



**HAL**  
open science

## Enrichment in a Fish Polyculture: Does it Affect Fish Behaviour and Development of Only One Species or Both?

Marielle Thomas, Jean-Gabriel Reynaud, Yannick Ledoré, Alain Pasquet,  
Thomas Lecocq

### ► To cite this version:

Marielle Thomas, Jean-Gabriel Reynaud, Yannick Ledoré, Alain Pasquet, Thomas Lecocq. Enrichment in a Fish Polyculture: Does it Affect Fish Behaviour and Development of Only One Species or Both?. *Applied Sciences*, 2022, 12 (7), pp.3674. 10.3390/app12073674 . hal-03673470

**HAL Id: hal-03673470**

**<https://hal.univ-lorraine.fr/hal-03673470>**

Submitted on 20 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Article

# Enrichment in a Fish Polyculture: Does it Affect Fish Behaviour and Development of Only One Species or Both?

Marielle Thomas , Jean-Gabriel Reynaud, Yannick Ledoré, Alain Pasquet  and Thomas Lecocq 

Unit Research Animal and Functionality of Animal Products (UR AFPA), INRAE—National Research Institute for Agriculture, Food and the Environment (France), University of Lorraine, 54000 Nancy, France; jean-gabriel.13@hotmail.fr (J.-G.R.); yannick.ledore@univ-lorraine.fr (Y.L.); alain.pasquet@univ-lorraine.fr (A.P.); thomas.lecocq@univ-lorraine.fr (T.L.)

\* Correspondence: marielle.thomas@univ-lorraine.fr

**Abstract:** Physical enrichment of structures has been used for the last decades in aquaculture to improve fish production and welfare. Until now, this enrichment has been practiced in fish monoculture but not in fish polyculture. In this study, we developed a polyculture of two freshwater species (pikeperch and sterlet) in recirculated systems (tank of 2.4 m<sup>3</sup>) with or without physical structures for enrichment. Two types of structures were used: a cover plank on a part of the tank decreasing the light intensity and vertical pipes modifying the water flow. The experiment was conducted in triplicate for a three-month period with juvenile fishes (143 ± 41 g and 27.3 ± 2.2 cm for pikeperch and 133 ± 21 g and 32.8 ± 1.6 cm for sterlet). Behavioural (space occupation and abnormal behaviours) and morphological (total length, final weight, Fulton condition factor, coefficient of variation of the final weight, percentage of biomass gain and specific growth rate) traits were measured. The pikeperch changed their space occupation and showed a preference for low light areas. Sterlet also changed their space occupation: they did not use the cover and occurred mainly in the part of the tank without enrichment. There was no difference for the frequency of abnormal behaviours for pikeperch and sterlet between the two sets (with or without enrichment). There was no statistical difference between the two sets for all the morphological and growth parameters no matter the species and the rearing modality.

**Keywords:** aquaculture; physical enrichment; interspecies relationships; freshwater fish; pikeperch (*Sander lucioperca*); sterlet (*Acipenser ruthenus*)



**Citation:** Thomas, M.; Reynaud, J.-G.; Ledoré, Y.; Pasquet, A.; Lecocq, T. Enrichment in a Fish Polyculture: Does it Affect Fish Behaviour and Development of Only One Species or Both? *Appl. Sci.* **2022**, *12*, 3674. <https://doi.org/10.3390/app12073674>

Academic Editor: Tamás Molnár

Received: 17 February 2022

Accepted: 2 April 2022

Published: 6 April 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

To respond to the increasing demand of human food needs, aquaculture takes a particular role [1]. In Europe and North America, aquaculture during these last decades has been increasingly based on intensive fish rearing, with the development of fish monoculture in restricted farming systems (cages or recirculating aquaculture systems; RAS). A high fish density and a poorly diversified environment, mostly represented by empty structures, characterise these systems. Although such devices allow maximising fish growth and production, most of them do not consider animal welfare [2]. Now animal welfare (i.e., the ability of an animal to adapt to its environment and remain in good health [3]) has become a societal question with the need to be better integrated into farm management. Therefore, new practices should be developed to ensure fish welfare, and physical enrichment of the rearing environment has been proposed as a possible solution. The environmental enrichment is defined as a voluntary addition of environmental complexity into the rearing environment [4] in order “to reduce the expression of the undesirable traits that fish develop in captivity” [5].

