New species for EU aquaculture

Deliverable Report

Deliverable No:	D16.4		**Delivery Month:**	56
Deliverable Title	Identification of optimal combinations of factors for 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 for pikeperch larval rearing			
Other beneficiaries:	P3.IRTA	P21.DTU	P39.F2B	
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Table of contents

Objective:
The overall 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 (*Sander lucioperca*) larval populations. The specific objective of ***D16.4 Identification of optimal combinations of factors for pikeperch larval rearing*** was to determine the success of a farm scale larval rearing based on the most beneficial rearing factors found in previous experiments and reported in previous deliverables (D16.1-D16.3).

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 freshwater species pikeperch 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 mainly due to cannibalism, (2) high rate of deformities and (3) a large size heterogeneity between larvae cohorts at various ontogenetic development stages. 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.

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***), two combinations of factors (see D16.3) appear particularly effective (swim bladder inflation rates: 90%, higher biomass gains). These combinations resulted from the identification of the main influential factors and their modalities or best practice. However, the last experiment showed that the main positive results were obtained with optimal combinations of factors that were independent of jumper sorting and the use of sibling populations. The main objective of this deliverable was to confirm the choice of tested combinations before validation under farm conditions. The second objective was to test the presence of potential prey on the incidence of cannibalism and on the relationships between individuals and group structure. It is expected that the presence of prey decreases the aggressiveness in the group and the level of cannibalism. The third objective was to estimate the cost of production of the juveniles in this experiment.

Material and Methods

Rearing System

The study was carried out in an experimental recirculating aquaculture system (RAS) with ten 700 l tanks. Eight of these tanks were used for fish rearing while one tank (1) served as a moving bed filter (Biological filter) and the last one was not used (water without bed filter). Water was also mechanically filtered and treated with an UV sterilization unit (**Figure 1**).

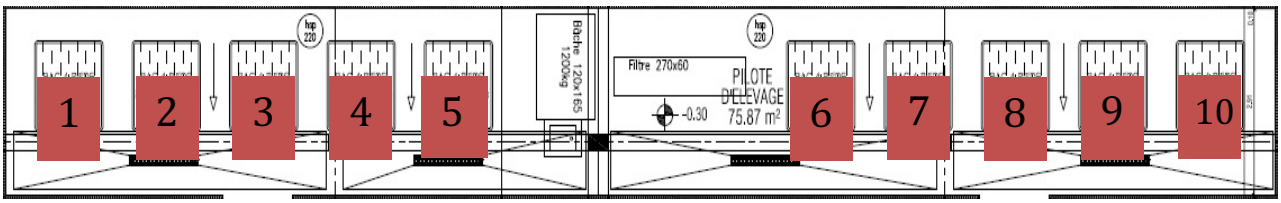

Figure 1: Diagram of the experimental facility. The closed loop consisted of 10 tanks with a central filter. Tanks 2 to 9 were used during the entire experiment, while tank 1 served as an additional moving bed filter.

Biological material: pikeperch larvae

Larvae (560.000) were obtained from a local broodstock maintained at SARL Asialor (Pierrevillers, France) and transferred to the UL experimental platform (UR AFPA, Vandœuvre-lès-Nancy, France). The broodstock was the same as used for the three previous experiments. Larvae hatched from February 19th, 2018 and were distributed to the 8 tanks, where water temperature was initially at 15-16°C. All larvae came from two females (3.90 and 4.47 kg body weight) where the eggs were stripped on February 10th, 2018 and then fertilized by mixing with sperm from 3 males. After hatching, the larvae from the two females were mixed and divided into 8 separate bags of 70000 larvae each for transportation to the research facility.

Standardized rearing conditions

The tanks were stocked with 70.000 larvae each (100 larvae l^{-1}). The photoperiod was fixed at 12h of light and 12h of darkness (Hamza et al. 2007) with a light intensity of 50 lx (see Deliverable D16.1) and a dimming period of 30 minutes in the morning and evening. Temperature was the same for all tanks ranging between 16 and 20°C. Temperature was initially increased incrementally by 1°C day^{-1} until 20°C. Dissolved oxygen was maintained above 6 mg l^{-1} and salinity was fixed below 1 ‰. In this experiment, environmental factors (light, water renewal rate, water current direction and cleaning period) were the same as for the second experiment. The feeding strategy and the population factors were carried out according to best protocol obtained in the second and third experiment (**Table 1**). From 0 to 25 dph a surface skimmer was placed in each tank to remove the oil on the water, which interferes with swim bladder inflation.

