Deliverable Report

Deliverable No:	D16.3		**Delivery Month:**	47
Deliverable Title	Determination of the effect of population factors on pikeperch larval rearing			
WP No:	16		**WP Lead beneficiary:**	P9. UL
WP Title:	Larval husbandry - pikeperch			
Task No:	16.1		**Task Lead beneficiary:**	P9. UL
Task Title:	Optimal combinations of factors to improve larval rearing			
Other beneficiaries:	P3.IRTA	P21.DTU	P39.F2B	
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Objective:
The aims of WP16. Larval husbandry-pikeperch were to identify the bottlenecks for larval survival and growth, and to establish an optimal combination of factors, which could give the best performance of pikeperch larval. The specific objective of ***D16.3 Determination of the effect of population factors on pikeperch larval rearing*** was to determine the effects of four population factors and their interactions on pikeperch larval rearing using a multifactorial approach. The present deliverable aims to investigate the effects of four population factors on the performance of pikeperch larvae. These include (a) initial larvae density (b) sorting out of fish jumpers, (c) sibling or not sibling populations and (d) spawning female size. This will standardize and improve the larval rearing protocols now employed in pikeperch larval rearing.

Introduction:
There exists 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 safe seafood of the highest quality and nutritional value. The European Project DIVERSIFY identified six new fish species, which could be eligible as potential future species for European fish farming. Among these candidates, the fresh water species pikeperch (*Sander lucioperca*) was selected.

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 the rearing of the 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. The group DAC of the unit UR AFPA (University of Lorraine, France) has lead the work studying the optimal combination of factors that can improve the production of larvae pikeperch.

Material and Methods

1 - Multifactorial experiment

A multifactorial experimental design was developed to study simultaneously the effect of selected factors and their interactions on pikeperch larval rearing using a factorial design. As this study aims to produce an optimal set of factors for the fish farmer, practical constraints are taken into account (P29 and P39) and the modalities for each of the four factors are in accordance with previous data given (**Table 1**).

1 - Initial larvae density: For the density, we have tested a high density and a low one (50 or 100 larvae. l^{-1}). In percidae, the effect of fish density on cannibalism is debated. Generally high fish density has been found to favour cannibalism (Baras, 2012), particularly in freshwater piscivorous fish like in Percidae (Cuff, 1980; Li and Mathias, 1982; Loadman et al., 1986). However in Eurasian perch *Perca fluviatilis*, which is another Percidae, cannibalism was lower in higher than at lower fish density (Mélard et al., 1996; Baras et al., 2003). The effect of fish density on cannibalism is highly related to the density range tested (Baras, 2012). In a number of studies on pikeperch larval rearing, a wide range of stocking densities were used (5 to 100 larvae.L^{-1}) while only two studies were designed to specifically tackle the density effect (Mamcarz et al., 1997; Szkudlarek and Zakes, 2007). These last authors concluded that growth and survival decrease when fish density is increased from 25 to 100 larvae.l^{-1}.

2- Sorting out fish'jumpers: In fish larvae culture, jumpers, which are often considered as cannibalistic individuals (Baras, 2012), are characterized by rapid growth, which induces size heterogeneity in the cultured population. In aquaculture size heterogeneity is most frequently estimated by the coefficient of variation (CV) for body length or mass. As demonstrated in several species high CV values generally result in higher risks of cannibalism and mortality (Folkvord and Otterå, 1993; Baras and d'Almeida, 2001; Chang and Liao, 2003; Babiak et al., 2004). Consequently size grading and jumper sorting are generally practiced in nursery management. However, Mandiki et al. (2007) indicated that the occurrence of few cannibalistic jumpers is not always detrimental as their presence inhibits the emergence of new jumpers. Thus the benefit of removing or leaving in the population the jumpers remains unclear.

3- Stocking Sibling or not sibling larval groups: In large scale aquaculture, the implementation of artificial spawning protocols using hormonal treatments is labor intensive and time consuming. Consequently, the use of all larvae batches available is common resulting in the stocking of mixed batches (spawning from different females), particularly when different spawning events occur close together. However, this practice encourages an increase of the initial size heterogeneity leading to potential cannibalism. Except for the studies of Mamcarz et al. (1997) and Skudlarek and Zakes (2007), who tested sibling and half-sibling populations, respectively, the majority of studies on pikeperch larval rearing (Ostazewka et al., 2005; Hamza et al., 2007, 2008; Kestemont et al., 2007; Lund et al., 2012, 2014; Krol and Zakes, 2016) never specified if the larvae originated from a couple of breeders (sibling) or several females and males (not sibling). In the present study, the performances of sibling and not sibling populations were compared.

4- Female weight: In general, the weight of spawning females is correlated directly with the size of the eggs and newly hatched larvae, which in turn, influences larval viability (Marteinsdóttir and Steinarsson, 1998; Kamler, 1992; Johnston, 1997; Raventos and Planes, 2008; Imanpoor et al., 2009). As the initial larval mouth size influences the fish's ability to consume live preys such as *Artemia nauplii*, and therefore affect indirectly larvae survival rate. Apart from the study of Skudlarek and Zakes (2007), the size or weight of breeders is rarely indicated. As a result, two modalities: larvae from large females (> 3 kg) or larvae from small females (< 3 kg) were tested.

Table 1. Applied modalities for each factor.

Factor	Modality 1	Modality 2
Density	50 larvae.L^{-1}	100 larvae.L^{-1}
Sorting of fish jumper	yes	no
Sibling or not sibling	Sibling	Not sibling
Female weight	Small (< 2.8 kg)	Large (> 3.3 kg)

Materials

The study was carried out in a ten 700 l tank recirculated aquaculture (RAS) experimental system using eight of these tanks which were equally divided into two rows and where the water was treated with mechanical and biological filters as well as a UV sterilization unit (**Figure 1**). The tanks were stocked with 35,000 or 70,000 larvae each, which corresponded to the 50 and 100 larvae l^{-1} treatments, respectively.