For fish rearing undergoing high restrictive conditions as in RAS, the environmental enrichment methods have already been shown to be effective in improving fish welfare [5].

Five categories are recognised for environmental enrichment [5]: physical, sensory, dietary, social and occupational enrichments. However, environmental enrichment may lead to conflictual results according to species and the level of complexity. Some studies have shown that environmental enrichment might have beneficial effects on fish growth [6–10] but also on neural structures [11], behaviours (aggressiveness, space occupation and dominance) [12–16] and cognitive abilities [17]. Other studies showed that the introduction of plants in the tanks or aquaria might be used by the fish as territories and lead to intraspecific conflicts for the space [18–20]. Therefore, physical enrichment could be a coveted resource for some fish species, especially if they are limited in number [19]. The beneficial effects of environmental enrichment on behaviour are thus not obvious and are species- and context-dependent. Moreover, all the studies were conducted for fish monoculture.

Polyculture is a fish farming practice defined as the rearing of two or more fish species in the same environment at the same time [21]. It is mostly applied in ponds but can also be applied in more artificial systems such as in RAS in order to optimise the resources (e.g., spatial and trophic) of the farming system [22,23]. As these rearing systems are very poorly diversified, physical enrichment may help improving the rearing conditions for fish species in polyculture [24]. However, two main problems remain: (i) the choice of the fish species, which may be associated in polyculture and (ii) the type of enrichment devices depending on the fish species requirements.

Here, we focused on physical enrichment of the structures for which different types of enrichment may be added to the tanks. Shelters or cover structures are known to reduce aggression, providing caches for subordinates when there is a high intraspecific pressure [25–32]. Introduction of structural objects can also reduce fin injuries [33–36] and improve growth, survival [37] and welfare [25,38,39]. Shelters can also reduce maintenance and metabolism, as observed in juvenile salmon [40]. However, shelters can have different effects depending on the fish species biology (e.g., cannibalism). Shelters reduced cannibalism in African sharp-tooth catfish (*Clarias gariepinus*) [41] but not in juvenile Asian redtail catfish (*Hemibagrus nemurus*) [42]. There are similar results concerning fish growth: shelters may have positive, negative or no effect on fish growth depending on the size or the species of the fish. A few studies tested several types of physical enrichment simultaneously; the use of a cover and vertical pipes in the same tank increased the biomass gain for juveniles of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) [10], but the physical enrichment of the rearing environment in fish polyculture is another challenge.

To the best of our knowledge, there is no study on the influence of enrichment in a polyculture farming system except studies that integrated the role of nutrient-enriched fertilisers on the growth of fish and shrimps reared in polyculture [42]. In this study, we introduced physical enrichments in a RAS polyculture of two fish species: pikeperch (*Sander lucioperca*) and sterlet (*Acipenser ruthenus*). These two species have an economic value due to the high quality of their flesh (see for pikeperch [43] and sterlet [44]). This fish association has been already used in RAS polyculture studies [22,23,43] because of their abiotic compatibility and complementarity in the exploitation of the same trophic resource. Pikeperch feed on the pellets in the water column whereas sterlet exploit the pellets found at the bottom of the tanks [22,23]. The responses of the fish can affect their behaviours and then also their development. Overall, we assessed whether physical enrichments similarly impact the behaviour and growth of both species.

## 2. Material and Methods

### 2.1. Fish and Environment

The experiment was carried out at the EPA (Experimental Platform for Aquaculture; registration number for animal experimentation D54-547-18) in the University of Lorraine (France). Fishes were juveniles: 15-month-old juveniles of pikeperch (*Sander lucioperca*, mean weight of  $143 \pm 41$  g, mean total length of  $27.3 \pm 2.2$  cm) were reared in our laboratory whereas juvenile sterlet (*Acipenser ruthenus*, mean weight of  $133 \pm 21$  g, mean total length of  $32.8 \pm 1.6$  cm) were obtained from a fishery (Fischzucht Rhönforelle GmbH & Co., Ltd.,

