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Improving pikeperch larviculture by combining environmental, feeding and populational factors

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ABSTRACT

Pikeperch (*Sander lucioperca*) has a high potential for inland aquaculture diversification in Europe. Bottlenecks (*i.e.* low survival, cannibalism and deformity rates) hamper further expansion of pikeperch culture, because of the weak production performances at the nursery level. To improve the production of pikeperch juveniles under recirculation system we used a pilot scale larval rearing system (700 L tanks) and multifactorial designs. Three successive larval rearing trials (duration: 35-49 days) were conducted to identify the best combination of environmental, feeding and population factors. Considering the main significant effects observed on survival, growth, swim bladder inflation rates and biomass gain, a favourable combination of twelve factor (F) modalities was proposed (F1- initial density: 100 larvae.L^{-1}, F2- no sorting of fish jumper, F3- no sibling population, F4- eggs from large females, F5- discontinuous feeding, F6- no co-feeding, F7- light intensity: 50 lx, F8- beginning of the weaning at 16 dph (days post-hatching), F9- weaning duration: 9 days, F10- water renewal rate of 100 % per hour, F11- tank cleaning during morning and F12- tank bottom-up water current). A final validation step was realized over a last trial (seven replicates, duration: 49 days), and validated with the best productive results obtained over the global experimental period (2015-2018). These results were: a final body weight of 815.64 ± 95.34 mg, a survival rate of 16.9 ± 1.7 %, a specific growth rate of 15.1 ± 5.9 %.d^{-1}, a final fish biomass of 9.55 ± 0.23 kg, a swim bladder inflation rate of 92.6 ± 3.2 % and a food conversion rate of 0.65 ± 0.02 (dry food). The final stocking density was 13.6 kg.m^{-3} of rearing volume. Authors were able to validate and provide a reliable basic protocol for pikeperch larval rearing using recirculating units.

1. Introduction

Pikeperch (*Sander lucioperca*) is considered to have a high potential for inland aquaculture diversification in Europe (Wang et al., 2009) and its demand has been strengthened by the strong decline of wild catches from 48.800 t in 1950 to 19.872 t in 2016 (FAO, 2019). To promote pikeperch intensive culture, numerous studies have been addressed on its biology and culture over the last decade (Kestemont et al., 2015). The bio-economic feasibility of pikeperch intensive rearing has been demonstrated (Steenfeldt and Lund, 2008; Steenfeldt et al., 2010; Dalsgaard et al., 2013), and extensive researches were conducted on this species. Several bottlenecks (low survival, high cannibalism and deformity rates) limit the further expansion of pikeperch culture inducing weak performances at the nursery level. The most critical period of early larval development occurs 10-15 dph (days post-hatching), affecting the survival rate of larvae between 25 and 50 % on day 35 post-hatching (Kestemont et al., 2015). Cannibalistic behaviour is very severe from 18 to 39 dph representing up to 80 % of all mortality at weaning time (19 to 23 dph) (Kestemont et al., 2007; Szkudlarek and Zakęś, 2011). Consequently, the provision of a reliable protocol for pikeperch larval rearing is necessary to secure the development of its culture.

The development of such reliable protocol in the short term is not easy because larval rearing is a complex phenomenon (Weisbuch, 2002; Gardeur et al., 2007). Numerous factors impact larval development as well as their behaviour, growth and survival (Kestemont et al., 2003). These impacting factors can be classified into three categories: environmental factors (*i.e.*, temperature, light intensity, photoperiod, water quality and tank cleaning), feeding factors (*i.e.*, food composition, feeding frequency and ratio, meal distribution timing and weaning

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Table 1
Schedule of available data in the literature about broodstock management, eggs incubation and larval rearing of pikeperch *Sander lucioperca* L. during pre-experimental periods. Different parameters were considered:, origin, waterbody, number of breeders and techniques of broodstock management for *broodstock management,* origin, waterbody, number of spawns and incubation conditions for *eggs incubation*, and, origin, waterbody, number of spawns and larval rearing conditions for *larval rearing*. The level of information correspond to: 1– No information; 2 – Very few information: only one information was given; 3 – Few information: two information were given; 4 – Enough information: three information were given and 5 – Many information: more than three information were given. * Studies which concern newly hatched larvae are not considered (Lund et al., 2012, 2014; Policar et al., 2016; Xu et al., 2017).

Level of information	Broodstock management	Eggs incubation	Larval rearing*
1 - No information	Ostaszewska, 2005, Hamza et al. 2007, 2008, Lund et al. 2014, 2018, 2019, Król and Zakęś 2016, El Kertaoui et al., 2019	Mamcarz et al., 1997, Colchen et al., 2019	Mamcarz et al., 1997
2 - Very few information	*Only breeders origin fish farmer, waterbody* ***or*** *number of breeders* ***or*** *technique of broodstock management* Mamcarz et al., 1997, Molnár et al., 2004, Ostaszewska et al., 2005, Lund et al., 2012, Schaefer et al., 2017, Tielmann et al. 2016, 2017, Colchen et al., 2019	*Only eggs origin fish farmer, waterbody* ***or*** *number of spawns used for reproduction* ***or*** *incubation conditions* Molnár et al., 2004, Ostaszewska, 2005, Ostaszewska et al., 2005, Hamza et al. 2007, 2008, Kestemont et al., 2007, Szczepkowski et al., 2011, Lund et al. 2012, 2014, Ljubobratović et al., 2015, Król and Zakęś 2016, El Kertaoui et al., 2019	*Only larvae origin fish farmer, waterbody* ***or*** *number of spawns* ***or*** *larval rearing system* ***or*** *conditions* Molnár et al., 2004, Ostaszewska, 2005, Ostaszewska et al., 2005, Szkudlarek and Zakęś 2007, Policar et al., 2016, Tielmann et al., 2016, 2017, Yanes-Roca et al., 2018, Lund et al., 2019
3 - Few information	*Breeders origin* ***and*** *technique of reproduction* Kestemont et al., 2007; Szczepkowski et al., 2011	*Eggs origin fish farmer, waterbody* ***and****incubation conditions* ***or*** *systems* Policar et al., 2016, Xu et al., 2017, Schaefer et al., 2017, Lund et al. 2018, 2019	*Only larvae origin fish farmer, waterbody* ***and*** *number of spawns* ***or*** *larval rearing system or conditions* Szczepkowski et al., 2011, Król and Zakęś 2016
4 - Enough information	*Breeders origin* ***and*** *technique of reproduction used* ***and*** *number of breeders used for reproduction* Ljubobratović et al., 2015, Xu et al., 2017	*Eggs origin fish farmer, waterbody* ***and****incubation conditions* ***and*** *systems* Tielmann et al., 2016	*Only larvae origin fish farmer, waterbody* ***and*** *number of spawns* ***and*** *larval rearing system or conditions* Hamza et al., 2007, 2008, Ljubobratović et al., 2015, Schaefer et al., 2017, El Kertaoui et al., 2019
5 - Many information	*Fish origin, size and weight, technique of reproduction, number of breeders used for reproduction* Szkudlarek and Zakęś 2007, Policar et al., 2016, Yanes-Roca et al., 2018	*Eggs origin fish farmer, waterbody* ***and*** *spawn number* ***and****incubation conditions* ***and*** *systems* Szkudlarek and Zakęś 2007, Tielmann et al., 2017; Yanes-Roca et al., 2018	*Only larvae origin fish farmer, waterbody* ***and*** *number of spawns* ***and*** *larval rearing system* ***and*** *conditions* Kestemont et al., 2007, Lund et al., 2018