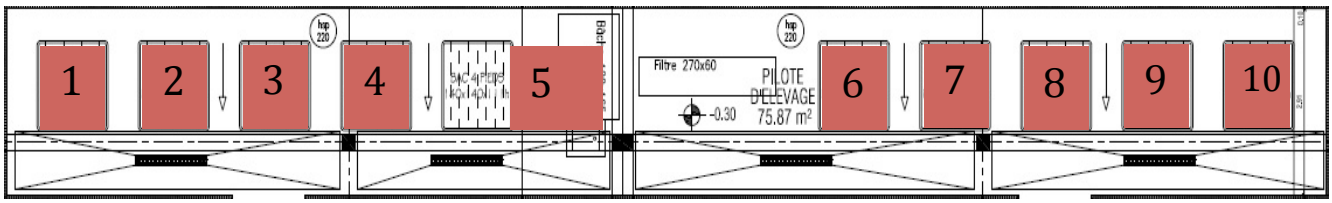

Figure 1. Diagram of the experimental facilities. The closed loop consisted of 10 tanks with a central filter. Tanks 2 to 9 were used during the entire experiment, while tanks 1 and 10 were employed as additional moving bed filters.

Biological materials: pikeperch larvae

Larvae (420,000) were obtained from a local brood stock maintained at SARL Asialor (Pierrevillers, France) and transferred to the UL experimental platform (UR AFPA, Vandœuvre-lès-Nancy, France). Larvae hatched from February 19-20th, 2017 and were distributed to the 8 tanks, where water temperature was initially at 15-16°C. All larvae came from four females stripped during two days (February 12-13, 2017), which were fertilized by mixed sperm from 3 males. The transfer of larvae was done using separate bags from each female. The distribution of larvae to the tanks is shown in **Table 2**.

Table 2. Larvae distribution according to female body weight and initial fish density.

Female number	Female weight	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7	Tank 8	Tank 9	Total
2	2.72 kg	17,500			35,000		70,000	35,000		157,000
53	2.35 kg	17,500			35,000					52,500
59	3.62 kg		35,000	17,500		35,000			70,000	157,500
67	3.00 kg		35,000	17,500						52,500

Standardized rearing conditions

The photoperiod was fixed at 12h of light and 12h of darkness (Hamza et al. 2007) with a progressive increase of light intensity (from 0 to 50 lx) during the first 30 min, stabilizing at 50 lx (see Deliverable D16.1) until the last 30 minutes of the photoperiod where the light intensity decreased from 50 to 0 lx. Temperature was the same for all tanks ranging between 16 and 20°C (Hamza et al. 2007; Kestemont et al. 2007; Szkudlarek and Zakęś 2007). Temperature was initially increased incrementally by 1°C day^{-1} until 20°C. Dissolved oxygen was maintained above 7 mg l^{-1} and salinity was fixed below 1 ‰. In the third experiment, environmental factors (light, water renewal rate, water current direction and cleaning period) were the same as for the second experiment. The feeding strategy was carried out according to best protocol obtained in the second experiment. This was comprised of a discontinuous distribution of food, a co-feeding period, initiating the weaning period at 16 dph and a weaning duration of 9 days (see Deliverable D16.2).

Distribution of tested factors in the tanks

The experimental combinations of factors were applied according to an experimental matrix (**Table 3**).

Table 3. Experimental matrix applied.

Tank	Combination	Density (larvae/L)	Sorting out	Number of sibling	Female weight
2	2	50	no	2	< 2.8 kg
3	1	100	no	2	> 3.3 kg
4	3	50	yes	2	> 3.3 kg
5	7	100	yes	2	< 2.8 kg
6	0	50	no	1	> 3.3 kg
7	4	100	no	1	< 2.8 kg
8	5	50	yes	1	< 2.8 kg
9	6	100	yes	1	> 3.3 kg

Measures, sampling and observations

The physical-chemical properties of the water were monitored regularly to ensure optimal conditions: Oxygen level was checked every morning (> 7 mg); ammonia and nitrite concentrations were titrated twice a week and pH was also measured twice a week. Water pH was corrected by regular inputs of NaHCO3.

The experiment lasted 53 days (from February, 19th until April, 13th 2017). Larvae were sampled every 7 days and designated as days after first feeding: T0, T7, T14, T21, T28, T35, T42 and T49. During sampling, larvae were siphoned into a basin then pipetted, counted and distributed in the different sampling tubes. Larvae siphoned in excess were returned to their original tank. This method allowed limited net sampling losses. Sampled larvae in the tubes were then sacrificed by a lethal dose of MS222 and excess water removed with a fine mesh dip net and placed in 4% buffered formalin.

The parameters measured included: (a) Morphometric (total length;TL, body weight; W, coefficient of variation of total length;CV TL) and coefficient of variation of weight (CV W)) and were carried out on 30 larvae $tank^{-1}$ sampling $date^{-1}$; (b) Observations during 5 min day^{-1} of each tank in order to detect cannibals.

Cannibals were identified and counted as individuals responsible for a direct attack or with a big abdomen (for the last weeks). Each cannibal was measured and weighted. (c) Every day, after tank cleaning, a sorting out of "big" fish called jumpers was done. An observation of 15 min per tank (with modality *Yes* for sorting out of fish's jumpers) was carried out and if a jumper was detected, it was caught with a net, measured and weighted. (d) At the end of experiment, the total fish biomass was weighed for each tank and the % swim bladder inflation measured. This was done by separating fish with or without a swim bladder and then lightly anesthetizing them with MS222 (70 mg l^{-1}) followed by transfer to salted water (20 g of salt l^{-1}) according to Jacquemond (2004a, b).