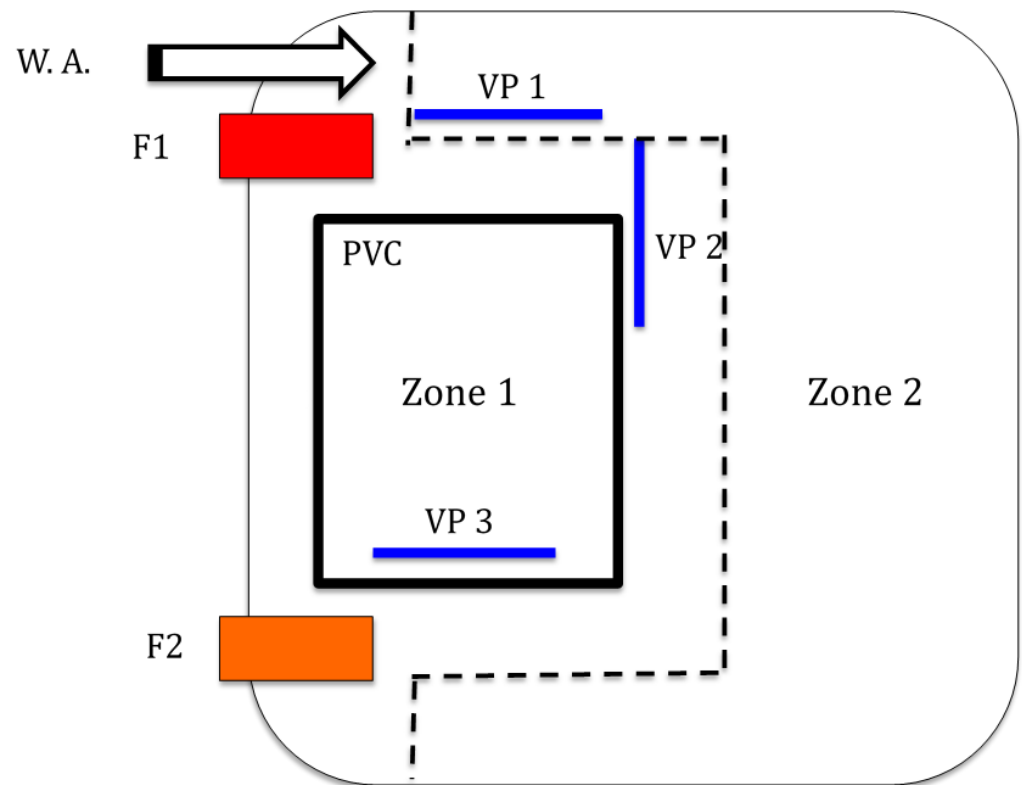
Gersfeld, Germany). A health assessment of the fish was carried out when they were admitted to the animal facility and we confirmed that the fish were in good health. The rearing conditions were defined according to the physiological needs of the two species (for pikeperch [22,45] and for sterlet [46,47]). Each experimental unit was an independent RAS with square tank (2 m × 2 m × 0.8 m) of 2400 L volume. For the physical conditions, the light was turned on for 10 h (from 8.30 a.m. to 6.30 p.m.) with a simulation of dawn and dusk of 30 mn each. The water temperature was stabilised at 20.8 °C (±0.2 °C), the dissolved O<sub>2</sub> at 7.2 mg/L (±0.4 mg/L) and the pH at 7.4 (±0.2). These data were controlled three times per week as were the levels of nitrites and ammonium (mean values for N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>2</sub><sup>-</sup> were respectively 0.06 ± 0.04 and 0.02 ± 0.01 mg/L during acclimatisation and the experimental period).