period), and population factors (*i.e.*, fish density, strain and domestication level). Like any complex biological system, the effects of the interactions between factors are often more important than their simple effects and the determinism of the performance is multifactorial. In this context, the use of factorial design experiments is particularly adapted to determine the optimal combinations of factors that would be required to improve the rearing system. In aquaculture, very few studies were designed using such multifactorial approach (Torstensen et al., 2001; Hamre et al., 2004; Gardeur et al., 2007; Teletchea et al., 2009; Trabelsi et al., 2011; El Kertaoui et al., 2019). In Eurasian perch *Perca fluviatilis*, this practical approach improved the quality of the aquatic production system (growth efficiency, size homogeneity, fillet quality; Gardeur et al., 2007). In pikeperch, a recent multifactorial study provided further insights into the optimal nutritional and husbandry rearing conditions (Lund et al., 2019).

Previous works on pikeperch larval rearing were mainly based on uni-factorial approaches and their application on farm conditions is not evident for several reasons. Many data are not specified, concerning the egg or larva origin (broodstock management) and the previous life of fish *prior* to the trials (Table 1). There is a lack of data concerning also the initial weight or size of larvae (Lund et al., 2012, 2014; Tielmann et al., 2017) and some technical variables (type and volume of the rearing tanks, wall tank colour, method for tank cleaning, use or not of a water surface skimmer, light spectrum or direction of the water flow) are missing. The technical practices were very variable; *i.e.* fish were fed *ad libitum* (Mamcarz et al., 1997; Molnár et al., 2004; Król and Zakęś, 2016; Policar et al., 2016) or fed in excess (Szkudlarek and Zakęś, 2007; Szczepkowski et al., 2011). According to their specific objectives and local constraints (equipment, eggs and/or larvae suppliers), the experimental designs were very heterogeneous.

The lack of data on multifactorial approaches, and the extreme heterogeneity in experimental designs (Table 1), limit the possibility to aggregate and integrate knowledge for the development of a reliable multifactorial protocol relevant in commercial conditions. Using a pilot scale larval rearing system and multifactorial design protocols, three successive experiments were conducted to identify the best combination of environmental, feeding and population factors to improve the production of pikeperch juveniles in Recirculating Aquaculture Systems (RAS). A favourable combination modalities of twelve factors was determined, and a fourth trial was conducted to validate such combination of modalities on the production (higher survival rates, swim bladder inflation, and growth rate) of pikeperch juveniles in RAS.

2. Material and methods

2.1. Fish origin and rearing conditions

For each experiment and the validation trial, newly hatched larvae

(500,000 in 2015, 240,000 in 2016, 420,000 in 2017 and 560,000 in 2018) were obtained from a same broodstock (Czech origin, 1-4 spawning seasons) maintained at the fish farm SARL Asialor (Pierrevillers, France). Larvae (age: 1 day post-hatching (dph)) were transferred to the Experimental Platform in Aquaculture (UR AFPA, http://www.urafpa.fr/main.php, Vandœuvre-lès-Nancy, France) located at the University of Lorraine. After their introduction in tanks, larvae were acclimated without feeding until 4 dph. Three successive experiments and the validation trial (January-March 2015, February-March 2016, February-April 2017 and 2018) were carried out in eight green (ref. RAL6021, CONREAU, France) 700 L indoor tanks associated in a unique RAS using common mechanical (sand Lacron filter 50 µm under pressure, 100 L, Waterco Europe Ltd, Sittingbourne, Kent, UK) and biological filters (piece of ground glass as bacterial support, under pressure, 300 L, Waterco Europe Ltd, Sittingbourne, Kent, UK), and UV sterilization unit. Each 700 L tank was equipped with an air surface skimmer to remove the oily layer. The RAS was located in a thermo-regulated room. According to fish farmer constraints, artificial photoperiod followed a 12 h light/12 h darkness cycle (light on at 8:00 and off at 20:00 with a progressive increase of light intensity from 8:00 to 8:30 (from 0 to 5 or 50 lx, simulating a dawn period) and a decrease of light intensity from 19:30 to 20:00 (from 50 or 5 to 0 lx, simulating a dusk period). Temperature was similar in all tanks and ranged from 16 °C (day 1) to 20 °C incrementally increasing by 1 °C per day (Hamza et al., 2007; Szkudlarek and Zakęś, 2007). Consequently, after 4 days, water temperature was maintained at 20 °C. The physico-chemical properties of the water were monitored once or twice per week in each experiment (Table 2). Water pH was corrected by regular inputs of $NaHCO_3$. Larvae were fed initially with *Artemia* nauplii (550-600 µm, Sep-Art *Artemia cysts*), then, with compound diets (Larviva PROWEAN 100, 300, 500, 700 µm and INICIOplus 0.8 mm, BIOMAR®, France) according to experimental modalities (Table 3). Fish were fed between 8:30 to 17:30 during the light period, seven times per day. Fish were reared from 1 to 39 dph for the first experiment (due to high mortality) and from 1 to 53 dph for the second and third experiments.

2.2. Factors and modalities tested

The experiments 1 to 3 based on factorial experimental designs were developed to study the effects of the twelve factors with two modalities each on pikeperch larval development (Table 3). The effects of the environmental (experiment 1), feeding (experiment 2) and populational (experiment 3) factors and their interactions were studied successively (years 2015, 2016 and 2017). After considering some constraints exposed above, the choice of factors and their applied modalities resulted from a bibliographical analysis (Table 3).

