net (*vs* no sorting) have no significant effect. At that level, we have to consider that all parameters tested were fixed for the whole experimental duration. The value applied for several factors in order to adapt it to the requirements of the different physiological stages can be a way to improve the protocol. Some parameters were fixed according to constraints of the SME (photoperiod fixed according to working period of employees) or related to the experimental facilities (the use of tanks all connected in a RAS didn't permit to apply several temperatures). Likewise it must be noted that in all experiments made in laboratory conditions (D16.1-4), only one type of *Artemia* nauplii (480 µm; Premium Artemia Cysts, Salt Lake Aquafeed, Utah, USA) was used and any *Artemia* nauplii enrichment was practiced. Concerning the formulated diet during and after the weaning period, we have always distributed the same diet food (Prowean 300, BioMar, Århus, Denmark). These initial choices can be reconsidered to improve the protocol.

Inputs from the WP10:

Dietary composition has a large influence on pikeperch larval growth and stress sensitivity as well as several physiological parameters. Results from WP 10 (Deliverable 10.1 and 10.2) have shown a significant importance of both long chain polyunsaturated fatty acids (LC-PUFAs) especially DHA, as well as a significant dietary requirement for phospholipids from start feeding until 40-50 DPH. Pikeperch larvae are incapable of desaturating and elongate shorter chain FA to longer chain LC- PUFAs (D10.2), meaning that these have to be supplied in the diets either in live feed or in the later formulated feeds until after metamorphosis (Lund et al. 2019). Based on experimental studies performed with formulated diets for pikeperch larvae reared at 18-19 °C and weaned from 15 DPH,- the optimal dietary LC-PUFA requirement has been estimated with a suggested DHA:EPA:ARA (D10.3). Phospholipid content should also respect a percentage of total lipid content and can be provided as soy lecithin. Several studies were undertaken to determine the optimal Ca/P ratio.

Based on these results a diet was formulated (D1 Diversify) and tested on pikeperch larvae at a commercial farm (Fish2B), against a high quality Otohime diet from Japan. Results showed a significant better growth (weight and length, $P<0.001$) of larvae fed the DIVERSIFY developed formulated extruded diet until 52 DPH (**Fig. 1**).