Table 1: Applied modality for each factor. This combination of factors was repeated in the 8 experimental tanks (n = 8).

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

Feeding

The first feeding occurred at 4 days post hatching (dph), where larvae received *Artemia* nauplii (480 µm; Premium Artemia Cysts, Salt Lake Aquafeed, Utah, USA) until weaning. Weaning occurred between 16-24 dph with mixed feeding using an inert food (Prowean 300, BioMar, Århus, Denmark) and live preys, where the proportion of live prey was decreased over a three day interval (**Table 2**). After this period, larvae were exclusively fed inert feed of increasing particle size (**Table 2**). After weaning, inert feed was provided in excess over the whole experimental period. At the end of the experiment (53 dph), a feeding ratio of 4% was applied.

Table 2: Ratio of *Artemia*/Prowean applied during weaning period and feed size.

Dph	16-18	19-21	22-24	22-36	37-47	48-52
Ratio *Artemia*: Prowean (%)	75:25	50:50	25:75	0:100	0:100	0:100
Size inert feed (µm)	300		500	500	700	800

Measures, sampling and observations

The physical-chemical properties of the water were monitored regularly to ensure optimal conditions: Oxygen level and pH were checked every morning 1.5 hours after the first feeding; ammonia and nitrite concentrations were titrated twice a week. Water pH was corrected by regular inputs of NaHCO3.

The experiment lasted 53 days (from February, 19th until April, 12th 2018). Larvae (n=30) were sampled every 7 days and designated as days after first feeding: T0, T7, T14, T21, T28, T35, T42 and T49. For sampling, a group of larvae were caught with a net and transferred into a small basin, counted and placed in sampling tubes. Larvae caught 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. After this, the water was removed by placing fish in a fine mesh dip net and then transferring the larvae to 4% buffered formalin.

The parameters measured included: (a) morphometric indicators (total length; TL, body weight; W, coefficient of variation of total length; CV TL and coefficient of variation of weight; CV W) carried out on 30 larvae tank^{-1} date^{-1} and (b) behavioural indicators derived from five-minute observations of each tank carried out twice a week at the same time in order to detect cannibals. Cannibalism was identified as partially swallowed conspecifics or attempted biting or charging at conspecifics. (c) At the end of the 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 by lightly anesthetizing them with a MS222 (70 mg l^{-1}) solution in salted water (20 g of salt l^{-1}) similar to the protocol of Jacquemond (2004a, b). Initial biomass was calculated with the average weight of sampled 4 dph fish and multiplied by the total number of initially stocked fish. The specific growth rate (SGR = $(\ln(W_{final})-\ln(W_{initial}))/days*100$) is calculated over the whole experimental period (4-53 dph). Feed conversion ratio (FCR = $W_{feed\ dry\ matter}/ W_{final\ fish\ fresh\ weight}$) is based on feed dry weight fed (Artemia and inert feed) divided by final fish fresh weight. All presented data was calculated and measured before swim bladder sorting.

In order to assess the effects of potential prey on cannibalism in pikeperch larvae, an additional experiment was performed. In 16 small enclosures (volume of 3l) belonging to two separated hatcheries, we tested the effect of potential prey on mortality rate, cannibalism, growth (total length and weight), and relationships between individuals in an enclosure. In a second experiment, we tested the influence of potential prey on the group structure through a behavioral assay. For the first experiment, 50 individuals were placed in a small enclosure under two conditions: with or without potential prey. Larval behavior was recorded on a daily basis and subsequently reviewed for analysis. For the second experiment, to study the group structure and the relationships between individuals, ten pikeperch larvae per enclosure were placed in small arena (30cm in diameter). After a 30-minute acclimatization phase their behavior was recorded for one hour. The group structure was characterized by the distances between individuals (closest distance to a conspecific, mean distance and the variance of the distances between individuals of the group): moreover, the activity (time of swimming) and relationships between individuals (contact or not) were analyzed

For the production cost analysis, costs for electricity, heating/cooling, *Artemia* production, feed, labor, general analytical costs (water analysis, small equipment), other expenses (water and sodium bicarbonate) and depreciation of facility and equipment (5% per year over 20 years) were calculated.

