Deliverable Report

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Deliverable Title	Determination of the effect of environmental factors on pikeperch larval rearing			
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Task Title:	Optimal combinations of factors to improve larval rearing			
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Objective:

The objective of this deliverable was to determine the effects of four environmental factors and their interactions on pikeperch larval rearing using a multifactorial approach. This is the first of four consecutive experiments. Each factor will be tested in two modalities. The objectives are to identify the bottlenecks for fish survival and growth and to establish the optimal combination of factors, which could give the best development of the fish populations.

Introduction:

There exists a strong incentive to increase the production of intensive aquaculture products as a response to the decrease of natural populations. In this context, the European aquaculture industry aims to promote efficient and sustainable production of safe aquatic food 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 fish farming. Among these species, a fresh water species, the pikeperch (*Sander lucioperca*), was selected.

Pikeperch has gained attention as a potential and promising new species in intensive fish farming (Nyina-wamwiza et al. 2005) having high commercial value. Until now several bottlenecks prevented the success of the rearing of the larvae. Three bottlenecks have been identified: a high rate of mortality due mainly to cannibalism, a high rate of deformities and a large growth heterogeneity characterized by important differences in size between larvae of various ontogenic development stages. The team DAC of the unit UR AFPA (University of Lorraine, France) will lead the work studying the optimal combination of factors that can improve the production of larvae of pikeperch. Four experiments were planned with four factors being tested in each experiment utilizing a multifactorial approach.

Description:

The present deliverable aims to investigate the effects of four environmental factors (light intensity, water renewal rate, water current direction and time of cleaning) on the development of the larvae of pikeperch. These results were those of the first experiment. After the end of this first multifactorial experiment, a behavioral experiment was carried out using juveniles at day 50 post-hatching.

Material and Methods

Multifactorial experiment

A multifactorial experimental design was developed to study simultaneously the effect of factors and their interaction on pikeperch larval rearing using a factorial design. Experimentally, the effects of the light intensity, water renewal rate, water current direction and time of tank cleaning have been studied. The choice of these factors was a trade-off between what is reported in the literature and the constraints of our system (*i.e.* the impossibility for varying the temperature in each tank). As this study aims to produce an optimal set of factors for use by the fish farmer, we take into account the constraints (costs in terms of economic efficiency of human resources) linked to the use of particular modalities of each factor (i.e. the cost of energy for lighting, which is a trade-off between the cost of the electricity and the optimal light for the development of the larvae). The modalities for each of the four latter factors are in accordance with the results of previous studies (**Table 1**).

Light intensity: according to several studies (Summerfelt 1996; Hamza et al. 2008; Lund and Steenfeldt 2011; Lund et al. 2012; Francesconi 2014) that showed that pikeperch is very sensitive to high light

intensities (above 200 lux), we chose the two following modalities: 5 lux and 50 lux. These modalities are in the range of previous studies.

Water renewal rate: based on previous publications (Szkudlarek and Zakes 2007; Lund and Steenfeldt 2011; Lund et al. 2012; Ott et al. 2012) that found that the water of the tanks should be recycled in accordance with the two following modalities: 50% and 100% per hour.

Water current direction: the water direction in the tank may be important because it may influence the position of the larvae in the water column and their swimming activity. Water current direction may influence mortality, deformities (related to the non-inflation of the swim bladder, Summerfelt 1996) and lower growth rate of the larvae. We chose to place the water inlet at the water surface or at the bottom in the tank.

Timing of tank cleaning: siphoning tank period can be considered as a stress period for larvae. It can influence the behavior of the larvae (foraging and swimming) but also the water quality. It will be done at two different times, in the morning after the first feeding or in the afternoon after the last feeding of the day.

Table 1. Applied modalities for each factor.

Factor	Modality 1	Modality 2
Light intensity (lux)	5	50
Water renewal rate (%)	50	100
Water current direction	At the water surface	At the bottom of the tank
Timing of tank cleaning	Morning	Afternoon

Materials

We used an indoor water recirculated aquaculture system (RAS) comprised of 10 tanks of 700 l each and distributed in 2 rows with a common water recycling sequence based on a mechanical filter, a biological filter and a UV sterilization unit (**Figure 1**). For our experiment, only 8 tanks (2 m^3) were used.

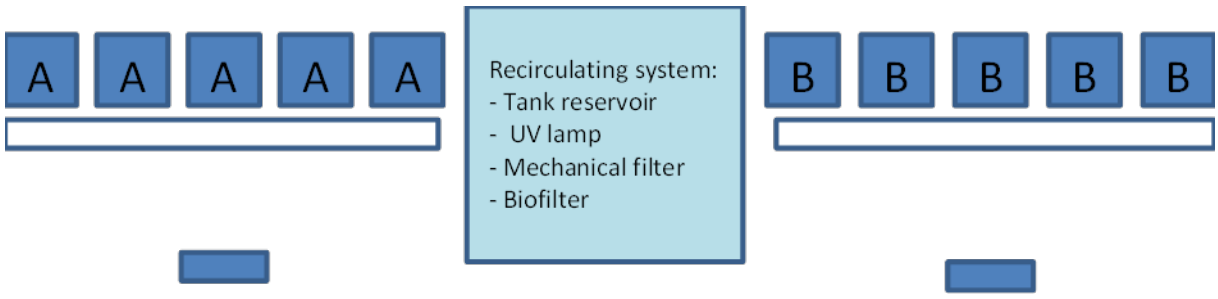

Figure 1. Diagram of the experimental facilities.

Biological materials: pikeperch larvae

From the spawn of a domesticated broodstock 500,000 newly hatched larvae (<1 dph) were obtained from Asialor (Pierrevillers, France) and transferred to the UL experimental facilities (UR AFPA, Vandœuvre-lès-Nancy, France). Then larvae were distributed into 8 tanks, where water temperature was initially kept at 15-16°C.

Standardized rearing conditions

Photoperiod was fixed at 12 h of light and 12 h of darkness (Hamza et al. 2007) with a progressive increase of light intensity (from 0 to 5 or 50 lux) from 07:30 to 08:00 and a decrease of light intensity (from 50 or 5 to 0 lux) from 19:30 to 20:00. Temperature was similar in all tanks and ranged from 16 ^{0}C and incrementally increasing by 1°C per day to 20°C (Hamza et al. 2007; Kestemont et al. 2007; Szkudlarek and Zakes 2007). The frequency of feeding was a meal every 1.5 hours during the light period (Hamza et al. 2007, 2008, 2010, 2012; Kestemont et al. 2007). Dissolved oxygen was maintained above 6 mg l^{-1}, corresponding to 64% and 66% of saturation at 19°C (9.26 mg l^{-1}) and 20°C (9.07 mg l^{-1}), respectively. The initial number of larvae per tank was around 62 500 (90 larvae l^{-1}). The experimental combinations of factors were applied according to an experimental matrix (**Table 2**).

Table 2. Experimental matrix applied.

Tank	Combination	Light intensity (lux)	Water renewal rate (% per hour)	Water current inlet	Timing of tank cleaning
A4	4	5	100	Surface	Afternoon
A3	6	5	50	Bottom	Afternoon
A2	8	5	100	Bottom	Morning
A1	2	5	50	Surface	Morning
B1	3	50	100	Surface	Morning
B2	1	50	50	Surface	Afternoon
B3	5	50	50	Bottom	Morning
B4	7	50	100	Bottom	Afternoon

Feeding

Live prey: the first feeding occurred at day 4 (post-hatch), where larvae received small size *Artemia* nauplii (430 µm; Catvis, Hertogenbosch, The Netherlands) until 6 dph. Then small nauplii were replaced by larger ones (550-600 µm; Catvis, Hertogenbosch, The Netherlands) until weaning. A co-feeding was carried out from 8-15 dph with a formulated diet (Prostart 100, BioMar, Århus, Denmark). Weaning occurred between 16-19 dph with mixed feeding using an inert food (Prowean 100, BioMar, Århus, Denmark) and live prey, which decreased (**Table 3**). The ration level of inert food decreased gradually until reaching a value of 3% at the end of the experiment (60 dph) (**Table 4**).