In the experiment 1 (larval density: 90 larvae.L^{-1}), four environmental factors (F) (F1: light intensity, F2: water renewal rate, F3: water current direction and F4: tank cleaning period) were tested (Table 3). In the experiment 2 (larval density: 43 larvae.L^{-1}), according to the results of the experiment 1, we fixed the light intensity at 50 lx, the tank water renewal rate at 100 % per hour, a water down-flow, and the cleaning period in the morning. Four factors related to feeding strategies were tested: F5: the beginning of weaning (relatively to the age of the larvae), F6: the mode of feed distribution, F7: the co-feeding and F8: the weaning duration (Table 3). Regarding the mode of feed distribution, we compared a discontinuous feeding (one meal every 90 min, seven meals/day) to a continuous one (using peristaltic pumps for live prey and belt feeders for artificial diet) over a 12 h period (photophase). The effect of a co-feeding or not on the larvae was tested with live food and a 100-µm inert diet (Larviva Pro-wean, BioMar, Denmark) at 3.5 g.day^{-1}, six days before the beginning of the weaning period. In the experiment 3 (larval density: 50 or 100 larvae.L^{-1}), environmental factors (light intensity, water renewal rate, water current direction and cleaning period) were those, which gave the best growth and survival results in the experiment 1. The feeding strategy was fixed in relation to the results of the experiment 2 with no co-feeding, a weaning of nine days beginning at 16 dph and a discontinuous feeding schedule. Four population factors were tested: F9: initial fish density, F10: jumpers sorting out, F11: sibling link and F12: female weight (Table 3).

After the experiments 1-3 and the analysis of the main significant results concerning the survival, growth, and development variables of pikeperch larvae and juveniles, a best combination of modalities of the 12 factors was proposed. Then, a fourth experiment (validation trial) was conducted to validate this combination with seven replicates.

2.3. Fish survival and growth

Larvae were sampled every seven days as follows: T0 (at first day of feeding, 4 dph), T7, T14, T21, T28, T35, T42 and T49 (53 dph). For each sample, larvae were siphoned into a bucket; then, pipetted, counted and distributed in the different sample tubes. This method allowed limiting losses due to sampling with a net. Sampled larvae in tubes were then sacrificed with lethal dose of MS-222 (300 mg.L^{-1}), drained of excess water (fine mesh dip net) and put in buffered formalin 4 %.

Several measurements were carried out, including: (1) morphometric measures (total length (TL); body weight (W); coefficient of variation of total length (CV TL); and coefficient of variation of body weight (CV W) using 60 larvae per tank per sampling date, and (2) skeletal deformity analyses: (e.g. axial skeleton and jaw deformities) using 60 larvae per tank per sampling date. At the end of the experiment, fish biomass was weighed for each tank and the number of fish with inflated swim bladder counted by separating fish with or without a swim bladder by lightly anesthetizing them with a MS-222 (70 mg.L^{-1}) in salted water (20 g.L^{-1} of salt) (Jacquemond, 2004). 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 = $(\ln(W_{final})-\ln(W_{initial}))/days*100$) was calculated over the whole experimental period (from 4 dph to 39 or 53 dph). For the validation trial, the Feed Conversion Ratio per tank (FCR = Feed given / Animal weight gain) was calculated. All presented data were measured and calculated before swim bladder sorting.

2.4. Data analysis and statistics

For the two first experiments, a multifactorial analysis was carried out. Active simple effects of tested factors and their interactions were given by Daniel's graphics (Half Normal probability plot of basal estimation function, Daniel, 1959) using an oversaturated model of variance analysis. . In Daniels graphs, the interactions between three or more factors were considered insignificant due to the impossible

Table 2
Mean ± Standard Deviation water quality for each experiment. The water quality was averaged for each experiment on the total period of experiment.

Experiments	Temperature (°C)	Dissolved oxygen (mg.L^{-1})	pH	Ammonium ion content (mg.L^{-1})	Nitrous nitrogen content (mg.L^{-1})
Experiment 1 (2015)	20.0 ± 0.3	7.6 ± 0.4	8.0 ± 0.2	0.20 ± 0.1	0.02 ± 0.02
Experiment 2 (2016)	20.0 ± 0.3	8.1 ± 0.5	6.9 ± 0.8	0.13 ± 0.1	0.08 ± 0.06
Experiment 3 (2017)	20.1 ± 0.14	6.9 ± 0.8	6.9 ± 0.7	0.07 ± 0.1	0.07 ± 0.05
Validation trial (2018)	19.9 ± 0.2	6.0 ± 0.7	6.9 ± 0.4	0.63 ± 1.06	0.21 ± 0.23

Table 3
The twelve factors (in three experiment) and their applied modalities tested in the framework of the three multifactorial experiments. For each factor, the cited literature justifies the choice of each tested modalities.

Factors	Modality 1	Modality 2	Literature
Experiment 1 - Environmental factors			
1 - Light intensity (lx)	5	50	Summerfelt, 1996; Hamza et al., 2008; Lappalainen et al., 2003; Lund and Steenfeldt, 2011; Lund et al., 2012
2 - Water renewal rate (%)	50	100	Szkudlarek and Zakęś, 2007; Lund and Steenfeldt, 2011; Lund et al., 2012
3 - Water current direction	Top-down	Bottom-up	Summerfelt, 1996
4 - Time of cleaning	Morning	Evening	Ostaszewska et al. 2005; Kestemont et al., 2007; Szkudlarek and Zakęś, 2007; Hamza et al., 2008; Ljubobratović et al., 2015; El Kertaoui et al., 2019
Experiment 2 - Feeding factors			
5 - Beginning of weaning	10^{th} day	16^{th} day	Steenfeldt, 2015; Hamza et al., 2007
6 - Mode of food distribution	Continuous	Discontinuous	Ostaszewska et al., 2005; Kestemont et al., 2007; Hamza et al., 2008, 2010; 2012; Ljubobratović et al., 2015; Król and Zakęś, 2016; Szkudlarek and Zakęś, 2007; Lund et al., 2012
7 - Co-feeding	Yes	No	Hamza et al., 2007; Szkudlarek and Zakęś, 2007; Ljubobratović et al., 2015; Król and Zakęś, 2016; Lund et al., 2012, 2014
8 - Weaning duration	3 days	9 days	Kestemont et al. 2007; Lund et al. 2014; Hamza et al., 2007; Lund et al., 2012
Experiment 3 - Populational factors			
9 - Density	Small	High	Hamza et al., 2007; Szkudlarek and Zakęś, 2007; Tielmann et al., 2016 ; Xu et al., 2017
10 - Sorting out of fish'jumper	Yes	No	Mandiki et al. 2007
11 - Sibling or not sibling	Sibling	Not sibling	Mamcarz et al., 1997; Skudlarek and Zakęś, 2007; Policar et al., 2016; Schaefer et al., 2017; Tielmann et al., 2017; Xu et al., 2017
12 - Female weight (kg)	Small (< 3)	Large (> 3)	Kamler, 1992; Johnston, 1997; Imanpoor et al., 2009; Raventos and Planes, 2008

biological explication. When an interaction or a simple effect has a potential effect on tested variables (read on Daniel's graphics), they were statistically tested with an ANOVA (R Core Team, 2017). When an interaction between two factors was found significant, the potential single effects of these factors alone were considered insubstantial and a test of Tukey was realized as post-hoc test. Data are presented as means ± standard errors of the means.