## 2.2. Experimental Design

During an acclimatisation period, the two species were separated in two different tanks. Fish were fed with Sturgeon Grower 3 (Le Gouessant; granule size of 3 mm; protein content of 47%, lipid content of 13% and a digestible energy of 17.1 MJ/kg). The daily feeding ration was 1.5% of the total biomass. After this acclimatisation period, they were distributed in six tanks, three with physical enrichment (noted E) and three without (noted NE). The study lasted three months (from May 2020 to August 2020). The attribution of the fish of the two species in each tank was done with respect to the size of the fish (same size in the different tanks) and in the number proportion of 60% of pikeperch and 40% of sterlet, a ratio between species justified by the feeding behaviour of the two species. Pikeperch had the first opportunity to access the food while the sterlet consumed the pellets reaching the bottom of the tanks. There was no difference in fish biomass between replicates at the beginning of the experiment. In these conditions, 45 pikeperch and 30 sterlet were placed in each tank for a total biomass of 10.425 g (±60 g) per tank. Fish were introduced on day (0) in the different tanks after sedation with MS222 (80 mg/L), weighted and measured. After that, all fishes were fed with two belt feeders per tank and food distributed continuously for three hours (from 9 a.m. to 12 a.m.). The feeding rate was 1.8% of the total biomass of each tank. Growth control (weight and total length) was conducted every two weeks in order to adjust the feeding rate. The type of feed was the same as during the acclimatisation period.

## 2.3. Physical Enrichment

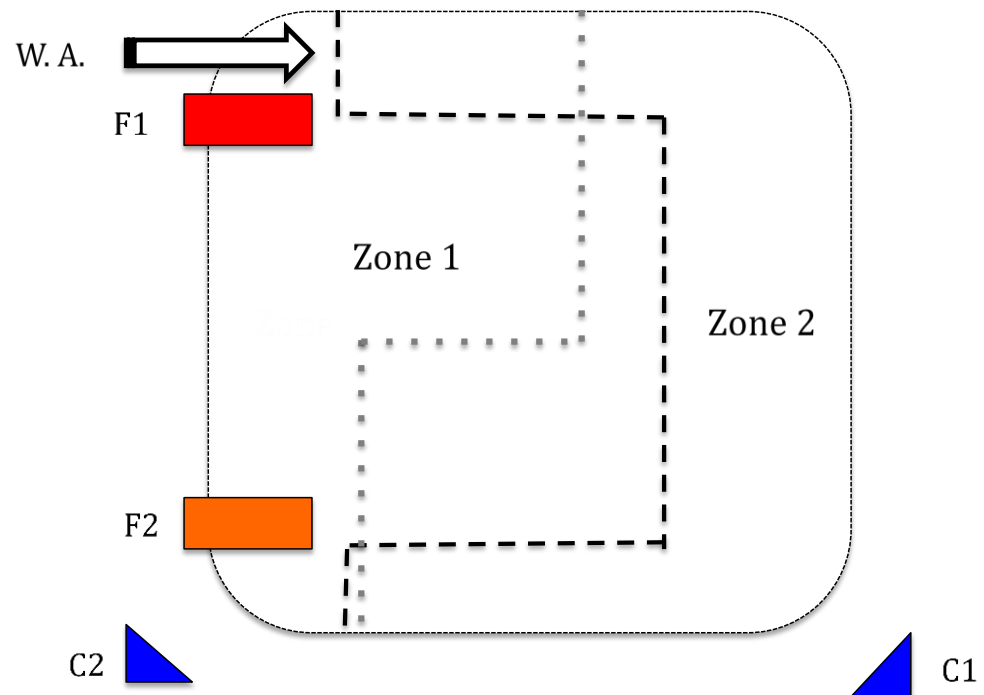
The physical enrichment was chosen in accordance with the preferences of each species. Three of the six tanks were randomly equipped with the physical enrichment. For each tank, the physical enrichments included a cover with a PVC plank and vertical pipes. The cover was chosen because it reduced the light intensity in a part of the tank, and low light is preferred by pikeperch [48]. The pipe positioning aimed to reduce the flow inside the tank and create some zones with lower flow [49]. The pipes were distributed in the tank in order to break the water current, which is dependent on the water arrival in the tank; three structures of five pipes were used (total length: 48 cm; diameter: 3 cm; length of each pipe: 49 cm for the four external pipes and 39 cm for the central pipe). These structures were vertically immersed in the tanks. This environment is suitable for sterlet which prefers stream water with low current [50] but also pikeperch [51]. The PVC planks (L: 90 cm, l: 75.5 cm and H: 1 cm) were put above the water (10 cm) in the left part of the tank where the belt feeders were disposed (Figure 1).