Results

Tank 4 was taken out of the experiment because of abnormal and unexplained low initial density. Consequently, the number of replicates was reduced to 7.

Water Quality

Average dissolved oxygen concentration was 6.08 mg/l. The lowest oxygen level measured was 4.13 mg/l in tank 5 at 40 dph but did not last longer than one day (**Table 3**). The average TAN concentration was 0.63 mg/l with a peak of 3.55 mg/l at 45 dph. The peak of 3.55 mg TAN /l was due to a mismanagement of the pH which dropped to 5.86. The low pH led to a drop in biofilter performance resulting in the increase of TAN. However, no increased mortality was observed in this period. The pH was gradually corrected up to 7.14 within 3 days to avoid ammonia toxicity due to a steep increase of pH. Average $N\text{-}NO_2$ was 0.21 mg/l with a maximum concentration of 0.75 mg/l. The pH was maintained on average at 6.98 with a minimum of 5.86 (at 45 dph) and maximum of 7.97 (**Table 4**).

Table 3: Average, lowest and highest measured oxygen concentrations for each tank and calculated overall tank average from 0 to 52 dph.

Tank	Average oxygen level (mg/l)	Lowest oxygen level (mg/l)	Highest oxygen level (mg/l)
2	6.00 ±0.84	4.46	7.87
3	6.34 ±0.62	5.01	7.62
5	5.95 ±0.73	4.13	7.30
6	5.97 ±0.85	4.45	7.72
7	6.11 ±0.94	4.58	8.03
8	5.95 ±0.81	4.22	7.30
9	6.20 ±0.67	4.85	7.73
Average	6.08 ±0.79	4.13	8.03

Table 4: Concentrations of total ammonia nitrogen (TAN), nitrite nitrogen ($N\text{-}NO_2$) and pH with their corresponding average, maximum (Max) and minimum (Min) values over the whole experiment (0-53 dph).

Tanks	TAN (mg/l)	$N\text{-}NO_2$ (mg/l)	pH
Average	0.63 ±1.06	0.21 ±0.23	6.98 ±0.46
Max	3.55	0.75	7.97
Min	0.00	0.00	5.86

Fish performance

At 4 dph, the mean body weight of larvae varied between 0.49 mg and 0.57 mg. Final mean body weight was between 710 and 940 mg at 53 dph (**Table 5**). The average 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, 53 dph) (**Table 5**). With the sampled individual weight over the whole period a growth curve was plotted (**Fig. 2**). Furthermore, the function equation was calculated with the help of a fitted trend line. The final biomass harvested varied between 9.075 kg (tank 3) and 9.754 kg (tank 5). Survival rate averaged 14.9% over a period of 49 days (**Table 4**). Highest survival was found in tank 2 with 19.2% and lowest survival was obtained in tank 9 and 6 (13.7%). The swim bladder inflation rate was on average 92.6% (**Table 5**). All tanks except tank 5 (88.1%) showed a swim bladder inflation rate above 90%. The feed conversion ratio (FCR) showed little variation between tanks and averaged at 0.66. The results in **Table 5** were calculated with values before the swim bladder sorting.