Table 3. Ratio of *Artemia*/Prowean applied during the weaning period (Kestemont et al. 2007).

DATES	**DPH16**	**DPH17**	**DPH18**	**DPH19**
RATIO OF ARTEMIA/PROWEAN	75 : 25	50 : 50	25 : 75	0 : 100

Table 4. Feeding rate.

Dates	**D16-D20 (Hamza et al. 2007)**	**D21-D37 (Hamza et al. 2007)**	**D38-D64 (BioMar)**
Feeding rate (%)	15	10	3

Measures, sampling and observations

The physical-chemical properties of the water were monitored regularly to ensure optimal conditions: oxygen level was checked every morning (> 6 mg); ammonia and nitrite concentrations were measured twice a week and maintained at a level lower than 0.05 and 0.01 mg L^{-1}, respectively while pH was stable at 8.00 ± 0.10. The experiment lasted 35 days (from the 30th of January until the 6th of March 2015). Larvae were sampled every 7 days as follows: T0 (at first day of feeding), T7, T14, T21, T28 and T35. For each sample, larvae were siphoned into a basin then pipetted, counted and distributed in the different sample tubes. Larvae siphoned in excess are put back into their original tank. This method allowed limiting losses due to sampling with a net. Sampled larvae in the tubes were then sacrificed (with lethal dose of MS222), drained of excess water (fine mesh dip net) and put in buffered formalin 4%.

Several measurements were carried out and included (1) Morphometric measures (total length TL, body weight W, coefficient of variation of total length [CV TL] and coefficient of variation of weight [CV W]) using 60 larvae per tank per sampling date. (2) Deformity analyses: (e.g. skeleton and jaw deformities) using 60 larvae per tank per sampling date. (3) Histological analysis (intestinal development, retina development and jaw development) using 40 larvae per day per sampling date and (5) Observations during 5 min/day of each tank in order to detect cannibalism.

Behavioural experiment

A complementary experiment was carried out in March 2015 in order to evaluate whether personality traits (Pasquet et al. 2015, Ferrari et al. 2014) could be linked to cannibalism. This experiment was made on fish, coming from the multifactorial experiment, which were tested between 50 and 64 dph. Overall, 42 pikeperch juveniles (5.78 ± 1.01 cm in total length) were submitted to two tests each day; cross-maze test and dyadic test. The cross-maze test allowed us to evaluate the activity level as well as the exploration and the boldness of fish (Ferrari et al. 2014). The fish was placed in a start zone, and after 5 min acclimatization period, the door was gently opened and video recorded during 30 min. For each fish, we took into account the duration of the total swimming, the latency to emerge from the start zone (s), the duration outside the start zone (s), the number of zones (among 5) visited. The dyadic test allowed us to evaluate the sociability and the aggression level of fish (Ferrari et al. 2014). This test was carried out in aquaria where fishes were left together during 30 min. The following behaviors were analyzed: number of orientations and approaches. The whole experiment was video recorded (camera HDR-CX550VE). Behavioral variables (activity, boldness, exploration) were analysed with The Observer XT software (Noldus, The Netherlands, version 10.0).

Statistical analyses

For the multifactorial experiment, statistical analyses were carried out with Analysis software (Kobilinsky, 2000). The detection of the potentially active effects of tested factors on the output variables was given by Daniel's graphics (Half Normal probability plot of basal estimation function, Daniel, 1959) using an oversaturated model of variance analysis. The interactions between 3 or more factors were considered insubstantial. When an interaction between two factors was found significant ($P < 0.05$), the potential single effects of these factors were also considered insubstantial. Data are presented as means ± confidence interval (95%) calculated with the standard errors of the means based on mean square errors. Confidence intervals have been calculated with the Statistical software (version 10). When a significant interaction was identified, a Tukey test was realized to compare all means.

For behavioural analyses, the statistical analyses were conducted in two steps. First, we characterized the personality traits of fish based on the results of the two behavioral tests (cross-maze test and dyadic test). We analyzed these data by using a principal component analysis (PCA). Second, a hierarchical ascending

classification (HCA) was performed on the scores of fish along the axis 1 of PCA (PC1 scores of PCA reduction). This analysis was done to identify groups of individuals displaying similar personality traits. The level of gro up discrimination was considered as the level 1 when the dissimilarity was higher (method used is the smaller distance between two individuals). To compare the behavior response in each test between the two extreme groups (reactive and proactive), a non-parametric test (Mann-Whitney U test) was used due to the imbalanced sample size of the two groups. All the statistical analyses were performed with *R* (version 3.2.3). Data and are presented as means ± SE.

Results

Multifactorial experiment

Effects on growth rate

The mean growth rate for all tanks was 16.16% between the first sampling date (T0, 4 dph) and the last one (T35, 39 dph). The tank A4 had the best growth rate with 17.21 % and the worst growth rate was observed in the tank A1 with 13.9%. Between T7 and T28, the growth seemed homogenous with a coefficient of variation of weight comprised between 23.12% (B4) and 31.98% (A4) for T7, 29.30% (A2) and 41.36% (A3) for T14, 20.70% (B3) and 45.10% (A4) for T21 and 51.12% (B1) and 82.11% (A4) for T28. However, at T35, the range of coefficient of variation of weight was higher: 36.40% (B1) and 120.60% (A3) (**Figure 2**).

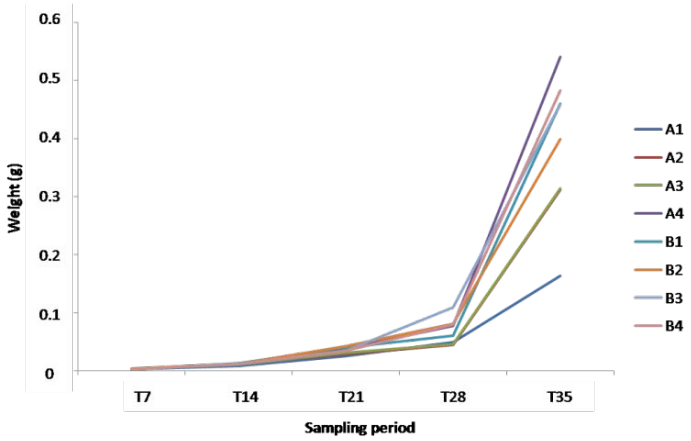

Figure 2. Growth curve of pikeperch larvae in the eight experimental tanks in function of sampling date.

Effects on total length, body weight and size heterogeneity

At T7 (10 dph), the multifactorial analysis provided two simple effects of light intensity and timing of tank cleaning on the total length. The mean total length was higher when the light intensity was 50 lux than 5 lux ($F = 9.7$; $p = 0.03$). The mean total length of larvae was also higher when the tank was cleaned in the evening than in the morning ($F = 53.9$; $p = 0.002$). No significant interaction is observed.

