New species for EU aquaculture

Deliverable Report

Deliverable No:	D16.5	**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:	16.1	**Task Lead beneficiary:**	P39. F2B
Task Title:	Optimal combination of factors for pikeperch larval rearing		
Other beneficiaries:	P9. UL		
Status:	Delivered	**Expected month:**	57
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Lead Scientist preparing the Deliverable: Fontaine, P. (UL)

Other Scientists participating: Bossuyt, J. (F2B)

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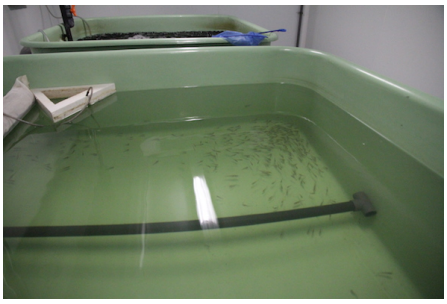

Objective:

The objective was to test the optimal combination of factors, previously identified for pikeperch larval rearing under laboratory conditions, in commercial conditions at a commercial situation, using the facilities of P39. F2B.

Introduction:

There is a strong incentive to increase the production of intensive aquaculture while limiting the impact of this industry on dwindling natural resources. To this end, European aquaculture aims to promote efficient and sustainable production of high quality seafood of high nutritional value. The European Project DIVERSIFY identified six new fish species, which could be eligible as new species for European fish farming. Among these candidates, pikeperch (*Sander lucioperca*), which is a freshwater species, was considered.

Pikeperch has gained attention as a promising new species in intensive fish farming (Nyina-wamwiza et al. 2005) with great economic potential. Until now several bottlenecks have prevented the success of larval rearing. Three major bottlenecks have been identified: (1) high mortality mainly due to cannibalism, (2) high rate of deformities and (3) a large size heterogeneity between larval cohorts at various ontogenetic development stages. The unit UR AFPA (P9. University of Lorraine, France) performed some studies in order to identify the optimal combination of factors which can improve the production of pikeperch larvae.

From previous studies (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**), an optimal combination of factors was identified and validated in laboratory conditions using an experimental recirculating aquaculture system (RAS) with ten 700 l tanks. However, such optimal combination of factors needed to be tested under commercial conditions in order to compare results obtained with those using fish farm current practices (which use different rearing parameters). Nevertheless, such comparison must consider the SME's constraints, such as their specific equipment as well as available broodstock and production planning. It was, therefore, impossible to test the exact optimal combination of factors identified and we had to perform the test with an "adapted" optimal protocol (see differences in the Material and Methods).

Material and Methods:

At the facilities of P.39 F2B, the improved protocol was tested in one 500 l circular black walled tank with a white bottom and compared to the F2B commercial protocol applied in two similar tanks. These three tanks (together with three other tanks) were part of a standalone filtration system (**Error! Reference source not found.**). Temperature was controlled by a heat pump (Fluidra Evoline 13) while temperature and oxygen were measured using an online controller (Tecnos Oxiwifi2 with a Tecnos galvanic oxygen and temperature probe).

The reproductive cycle of the broodstock was controlled by environmental manipulations for 6 months following a standard protocol similar to those described by Teletchea et al. (2009). Breeders were injected with hCG (Zakęś 2007; Zakęś and Demska-Zakęś 2009; Zakęś et al. 2013; Křišťan et al. 2013) on 27/06/2018 and stripped 3 – 7 days later, depending on the stage of oocyte maturation at 12°C. Eggs from 4 females of 1.1 - 1.8 kg, were fertilized with sperm from 5 males and were later incubated in Weiss jars for 7 days at 14 – 16 °C. They were hatched on the 8th of July. Approximately 50 000 larvae were stocked in tank T3 ("Diversify protocol"), 100 000 in tanks T1 and T2 (F2B commercial Protocol). This resulted in concentrations of 200 larvae $\cdot$ l^{-1} in tanks T1 and T2 and 100 larvae $\cdot$ l^{-1} in tank T3. Concerning T3, only eggs from two large females (1.7-1.8 kg) were used. **Table 1** summarizes the different experimental conditions applied in the two protocols.