Table 5: 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	0.48±0.13	816.0 ±248.8	14.9	15.1	0.66

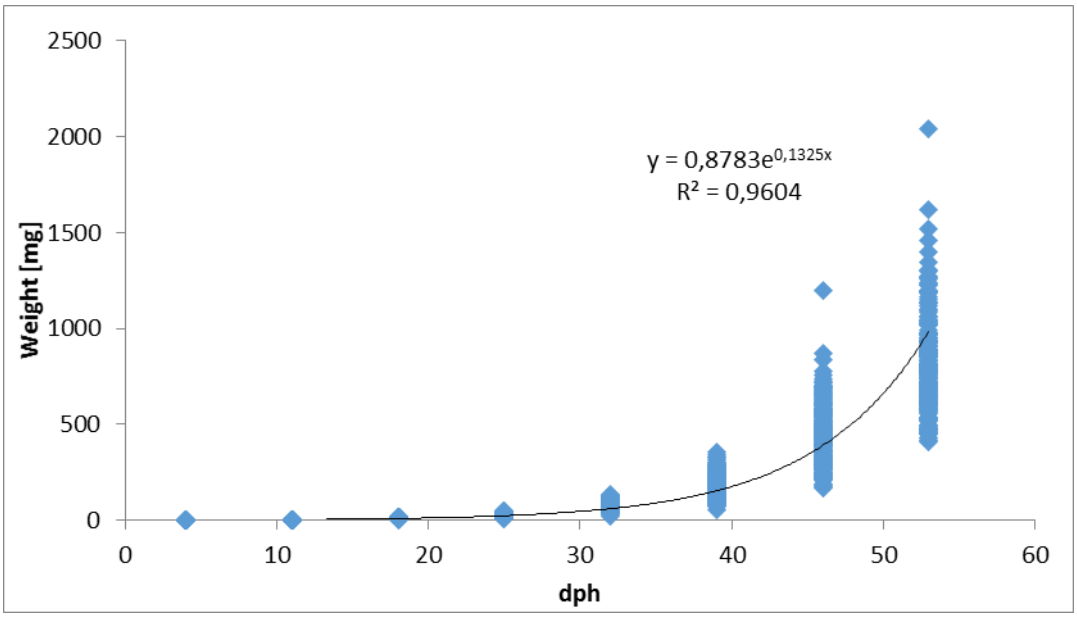

Figure 2: Average growth curve (black) of juvenile pikeperch larvae and juveniles reared in RAS (n = 210).

Cannibalism

An increase in cannibalistic attempts (attacks and bites) was observed at 19 dph, they peaked between 19 and 25 dph while first observed cannibalism was at 33 dph and peaked between 40 and 44 dph. **(Fig. 3**). The coefficient of variation (CV) for size and weight for each sampling day was plotted and is represented on the secondary axis of **Fig. 3**. The CV for weight was, apart from 18 dph, consistently increasing over the experimental period to peak at 39 and 46 dph (**Fig. 3**).

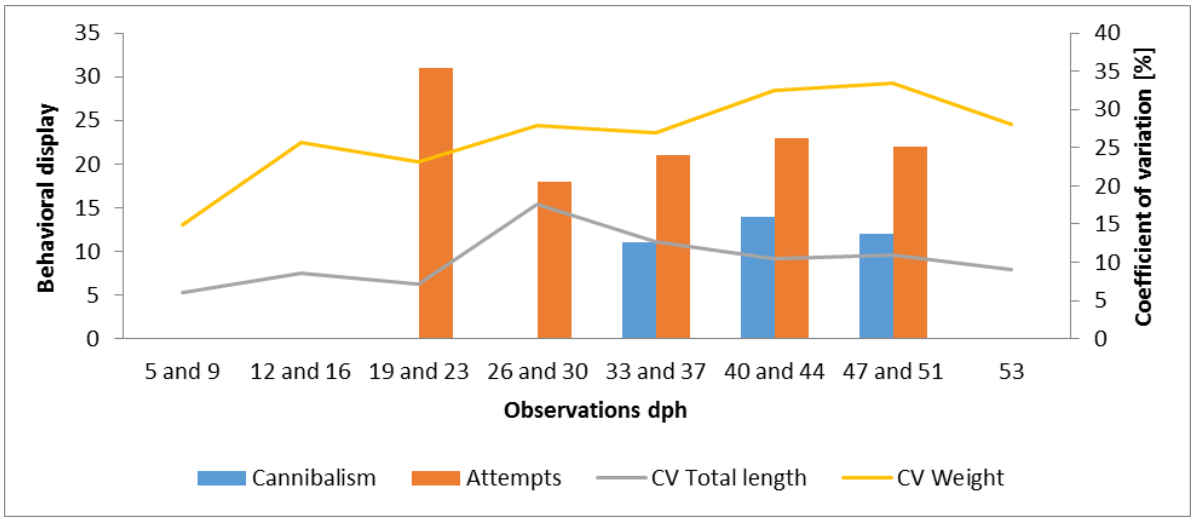

Figure 3: Overview of cannibalistic behaviour and coefficient of variation in size and weight from 5 dph to 53 dph.