At T14 (17 dph), a significant effect of the light intensity is again observed on size heterogeneity. The coefficient of variation of total length is higher for larvae exposed to 50 lux than for larvae exposed to 5 lux ($F = 23.9$; $p = 0.02$). Consequently, size of larvae was more homogenous at 5 lux. Moreover, the multifactorial analysis shows a significant interaction between light intensity and water renewal rate for the coefficient of variation of weight ($F = 45.20$; $p = 0.04$). The larvae weight was more homogenous at 5 lux with a renewal rate of 100%. It must be noted that the cleaning time has no significant effect, but it is at the limit of significance ($p = 0.06$).

For T21 (24 dph) and T28 (31dph), there was no interaction and no simple effect provided by multifactorial analysis. However at T21, the effects of the timing of tank cleaning ($p = 0.07$) and the interaction between light intensity and water renewal rate ($p = 0.08$) are just at the limit to be significant ($P<0.05$) on larvae total length.

At T35 (39 dph), the multifactorial analysis shows that the interaction between light intensity and water renewal rate is significant for total length ($F = 35.1$; ddl = 1; $p = 0.027$). Pikeperch larvae have a significant lower size with a light intensity of 5 lux and a 50 % water renewal rate compared to the three other combinations (Figure 3A). It also shows that the interaction between light intensity and timing of cleaning tank is significant for total length ($F = 35.4$; ddl = 1; $p = 0.027$). Pikeperch larvae are smaller with low light intensity and a tank cleaning during the morning (Figure 3B). The multifactorial analyze shows also simple effects of light intensity and water current direction on the coefficient of variation of total length (**Figure 4**). The coefficient of variation of total length is higher when the water flow is bottom-up ("water inlet at depth") than when the water flow is top-down ("water inlet at the water surface") ($F = 31.1$; $p = 0.01$). Larvae size was more homogenous when water flow was top-down (**Figure 4A**). Furthermore, the coefficient of variation of total length is higher for larvae exposed at 5 lux than for larvae exposed at 50 lux ($F = 21.8$; $p = 0.02$). Consequently, the total length of larvae was more homogenous at 50 lux (Figure 4.B). There were observed tendencies ($P>0.05$) of the effects of light intensity ($p = 0.052$), water renewal rate ($p = 0.055$) and the interaction between light intensity and timing of cleaning tank ($p= 0.066$) on larvae body weight.

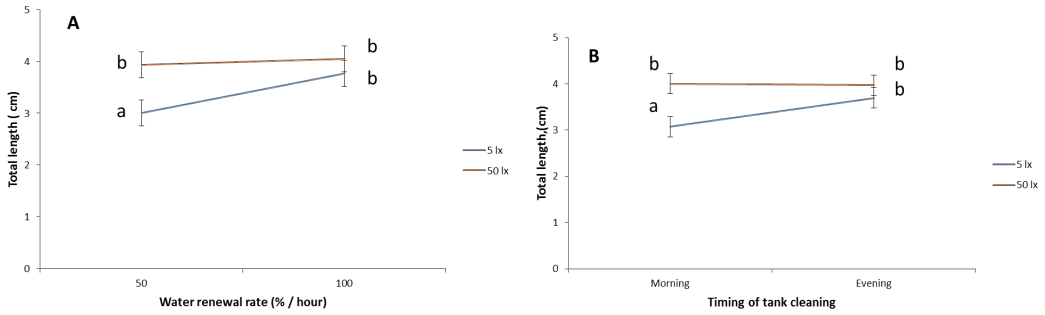

Figure 3. Effect of the interactions of water renewal rate * light intensity (A) and timing of tank cleaning * light intensity (B) on the total length of pikeperch larvae at day 35 ($p < 0.05$).

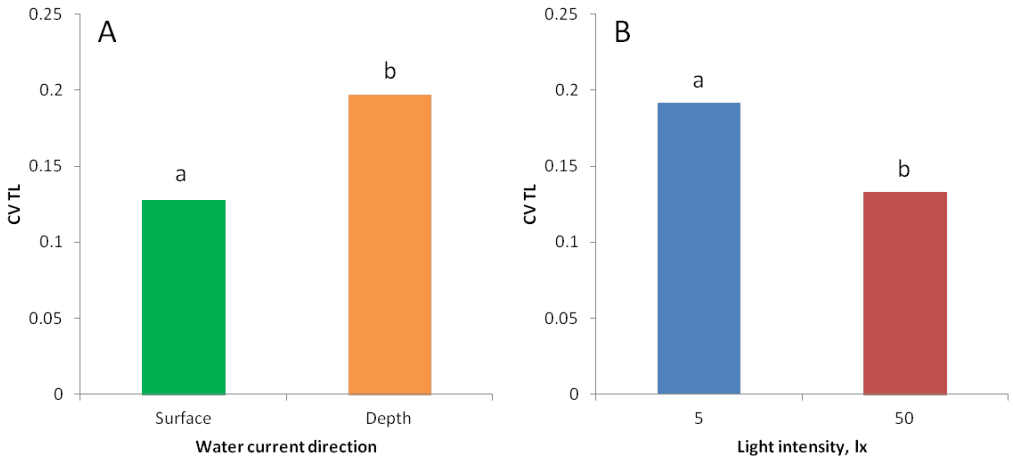

Figure 4. Impact of water current direction and light intensity on the coefficient of variation of total length at day 35 ($p < 0.05$).

No significant effects were observed on survival, deformities, swim bladder inflation and cannibalism rates (n = 142 cases of cannibalism observed). However the global survival rates were very low from 0.3% in A3 tank to 2.6% in A1 tank.

Histological assessment report of pikeperch larval development from the trial T35

The following results are presented per tank.

T35, tank A1:
Mouth: taste buds detected lining the multilayered flat epithelium of the buccopharynx (mid/posterior region), many canine-like teeth (>5) were detected in the posterior region (dorsal & ventral pharyngeal epithelia) of the buccopharynx close to the beginning of the oesophagus; tongue further developed with regard to previous ages (**Figure 5**).

Oesophagus: elongation of the oesophagus with regard to previous ages, as well as thickening of the muscular layers surrounding it; large and abundant mucous cells lining along the length of the oesophagus; longitudinal and transversal folds are visible along the length of the oesophagus. The histological organization of the oesophagus with regard to T7 was similar and only differing in the size of structures (**Figure 5**).

