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The effects of polyculture on behaviour and production of pikeperch in recirculation systems

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ABSTRACT

Recirculating aquaculture systems (RASs) are seen as a promising technology to address the societal and environmental challenges of aquaculture. However, this technology is mainly used in intensive monoculture and little knowledge is available in polyculture approaches. In this study, we studied survival, growth performance, and behaviour of juvenile pikeperch *Sander lucioperca* reared in RAS either in monoculture or in polyculture (associated with sterlet *Acipenser ruthenus* or tench *Tinca tinca* or both sterlet and tench). After 30 days, the survival rate was 100 % for both monoculture and polyculture reared pikeperch. The mean final weights and the biomass gain of pikeperch were significantly lower for pikeperch alone (respectively 75.7 ± 2.7 g and 25.2 %) or with sterlet (respectively 81.0 ± 3.2 g and 38.3 %) than in combination with tench (respectively 85.7 ± 8.1 g and 50.1 %) or with sterlet and tench (respectively 90.3 ± 16.4 g and 51.5 %). Behavioural changes were also detected depending on the fish combination: there are fewer interactions between individuals of pikeperch when reared alone (0.34 ± 0.10) or in polyculture with sterlet (0.40 ± 0.02) in comparison to the other polyculture conditions (with tench [0.80 ± 0.20], or the two other species [0.70 ± 0.15]). The group structure also differed with a lower cohesion and homogeneity of the pikeperch group when they were reared in monoculture compared to polyculture modalities. No aggressive interaction was detected between pikeperch regardless of rearing modalities. In conclusion, due to positive effects on growth parameters and few behavioural changes in pikeperch, this study highlights that RAS polyculture is a relevant alternative production strategy for pikeperch compared to monoculture.

1. Introduction

Aquaculture has been experiencing exponential development over the past decades to ensure human food demands, while capture fishery has remained stagnant (FAO, 2018). Nowadays, aquaculture uses several different rearing environments such as cages, ponds or yet Recirculating Aquaculture Systems (RASs) (FAO, 2018). The latter operates by treating water from one or several tank(s) prior reusing it in the same tank(s) (Martins et al., 2010). RAS is mainly used to intensively produce a single species (monoculture), mostly fish having a high commercial value such as *Salmo salar* smolts (Bergheim et al., 2009), flatfish as *Scophthalmus maximus* (Liu et al., 2017a) or catfish (Besson et al., 2014). RASs offer the following advantages: reduction in the

amount of water required to produce aquatic food, minimal space requirements for aquatic species production, higher control on rearing conditions compared to other systems, limitation of environmental deleterious consequences of aquatic food production by facilitating the treatment of effluents and minimising the risk of escape of domesticated individuals to the wild (FAO, 2018). However, these advantages are achieved through massive use of energy inputs (FAO, 2018). RASs are also very restrictive for fish that are subject to stress through handling, containment and high density (Liu et al., 2017b). Because of a high dependency on the inputs, and intensive farming conditions, RASs could be regarded as a poorly sustainable aquatic food production approach. It is also generally considered that monoculture systems are poorly robust, with a reduced viability and ability to withstand pest

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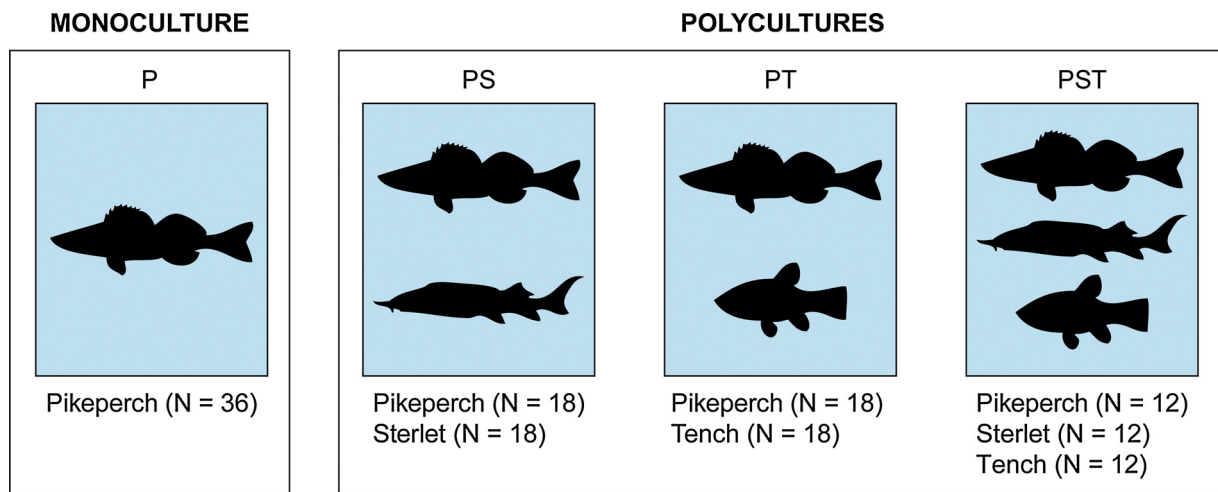