**Figure 1.** Top view of the tank with physical enrichment and showing parts of the tank that differ by the luminosity (zone 1–5 Lux vs. zone 2–20 Lux) due to the cover with PVC plank (PVC). The zones are delimited by the dash line. The surface of zone 1 was larger than the surface of the planks because it projects shadow on the bottom of the tank. W.A. = water arrival; F1, F2 = automatic feeders; VP1, VP2, VP3 = vertical pipes.

#### 2.4. Behavioural Parameters

The behavioural parameters focused on space occupation and abnormal behaviours of the two species. These behaviours were chosen because space occupation can be directly linked to the presence of physical structures in the tanks and abnormal behaviours are related to stress (e.g., stereotypies or behaviours not expressed in the wild). Therefore, these behaviours are indicators of behavioural changes due to environmental enrichment. These behaviours were extracted from videos that were realised with two cameras (Sony HDR CX550 VE) for each tank. To compare the space occupation by the two fish species, we divided each tank into two zones (Figure 1). The first zone (zone 1) matched with the low light intensity (5 lux) zone defined by the projection of the shadow due to the presence of the plank and the two belt feeders for the three tanks with physical enrichment. This zone 1 is drawn virtually for the tanks without enrichment. The zone 2 was the high light intensity (20 lux) zone. This luminosity was the same everywhere for the tanks without enrichment. Even with the two cameras, all the surface of the tanks was not visually accessible. Only 54% of the total surface of each tank was in the field of the cameras, and this part was equally shared in zones 1 and 2 (Figure 2).



**Figure 2.** Top view showing part of the tank with the physical enrichment (PVC planks and pipes) and feeders that were covered by the field of the video cameras. C1 and C2 indicate the position of the video cameras. The dotted line indicated the parts, which are visually accessible by the cameras in zones 1 and 2, respectively. Zone 1 = the low light intensity zone (5 lux); Zone 2 = the high light intensity zone (20 lux); W.A. = water arrival; F1, F2 = automatic feeders.

We recorded fish behaviours (space occupation and abnormal behaviours) during two periods: first between day 19 and day 22, and second between day 39 and day 42. Each recording sequence lasted 30 min, and there was a sequence in the morning after the feeding phase and another during the afternoon three hours before the dawn when all the feed was eaten. Each day, a couple of tanks with and without enrichment were recorded. The association between the two tanks was realised at random. The six tanks were recorded one time. During a recording session, we stopped the air diffusion to facilitate the fish view and their counting. This short pause of the air diffusion had no consequence on the dissolved oxygen concentrations. The two cameras were synchronised.

All the 30 min of video recordings were analysed. For that, 20 images were extracted from the video every one minute and a half. On each image, we counted the number of fishes of the two species in each zone (see above for the delimitation of the two zones, shaded or not). These two zones were reported on the screen of the computer and the position of each fish was pointed.

For the two species, abnormal behaviours were defined as:

- Particular swimming. Two different behaviours were observed: (i) the fish swam on the back (SB) with the ventral body above, and (ii) the fish swam vertically (SV) with the head above the water surface.
- Jumps (J) were also observed; the fish was out of the water with its entire body above the surface. This behaviour was observed mainly along the wall of the tank.

### 2.5. Growth Characteristic Assessment

We recorded the fish mortality daily and assessed the final survival rate for each tank and for each fish species at day 90. Fish mortality also included individuals excluded from the experiment because of their thinness (limit point). This was done by visual control, with confirmation based on the value of the Fulton condition factor (see below) less than 0.27 and 0.60 for sterlet and pikeperch, respectively. For fish development, two vari-