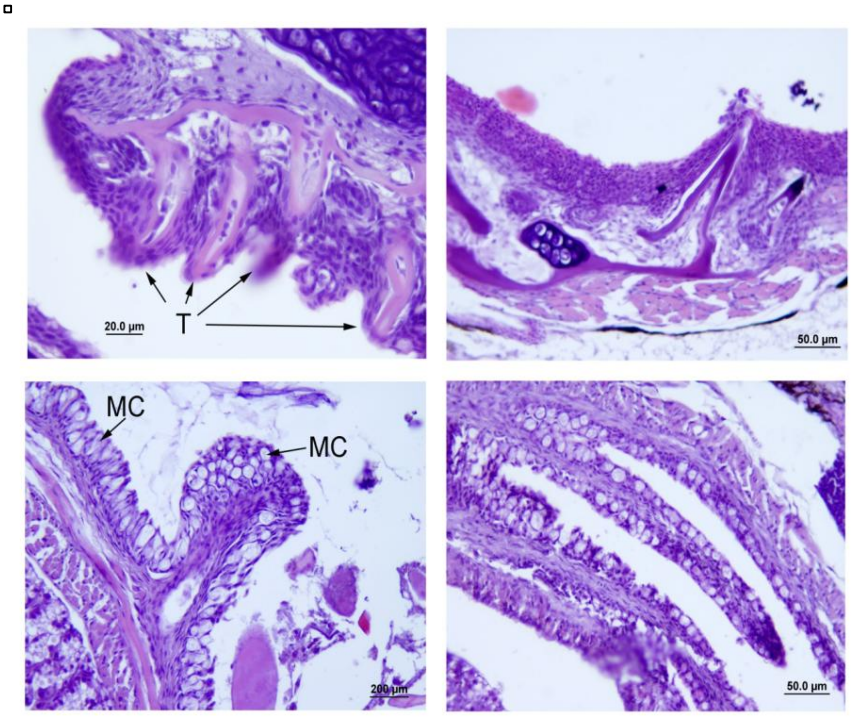

Figure 5. Images of the mouth and oesophagus of the pikeperch larvae. Detail of canine-like teeth (T) in the posterior regions of the buccopharynx close to the connection between the pharynx with the oesophagus. Note the multistratified flat epithelium lining the pharyngeal epithelium. Lower images: left image; detail of a transversal oesophageal fold covering with large mucin-producing cells (MC). Right image; detail of a longitudinal oesophageal fold connecting with the cardiac region of the stomach. The mucins produced by these cells serve as lubricant since fish do not have salivary glands, as well as facilitate the absorption of simple nutrients in other regions of the gut and even be involved in protecting the oesophageal mucosa from bacteria and virus infections. Note: it is not possible to get a general overview of the buccopharyngeal cavity due to its large size, so just the most relevant anatomical feature (teeth) is shown. General views of the oesophagus can be seen in histological sections of other regions of the digestive system.

Intestine: the intestine is coiled with several loops in order to accommodate its length to the small abdominal cavity. The three regions in which the intestine is divided are clearly differentiated, being the intestinal villi (folds) longer in the anterior and mid intestine in comparison to the posterior region; the rectum is short and in some specimens almost nonexistent. Lipids are mainly detected in the posterior intestine; although the level of lipid inclusions in enterocytes is low in this group (fat is detected as an unstained area within cells, since lipids are washed out from the tissue during the paraffin embedding process). Several pyloric caeca with long mucosal folds are visible at the connection between the stomach and anterior intestine. Inert feed is visible in the gut of all examined specimens (**Figure 6**).

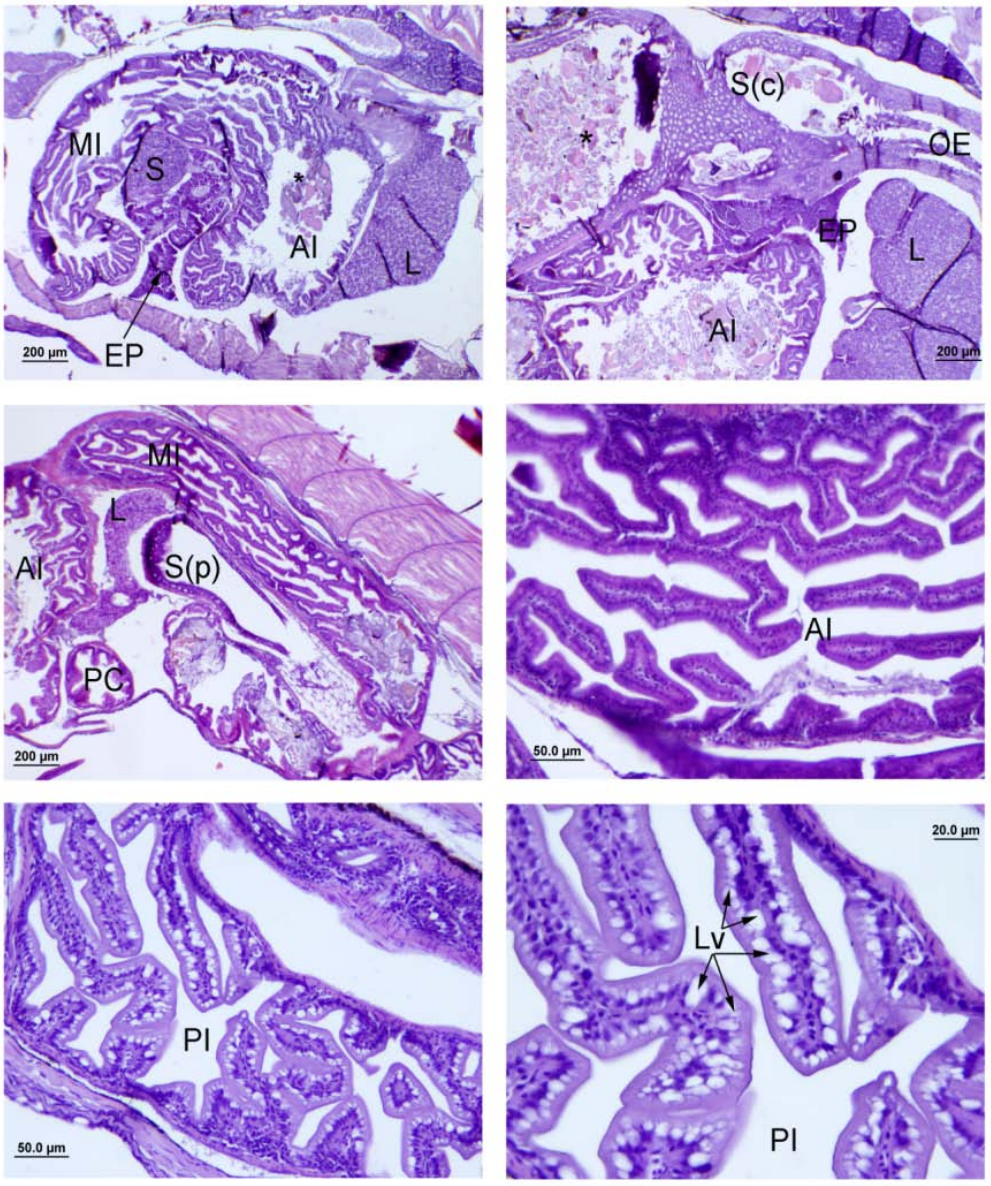

Figure 6. Images of intestine of pikeperch larvae. General view of the abdominal cavity showing the coiling of the intestine and the absence of lipid inclusions in the anterior and mid intestinal segments (upper and mid images). The lower images show the posterior region of the intestine with supranuclear lipid vacuoles (Lv) inside enterocytes. Abbreviations: AI, anterior intestine; EP, exocrine pancreas; L, liver; MI, mid intestine; PC, pyloric caeca; PI, posterior intestine; S, spleen; S(c), stomach – cardiac region; S(p), stomach – pyloric region; asterisks show inert feed particles.

Stomach: all examined fish showed a large and well-developed stomach with abundant tubular gastric glands occupying most part of the abdominal region (it extends almost to the mid part of the posterior intestine). The stomach is Y-shaped and consists of cardiac, pyloric and blind sac regions, as it is described by Ostaszewska (2005; Electronic Journal of Ichthyology 2: 65-78) and Hamza et al. (2015; Biology and Culture of Percid Fishes, Chapter 8). In this sense, the pyloric region is situated between the cardiac and the blind sac. The stomach is surrounded by a thick muscular layer (circular muscular fibers were more abundant than longitudinal ones) (**Figure 7**).