Statistical analyses

For this multifactorial experiment, a statistical software package (Analysis; Kobilinsky, 2000; Gardeur et al., 2007) was used for the deliverables D16.1 and D16.2. The unexplained high mortality in tank 7 was not considered in the analysis. All statistical analyses were performed using the free software R version 3.2.4 (R Core Team 2016). For all the dependant variables, homogeneity of variances was previously tested using the Levene test (leveneTest, package 'car', Fox and Weisberg 2011). Analysis of variance (ANOVA) using aov function (R Core Team 2016) with the FACTOR 1 and the FACTOR 2 as fixed effects (aov;Y~FACTOR 1*FACTOR 2) with Y the dependant variable. For ANOVA validation, residuals were tested for homogeneity and normality using residuals vs fitted values and sample vs theoretical quantiles (Q-Q) plots respectively (plot, R Core Team 2016) followed by a Shapiro-Wilk test for normality (shapiro.test, R Core Team 2016). If necessary, data were log-transformed. When the data met the ANOVA assumptions, an ANOVA Type I was performed to calculate F-tests (ANOVA, R Core Team 2016) followed by a Tukey's range test based on Tukey–Kramer method for multiple comparisons as post-hoc test (TukeyHSD, R Core Team 2016). When data, including transformed, did not meet the assumptions for ANOVA (homogeneity of variance and normality), the aligned rank transformation was used for nonparametric factorial analysis (aligned.rank.transform, package 'ART', Villacorta 2015) followed by a pairwise comparison using Tukey and Kramer test (posthoc.kruskal.nemenyi.test, package 'PMCMR', Pohlert 2014). The level of significance used in all tests was $p<0.05$.

2 – Comparison of the predatory behaviour of cannibals *vs* non-cannibals

For this additional experiment, fertilized eggs were obtained from one female and one male (supplied by the SARL Asialor, Pierrevillers, France). Larvae hatched on February, 1st, 2017and were then transferred to and reared at the UR AFPA (Unit of Animal Research and Functionality of Animal Products - University of Lorraine). The larvae were stocked in four independent incubators (110 × 64 × 186 cm, 300 L) each including nine racks (45 x 7 x 12 cm), in the same hatchery room (**Figure 2A**). Each incubator had a flow rate of 4 $m^3.h^{-1}$ of recirculated water which was U.V. treated and its own temperature control system (T = 20°C; **Figure 2B**). The physico-chemical properties of the water were monitored twice per week to ensure optimal conditions: oxygen saturation (82.8% ± 9.8%); ammonia and nitrite concentrations were lower than 0.5 and 0.01 mg l^{-1}, respectively; pH was controlled at 7.83 ± 0.05. Incubators were kept under a photoperiod of 10h light/14 h dark. At 10 dph, approximately 100 larvae were put in each of three racks of each incubator while other larvae were divided in groups of four (12 larvae in each incubator; 4 in one rack) and reared in booths (**Figure 2C, D, E**).

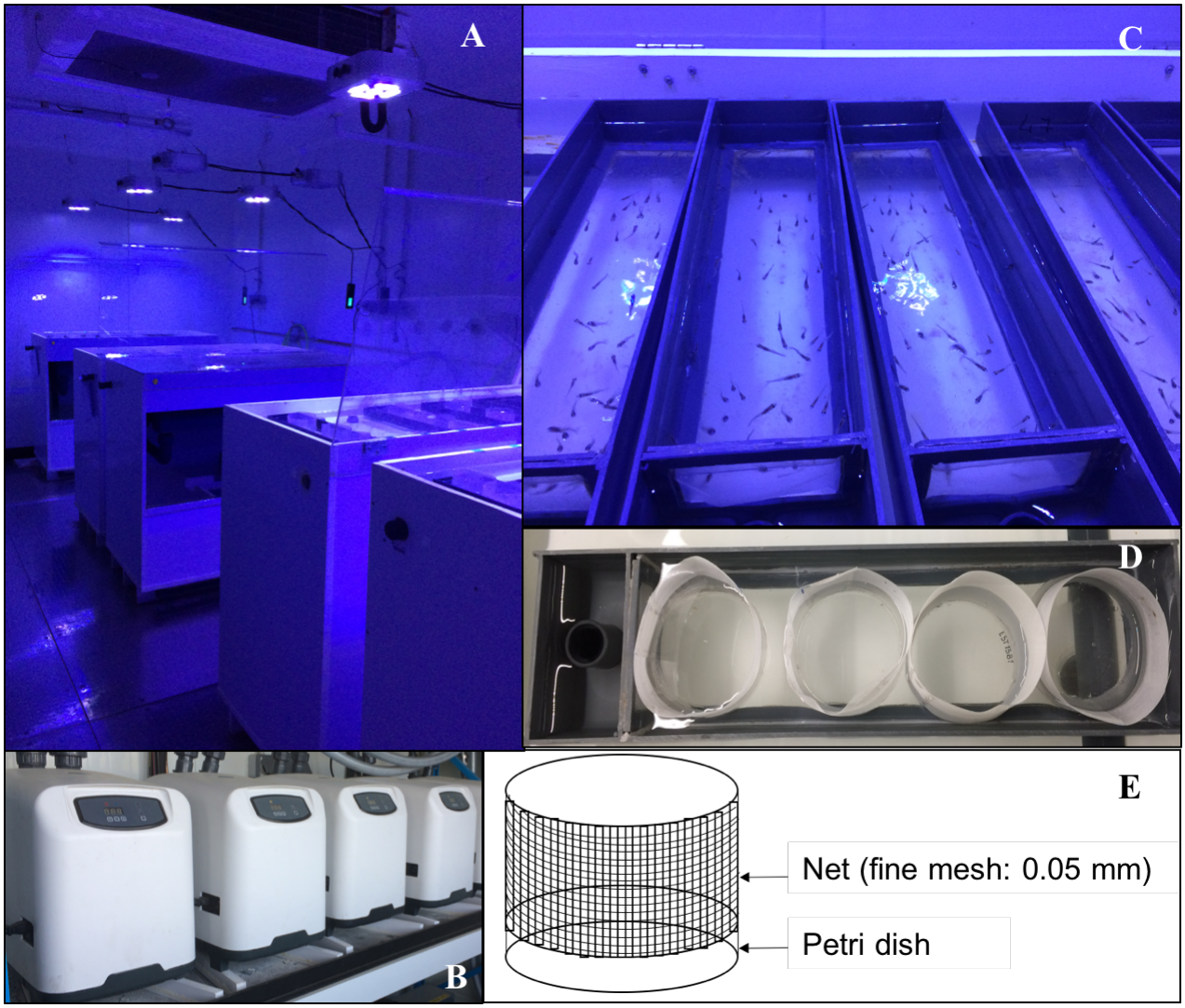

Figure 2. A. Room with four incubators; B. Cooling system to maintain temperature at 20°C; C. Racks with 100 larvae; D. One rack with four booths. E. A booth.