For the experiment 3, it was expected to apply similar multifactorial analyses (Kobilinsky, 2000). However, we observed a very high mortality level in tank 7 the February 22^{th} 2017, consequently we removed this tank from the analysis. A two-way ANOVA (aov, R Core Team, 2017) was performed to calculate F-tests followed by a Tukey's range test based on Tukey–Kramer method for multiple comparisons as post-hoc test (TukeyHSD, R Core Team, 2017). When data, even log-transformed, did not meet the assumptions for ANOVA (homogeneity of variance and normality), we used the aligned rank transformation for non-parametric 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).

All statistical analyses were performed using the free software R version 3.2.4 (R Core Team, 2017 except for Daniel's graphics, which were performed with ANALYS software (Kobilinsky, 2000; Gardeur et al., 2007). For ANOVA validations, residuals were tested for homogeneity and normality using residual *vs*. fitted value and sample *vs*. theoretical quantile (Q-Q) plots, respectively [plotresid, package 'RVAideMemoire' (Hervé, 2020)]. The level of significance used in all tests was $P < 0.05$. Results were presented as Mean ± Standard Deviation. In this study, all results presented were final results at 39 dph for the experiment 1 and 53 dph for the experiment 2 and 3 and validation test.

2.5. Ethical note

During all procedures, we took care to minimize handling and stress as much as possible for the experimental animals. All fish treatments and procedures used in this study were in accordance with the guidelines of the Council of European Communities (2010/63/UE) and approved by the French Animal Care Guidelines (agreement number: C54-547-18).

3. Results

3.1. Experiment 1: Effects of environmental factors

For this experiment 1, final survival rates were very low, 1.1 ± 0.8 % per tank. The mean SGR for the eight tanks was 16.2 ± 1.1 %.d^{-1} between the first sampling date (T0, 4 dph) and the last one (T35, 39 dph). At 39 dph, juveniles were smaller with a light intensity of 5 lx and a 50 % water renewal rate compared to the three other combinations ($F_{(1,6)}$ = 35.1; p = 0.027; Fig. 1A). It was identical with a light intensity of 5 lx and a morning cleaning time compared to the three other combinations ($F_{(1,6)}$ = 35.4; p = 0.027; Fig. 1B). There were significant simple effects of light intensity and water current direction on the CV TL (Fig. 2). CV TL was higher when the water flow was bottom-up ($F_{(1,6)}$ = 31.1; p = 0.01; Fig. 2A), and these values were higher for fish exposed at 5 lx than for those exposed at 50 lx ($F_{(1,6)}$ = 21.8; p = 0.02; Fig. 2B).

3.2. Experiment 2: Effects of feeding factors

At the end of the experiment, the swim bladder inflation rate was 43.0 ± 35.9 % per tank, the final biomass harvested was 1.5 ± 0.6 kg per tank, and survival rates was 7.9 ± 3.5 % per tank. The mean SGR for all tanks was 15.6 ± 0.5 %.day^{-1} between the first sampling date (T0, 4 dph) and the last one (T49, 53 dph). At 53 dph, there were two significant interactions between the beginning of weaning with the weaning duration on W ($F_{(1,6)}$ = 11.8; p = 0.041) and TL ($F_{(1,6)}$ = 214; p = 0.005). Post-hoc tests did not show any significant differences between the four combinations of tested parameters, but with weaning beginning at 16 dph and lasted nine days, juveniles tended to be heavier and larger (Fig. 3A, B). Furthermore, there was a significant interaction between the weaning duration and the mode of food distribution on fish size ($F_{(1,6)}$ = 63.8; p = 0.015; Fig. 3C), and between the beginning of weaning and the co-feeding on the CV W ($F_{(1,6)}$ = 24.8; p = 0.016; Fig. 3D). For both interactions, post-hoc tests did not demonstrate significant differences between the four combinations, but larvae tended to be larger with a continuous distribution of food and nine days of co-feeding and the CV W tended to be lower with a beginning of weaning at 16 dph with or without co-feeding. With discontinuous feeding, CV W was significantly lower (30.5 %) than with continuous

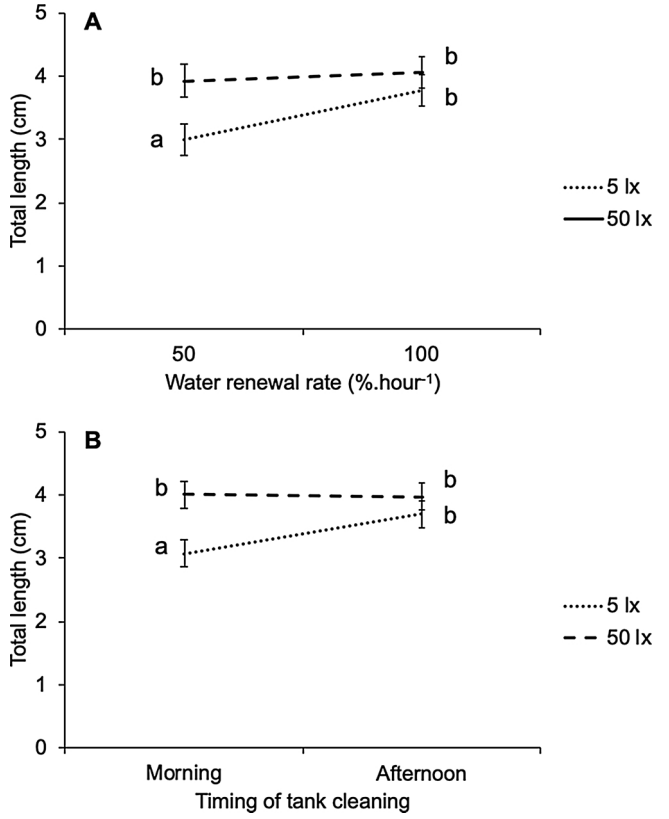

Fig. 1. Effect of the interactions between: (A) water renewal rate and light intensity and (B) timing of tank cleaning and light intensity on the pikeperch total length at 39 days post-hatching. Different letters indicate a significant difference ($p < 0.05$)