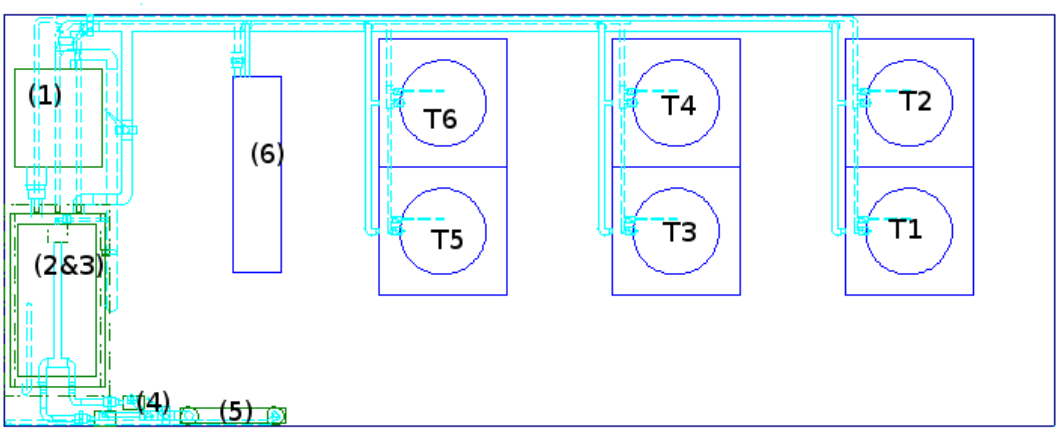

Figure 1: Hatchery layout at F2B, 6 tanks (T1-T6), (1) drumfilter Trome DF60 with 30µm mesh, (2) moving bed biomedia reactor 1350 l, (4) pumps 2 times Messner Powertec2 8 000, (5) UV VGE Pro UV INOX 200-154, Heat pump Fluidra Evoline 13, (3) static bed aerated biofilter 200 l and (6) rack of Weiss Jars.

Table 1: Experimental conditions applied for the two protocols (Diversify *vs* F2B).

Parameters	**Diversify protocol (T3)**	**F2B protocol (T1, T2)**
Stocking Density	100 larvae · l^{-1}	200 larvae · l^{-1}
Sorting of large larvae	No	yes
Only siblings or mixed offspring	Mixed offspring	Mixed offspring
Female weight	Medium - Large (> 1.7 kg)	any
Feeding schedule	Discrete (every 2 hours)	Semi-continuous (every 10 – 15 min)
Light regime	12:12	12:12
Light intensity	50 lx	300 – 500 lx
Weaning start	16 dph	14 dph
Weaning duration	9 days	7 days
Water renewal rate	1 tank volume h^{-1}	0.5 – 1.5 tank volume h^{-1}
Tank cleaning period	Morning	Evening
Tank current direction	Bottom to top	Bottom to top

Concerning the feeding strategies, the protocol used under laboratory conditions at the University of Lorraine had to be modified. Artemia distribution was done mechanically using automatic hoppers made by FFAZ (FFAZ Feeding Systems, Germany) which were filled twice a day with chilled concentrated Artemia. For dry

feed, feeding was performed using T-drum 2000 feeders (Arvo-Tec, Finland). All feeders were controlled by an arser-wolf feed-controller made by Arvotec. Furthermore, during the first 4 days of Artemia feeding, larvae were all fed with AF Sep-Art Artemia cysts from INVE, followed by 24hr enriched (Easy DHA selco by INVE) Artemia (Salt Lake Aquafeed premium Artemia cysts) for the remainder of the time. During weaning, diet used was Otohime B1 and later on B2 (Marubeni Nisshin Feed Co, Japan). The different weaning protocols used are detailed in **Error! Reference source not found.**.

Table 2: Weaning protocols applied for the two pikeperch larval rearing protocols (Diversify *vs* F2B).

Age (dph)	**Diversify Protocol (T3)**		**F2B protocol (T1, T2)**	
	Ratio Artemia – dry feed (%)	*Dry diet size*	*Ratio Artemia – dry feed (%)*	*Dry diet size*
14 - 15	100 – 0		80 – 20	250 – 350 µm
16 - 18	75 – 25	250 – 350 µm	60 – 40	250 – 350 µm
19 - 21	50 – 50	250 – 350 µm	30 – 70	250 – 350 µm
21 - 24	25 – 75	450 – 580 µm	0 – 100	250 – 350 µm
24 - 36	0 – 100	450 – 580 µm	0 – 100	450 – 580 µm
37 - 52		650 – 850 µm		650 – 850 µm

Concerning physicochemical environment and water quality, the DIVERSIFY protocol was slightly changed compared to what was used in laboratory conditions. Indeed, light intensity was slowly increased and decreased in the tanks over a period of two hours in the morning and evening, respectively, instead of just half an hour as described in D16.4. As performed in laboratory conditions, temperature was increased from 16°C to 20°C by 1°C per day and maintained at 20°C ± 1°C for the whole period. Oxygen was 10.0 ± 1.04 $mg{\cdot}l^{-1}$ and maintained at or above 8.5 $mg{\cdot}l^{-1}$, for all tanks. Total Ammonium Nitrogen (TAN) was 0.16 ± 0.07 $mg{\cdot}l^{-1}$ and $NO^{2-}N$ was 0.14 ± 0.11 $mg{\cdot}l^{-1}$. All other changes between the protocol used in laboratory conditions at the University of Lorraine and the protocol applied in the SME, due to the SME's constraints, are referenced in **Table 3**.

Table 3: Major divergences between the original Diversify (as presented in the deliverable D16.4) and the SME (F2B protocol) experimental conditions.