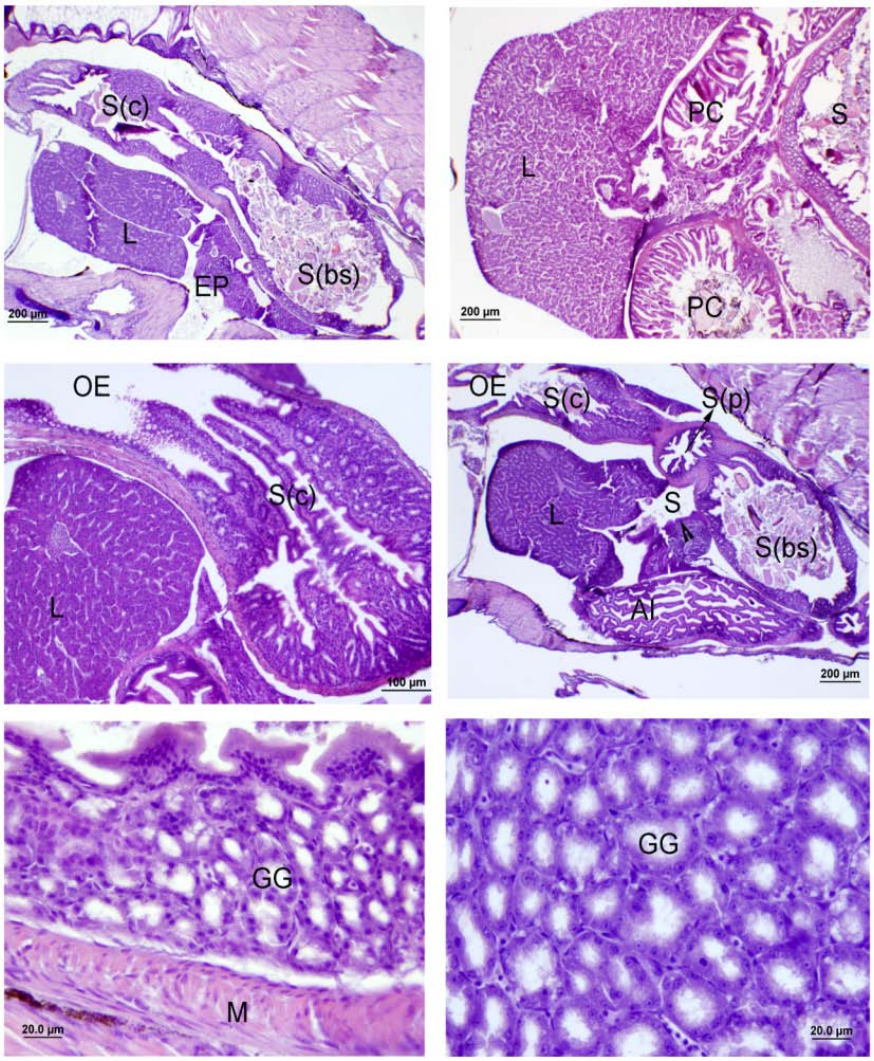

Figure 7. Images of the stomach from pikeperch larvae. Abbreviations: AI, anterior intestine; EP, exocrine pancreas; GG, gastric glands; L, liver; OE, oesophagus; M, muscular layer surrounding the stomach; PC, pyloric caeca; S, spleen; S(c), stomach – cardiac region (the tubular shape of gastric glands is visible); S(bs), stomach – blind sac; S(p), stomach – pyloric region (connection with the anterior intestine and pyloric caeca).

Liver: moderate accumulation of lipid deposits in hepatocytes 30% of the examined animals (fat is detected as an unstained area within cells, since lipids are washed out from the tissue during the paraffin embedding process); 70% of fish with a no accumulation of lipids in the liver (the absence of lipids cannot be attributed to a fasting status of the specimen, since inert feed particles were seen in the gut). Those livers containing a

moderate level of lipid inclusion had their nuclei displaced to the periphery of the hepatocyte as a consequence of lipid deposition, but maintaining their round shape (**Figure 8**).

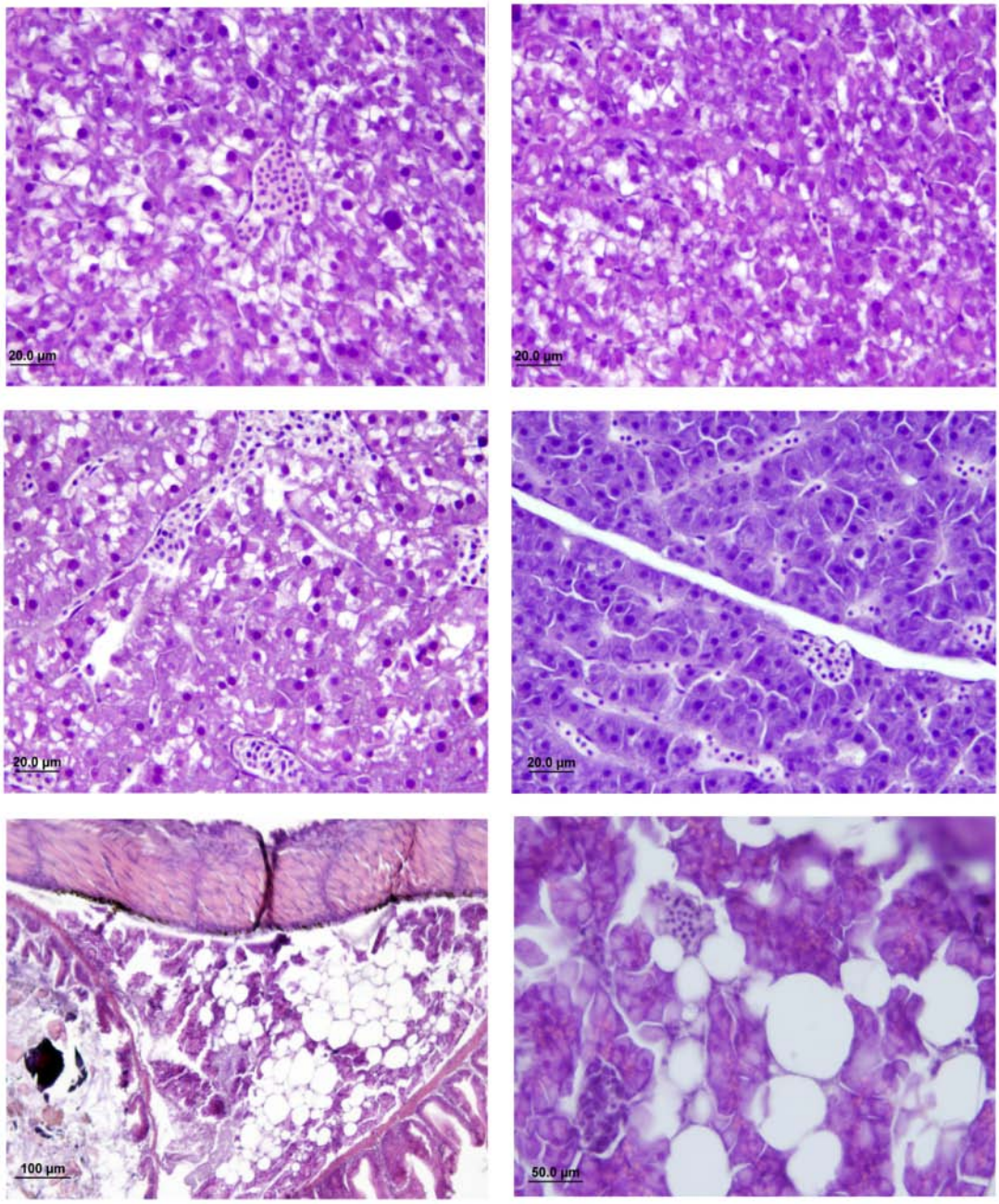

Figure 8. Images of liver from pikeperch larvae. The mid right image shows a liver devoid of lipid accumulation within hepatocytes and hepatocytes with eosinophilic cytoplasm that mostly corresponded to glycogen deposits. The rest of the upper and mid images are from livers with a moderate level of inclusion of lipids, but also showing a noticeable quantity of glycogen within hepatocytes. The lower images showed the accumulation of fat deposits (round adipocytes) surrounding and between the pancreatic tissue (acini).