ables were used: individual measures when it was possible and experimental unit variables on all the individuals of each experimental unit. At the beginning of the experiment (day 0), all fishes ( $n = 450$ ) were measured (total length with a precision of 1 mm) and weighed (precision of 1 g). To perform the measurements, fish were sedated with a Tricaine solution (MS222, 80 mg/L). From these measurements, we calculated the coefficient of variation of the initial weight (CV<sub>i</sub>) and the Fulton condition factor (FCF<sub>i</sub>) at day 0. For fish growth, individual parameters of mass and total length were measured at Day 90 in the same conditions as the beginning of the experiment. From these measures, we calculated the coefficient of variation of the final weight (CV<sub>f</sub>) and the Fulton condition factor (FCF<sub>f</sub> at day 90). FCF was computed as:  $FCF_f = ((W_{if} \times L_{if}^{-3}) \times 100)$ , where  $W_{if}$  was the weight of each fish at the end of the experiment and  $L_{if}$  was total length. The FCF was then averaged for each experimental unit. The percentage of biomass gain (BG) was also calculated at the level of the experimental unit according to the following formula:  $BG = (BM_f - BM_i) / BM_i \times 100$ , where  $BM_i$  and  $BM_f$  were the initial and final biomass in each experimental unit, respectively. Lastly, the specific growth rate (SGR) was also calculated for each species, according to the following formula:  $SGR (\% \cdot \text{day}^{-1}) = [(\ln W_f - \ln W_i) / T] \times 100$ , where  $W_f$  was the average of the final body weight (g) of all individuals of a given species in a tank,  $W_i$  was the average of the initial body weight (g) of all individuals of a given species in the same tank and  $T$  was the time of the experiment (days).

### 2.6. Statistical Analyses

The survival rate was compared for each species between the two modalities using a Chi square test. The growth and behavioural characteristics were tested for normality (Kolmogorov–Smirnov test) and for variance homogeneity (Barlett Test). When these assumptions were met, data were compared with the generalized linear mixed model, with the modalities as fixed variables, the replica (ecotron) as the growth variables and the recording day (first period (days 19–22) or second period (days 39–42)) as random variables for the behavioural variables (R packages used: lme4, lmerTest and MnMin). The different models were compared using an Akaike information criterion for small samples (AICc). When the global comparison was significant, pairwise comparisons between modalities inside a given species were done with a protected least significant difference test (PSLD). When the data did not fit normality and variance homogeneity, a non-parametric test of Kruskal–Wallis (K–W) was used, and when it was significant, pairwise comparisons were done with the R package pgirmess [52]. All statistical analyses were done with R software (R version 3.6.1, 2019) and the significance threshold was set at  $p < 0.05$ .

## 3. Results

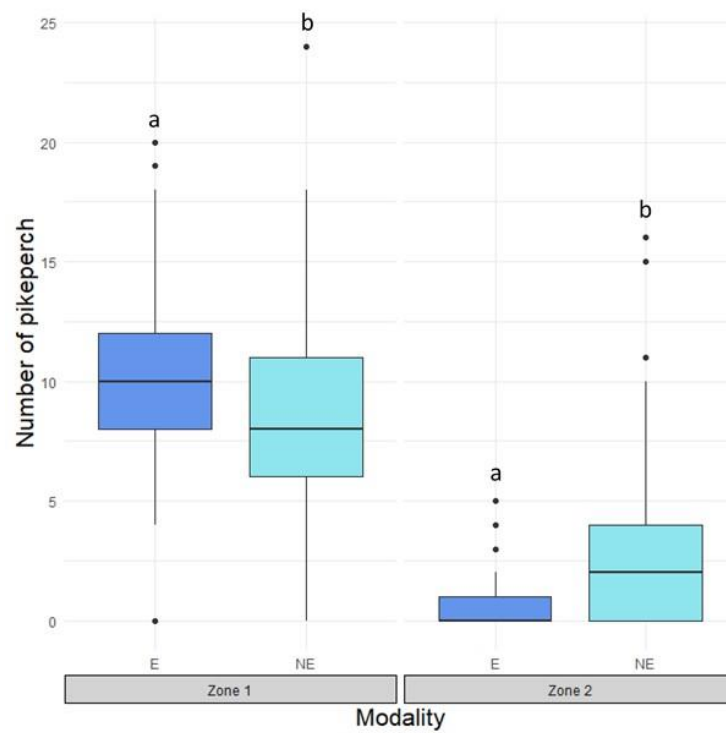
### 3.1. Survival

Fish were observed daily, and no pathology was reported for the two species. There were less than 14% of dead fish for the pikeperch (no individual excluded from the protocol), while the rate of death for sterlet was higher (25%, corresponding to 4% of mortality and 21% of excluded lean fish) and significantly different ( $X^2 = 7.86$ ,  $p < 0.05$ ). For pikeperch and sterlet, there was no statistical difference (for pikeperch:  $X^2 = 0.00$ ,  $p = 1$  and for sterlet:  $X^2 = 0.12$ ,  $p = 0.73$ ) of survival rate between the two modalities (with and without enrichment).