Fig. 1. Description of the experimental design. Four modalities were tested in triplicate: one modality in monoculture: P (pikeperch) and three modalities in polyculture: PS (pikeperch and sterlet), PT (pikeperch and tench) and PST (pikeperch, sterlet and tench). The initial fish number was 36 (with an adjustment of fish number by species according to the modality).

attack (e.g. Dahlberg, 1979).

Although monoculture is the prevailing approach in RAS, polyculture could theoretically overcome shortcomings of this production system. Polyculture has been proved to be a valuable option to increase the efficiency and the sustainability of production systems (Dumont et al., 2013). Polyculture can improve the system functioning by taking advantage of coexistence and interactions between species (Altieri et al., 2014). The efficiency of the polyculture systems could be improved thanks to animal diversity (Zimmermann and New, 2000; Gupta and Rab, 1994). First, it ensures a better food resource utilisation and limits food losses (Milstein, 1992; Rahman et al., 2008; Bregnballe, 2015; Thomas and Piedrahita, 1998). Second, species diversity allows a lower level of waste by the recycling of co-products provided by the other species or by optimal utilisation of space (Zimmermann and New, 2000). Polyculture can also offer interesting alternative to drug treatments by promoting direct biotic interactions (e.g. the use of cleaner fish; Brooker et al., 2018). However, potential polyculture advantages for RAS remain theoretical since very few studies have begun to scratch polyculture implementation in RAS. To fill this gap, we conducted here the first multi-trait assessment of the consequences of RAS polyculture by comparing survival rates, growth performance and behaviour of the pikeperch (*Sander lucioperca*), a freshwater carnivorous species, in monoculture or in polyculture with two other species, the sterlet (*Acipenser ruthenus*) and the tench (*Tinca tinca*). We chose these species because their polyculture could improve pikeperch RAS productions. Pikeperch is a valuable freshwater fish (Kestemont et al., 2015) mainly produced in RAS monoculture (Molnár et al., 2004). This production strategy faces two main challenges: steady supply of good-quality water and fish stress reduction. As pikeperch does not take the feed on the bottom of the production tanks (Kozłowski et al., 2008, 2014), its monoculture gives rise to poor water quality issues (Thomas and Piedrahita, 1998; Zakęś, 2012). Therefore, associating pikeperch with bottom-feeder species such as sterlet and tench (Kottelat and Freyhof, 2007) could improve the water quality and therefore the fish production. Moreover, pikeperch is very sensitive to stress occurring in rearing systems (Milla et al., 2015). Although no scientific assessment is available to date, fish farmers to stocks of other fish species commonly add tench during transportation because of their known soothing effect on the other fish species. Therefore, co-rearing of tench and pikeperch could mitigate the pikeperch stress issue. Although pikeperch and sterlet co-rearing in RAS has already been investigated (Kozłowski et al., 2014), its production consequences are not clear, and its behavioural consequences are still unknown. The aim of this study was to compare survival rates and growth and behaviour of pikeperch reared

alone and with other fish species.