Exocrine pancreas: large exocrine pancreas with abundant of zymogen granules (precursors of pancreatic digestive enzymes); abundant mesenteric fat (adipose tissue) surrounding the pancreas or other organs is seen in the abdominal cavity.

Other organs: swim bladder, completely inflated; kidney, pronephros, meso- and metanephros very well developed; gall bladder, visible; endocrine pancreas, islet of Langerhans visible; spleen, well developed.

Eye: well differentiated, all layers of the retina are clearly visible; it seems that rods (detection of light intensity in black and white) and cones (color vision) were detected (**Figure 9**).

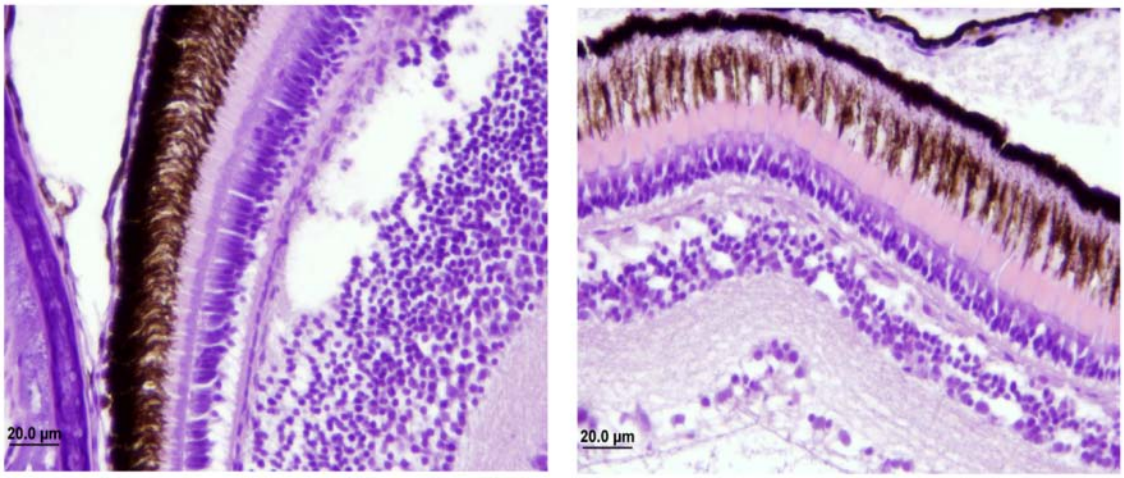

Figure 9. Images of retina from pikeperch larvae. Detail of the retina (photoreceptor layer) in order to evaluate the presence of the two types of photoreceptors (cones and rods).

T35, tank A2: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1. The level of fat inclusion in the hepatic parenchyma is similar to that observed in the T35A1 group, even though the proportion of animals with no signs of lipid accumulation and a moderate level of fat accumulation in the liver was similar (50%). The level of fat accumulation in the posterior intestine was similar to fish from the T35A1 group.

T35, tank A3: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1 and T35A2. 30% of the examined specimens showed a large level of fat accumulation in the liver, 20% of fish showed a moderate level of fat deposits and 50% of them did not show any sign of lipid deposition in hepatocytes. The level of fat accumulation in the posterior intestine was similar to fish from the T35A1 group.

T35, tank A4: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1 and the rest of T35A2 and T7A3 groups. Most of examined fish show a large/moderate accumulation of lipids within hepatocytes, whereas no fish were found without fat deposits in the liver. The level of fat accumulation in the posterior intestine was similar to fish from the T35A1 group.

T35, tank B1: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1. The level of stomach development seems similar than in the T35A(1-4) groups. All examined fish (100%, 8/8) show a large accumulation of lipids within hepatocytes (round nuclei displaced to the periphery of the cell; see image). Large accumulation of lipids in the posterior intestine similar or slightly higher in magnitude to those of the T35A1 group. The level of perivisceral fat, even though difficult to quantify, seems to be higher in comparison to all the fish from the T35A(1-4) groups (**Figure 10**).

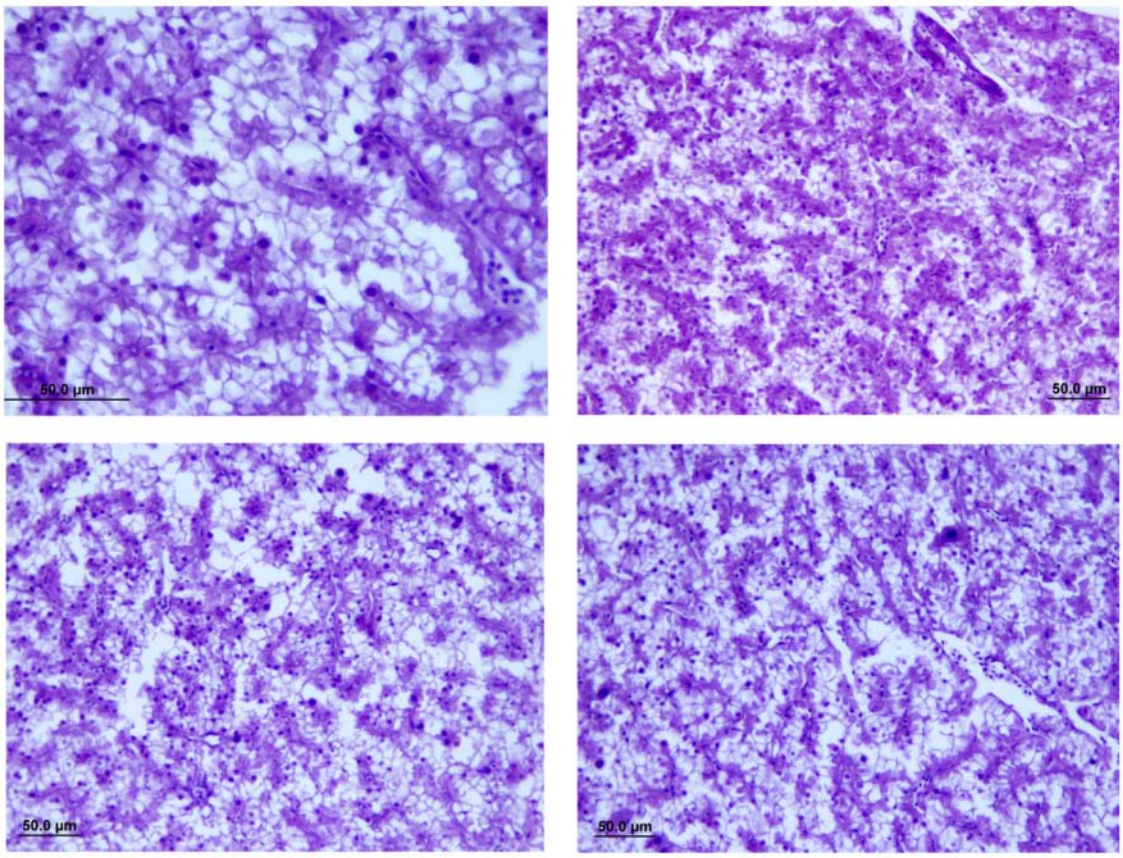

Figure 10. Images of liver (hepatic parenchyama) for pikeperch larvae from tank B1. Different livers showing the large accumulation of lipids in comparison to the T35A1 group (group chosen as reference for comparative purposes). These images are similar to those seen in T35B2 and T35B4 groups, so those for the former groups have not been included.