### 3.2. Space Occupation

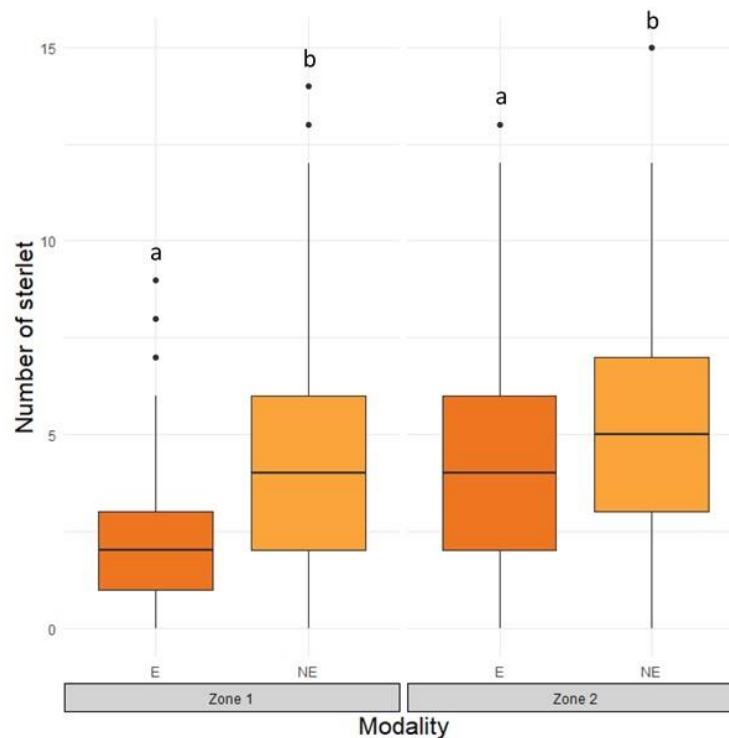
For both species, there was no effect of the recording day on the results, so we combined the data of the two periods (days 19–22 and days 39–42).

Pikeperch were more distributed in zone 1 in the modality with enrichment (K-W test,  $X^2 = 25.9$ ,  $p < 0.05$ ). Pikeperch were more distributed in zone 2 in the modality without enrichment (K-W test,  $X^2 = 117.1$ ,  $p < 0.05$ ) (Figure 3).



**Figure 3.** Median (quartiles) of pikeperch numbers in the different zones (1 and 2). Two different letters mean a significant difference ( $p < 0.05$ ). E: enrichment, NE: no enrichment.

Sterlet were more distributed in zone 1 in the modality without enrichment (ANOVA,  $F_{1,4} = 92.2$ ,  $p < 0.05$ ). Sterlet were more distributed in zone 2 in the modality without enrichment (K-W test,  $X^2 = 8.5$ ,  $p < 0.05$ ) (Figure 4).



**Figure 4.** Median (quartiles) of sterlet numbers in the zones (1 and 2). Two different letters mean a significant difference ( $p < 0.05$ ). E: enrichment, NE: no enrichment.



### 3.3. Behaviours

Only sterlet exhibited abnormal behaviours. There was no difference in the frequencies of these behaviours (SB, ANOVA,  $F_{1,24} = 0.73$ ,  $p = 0.40$ , SV, ANOVA,  $F_{1,24} = 0.16$ ,  $p = 0.70$ , and J, ANOVA,  $F_{1,24} = 0.87$ ,  $p = 0.36$ ) between the two modalities.