From 17 dph to 33 dph, 2 hours each day were dedicated to observe booths and racks. When a cannibalistic larva was found, it was isolated in a booth and its behaviour monitored the next day. Non-cannibalistic larvae were obtained and groups of 4 larvae were stocked in booths. Indeed, if there did not missed larvae in a booth, there were 4 non-cannibals to test at a given time. Larvae were evaluated with two different tests each separated from the other by 24 h (**Figure 3**). In the first test of predation, larvae were confronted with three prey (zebrafish larvae). In the second test of cannibalism larvae were confronted with three congeners (pikeperch larvae). For each test, each larva was transferred from the booth to a device (20x7x4 cm with 2 cm of water height), which was placed above a translucent table with a light (50 lx) placed below. The device was divided into two equal parts separated by a divider. The pikeperch larvae (cannibal or non-cannibal) were put in one compartment and prey or congeners in the other one. After 30 min of acclimatization for both tested larva and prey or congeners, the divider between the two parts of the device was removed and behaviours of pikeperch larvae and prey were observed during 20 min. All behaviours were video recorded with a camera (Sony Handycam, DCR-SR72) positioned 80 cm above the device. The water in the device was the same as in the incubator and renewed between each 20 min trial.

The following behaviours were analysed:

1. Fish orientation where tested larva turned its body head-first towards prey or congener and visually tracked it (Bell and Sih, 2007).
2. Fish approach, was defined as the movement of the tested larva towards the other larva with slow swimming.
3. Fish attack, characterized by a rapid movement of the pikeperch towards the zebrafish larvae or congeners, with the mouth open. This behaviour is easily identifiable: just before the attack, the larva stopped and adopted an "S" position (Houde, 2001, Turesson et al., 2002), or just modified the orientation of its caudal fin.

- Fish capture, which corresponded to the bite of the prey by the pikeperch.

Statistical analyses

All statistical analyses were performed using the free software R version 3.2.4 (R Core Team, 2016). For all the dependant variables, homogeneity of variances was previously tested using Levene test (leveneTest, package 'car', Fox and Weisberg 2011) and normality was tested by a Shapiro-Wilk (shapiro.test, R Core Team, 2016). Data were analysed by a Mann-Whitney-U-test (wilcoxon.test, R Core Team, 2016). The level of significance used in all tests was $p<0.05$.

Results

Multifactorial experiment

Effects on growth rate

At T0, the mean body weight of a larva varied between 0.79 mg (tanks 5 and 6) and 1.02 mg (tank 3). The mean specific growth rate for all tanks was 15.1 $\%.d^{-1}$ between the first sampling date (T0, 4 dph) and the last one (T49, 52 dph). Tank 6 had the best growth rate with 15.9 $\%.d^{-1}$ and the lowest growth rate was observed in tank 3 with 14.1%.d^{-1}. At the end of the experiment, larvae of tanks 6 and 2 had higher mean weights than the other tanks (1905.7 ± 398.1 mg and 1896.4 ± 733.9 mg, respectively) (**Figure 3**).

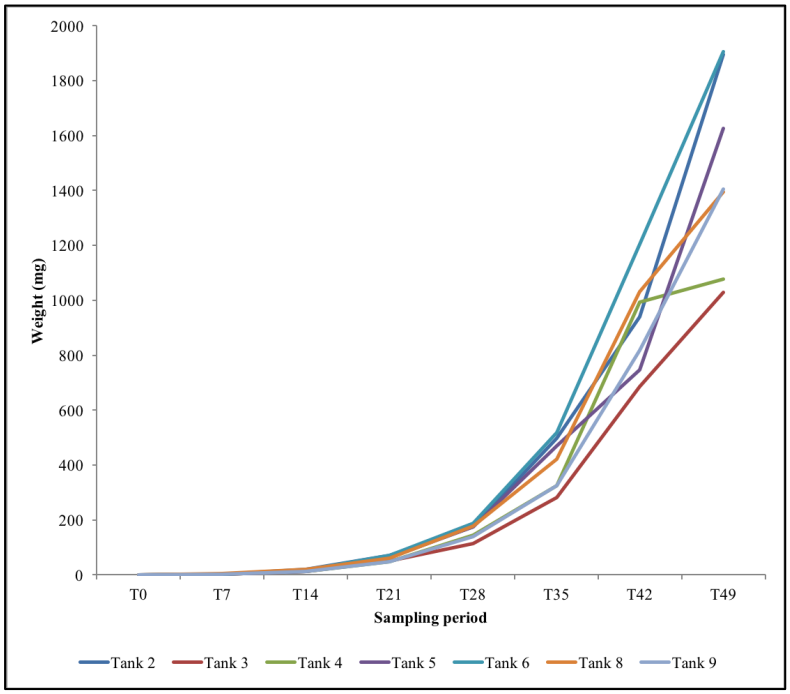

Figure 3. Growth curve of pikeperch larvae submitted to the seven combinations of factors.

Effect on body weight and size heterogeneity

At T49 (52 dph), we compared the effect of factors on weight and weight heterogeneity.

Effects of the interaction sorting of jumpers * initial density:
The statistical analysis shows a significant interaction between both factors on final mean body weight ($F = 91.34$; $p < 0.0001$; **Figure 4A**) but no significant interaction on the coefficient of variation for weight ($F = 0.3$; $p = 0.61$). The results show that a low larval density with no removal of jumpers produced significantly ($P<0.05$) heavier juveniles (**Figure 4A**). In contrast, a high larval density with removal of jumpers resulted in a significantly ($P<0.05$) higher weight gain compared with no removal of jumpers.

Effect of the interaction female weight * initial density:
There is a significant interaction between both factors on final mean body weight ($F = 4.85$; $p = 0.03$; **Figure 4B**) but no significant interaction on the coefficient of variation for weight ($F = 0.29$; $p = 0.62$). The results show that with a smaller female, larvae density has no effect, but with a larger female, a decrease of initial larvae density is associated with heavier juveniles (**Figure 4B**).