At 53 dph the CV of weight decreased again to 28%. At the beginning of the experiment, from 4-22 dph the CV of length varied between 6-9% (**Fig. 3**). A peak of 18% was observed at 25 dph. Thereafter, CV of length was almost continuously decreasing down to 9% on 53 dph. The absence of cannibalistic behaviour at 53 dph was because this was the end of the experiment and no behavioural observations were carried out that day. However, data on size and weight were evaluated.

The results of the experiment with the perch larvae as prey showed that there was no significant difference in mortality rate between the presence or absence of prey but the rate of cannibalism was higher when the prey were present (**Fig. 4**) as well as the length and biomass (**Fig. 5**). There was no significant difference in relationship parameters between individuals (attacks, pursuit, bite and ingestion) or for parameters characterizing the group structure of larvae (closest distance to conspecific, mean distance and its variance between individuals of the group) as a function of prey presence.

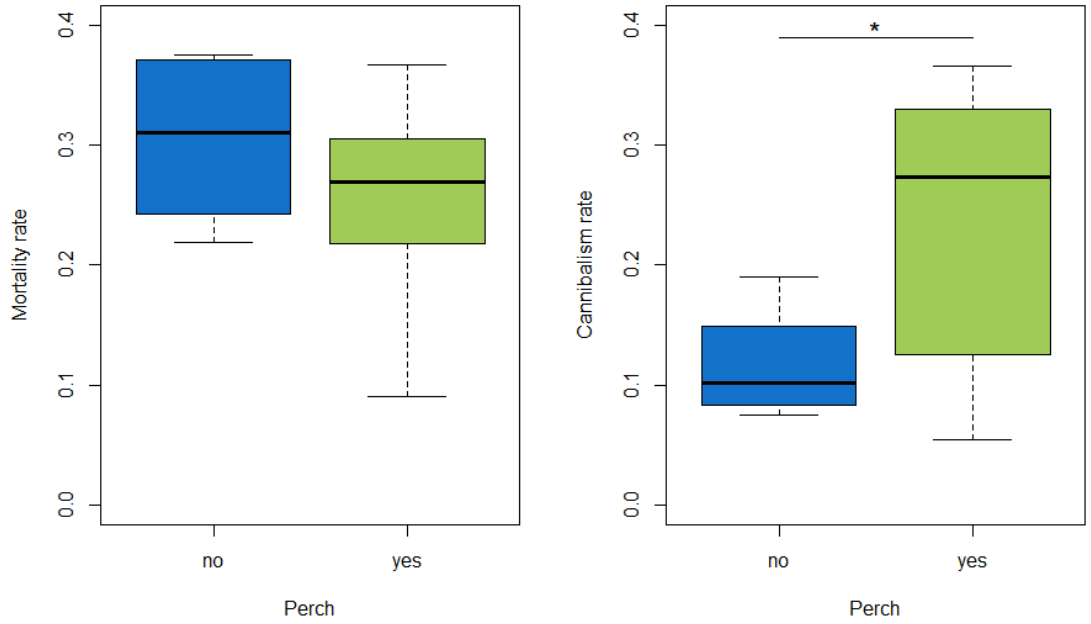

Figure 4: Rate of mortality and cannibalism in the presence or absence of prey. Box plots indicate the median, the1st and 3th quartiles. The * indicates a significant difference at the level of $p<0.05$.