2. Material and methods

2.1. Experimental environment

The experiment was carried out at the Experimental Platform for Aquaculture (registration number for animal experimentation C54-547-18) belonging to the UR AFPA lab located at the Faculty of Sciences and Technologies of the University of Lorraine (France). Juvenile pikeperch (58 ± 10 g) were reared in our facilities, whereas juvenile sterlet (17 ± 4 g) and tench (40 ± 6 g) were obtained from the Fisheries Cooperative Győr (Hungary). Before the beginning of the experiment, fish were transferred into indoor aquariums ($100 \times 60 \times 50$ cm). Each aquarium was an independent RAS. The temperature ($21 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$) and luminosity (20 lx) were continuously controlled during the acclimation and experimental periods. The light/dark period was 10 h/14 h, using light-emitting diodes (LEDs of 4.000 °K). The water quality (ammonia, nitrites, pH and dissolved oxygen) was monitored three times per week (Supplementary data S1). For the pH maintenance, sodium bicarbonate (NaHCO_3) was used. Fish were fed manually with a commercial diet (Sturgeon Grower, semi-floating, 3 mm, Le Gouessant France), three times per day (at 9 a.m., 12 a.m. and 3 p.m.) with a standardised food ration (i.e. 1.5 % of the total fish biomass of each aquarium). Aquarium cleaning was performed once a week. These rearing conditions (Tab. S1) were based on fish farmer practices and scientific literature (Akbulut et al., 2013; Baekelandt et al., 2018; Bayrami et al., 2017; Pula et al., 2018). Four modalities (P, PS, PT, and PST) were tested, each in triplicates, as described in Fig. 1. The total initial biomass thus differed between aquariums. We chose to work with a fixed number of fish per aquarium instead of uniform biomass, because the number of fish could influence the behaviour and particularly the relationships.

All fish treatments and procedures used in this study were in accordance with the guidelines of the European Parliament and of the Council (2010/63/EU) and the French Animal Care Guidelines.

2.2. Production parameter assessment

Before all manipulations, all fishes were anaesthetised with a Tricaine solution (MS 222 , 80 mg L^{-1}). At the beginning and at the end (after 33 days) of the experiment, all fishes ($n = 432$) were individually weighed (precision of 1 g) allowing estimation of growth parameters (i.e. the mean final body weight, the weight heterogeneity (CV), the

biomass gain (BG) and the specific growth rate (SGR)) (Supplementary data S2 and S3). The survival rate was also recorded. All these parameters were recorded to determine fish growth performance (e.g. Rapp et al., 2019; Rożyński et al., 2017). In order to compare production performances between the four modalities, a random sampling of 12 pikeperch per aquarium was first achieved. The same statistical procedure was repeated for all parameters during the experiment. Production parameters (i.e. survival rates and growth) were compared by using a general linear model (Bates et al., 2015), with the replicate as random variable and the modalities as categorical variable. Prior log transformations were performed when the data did not fit the normality. All statistical analyses were performed using R version 3.6.1 (R Development Core Team, 2019), and the significance threshold was set at $p < 0.05$.

2.3. Behavioural assessment

Periods of video recording were defined from day 32 to day 33 after weight control (day 30), during feeding. Each day, four videos (one per modality) were made and the recording order of the modalities and aquariums was chosen at random. The total video recording lasted two days. Each video recording started at least 30 min before a feeding period (the 9.00 a.m. feeding period was not considered because it took place just after the light was turned on) and stopped at least 50 min after. Two cameras (Sony Handycam DCR-SR72E) used simultaneously were placed in front of the aquarium (at a distance of 0.80 cm). For each modality, videos were recorded in two replicates.

To follow fish behaviour, observations from the videos were limited to what was happening within the first 15 cm from the front window as feed was distributed in that area, through a small slot (3 × 50 cm) 2 cm from the front window. Fish behaviours were apprehended throughout: (i) the interactions between pikeperch, and (ii) the structure of the pikeperch group based on the distances between individuals. To analyse these parameters, three periods were considered; (i) 30 min before introduction of the feed, (ii) 20 min immediately after introduction of the feed and (iii) 30 min after the feeding period.

2.3.1. Interindividual interactions

The videos were displayed using VLC Media Player 3.0.7.1 (VideoLAN, Paris, France). Only pikeperch behaviours were noted. The three periods (see above) were analysed: to that end, bouts of two minutes were chosen every five minutes of the videos; so six bouts of two minutes were analysed for the two periods, which lasted 30 min before and after the feeding period, and four bouts for the feeding period, which lasted 20 min. No aggressive behaviours (i.e. attack, bite) were noticed between pikeperch, so only the following behaviours were noted: orientation-advance, contact and consequence of a contact (for definitions, see Colchen et al., 2017). During each two minute-bout, the numbers of behaviours as a function of the number of pikeperch present during the observations were recorded. The numbers of behaviours by fish were compared between the four modalities, using a linear model (Bates et al., 2015; Kuznetsova et al., 2017), where the number of behaviours by fish was the fixed variable, the period (relatively to feeding), the time of the day (morning or afternoon) and the replicates were the random variables, and the modalities were the categorical variable. When the data did not fit the normality, a square root transformation was performed. As no effect of the period or of the time of the day was noticed, all the data were combined for further analyses. Then, a one-way analysis of variance (ANOVA) was carried out to compare behaviours between the four modalities, with Tukey's pairwise comparisons when the result was significant. These statistical analyses were conducted with R and the significance threshold was set at $p < 0.05$.