Effect of the interaction female weight * sibling link:
There is a significant interaction between both factors on final mean body weight ($F = 68.67$; $p < 0.0001$; **Figure 4C**) but no significant interaction on the coefficient of variation ($F = 5.85$; $p = 1$). A significant mean body weight was recorded when the female body weight was lower and no sibling population were applied.

Effect of the interaction sorting out of jumpers * sibling link:
The statistical analysis show a significant ($P<0.05$) interaction between both factors on weight ($F = 10.58$; $p = 0.0001$; **Figure 3D**) but no significant interaction on the coefficient of variation ($F = 1.38$; $p = 0.32$). The results show that without a sorting out of jumpers, juveniles were heavier in sibling population (**Figure 4D**).

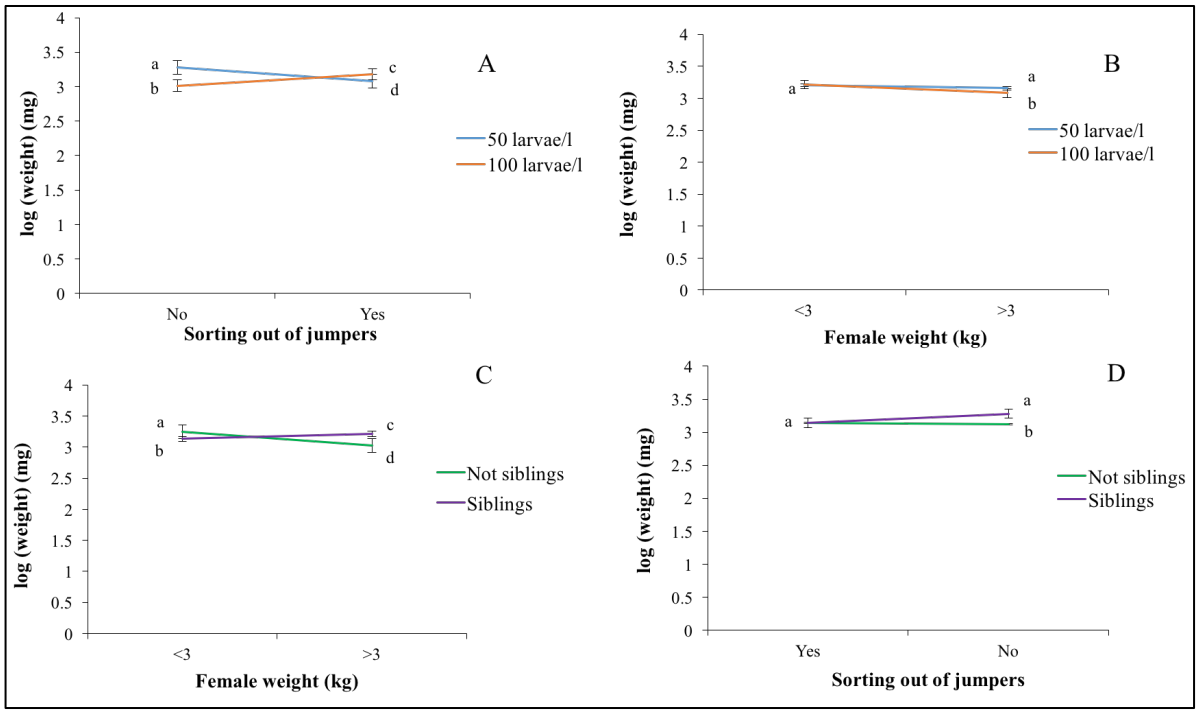

Figure 4. Significant interactions on final body weight of pikeperch juveniles recorded at T49 (52 dph)

Effect of sorting out of jumpers and female weight:

There is no significant effect of the interaction between both factors on weight (F = 0.27; p = 0.6) and the coefficient of variation (F = 8.4; p = 0.06). However, there are simple effects of sorting out of jumpers (F = 13.9; p < 0.0001; **Figure 5A**) and female weight (F = 21.4; p < 0.0001; **Figure 5B**) on the final mean body weight of juveniles. The final mean body weight of juveniles was significantly higher when the jumpers were not sorted and larvae came from smaller females.

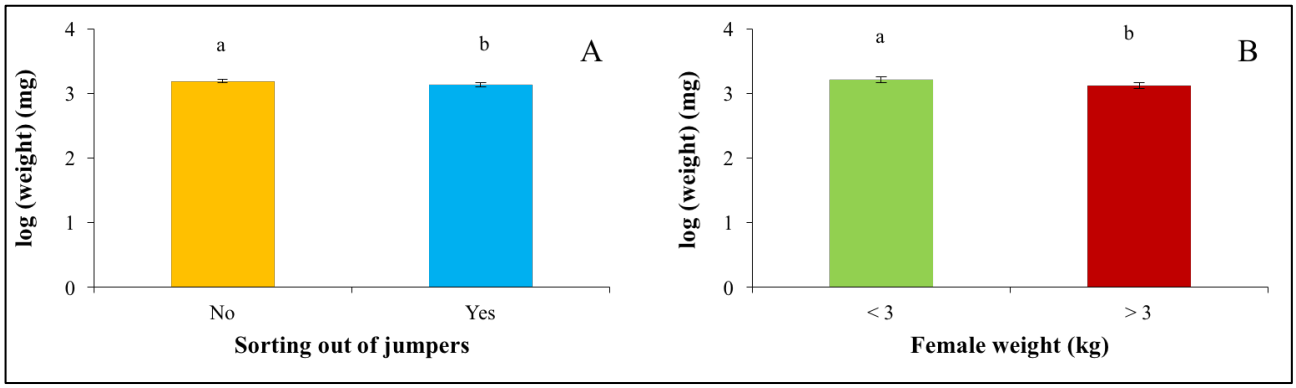

Figure 5. Simple effects of sorting out of jumpers and female weight on final mean body weight of juveniles recorded at T49 (52 dph).