Parameters	**UL Laboratory**	**SME (FISH 2 B)**
Facilities		
Form	Sub-squared	Circular
Tank wall color	Green	Black (walls) and white (bottom)
Broodstock		
Female weight	3.8 – 4.4 kg	1.1 – 1.8 kg
Feeding schedule	Discontinuous (every 2 hours)	Semi-continuous (every 10 – 15 min)

The trial lasted for 49 days (from the 8th of July until 26th of August 2018). During this period, according to the SME practices, larvae from the tank T1 were regularly size graded since the age of 26 dph (first time grading), two other size grading occurred at 39 dph and 49 dph. After each size grading, the larger fish were introduced in another tank, then reared separately to the smaller ones.

Results:

The swim bladder inflation was around 95% for both tanks. In tank T2 (F2B commercial protocol), almost all larvae died without any explanation during the weaning period. Therefore, the trial was stopped at day 20 in this tank and this replicate (Fish 2 B protocol) could not be used. In tank T3 (Diversify protocol), 2035 juveniles were harvested at 49 dph, which corresponds to a final biomass of 1363 g (survival rate: 4,07%, final density: 2,72 $kg.m^{-3}$). The mean body weight was 0.669 g. Finally, for tank T1 (F2B protocol), 7688 juveniles (final biomass: 4400 g, survival rate: 7,68%; final density: 8,80 $kg.m^{-3}$) were produced with a final mean body weight of 0,572 g at 49 dph. In this second tank, the total biomass integrated the biomass of the larger ones reared separately, these fish represented 15% of the total number. The major cause of mortality was cannibalism.

Discussion:

Due the operational constrains of the partner SMEs related to its own production (spawning time of their broodstock and reproductive success) and the successive delays we met during the previous experiments done in the WP16 (see D16.1-4), it was not possible to implement this trial as foreseen initially. Furthermore, the abnormal mortality observed in T2 reduced the power of this trial. Consequently, the present results must be considered as preliminary (no replicate). Secondly, we were not able to apply faithfully our optimal combination due to the limitations of the technical facilities and pikeperch broodstock of the SME (see **Table 3**), thus the efficiency of our optimal combination of factors (see D16.4) cannot be fully discussed. It means that we have applied an "adapted DIVERSIFY protocol" and a real validation of our optimal combination of factors in SME conditions remains to be confirmed. The better results obtained here in tank T1 *vs* T3 could be mainly related to the higher initial larval density introduce in the tank (200 $larvae.l^{-1}$ vs 100 $larvae.l^{-1}$). This factor was shown previously to be very important (see D16.3).

Comparing the results obtained here in T1 (SME protocol) with those obtained previously using the optimal combination of factors in a laboratory pilot-scale RAS (D16.4), we observed that we obtained better results with a higher final mean body weight (0.816 mg vs 572 mg), a better survival rate (14.9% vs 7.8%) and higher final fish density (13.6 kg/m^3 vs 8,80 $kg.m^{-3}$) at day 53. The disappointing results obtained in T3 (adapted Diversify protocol) could indicate that the UL trial (D16.4) may be related to the characteristics of the rearing facilities used (i.e. tank shape and wall color), which must be tested and confirmed.

Taken together, the present comparison between the Diversify and commercial protocols suggests that the continuous production of pikeperch juveniles using RAS in SME conditions continues to be limited and requires further research in order to improve survival and growth.

Deviations:

Apart from what was already explained in the Discussion, the deliverable was completed with some delay, which was due to previous delays met for the laboratory experiments (see D16.1-4) and spawning time of the SME broodstock.

References:

Křišťan J., Alavi S.M.H., Stejskal V., Policar T. (2013) Hormonal induction of ovulation in pikeperch (*Sander lucioperca* L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue. Aquaculture International 21 (4): 811–18.

Nyina-wamwiza L., Xu X. L., Blanchard G., Kestemont P. (2005) Effect of dietary protein, lipid and carbohydrate ratio on growth, feed efficiency and body composition of pikeperch *Sander lucioperca* fingerlings. Aquaculture Research 36(5), 486-492.

Teletchea F., Gardeur J.N., Psenicka M., Kaspar V., Le Doré Y., Linhart O., Fontaine P. (2009) Effects of four factors on the quality of male reproductive cycle in pikeperch *Sander lucioperca*. Aquaculture 291 (3–4): 217–23.

Zakęś Z. (2007) Out-of-season spawning of cultured pikeperch (*Sander lucioperca* (L.)). Aquaculture Research 38 (13): 1419–27.

Zakęś Z. & Demska-Zakęś K. (2009) Controlled reproduction of pikeperch *Sander lucioperca* (L.): A review. Archives of Polish Fisheries 17 (4): 153-170.

Zakęś Z., Szczepkowski M., Partyka K., Wunderlich K. (2013) Effect of gonadotropin hormonal stimulation on out-of-season propagation success of different year classes of indoor-reared pikeperch (*Sander lucioperca* (L.)). Aquaculture International 21 (4): 801–810.

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