2.3.2. Group structure

The group structure was based on three types of interindividual distance measurements within a group: (i) the distance to the nearest

neighbour (DNN) for each pikeperch of the group, which is an index of the group aggregation, (ii) the mean distance (MD) for each fish to all the other members of the group, which is correlated with the group cohesion, and (iii) the variance of this mean distance (VMD), which is a parameter of the group homogeneity (Buske and Gerlai, 2011). These measurements were based only on the subpopulation of pikeperch that was observable. These three group structure parameters were compared before and after feeding periods in each modality and between modalities, using general linear models (Bates et al., 2015). The distance to the nearest neighbour, the mean individual distances and the variance of the latter were the fixed variables, the period and the replicate were the random variables, and the modalities were the categorical variable. A one-way ANOVA was carried out to compare group structures between the four modalities, with Tukey's pairwise comparisons. When the data did not fit variance homogeneity and a normal distribution even after transformation (ln or square root), non-parametric Kruskal and Wallis test (KW) was used. Statistical analyses were performed with R and the significance threshold was set at $p < 0.05$.

3. Results

3.1. Production parameters

The survival rate was 100 % for pikeperch after one month in all modalities. At the beginning of the experiment, pikeperch weights were similar between the four modalities (ANOVA, $F_{3,8} = 0.81$, $p = 0.48$). The mean final weight for this species between the four modalities significantly differed (ANOVA, $F_{3,8} = 7.10$, $p < 0.05$) (Fig. 2A). Pikeperch alone were of lower weight than those with tench ($p < 0.05$) and those with both tench and sterlet ($p < 0.05$) (Modality P: 75.7 ± 2.7 g; Modality PT: 85.7 ± 8.1 g; Modality PST: 90.3 ± 1.6 g). Moreover, juvenile pikeperch weight was lower when fish were reared with sterlet than those reared with both tench and sterlet ($p < 0.05$) (PS: 80.1 ± 3.2 g). After one month, there was no difference in CV between the pikeperch of the four modalities (ANOVA, $F_{3,8} = 1.34$, $p = 0.33$). Pikeperch BG also differed between the four modalities after one month (ANOVA, $F_{3,8} = 6.58$, $p < 0.05$) (Fig. 2B). The pikeperch alone had lower BG than those reared with tench ($p < 0.05$) and those reared with both sterlet and tench ($p < 0.05$) (P: 25.2 %; PS: 38.3 %; PT: 50.1 %; PST: 51.5 %).

All the data for tench and sterlet were hosted in the TOFF database and can be directly consulted through an online system with open-source access (Lecocq et al., 2019). In particular, the survival rates were 100 % and always greater than 97 % for tench and sterlet respectively, regardless of the modalities. BG varied between 13.5 % and 15.2 % for tench (respectively for PTS and PT modalities) and between 49.8 % and 71.7 % for sterlet (respectively for PS and PST modalities).

3.2. Behavioural parameters

3.2.1. Interindividual relationships

The 'orientation-advance' and 'consequence of a contact' behavioural parameters did not differ significantly between modalities. The 'contact' behavioural parameter exhibited significant differences between modalities (ANOVA, $F_{3,8} = 15.5$, $p < 0.05$) (Fig. 2C). The number of 'contacts' was lower when juvenile pikeperch were alone or reared with sterlet, than for those reared with tench ($p < 0.05$) or with both tench and sterlet ($p < 0.05$).

3.2.2. Group structure

After one month, DNN significantly differed between the four modalities (KW, $X^2 = 12.6$, $df = 3$, $p < 0.05$) (Fig. 2D). DNN values were higher for pikeperch reared with tench than for those reared alone ($p < 0.05$). No significant differences were observed between the other modalities. Moreover, significant differences in MD were observed between the four modalities (KW, $X^2 = 55.2$, $df = 3$, $p < 0.05$) (Fig. 2E).