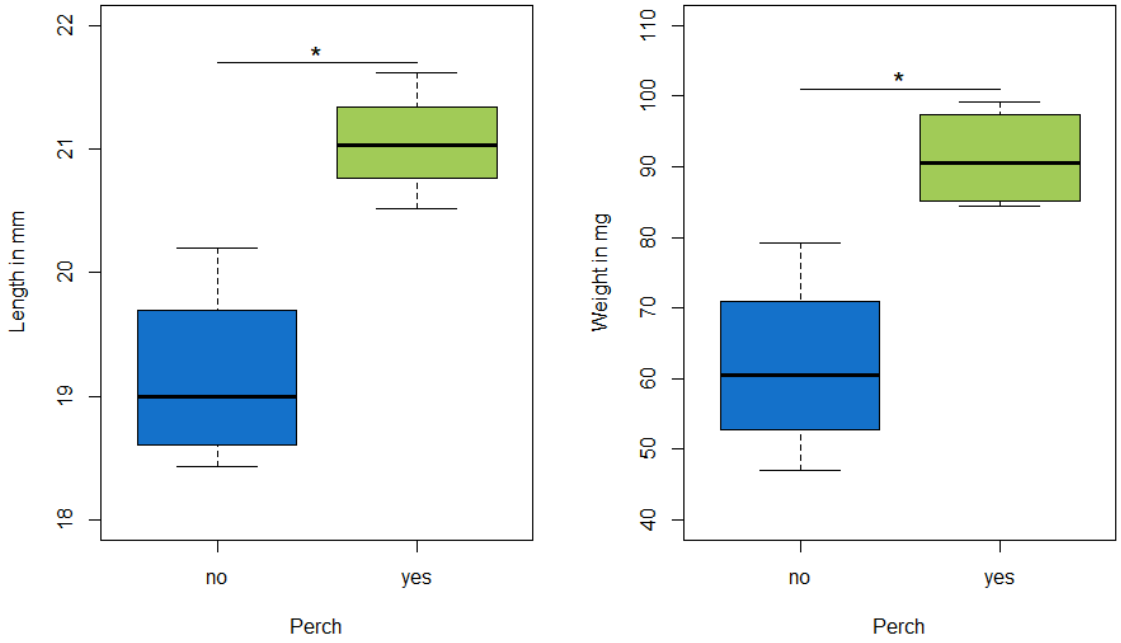

Figure 5: Length and mass of pikeperch larvae in the presence or absence of prey at the end of the experiment. Box plots gave the median and 1st and 3th quartiles. The * indicates a significant difference at the level of $p<0.05$.

Production costs

In our experiment we produced juveniles for 249 € per kilogram (**Fig. 6**). This results in a price of 0.20 € per juvenile with an average weight of 816 mg. The highest expenses were labor costs with 90 € per kilogram (36%). Second and third largest expenditures were the purchase of eggs and general costs, which were 50.5 and 48 € respectively (20% each). Other costs were depreciation (24.5 €), energy (21.6 €), feed (12.7 €) and miscellaneous expenses (1.70 €).

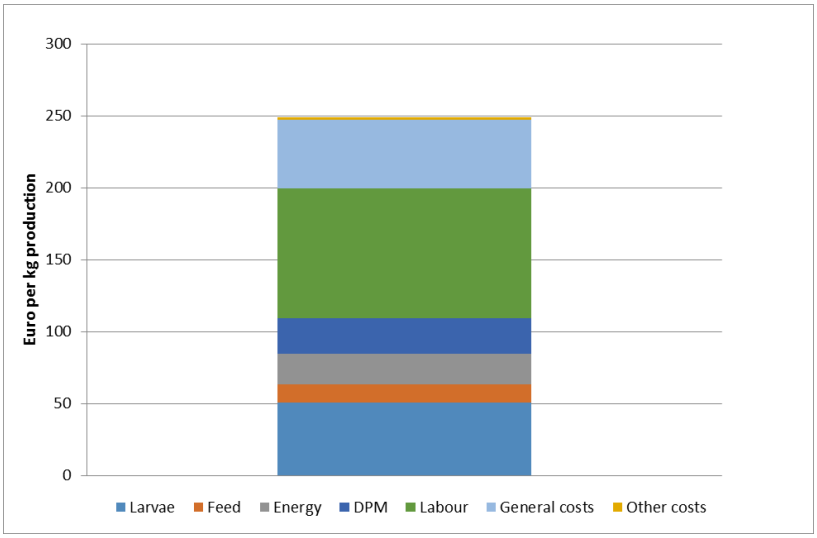

Figure 6: Overview of production costs per kilogram of juveniles produced.