T35, tank B2: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1 or T35B1. The level of stomach development seems similar to that in the T35A(1-4) groups and T35B1. Most of the examined fish (11%, 8/9) show a large accumulation of lipids within hepatocytes (round nuclei displaced to the periphery of the cell) similar to those from T35B1 (see image), whereas only 11% (1/9) of fish showed a moderate of accumulation of lipids in the liver. Large accumulation of lipids in the posterior intestine similar or slightly higher in magnitude to those of the T35A1 group. The level of perivisceral fat seems similar to those animals from T35B1, but it seems to be higher in comparison to all the fish from the T35A(1-4) groups.

T35, tank B3: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1 or T35B1. The level of stomach development seems similar than in the T35A(1-4) groups and T35B1. All examined fish (8/8) show a moderate accumulation of lipids within hepatocytes (round nuclei displaced to the periphery of the cell) similar to those from T35A1, but lower than T35B1 and T35B2 groups. There was a large accumulation of lipids in the posterior intestine that was higher in magnitude to those of the T35A1 group. The level of perivisceral fat seems similar to those animals from T35B1, but it seems to be higher in comparison to all the fish from the T35A(1-4) groups.

T35, tank B4: no differences with regard to the histological organization of target organs are seen in comparison to the reference group T35A1 and T35B1. The level of stomach development seems similar in the T35A(1-4) groups and T35B1. All examined fish (100%, 8/8) show a large accumulation of lipids within

hepatocytes (round nuclei displaced to the periphery of the cell). A large accumulation of lipids in the posterior intestine appears similar or slightly higher in magnitude to those of the T35A1 group and similar to T35B1. The level of perivisceral fat, even though difficult to quantify, seems to be higher in comparison to all the fish from the T35A (1-4) groups.

Behavioural experiment

The first two axes of the PCA represented 74.9% of the total variability, thus they were the only ones considered further. On the first axis, activity was positively associated with the exploration ("number of zones visited" and "duration outside the start zone") and the dyadic test variables ("orientation" and "approaches"). All these variables were opposite to the "latency to emerge from the start zone". The second axis represented more the result of the dyadic test ("orientation" and "approach"). Three main groups were found in the hierarchical ascending clustering (**Figure 11**). The limit of dissimilarity between the three groups was clearly established. The first group gathered 9 individuals (G1), the second group 18 individuals (G2) and the third group 15 individuals (G3). G1 and G3 represent extreme personality in the studied population. Pikeperch of G1 were more active (428.78 ± 28.42 s) than pikeperch of G3 (19.53 ± 8.82 s) (Mann Whitney U test for all comparison, $U = 0$, $p < 0.0001$, **Figure 12**), explored more their environment (number of visited zone: G1 = 454.22 ± 37.17; G3 = 3.40 ± 0.88; $U = 0$, $p < 0.0001$) and had more aggressive behavior (number of approaches: G1 = 3.44 ± 0.65; G3 = 0.53 ± 0.29; $U = 15$, $p = 0.0009$). Therefore, G1 regrouped proactive fish and G3 was reactive ones.

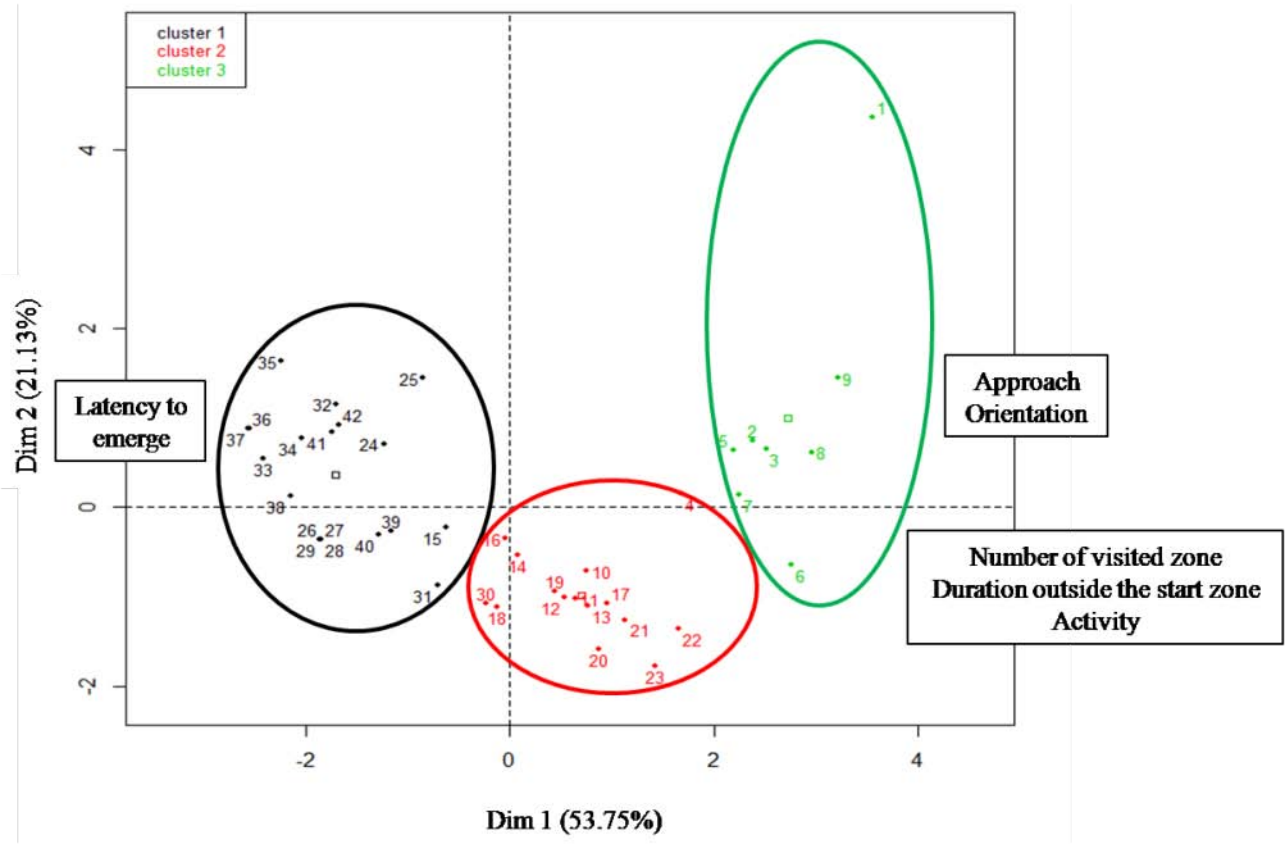

Figure 11. Projection of individuals on the PCA with the three groups determined by HCA realized with the six behavioral variables.