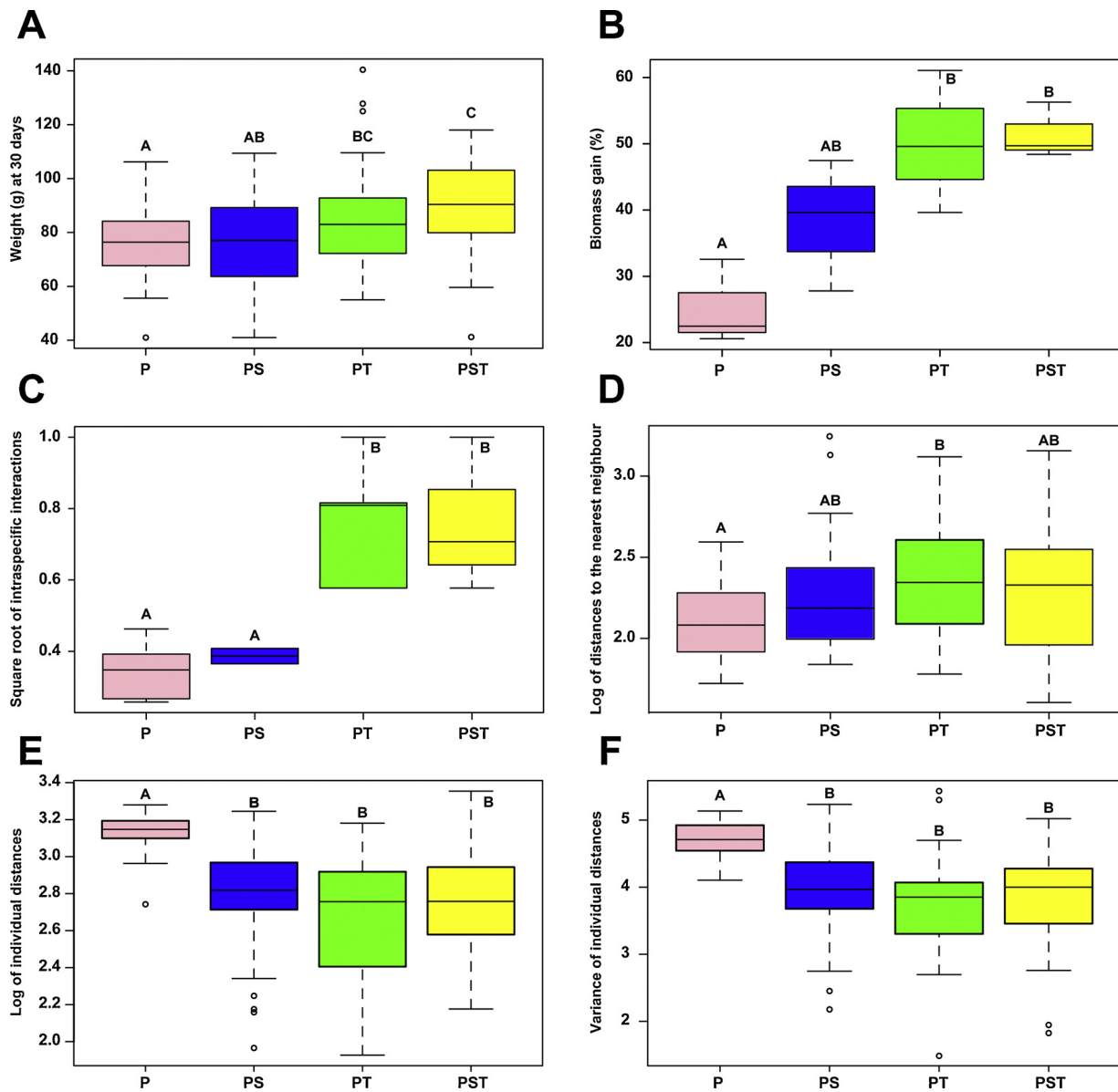


Fig. 2. Growth and behavioural parameters of pikeperch: mean final weight (A) and biomass gain (B) of the juvenile pikeperch after 30 days of experiment, numbers of interindividual relationships (C), distance to the nearest neighbour (D), mean interindividual distance (E) and variance of the mean interindividual distance (F). The different modalities are: P: pikeperch alone, PS: pikeperch with sterlet, PT: pikeperch with tench, and PST: pikeperch with tench and sterlet. Different letters near the top of boxplots indicate statistical differences between the modalities.