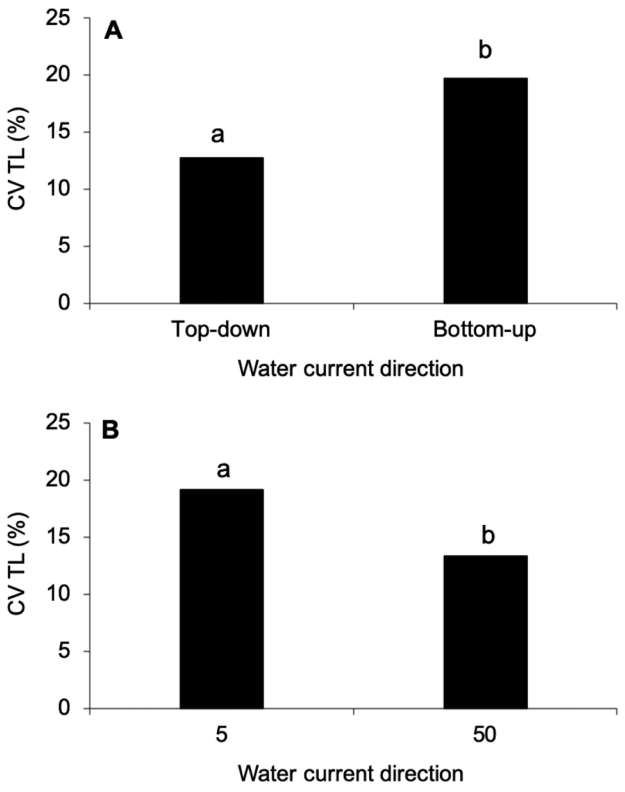

Fig. 2. Effect of: (A) water current direction and (B) light intensity on the coefficient of variation of total length (CV TL, %) of pikeperch juveniles at 39 days post-hatching. Different letters indicate a significant difference ($p < 0.05$).

feeding (33.8 %) ($F_{(1,6)} = 24.8$; $p = 0.02$). There was a significant effect of the interaction between the beginning of weaning and the weaning duration on SGR ($F_{(1,6)} = 18.8$; $p = 0.023$; Fig. 4). In addition, the final swim bladder inflation rate was lower (18.2 %) with three days than with nine days of weaning (67.8 %) ($F_{(1,6)} = 12.4$; $p = 0.024$). Finally, mean final biomass was significantly lower with a continuous food distribution (1.1 ± 0.6 kg) than with a discontinuous one (1.8 ± 0.7 kg) ($F_{(1,6)} = 12.9$; $p = 0.037$) and significantly lower after three days of weaning (1.1 ± 0.4 kg) than after nine days (1.8 ± 0.7 kg) ($F_{(1,6)} = 10.9$; $p = 0.046$).

3.3. Experiment 3: Effects of populational factors

At the end of the experiment 3, the swim bladder inflation rate was very high in all tanks, 92.9 ± 4.5 %, the final biomass harvested 3.6 ± 1.7 kg per tank, and survival rates was 5.2 ± 2.6 % per tank. The mean SGR for all tanks was 14.3 ± 0.5 %.d^{-1} between 4 dph (T0) and 53 dph (T49). At 53 dph, a significant effect of the interaction between the sorting of jumpers and initial density on final mean W ($F_{(1,5)} = 91.3$; $p < 0.0001$) was observed with heavier juveniles with a low larval density and no removal of jumpers (Fig. 5A). Moreover, a high larval density with removal of jumpers resulted in a significantly higher W gain compared with no removal (Fig. 5A). Larvae density had no effect on W gain when they came from smaller females, but, a decrease of initial larvae density was associated with heavier juveniles when larvae came from larger females ($F_{(1,5)} = 4.8$; $p = 0.03$; Fig. 5B). A significant mean W juveniles' difference was recorded when fish were originated from smaller females and sibling were not mixed compared to the three others combinations ($F_{(1,5)} = 68.7$; $p < 0.0001$; Fig. 5C). Without sorting out of jumpers, juveniles were heavier in sibling population ($F_{(1,5)} = 10.6$; $p = 0.0001$; Fig. 5D). Simple effects of sorting out of jumpers ($F_{(1,5)} = 13.9$; $p < 0.0001$), female W ($F_{(1,5)} = 21.4$; $p < 0.0001$), sibling mix ($F_{(1,5)} = 5.5$; $p = 0.02$) and initial larvae density ($F_{(1,5)} = 6.7$; $p = 0.01$) on the final W of juveniles were shown. The final mean W of juveniles was significantly higher when the jumpers were not sorted, the fish came from small females, populations were not mixed, and initial larval density was lower (50 larvae.L^{-1}).

3.4. Validation of the best combination of factors

In the validation test, a best combination of factors was chosen in function of the results of the three experiments previously conducted, and tested in eight tanks (Table 4). Final mean W of juveniles was 815.6 ± 95.3 mg at 53 dph per tank. The SGR was 15.1 ± 5.9 %.d^{-1} per tank between 4 (T0) and 53 dph (T49). The mean sampled individual W increased exponentially during the period (Fig. 6). The final biomass harvested at 53 dph was 9.5 ± 0.2 kg per tank. Final survival rate averaged 16.9 ± 1.7 %. The mean swim bladder inflation rate was 92.6 ± 3.2 %. FCR values showed little variation between tanks and averaged at 0.6 ± 0.02.

4. Discussion

Our study confirmed that the use of a multifactorial approach is an efficient methodology to identify the best combination of abiotic, feeding and biotic factors in order to optimize the husbandry process in pikeperch. This method was already used to improve protocols related to the growth and flesh quality in Eurasian perch (Gardeur et al., 2007), the weaning of burbot *Lota lota* juveniles (Trabelsi et al., 2011) or more recently the nutrition of pikeperch larvae (El Kertaoui et al., 2019). In our case, we have identified and validated with eight replicates a combination of factors, which secured pikeperch larval rearing under RAS conditions (Table 4). This combination corresponded to a trade-off between the modalities of the twelve factors, which ensured acceptable