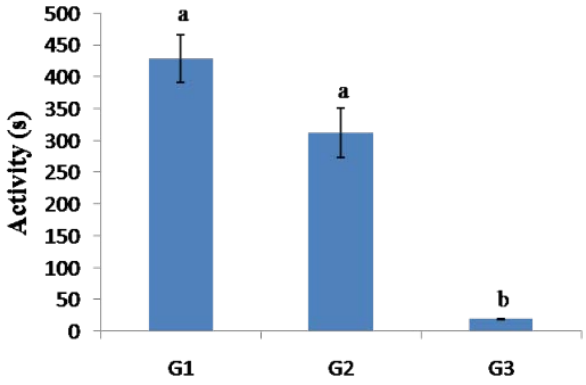

Figure 12. Activity in each group (mean± SE) characterized by HCA (G1, G2 and G3) ($p < 0.05$).

Discussion

Zootechnical performances

In this experiment, it was demonstrated that weaned juveniles of 0.5±0.06 g mean body weight can be produced in 5 weeks, but survival rates (0.3-2.6%) were very low. There are no references supporting these results that consider pikeperch larval rearing in large tanks (700 l) and over a long period (39 dph), including the initial phase of feeding on live prey and the weaning period. In the literature, authors related to very short experimental durations (2-3 weeks), very low tank volume (20-60 l) or a specific rearing phase (weaning). Kestemont et al. (2007) recorded survival rates between 13 and 24 % using 11 dph larvae and 20 l tanks. Ostaszewska et al. (2005) used a similar tank volume, but observed survival rates of 50-54%, using 5 dph larvae, after 5 weeks. Lund et al. (2014) used 40l tank volume. Hamza et al. (2008) observed an average survival rate of 35% from 10 to 34 dph using 60 l cylindro-conical tanks. Szczepkowski et al. (2011) recorded survival rates of 39-59% using 48 dph larvae weighing initially 40 mg and 40 l tanks after a 3 week experiment (from 48 dph to 69 dph). Szkudlarek and Zakęś (2007) indicated survival rates of 72-79% after a 14 days experiment from 4 dph to 18 dph using 200 L tanks. In a second experiment these authors observed survival rates of 45-56% over a 3-week trial (from 18 dph to 39 dph) with a mortality rate mainly due to cannibalism (27-35% of total). More recently, Ljubobratović et al. (2015) carried out an experiment focusing only on the weaning strategy using 11 dph larvae (20 L circular tanks, from 11 dph to 27 dph) and calculated survival rates between 21 and 43% with cannibalism rates of 25-45% according to various weaning strategies. Krol and Zakęś (2016) recorded survival rates of 20-30% with larvae (15 dph) rearing during 28 days. In other studies, survival rates are not mentioned (Hamza et al., 2007). Although survival rates were very low, results from this first experiment demonstrate for the first time growth and survival of pikeperch larval rearing approximating commercial hatchery conditions with large tank volumes and RAS conditions over a long period (5 weeks) and from newly hatched larvae. The fact that we used newly hatched larvae and not larvae of several days of age, as in other studies, might have contributed to increased mortality rates. This fact might be due to the presence of newly hatched larvae with potential congenital developmental defects that could have died during the lecithotrophic stage. This conclusion is confirmed by a lecture by Steenfledt (2015).

Considering the effect of the environmental factors tested, light intensity appeared to be the most important among the four factors evaluated. Compared to 5 lux, a light intensity of 50 lux allows obtaining larger larvae in size. The negative impact of the low light intensity is dependent on the value of the other factors. In particular, mean pikeperch larval size is lower when the water renewal rate is also low (50%) or when the tanks are cleaned early morning as observed at T35. A higher light intensity seems to allow a better expression of the growth potential of pikeperch larvae, which may be linked to the carnivorous and predatory behavior of this species at larval stages and the importance of proper light conditions for detecting prey in

the water column (Kozłowski et al. 2010). Consequently, as pikeperch is a species recently domesticated (Teletchea and Fontaine, 2014), under higher lighting conditions, the individual growth potential is more expressed. Therefore, under a low light intensity, pikeperch larvae are more homogeneous in size or weight as observed at T14. At T35, an inverse effect is observed where populations of pikeperch larvae are more homogeneous in size under a higher light intensity. However, at that time, cannibalism also contributes to explain this state with the elimination of the smaller fish from the population. Our results show also that it is better to clean the tank during the afternoon rather than at morning hours. A negative effect of cleaning the tank in early morning is observed at T7 and T35 (in interaction with light intensity). That could be explained by a stress effect on larvae or a disturbance of the first feeding/meal of the day, which can progressively induce stress and result in a delay in growth. In many fish species described so far, the hypothalamus–pituitary–interrenal axis is already functioning at hatching, and a stress response (increasing body cortisol levels between 1 and 3 h after exposure to the stress event) can be induced at early developmental stages (Pittman et al. 2013). Thus, it seems plausible that tank cleaning operations before the first meal of the day induced stress in pikeperch larvae that compromised their performance. Our experiment also indicates a role of the water renewal rate on pikeperch larval growth. A higher renewal rate (100%) improves larvae growth with lower weight heterogeneity (T14) or larger larvae (T35). Finally, it appears that a water inlet at the bottom of the tank is better to reduce size heterogeneity. Considering all these results, **we recommend to apply a light intensity of 50 lux, a water renewal rate of 100%, a cleaning of the tank during the afternoon and an inlet of the water at the bottom level**.

Histological analysis

The timing of organ development and its associated physiological functions are affected by the general life history and reproductive guild of each species, as well as by a variety of abiotic and biotic factors. Among them, temperature, water quality, food availability and composition have been generally considered as some of the most important ones (Gisbert et al. 2008). In this sense, changes in the histological organization of the liver, the exocrine pancreas, the intestine, the visual organ and muscular fibers have been used on a regular basis as histological targets to analyze the nutritional condition of fish larvae and elucidate the effects of different dietary regimes or rearing conditions on larval physiology, nutrition and early development. These tissues and organs are especially sensitive to non-optimal feeding and rearing conditions during larval development, because they are under progressive and intensive morphogenesis and respond rapidly and are sensitive to nutritional disorders and environmental stressors (Gisbert et al. 2008; Cahu et al. 2009). Under present experimental conditions, none of the environmental factors tested by means of this multifactorial trial had any marked effect on the histological organization and morphogenesis of the different examined organs (digestive tract, accessory digestive glands). The absence of these differences were not only observed in samples from T35, but they were also noted at earlier ages of development (T7, T14, T21 and T28; data not shown). In general terms, the development of the digestive system and eye in pikeperch from this study was similar to that described by other authors (Ostaszewska 2005; Kolawska et al. 2006; Kamaszewski and Ostaszewska, 2015).

The lack of marked differences in terms of the histological organization of selected organs and tissues may be linked to the fact that Although significant differences were found in larval size at different sampling ages (*i.e.* T7, T35), depending on rearing and husbandry conditions, the differences in body size among groups were not sufficiently large enough to have resulted in a differential morphogenesis rate among them. Finally, it should be noted that regardless of the differences in growth, none of the tested rearing conditions negatively affected the formation and histological organization of examined tissues in pikeperch during larval development, nor their nutritional condition assessed by the level of fat accumulation in target tissues like the intestine and liver.

Behavioural experiment

One of the most important results of the present study is that different behavioral traits were observed in very young pikeperch juveniles using maze and dyadic tests. This implies that personality traits appeared very

early in the life of pikeperch, and personality could be an inheritable character (Wright et al. 2003). We know, for instance, that a gene mutation linked to growth factors may modify fish personality in zebrafish *Danio rerio* (Norton et al. 2011). It is also known that domestication may also act as a selection process for personality traits (Moretz et al. 2007). This first experiment allowed us to know that it is possible to determine the personality on pikeperch juveniles and maybe highlight in a future experiment the link between personality and cannibalism.

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