Effect of initial density and sibling link:

There is no significant effect of the interaction between both factors on final mean body weight (F = 0.67; p = 0.4) and coefficient of variation for weight (F = 8.4; p = 0.06). However, there are simple effects of sibling link (F = 5.5; p = 0.02; **Figure 6A**) and initial larvae density (F = 6.7; p = 0.01; **Figure 6B**) on the final mean body weight of juveniles. The final mean body weight of juveniles was significantly higher when sibling populations were reared and initial larvae density lower (50 larvae.l^{-1}).

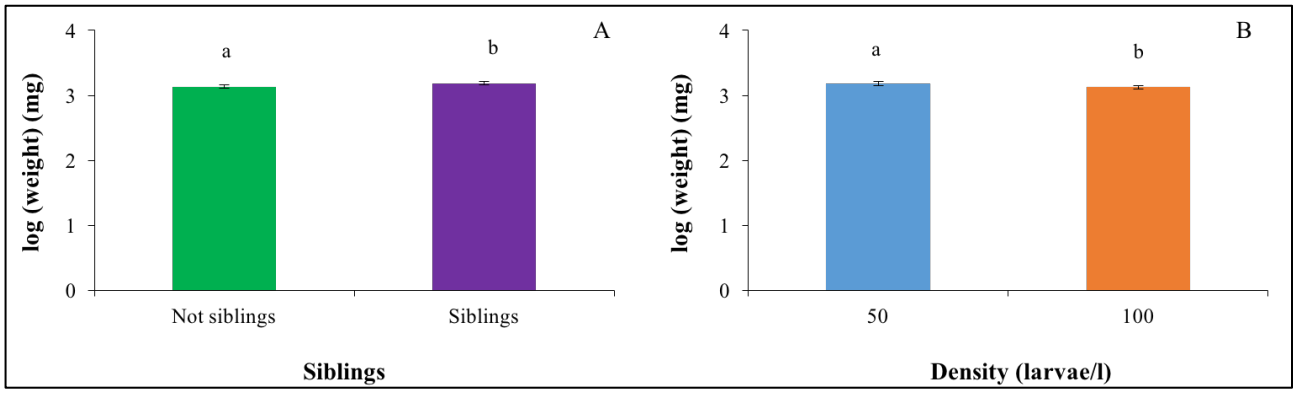

Figure 6. Simple effects of sibling link and initial larvae density on the final mean body weight of juveniles recorded at T49 (52 dph).

Comparison between jumpers, cannibals and fish took randomly each week

The first cannibal was observed at 17 dph. Consequently, the sorting out of fish' jumpers was started at 18 dph. At the end of the experiment, we have compared the weight of cannibals and jumpers with those of fish sampled weekly for growth study (T14, T21, T28, T35, T42, and T49) (**Figure 7**). This post-analysis allows us to note that jumpers who were sorted out were always bigger than other fish in tank. Non-cannibalistic jumpers were visually larger than sampled and cannibalistic cohorts, while the cannibalistic fish were larger than the sampled fish.

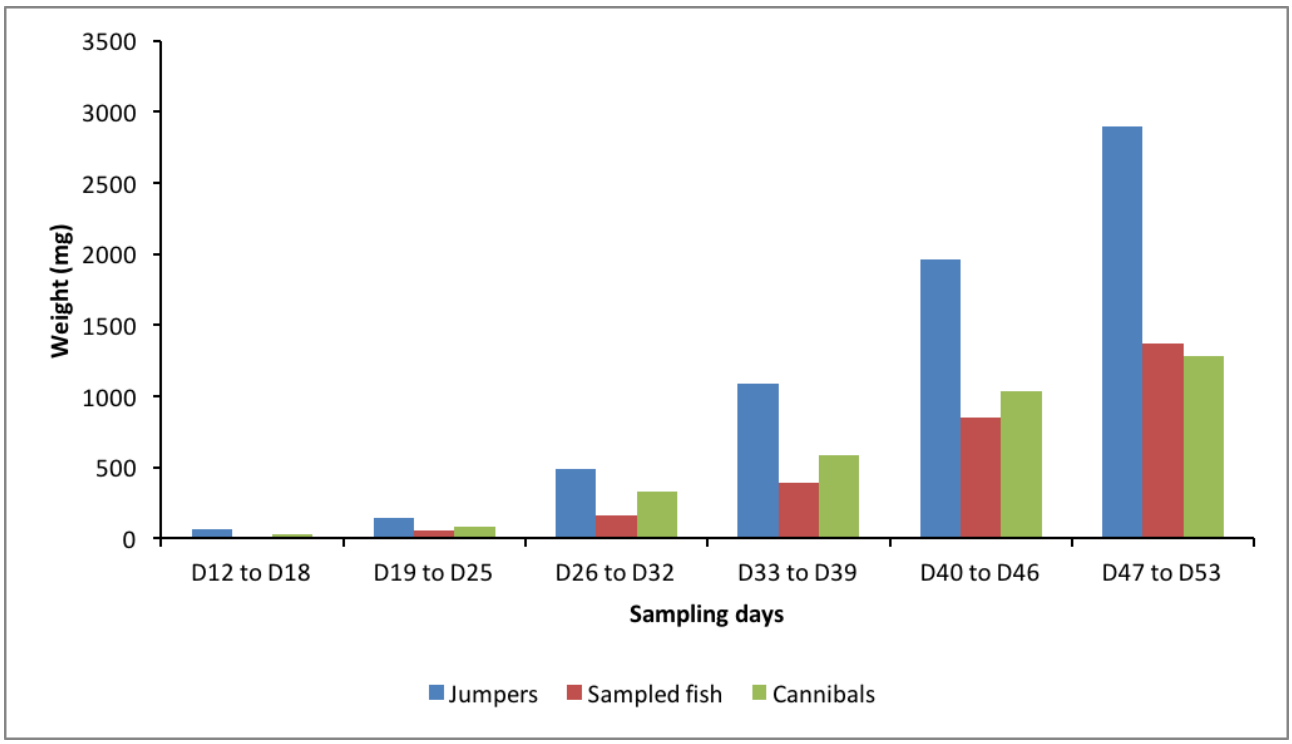

Figure 7. The mean body weight of cannibals, jumpers and randomly sampled fish taken each week.