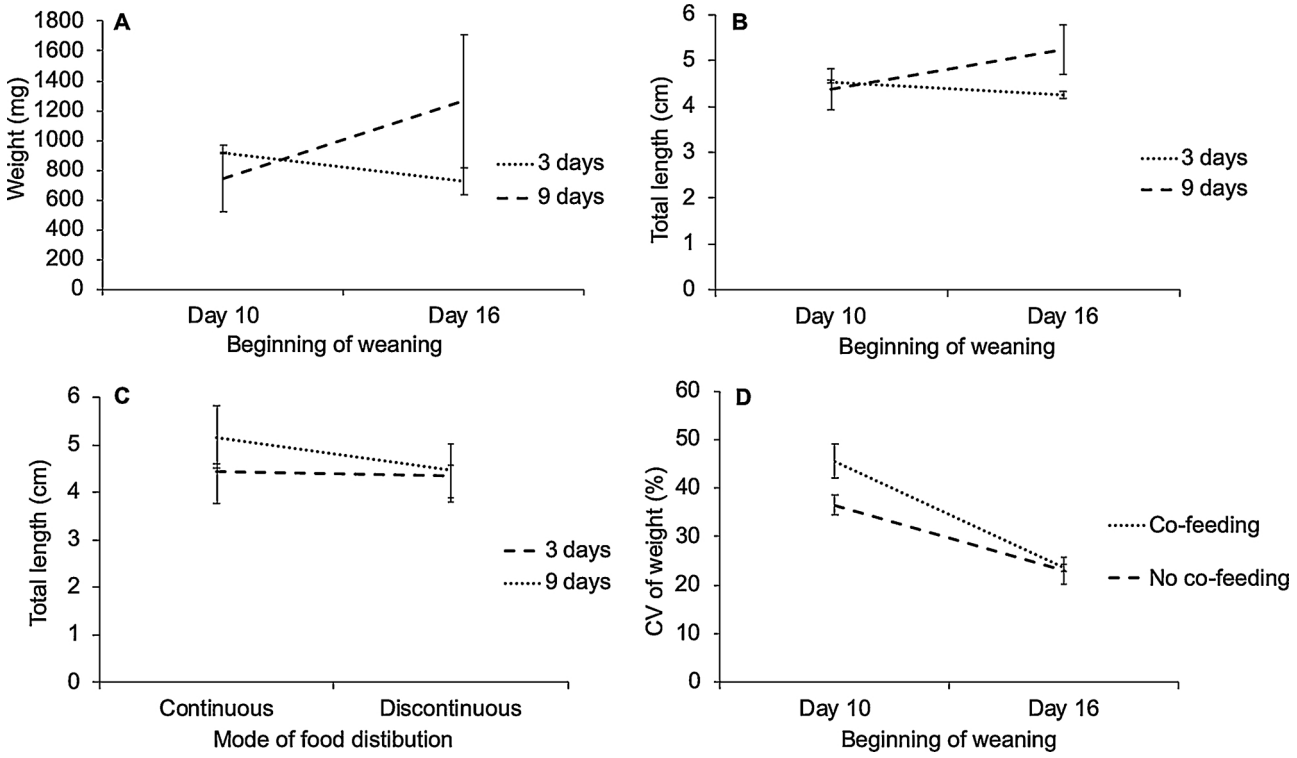

Fig. 3. Effect of the interactions between: (A) beginning of weaning and weaning duration on body weight, (B) beginning of weaning and weaning duration on total length, (C) weaning duration and mode of food distribution on total length, and (D) beginning of weaning and co-feeding on the coefficient of variation of weight on the pikeperch at 53 days post-hatching.

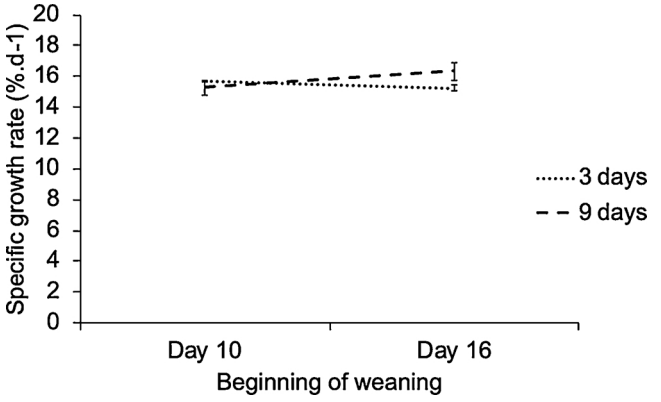

Fig. 4. Effect of the interaction between beginning of weaning and weaning duration on the pikeperch specific growth rate from 4 to 53 days post-hatching.

growth and survival rates on the overall larval production system.

In large tank (700 L) in RAS, raising pikeperch juveniles of 0.7-0.9 g body weight (53 dph) may be conducted with a final average survival rate of 16.9 %, a final stocking density (13.6 $kg.m^{-3}$), a very high level of swim bladder inflation (92.6 %) and a mean SGR of 15.1 $\%.d^{-1}$. The comparison of our results to previous studies was not evident. Few studies had considered pikeperch larval rearing in large tanks and over a similar period (4 to 39-53 dph), including the initial phase of feeding and the weaning period. Studies on pikeperch reported in the literature were mostly carried out for relatively short durations (2-3 weeks) and in small water volumes (20-60 L). It focussed often a specific rearing phase (e.g. weaning) (*i.e.* Hamza et al., 2008; Lund et al., 2014; Ljubobratović et al., 2015; Król and Zakęś, 2016). In fact, only Policar et al. (2016) conducted a comparable study by using also large tanks (360 L, RAS) and a long period (123 days).

In literature, survival rates were very contrasted: from 13 and 24 % after 26 days with 11 dph larvae in 20 L tanks (Kestemont et al., 2007) to 72-79 % after a 14 day experiment from 4 to 18 dph in 200 L tanks Szkudlarek and Zakęś, 2007). In a second experiment, these authors observed survival rates of 45-56 % over a three week trial (from 18 dph to 39 dph) with a mortality rate mainly due to cannibalism (27-35 % of total). It was confirmed with survival rates of 21-43 % by carrying out an experiment focusing only on the weaning strategy using 11 dph larvae (from 11 dph to 27 dph) in 20 L circular tanks with cannibalism rates ranging from 25 to 45 % (Ljubobratović et al., 2015). Many times, the survival rates observed in these studies were higher than under our best combination of factors (16.9 %, *i.e.* 0.3 % $survival.day^{-1}$), but these studies concerned shorter experimental periods (14-35 days *vs* 53 days) and did not integrate critical biological steps like first feeding (transition from endogenous to exogenous feeding) or the period with intense cannibalism which highly increase between weeks 3 and 4 after hatching (Hamza et al., 2008; Colchen et al., 2019).