MD values were higher for pikeperch alone than for those reared with other species ($p < 0.05$, either tench or sterlet, or both tench and sterlet). Furthermore, significant differences in VMD were also observed between the four modalities (KW, $X^2 = 43,1$ $df = 3$, $p < 0.05$) (Fig. 2F). In all cases, VMD values were higher for pikeperch reared alone than for those reared with other species ($p < 0.05$ either tench or sterlet, or both tench and sterlet).

4. Discussion

Our results show that the RAS polyculture of the pikeperch positively affects juvenile fish rearing performance without affecting deeply their behaviour. Indeed, although the number of contacts between pikeperch juveniles was lower under monoculture than under polyculture conditions, we detected no aggressive behaviours between conspecifics. Furthermore, RAS polyculture seems beneficial for fish farming production since we observed an increase in pikeperch weight from 25 % in monoculture to 51 % in polyculture. This result is

inconsistent with a previous experiment of pikeperch-sterlet RAS polyculture that did not highlight better growth indices for pikeperch juveniles (Kozłowski et al., 2014). The better growth rate measured in our study could result from using different pikeperch individual densities between the four modalities. In contrast, pikeperch individual densities were similar in monoculture and polyculture modalities in Kozłowski et al. (2014), since density can change feed availability and/or intraspecific relationships for pikeperch individuals. First, pikeperch ate the feed in the water column and a very low percentage of the pellets on the bottom (Kozłowski et al., 2008, 2014). Therefore, even though feed quantities were lower when pikeperch juveniles were associated with the other species due to the lower biomass of these species, pikeperch benefited from the totality of the feed (for all the species) during its presence in the water column. This meant that there could have been less competition between pikeperch as they had larger amounts of food per individual by taking the feed before the other two species. This hypothesis was also supported by the results from the behavioural analysis, which revealed lower cohesion and homogeneity

of the pikeperch group when they were reared alone compared to polyculture modalities. Second, intraspecific competition is known to be a key characteristic of the social life in pikeperch, and generally leads to the establishment of hierarchy with dominant fish (Molnar et al., 2018). Since this social relationship depends on the fish density (i.e. the domination phenomenon increases at higher density (Ellis et al., 2002)), the loss of feed due to intraspecific competition, and subsequent lower growth is more likely in the pikeperch alone modality. Our results suggest that the implementation of polyculture in RAS could be an interesting option for the rearing of juvenile pikeperch. Nevertheless, special attention should be paid to the limits of the experiment before projecting our results to the economic scale. On the one hand, we used low fish densities ($\sim 7 \text{ kg m}^{-3}$ in the modality pikeperch alone and lower in the modalities with the other two species), which is quite far from RAS pikeperch monoculture industry conditions (i.e. $80\text{--}100 \text{ kg m}^{-3}$) (Dalsgaard et al., 2013). Since the fish density could influence directly the growth through the interindividual competition for food, our results could not be transposable to industrial fish farming contexts. On the other hand, the experiment lasted only 30 days, while the rearing period of juveniles is one year in the pikeperch industry (Dalsgaard et al., 2013). However, growth parameters measured in this study can be projected over the entire juvenile growing period since juvenile growth is a continued function during this early life stage (Soriano et al., 1992). More generally, this study should be considered as a first step, showing the feasibility and the potential of RAS polyculture for fish production. It opens up great prospects for future studies on RAS polyculture with conditions closer to commercial-scale aquaculture in order to provide a comprehensive assessment of the potential of polyculture in intensive indoor fish farming.

In conclusion, due to positive effects on growth and few behavioural changes in pikeperch, this study highlights that the implementation of RAS polyculture is a relevant alternative option for the rearing of juvenile pikeperch compared to monoculture. This work opens up new prospects for the production of high-value species such as pikeperch, with the need to move towards more efficient and sustainable farming systems.

Author contributions

M. Thomas, T. Lecocq, S. Nahon, J. Aubin, C. Jaeger, A. Wilfart, L. Puillet and A. Pasquet conceived the idea and the design of experimental methodology. A. Pasquet, T. Lecocq, M. Thomas and L. Schaeffer conducted the behavioural study. M. Thomas, A. Pasquet, T. Lecocq and Y. Ledoré monitored the experimental progress. T. Lecocq, A. Pasquet and C. Abregal worked on data analysis. All authors jointly wrote the article.

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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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.aqrep.2020.100333>.

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