Discussion

In this study it was confirmed that raising juveniles of 0.7-0.9 g mean body weight can occur in 52 days. The mean specific growth rate (15.1% d^{-1}) was close to the values obtained in previous experiments with the same duration (specific growth rate: 15.6 and 15.1% d^{-1} for D16.2 and D16.3, respectively). The final mean individual body weight (816 mg) was lower than those of previous trials (average body weight: 911 and 1471 mg for D16.2 and D16.3, respectively). However, the average survival rate of 14.9%, final biomass harvested of 9.6 kg and the final stocking density (13.6 kg/m^3) were the highest of all our experiments (**Table 5**). A trade-off between individual growth performance (final individual weight) and overall tank performance (survival, final stocking density) seems evident. The increased survival rate and the resulting high final stocking density obtained in this experiment could have negatively impacted the individual final weight and length. Szkudlarek and Zakes (2007) also found a negative relationship between individual growth performance and stocking density. However, they found a negative effect of higher stocking densities on survival rate which was not the case in this experiment. Due to the increased numbers of individuals, access to feed might have posed a problem resulting in a decreased growth performance. The foraging area in the tank was limited since the automatic feeder was placed on the side of the tank and not above. This could have influenced feed acquisition due to high fish densities below the feeder and unfavourable larval distribution in the tank. Furthermore, in the last week a small amount of feed was observed on the tank bottom indicating possible underfeeding but increasing the ration was avoided as a serious deterioration of the water quality was feared as the mechanical

filter was at its operational limit. The approach to the maximum systems carrying capacity was also reflected by the oxygen saturation and the total suspended solids (TSS) in the water. Oxygen level was on average 6 mg/l which is below the recommended minimum of 7 mg/l (Szkudlarek and Zakes, 2007). This in return could have lowered individual feed intake resulting in lower growth and final weight (Kestemont and Baras, 2001; Glencross, 2009). We did not measure total suspended solids in the water but by observations it was evident that TSS steeply increased towards the end of the experiment. This could have had negative effects on fish growth performance and survival. However, the effects of TSS concentrations on fish health are still a matter of debate but are generally linked to harmful effects on gill structures and elevated stress levels (Becke et al., 2018). On the other hand, in walleye (*Sander vitreus*) it has been shown that turbid water has a positive effect on growth and survival (Bristow and Summerfelt, 1994). Nevertheless, we are certain that growth results obtained in this experiment would be higher with an appropriate mechanical filtration unit.

The higher survival rate obtained in this experiment might have been influenced by some external factors such as the age and size of the broodstock. For all four experiments the same broodstock was used. Subsequently the broodstock aged and grew in size over this time. Furthermore, in the first experiment (D16.1) the broodstock's first spawning was used. In previous studies the weight of spawning females was found to be directly correlated with the size of the eggs and newly hatched larvae, which in turn, influences larval viability (Marteinsdóttir and Steinarsson, 1998; Johnston, 1997; Kamler, 2005; 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. Lastly, the company we obtained the larvae from gained valuable experience during the years of the Diversify project. This resulted in improved rearing protocols for the broodstock which most likely had a positive effect on the larval quality (e.g. survival rate, deformities).

Although facing some difficulties regarding the systems carrying capacity, the results in this experiment constitute a marked improvement of pikeperch juvenile production in RAS conditions. This indicates that the multifactorial approach used in our previous experiments was successful in identifying beneficial combinations of factors required to improve pikeperch larval rearing under RAS conditions. That point is strengthened by the fact that results are relatively homogenous between all the seven replicates.