Growth (SGR) values recorded in the present study were similar to those of different studies: 16.4 to 18.5 $\%.d^{-1}$, measured in 11 to 37 dph fish reared at 20-21 °C (Kestemont et al., 2007), and 12.1 to 16.1%.d^{-1} in 10 to 34 dph fish reared at 21-23 °C (Hamza et al., 2015). These results were higher than those reported by Hamza et al. (2007) who obtained 5.9 – 12.8 $\%.d^{-1}$ in 4 to 36 dph larvae reared at 19-20 °C and Król and Zakęś (2016) that found 12.2 to 12.8 $\%.d^{-1}$ in 15 to 43 dph larvae reared at 20.0 °C. Higher SGR values (19.3-21.6 $\%.d^{-1}$) have been reported in 1-27 dph pikeperch reared in cooler water (16-18 °C) (Lund et al., 2012, 2014). Among the environmental factors (experiment 1), light intensity appeared to be the most influential. Compared to 5 lx, a light intensity of 50 lx allowed obtaining larger larvae. The negative impact of the low light intensity was dependent on the other factors. Mean pikeperch larval size was lower under a low light intensity when the water renewal rate was also low (50 %) or when the tanks were cleaned early morning. A higher light intensity could allow a better growth of pikeperch larvae. This may be linked to the predatory behaviour of this species at larval and juvenile stages. It could reflect the

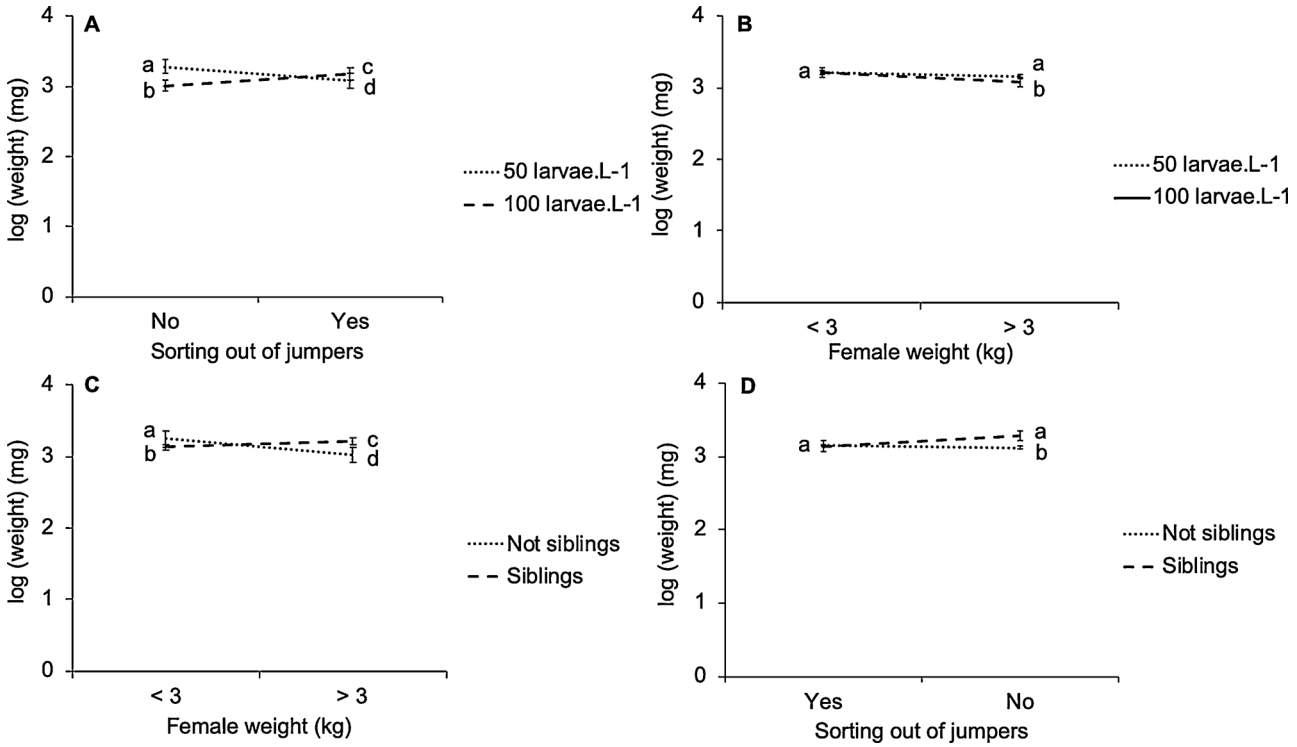

Fig. 5. Effect of the interactions between: (A) sorting of jumpers and initial density, (B) female weight and initial density, (C) female weight and sibling link, and (D) sorting out of jumpers and sibling link on the pikeperch body weight at 53 days post-hatching. Different letters indicate a significant difference ($p < 0.05$).

Table 4
Applied modality for each factor in the validation experiment. This combination of factors was repeated in the experimental tanks (n = 7).

Factors	Modality
1-Density	100 larvae L^{-1}
2-Sorting of fish jumper	No
3-Sibling or not sibling	Not sibling
4-Female weight	Large (> 3.3 kg)
5-Feeding schedule	Discontinuous
6-Co-feeding	No
7-Light intensity	50 lx
8-Weaning start (dph)	16
9-Weaning duration (days)	9
10-Water renewal rate (tank vol./h)	1
11-Tank cleaning period	Morning *
12-Tank current direction	Bottom-up

* The choice of a tank cleaning during the morning period was decided according to working constraints for technical workers.

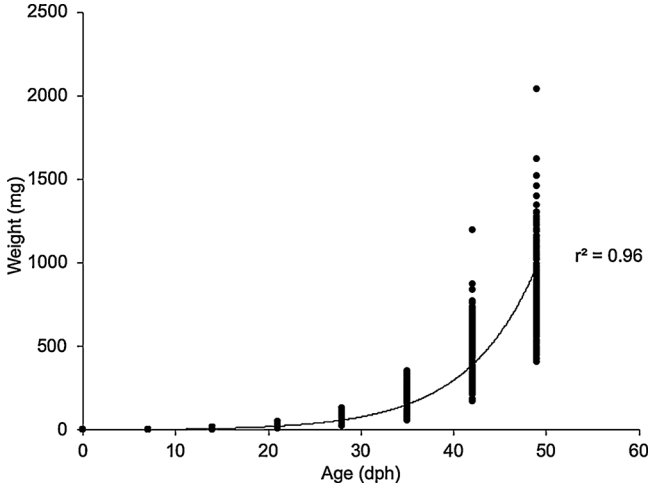

Fig. 6. Average growth curve of pikeperch larvae and juveniles reared in Recirculated Aquaculture Systems until 53 days post-hatching (n = 210, 30 larvae per tank, one individual = a black point).

importance of proper light conditions for detecting prey in the water column (Kozłowski et al., 2010). Pikeperch like the Eurasian perch is very sensitive to lighting conditions (Jourdan, 1999; Kestemont et al., 2003), and the larger size of fish in the 50 lx condition demonstrate an increase of feed intake. It could also explain why the size of larvae was higher in lighter tanks (Strand et al., 2007). The negative effect of cleaning the tank in early morning could be explained by a stress on larvae due to a disturbance during the first feeding. Our study also indicated that a 100 % of water renewal rate promoted larval growth could be explained by the swim training induced by a higher water current (Fiaz et al., 2012).