Effects on cannibalism, swim bladder inflation rate and final biomass

Two distinct periods of cannibalism were observed where there is a high increase from 28 to 34 dph but a decrease from 35-52 dph (**Figure 8**). Furthermore, two types of cannibalism were noted: (1) attacking the tail or head of the larvae or (2) attacking the flank of a fish (**Figure 8**). There was no cannibalism observed before 17 dph.

At the end of the experiment, the swim bladder inflation rates were very high in all tanks, between 90% and 100% except for the tank 8 with a rate of 86% (**Table 4**). The final biomass harvested varied between 1.345 kg (tank 8) and 5.596 kg (tank 3) whereas survival rates oscillated between 2.7 % and 9.5 %.

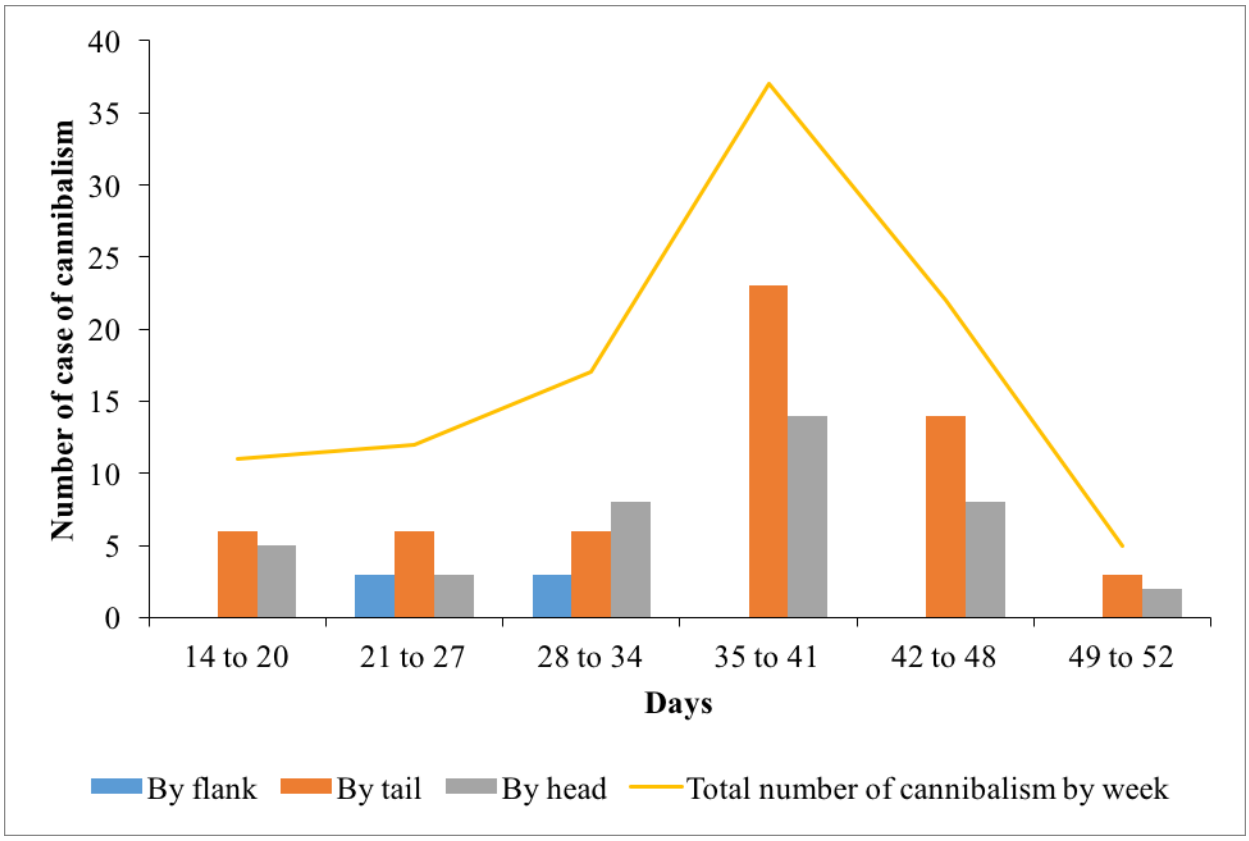

Figure 8. Evolution of the cannibalism intensity from 14 dph to 52 dph.

Table 4: Summary of swim bladder inflation rate, mean body weight, fish biomass and survival rate recorded in all tanks at 52 dph.

Tanks	Swim bladder inflation (%)	Final biomass (g)	Mean weight (mg)	Survival (%)
2	96.66	2073	1896.37	3.1
3	90.00	5596	1029.58	7.7
4	93.33	3606	1076,00	9.5
5	100.00	3527	1626.94	3.1
6	93.33	3046	1905.66	4.5
8	86.66	1345	1395.8	2.7
9	90.00	5837	1406.90	5.9

Comparison predatory abilities between cannibals and non-cannibals

Results of behavioural analyses are presented in **Figure 9** for predation and cannibalism tests. Cannibals showed better performances in the test of predation than non-cannibals' larvae. It means significant lower numbers of orientation changes, prey approaches and attacks, but significant higher captures of zebrafish larvae. Concerning the predation test, results a less clear, any significant difference was observed for the three first tests, however again cannibals had significant higher levels of congener captures.