For the cannibalism study, the first cannibalistic attempts were observed at 19 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, D16.2 and D16.3), we observed a similar level of cannibalism when pooling the cannibalistic behavior and the attempts. However, the peak of cannibalism was found to be between D40-D46 which is two weeks delayed compared to the first two trials (Deliverables 16.1 and 16.2 at D28-D34) and one week later than the third trial (Deliverable 16.3 at D35-D41). The later appearance of the cannibalism increase in pikeperch could be a consequence of higher fish density. This hypothesis is in accordance with studies done on Eurasian perch in which cannibalism was lower at high fish density (Baras et al., 2003). However, since stocking density and growth performance appear to be negatively interlinked with each other, individual's weight and size might also play a role in the exhibition of cannibalism. This is supported by the observed one-week delay of growth compared to the previous trial (D16.3). Furthermore, a delay in growth performance might be accompanied with lower size heterogeneity since heterogeneity increases over time. This might lead to a delay of cannibalism because increased size heterogeneity encourages this phenomenon (Kestemont et al., 2003). Observations on cannibalistic behavior appeared to be more difficult than in our previous trials due to the high stocking density in the tanks interfering with observing fish at depth, which worsened towards the end of the experiment. The higher TSS towards the end of the experiment resulted in

an increased turbidity of the water, which was a further impediment observing the fish. Thus, it is possible that we underestimated cannibalism especially towards the end of the experiment (40-51 dph).

Concerning the behavioral study, the results of the experiment where food was supplemented with live prey (perch larvae) were not as expected. Previous results (see deliverables 16-2 and 16-3) suggested that cannibals were individuals that developed their predatory behavior earlier than their conspecifics. In monoculture farming, there is a high density of individuals and the only prey available are conspecifics which leads to cannibalism. The hypothesis was that the introduction of real prey in the enclosures might lead to a decrease of the cannibalism rate. This was not the case. It is possible that the prey density (ten larvae per day for 50 pikeperch larvae) was not sufficiently high enough since other authors (Brownell, 1985; Macpherson and Gordoa, 1994; Fortier et al., 1996; Bell et al., 1999; Baras et al., 2000) found a reversed result by having large numbers of prey. Another possibility is that the presence of prey in the rearing environment generally improves the predatory capabilities of the cannibals. Thus, they become more efficient in capturing all prey types (perch and conspecifics). Lastly, the presence of perch larvae could have stimulated predatory behavior. This combined with a possible shortage of prey might have resulted in the observed increase of cannibalism.

Regarding the production costs it has been shown that juveniles of 0.8 g average can be produced for 0.20 €. However, this price is based on rearing in an experimental facility which was designed for task efficiency and not cost efficiency (for example, the biofilter was not designed for mass and intensive production). Thus, the production costs in a farm might be lower in terms of general costs and energy. Important to mention here is that the energy (electricity and cooling) cost was calculated for the spring but can vary seasonally. Furthermore, labor was not optimally used since the workload was low especially in the afternoon. However, one person was needed to ensure regular feeding throughout the day and be present in case of emergency. With a larger volume of fish produced, workforce probably could be scheduled more efficiently, resulting in a lower labor cost per kilogram of juveniles. To the best of our knowledge the current most requested marketable size for juveniles is 10 g which is much larger than the juveniles harvested in the current experiment. To reach this size it would need an estimated extra growth period of 60 days. We do not recommend extrapolating our results to this period due to various reasons. First, a bigger facility would be needed to redistribute the growing juveniles in order to maintain a beneficial stocking density and ensure water quality. Second, different feed types (*Artemia* and inert feed) and feed sizes (300, 500, 700, 800µm) were used throughout the experiment with each having a different price. Usually with decreasing feed prices for larger pellet sizes which would affect the feed costs. Lastly, seasonally variable energy expenses will also impact on production costs if the growth period is extended to 10 g juveniles.

To conclude, this fourth experiment allowed us to identify an optimal and reliable combination of environmental, nutritional and population factors that improves pikeperch larvae survival and final juvenile biomass in rearing tanks with very high swim bladder inflation and low deformities rates.

Deviations: Initially, the deliverable D16.4 was expected on month 48, which means a delay of 8 months. This delay is explained by the fact that we have repeated previous experiments, because of a very high mortality caused by perch *perhabdovirus*, observed in the first attempt in May 2016. This was the first time that this pathology was observed on pikeperch larvae (Bigarré et al., 2017). As a consequence, the present experiment was also delayed.

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