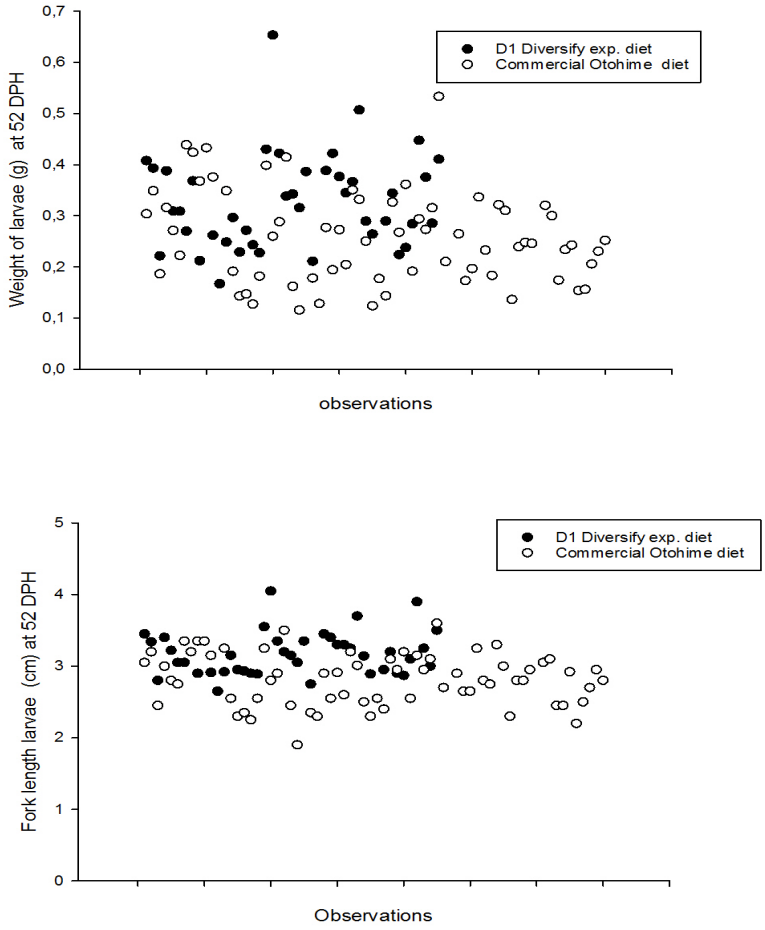

Figure 1: Weight and length of larvae fed either D1 Diversify exp. diet or a commercial Otohime diet.

Inputs from the SME (P39) experience:

Our industry protocol is already similar to some of the recommendations set out in D 16.4. On the other hand there is already a system installed, with different size and shapes of tanks compared to the experimental facilities in Nancy. All these tanks are part of one system, instead of several.

Light intensity is one of the factors that is a discussion topic. In the SME facility a common practice is to use LED-light bulbs of 3 – 5 W, but they are placed directly over the tanks. Resulting in light-intensities on the surface of over 300 lx. Attention is also directed to use of light sources with a warm color, to simulate the light color at dusk and dawn. At the same time the SME aims to have enough light for the operators to see any behavioural changes. Also from a practical perspective, it is more convenient to clean the tank bottoms in the evening rather than the morning, as there is a smaller risk of overflow due to waste blocking the filters during the nighttime. The use of fewer broodstock has the advantage over larger better performing parental fish by having more genetic variation in the offspring and especially a faster turnover in new and better performing broodstock.

In terms of feed usage, it is highly important to have feed that behaves in the best possible way in the water column, but is optimized for reducing malformities (such as described in D 16.2).

Recommendations for an industrial protocol:

In order to secure the production of juveniles of pikeperch using RAS and controlled conditions from hatching until 50 days post hatching (DPH), we suggest major recommendations according to results obtained in the WP10 and WP16, or recently published during the conduct of the DIVERSIFY project:

1 - To apply the following constant environmental conditions: light intensity of 50 lux, a water renewal rate of 100% per hour, a cleaning of the tank during the afternoon and an inlet of the water at the bottom level (see D16.1).

2 – To use a feeding strategy based on a later onset (starting at 16 DPH) and longer (9 days) duration of weaning followed by discontinuous feeding of the formulated diet after the weaning period (see D16.2).

3 – To feed larvae with enriched live preys (approximatively 3 first weeks including weaning period) and a formulated diet guaranteeing an adequate supply of LC-PUFAs and phospholipids (supplied as soy lecithin) (see D10.1-2).

4 – To mix rotifers (*Brachionus plicatilis*) and *Artemia nauplii* during the initial live preys feeding period in order to increase the survival rate during the transition from an endogenous to an exogenous feeding (Policar et al., 2018).

5 – To favour larvae from big females (> 3 kg) in order to obtain initially larvae of larger size (see D16.3).

6 – To rear larvae at high initial density (100 ind.L^{-1}) (see D16.3).

Discussion:

The recommendations cited above are mainly issues from experiments done in a specific rearing system (RAS, sub-squared tanks of 700 L each, green tank wall color …) (see D16.1-4), however the optimal combination of factors identified (D16.4) was not confirmed in the SME (P39) facilities which are very different to those used in laboratory conditions (see D16.5). As a result all these recommendations have to be validated according to the rearing system mobilized for pikeperch larvae culture. Another important point that must be considered is the production cost of a pikeperch juvenile, which is an important issue for fish farmers and a real bottleneck for their future success (see D31.31). In laboratory conditions, this cost was evaluated at 0.20 euro per juvenile (see D16.4). Some recommendations recommended here (new diet, use of rotifers …) could have negative consequences on this cost, which needs further evaluation.

Deviations: Initially, the deliverable D16.5 was expected for month 57.

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