Feeding factors (experiment 2) have logically a strong influence on pikeperch growth (Kestemont et al., 2015). The interaction between the onset of weaning (10 *vs* 16 dph) and its duration (3 *vs* 9 days) acted significantly on the mean larval TL and W. These variables were higher when fish were weaned later with a longer weaning duration. Similar results (weaning should take place at 19 dph) were found at the same rearing temperature (Kestemont et al., 2007). This emphasizes the importance of the timing of weaning besides the ontogeny of the digestive tract and the appearance of a functional digestive activity (Dabrowski, 1992; Cuvier-Pérès and Kestemont, 2002; Hamza et al., 2015). Pikeperch larval growth was also influenced by the interaction between the types of food distribution in interaction with co-feeding. When co-feeding was applied, no effect of the type of food distribution was observed, whereas in absence of co-feeding, pikeperch larvae were heavier and larger when reared under continuous feeding. This effect was not observed after 25 dph. The use of larger females and subsequent larger eggs is correlated to higher juvenile weight in sibling populations while an inverse result is obtained with mixed population. A positive effect of jumper sorting was observed when larval density was high and a negative effect when the density was low. No effect of

the type of population (sibling or mixed) was observed when the jumpers were sorted, whereas larger juveniles occurred in sibling populations *vs* mixed, if the jumpers were not sorted.

The regulation of TL or W heterogeneity can contribute to decreasing or stabilizing cannibalism (Kestemont et al., 2003). This behavioural issue has major consequences in pikeperch larval rearing (Colchen et al., 2019; Cortay et al., 2019). Numerous biotic and abiotic factors influence the size heterogeneity in a population. In our study, pikeperch larvae were more homogeneous in size under the following rearing variables: i) low light intensity, ii) tank cleaning during afternoon, iii) higher renewal rate (100 %) or iv) bottom-up water current. Our results also suggested that a later weaning increased W heterogeneity. Concerning feeding factors, significant lower coefficients for W heterogeneity were noted at 53 dph when larvae were weaned at later ages, which confirmed the effect of the weaning time on fish size heterogeneity (Hamza et al., 2007; Kestemont et al., 2007). Pikeperch batches were more homogenous at 53 dph with a discontinuous feeding. The abiotic conditions and the feeding strategy have a major impact on growth heterogeneity in pikeperch larval rearing (Table 4).

Differential survival and growth rates observed during this study explain the final biomass and fish density observed. The higher survival rates resulted in higher final stocking density, but it negatively affected the individual final W and TL. A negative relationship between individual growth performance and stocking density was also found (Szkudlarek and Zakęś, 2007). Under our experimental conditions, final biomass values were also related to a longer weaning duration and a discontinuous feeding. High final biomass was also positively correlated to a higher initial larvae density as it was previously observed (Szkudlarek and Zakęś, 2007), and to the use of larvae of bigger females. However, it was independent of jumper sorting and of the use of a sibling population. A high initial larvae density (85 larvae.L^{-1}) was already applied in pikeperch larval rearing in similar conditions (Policar et al., 2016). These authors obtained a final fish biomass of 9.6 kg per m^3, which is very close to our results. In our study, some effects have been also observed on the swim bladder inflation. As in our study, in Eurasian perch (*Perca fluviatilis*), variable levels of swim bladder inflation rates were recorded at 53 dph, ranging from ca. 10 % to 100 % (Jacquemond et al., 2004). The swim bladder inflation had a direct link with the food intake abilities of fish and could explained why the mode of food distribution affected the level of swim bladder inflation (Rinchard et al., 2008).

We have identified and validated a combination of modalities of twelve abiotic and biotic factors that ensures the production of pikeperch juvenile with reliable performances. The proposed protocol has some limits for know-how transfers at the fish farmer level, and it may be easily improved. Our results are highly linked to some parameters that have been voluntary fixed like the rearing system (tank wall colour, volume or shape), the broodstock characteristics and management, the commercial diets or some abiotic factors (*i.e.* photoperiod and temperature). That means that our results could be somewhat different if one or several of the fixed factors were modified. We have always used the same broodstock; however, we have not exactly used the same breeders during the three years. Reproductive traits of these breeders may have changed during these years (*i.e.* age, spawning rank). These biological traits could have taken part in the progressive improvement of the rearing protocol. However, we must also consider that the prospects of improvement are real and numerous indicating that their associated performances can be significantly increased. Firstly, progressive adaptation of the abiotic environment (*i.e.* lighting conditions, temperature) according to the fish ontogeny (retina) or behaviour (shoaling) could take place. In fish, the sensory system begins to develop during embryogenesis and evolves over the larval and juvenile stages (Kamaszewski and Ostaszewka, 2015). The development and functionality of digestive tract and structures occur progressively over the two first months (Hamza et al., 2015). For example in Eurasian perch, larvae (0.8 mg, 1 dph) or post-larvae (45.9 mg, 47 dph) have different *preferendum* for light intensity (Jourdan, 1999; Kestemont et al., 2003). Secondly, the use of a photoperiod based on a longer photophase could allow the application of more efficient feeding strategies to improve growth and survival as observed in Eurasian perch (Migaud et al., 2001; Kestemont et al., 2003). Thirdly, the use of smaller size prey like rotifers during the first days of the exogenous feeding period could improve the survival rates (Yanes-Roca et al., 2018).

5. Conclusion

Concluding, the use of a pilot scale larval rearing system for three successive experiments based on multifactorial designs allowed us to identify a best combination of twelve abiotic, feeding and biotic factor modalities for pikeperch larval rearing in RAS: F1 - initial density: 100 larvae.L^{-1}, F2 - no sorting of fish jumper (the biggest fishes), F3 - no sibling population, F4 - eggs from large females, F5 - discontinuous feeding, F6 – no co-feeding, F7 - light intensity : 50 lx, F8 - beginning of the weaning at 16 dph, F9 - weaning duration: 9 days, F10 - water renewal rate of 100% per hour, F11 - tank cleaning during morning and F12 - tank bottom-up water current. The validation of this combination by an additional trial confirmed the reliability of the protocol proposed and secure further know-how transfer to fish farmers.

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Author Contributions

All authors have given their approval to the final version of the manuscript. TC EG PF AP: conceived and designed the experiments. TC YL DK AP PF: performed the experiments. PF: supervised the work. TC EG PF AP: wrote the first draft of paper. TC AP: reviewing and editing the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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