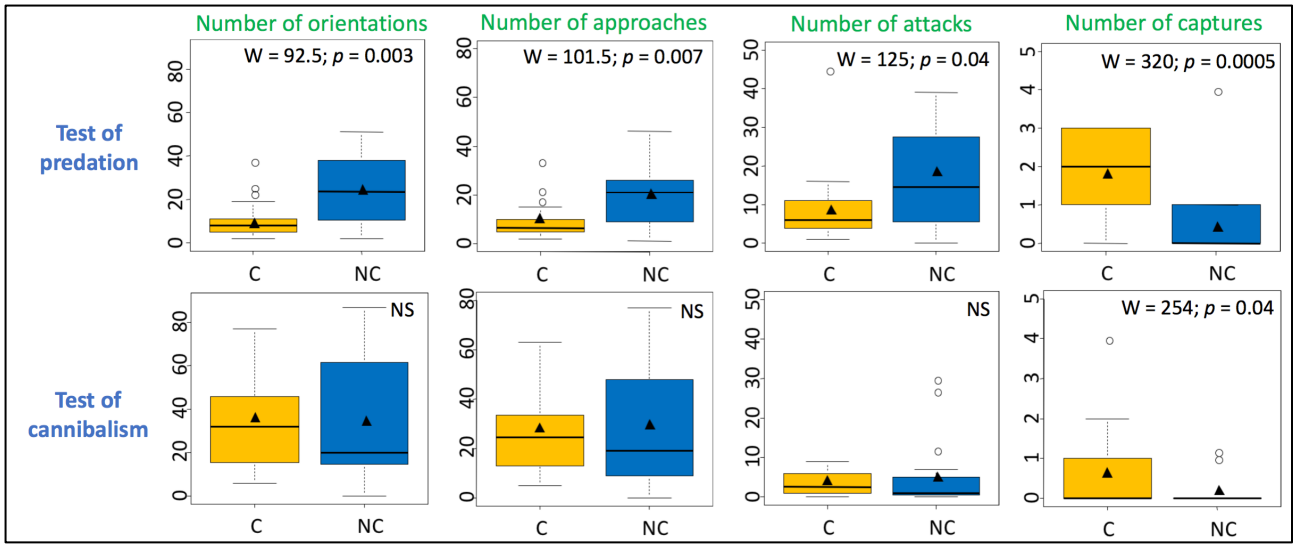

Figure 9. Comparison of the behavioural profile of cannibals (C, yellow) and non-cannibals (NC, blue) over the period 17-33 dph. The black line is the median, the black triangle is the mean, white dots are outsiders and top and bottom lines are first and third quartiles.

Discussion

In these studies, when compared to results obtained in the first (see D16.1 ***Determination of the effect of environmental factors on pikeperch larval rearing***) and second trials (see D16.2 ***Determination of the effect of nutritional factors on pikeperch larval rearing***), it was demonstrated that weaning juveniles of 1.8-1.9 g mean body weight can occur in 52 days. The mean specific growth rate (15.1 $\%.d^{-1}$) was similar to those previously calculated (16.1 and 15.6 $\%.d^{-1}$ for D16.1 and D16.2, respectively). However, the main results reported here are (1) the very high levels of swim bladder inflation (92.8% in mean) and (2) the high final biomass harvested in some tanks (tanks 3 and 9). In these tanks, the final densities were respectively (7.9 and 8.3 kg per m^3). These results constitute a marked improvement of pikeperch juvenile production in RAS conditions. To compare, in tank 9 of the D16.2 study a biomass of 3.5 kg per m^3 with 70.8% of swim bladder inflation was achieved. In term of biomass gain, the current results in tanks 3 and 9 demonstrated an increase of 130-140%. Consequently, according to previous works using similar methodology (Gardeur et al., 2007; Teletchea et al., 2009; Trabelsi et al., 2011), our multifactorial approach allows determining the combinations of factors that would be required to improve pikeperch larval rearing in RAS conditions. Two combinations (1 and 6, see table 3) appear particularly effective. 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. A similar positive effect of larval density was previously observed by Szkudlarek and Zakęś (2007), whereas Mamcarz et al. (1997) recorded negative effects on growth and survival. A high larvae density (85 larvae.l^{-1}) was also recently applied in pikeperch larval rearing in a larger tank (360 L) in a RAS system and over a longer period of time (123 days) resulting in a final mean body weight of 9.5 g (Policar et al., 2016). These authors maintained a final density of 1.1 fish.l^{-1} which corresponded to a final fish biomass of 9.6 kg per ^{m3}, which is similar to our results.
Contrary to the final weight heterogeneity, the final mean weight of juveniles (at 52 dph) depended on several interactions between factors. Firstly, a positive effect of jumper sorting was observed if larvae density is high, whereas its effect is negative if the density is low. Secondly, no effect of the type of population (sibling or mixed) was observed if the jumpers are sorted, whereas larger juveniles occur in sibling populations vs mixed if the jumpers are not sorted. The jumpers demonstrated a rapid weight gain (Figure 7) although a specific predatory activity was not exhibited and suggests why there is frequent observations of bimodal populations in tanks as in natural conditions (Mamcarz et al., 1997; Kestemont et al., 2003). As the fish density influences the cannibalism rate (Baras, 2012), this, in turn, indirectly influences the mean biomass as smaller individuals are eliminated. This impact of jumpers is strengthened in mixed populations.

The effect of larger females and the subsequent larger eggs, increases juvenile weight in sibling populations while an inverse result is obtained with mixed population. Nevertheless, weight heterogeneity was constant in all tanks and final mean weight was highly variable according to many interactions between the four factors tested.

In the cannibalism studies, the first cannibalistic behavior was observed at 17 dph which generally agrees with 14-17 dph found by Mamcarz et al. (1997) and Hamza et al. (2007, 16 dph). In comparison with our previous studies (see deliverables D16.1 and D16.2), we noted a similar level of cannibalism when considering the various types of attacks (by flank, tail or head) but differed when the peak of cannibalism occurred (D35-D41 *vs* D28-D34 in the two first experiments). This means that the number of attacks may be a function of fish density but may also be related to tank characteristics (e.g. volume, size etc.). The later appearance of the cannibalism increase in pikeperch could also be a consequence of higher fish density. Such hypothesis is in accordance with studies done on Eurasian perch in which cannibalism was lower at high than at lower fish density (Baras et al., 2003). Our behavioral study carried out some inputs to more understand the emergence of cannibalistic behavior in pikeperch. In fact predation tests revealed that cannibals show less predatory behaviours than non cannibals (less orientation changes, approaches and attacks on prey), but they are more efficient in prey capture; this last point was confirmed during the test of cannibalism. There was no effect of the tested factors on the rate of cannibalism.

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Deviations: Initially, the deliverable D16.3 was expected on month 36. This delay is explained by the fact that we have repeated this experiment, because of a very high level of mortality, due to perch rhabdovirus, observed in the first attempt in May 2016. This was the first time that this pathology was observed on pikeperch larvae (Bigarré et al., in press).

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