### 3.4. Growth of the Fish

There was no difference in the initial weight of the fish and length of the two species no matter the modality (with or without enrichment; Table 1) for pikeperch (weight ANOVA,  $F_{1,268} = 0.72$ ,  $p = 0.79$ ; length ANOVA,  $F_{1,268} = 10^{-4}$ ,  $p = 1$ ) and sterlet (weight ANOVA,  $F_{1,178} = 0.00$ ,  $p = 1$ , length ANOVA,  $F_{1,178} = 0.10$ ,  $p = 0.75$ ). After 90 days, there was also no statistical difference for the weight and length of pikeperch (weight ANOVA,  $F_{1,229} = 0.57$ ,  $p = 0.45$ ; length ANOVA,  $F_{1,229} = 0.44$ ,  $p = 0.51$ ) and sterlet (weight ANOVA,  $F_{1,132} = 0.00$ ,  $p = 1$ , length ANOVA,  $F_{1,132} = 0.45$ ,  $p = 0.50$ ). For the final weight, the CVf did not differ between the modalities for pikeperch and sterlet; it was the same for the FCFf.

**Table 1.** Fish growth data (mean  $\pm$  standard deviation) for the two species, sterlet (*Acipenser ruthenus*) and pikeperch (*Sander lucioperca*), reared with or without enrichment (E and NE, respectively).

		Enrichment (E)						
Species	Wi (g)	FCFi	Wf (g)	FCFf	BMi (g)	BMf (g)	BG (%)	SGR (%/day)
Sterlet	133.1 $\pm$ 21.2	0.40 $\pm$ 0.00	324.7 $\pm$ 65.2	0.4 $\pm$ 0.0	3993.2 $\pm$ 17.6	7472.2 $\pm$ 674.3	87 $\pm$ 17.5	0.99 $\pm$ 0.08
Pikeperch	142.0 $\pm$ 38.1	0.7 $\pm$ 0.1	381.9 $\pm$ 88.4	0.8 $\pm$ 0.1	6373.6 $\pm$ 39.7	14763.5 $\pm$ 303.1	131.6 $\pm$ 3.4	1.10 $\pm$ 0.03
		No Enrichment (NE)						
Species	Wi (g)	FCFi	Wf (g)	FCFf	BMi (g)	BMf (g)	BG (%)	SGR (%/day)
Sterlet	132.6 $\pm$ 21.1	0.38 $\pm$ 0.04	337.1 $\pm$ 51.4	0.46 $\pm$ 0.05	3979.0 $\pm$ 11.7	7434.5 $\pm$ 941.6	87 $\pm$ 23.2	1.04 $\pm$ 0.05
Pikeperch	143.4 $\pm$ 44.6	0.7 $\pm$ 0.1	369.7 $\pm$ 94.0	0.8 $\pm$ 0.1	6452.4 $\pm$ 92.5	14412.0 $\pm$ 617.0	123.3 $\pm$ 7.8	1.05 $\pm$ 0.03

Wi: initial weight; FCFi: Fulton condition factor at day 0; Wf: final weight; FCFf: Fulton condition factor at day 90; BMi and BMf: initial and final Biomass, respectively; BG: biomass gain; SGR: specific growth rate.

The percentage of biomass gain (BG) did not differ between modalities no matter the species (pikeperch,  $F_{1,4} = 2.95$ ,  $p = 0.10$ ; sterlet,  $F_{1,4} = 0.0$ ,  $p = 1$ ). In the same way, the specific growth rate (SGR) did not differ between modalities no matter the species (pikeperch,  $F_{1,4} = 2.72$ ,  $p > 0.05$ ; sterlet,  $F_{1,4} = 0.77$ ,  $p > 0.05$ ).

## 4. Discussion

Our results showed that (i) there was no effect of physical enrichment on the survival, growth and morphological development of the two species, (ii) space occupation differed between the modalities (with and without enrichment) for the pikeperch and the sterlet and (iii) there was no influence of enrichment on abnormal behaviours exhibited by the sterlet. Mortality rates should be considered differently according to the two species. They corresponded to dead fish or fish that were not found at the end of the experiment for pikeperch. The loss of individuals could be linked to cases of cannibalism, a common behaviour for this species [53] and observed twice in our experiment. For the sterlet, the mortality rates were mainly explained by fish reaching the limit point defined in the study (FCF less than 0.27), which refers to lean fish and are thus out of the experimental protocol.

Pikeperch switched its space occupation under the cover places due to the presence of PVC planks, which also corresponded to the part of the tank where the pipes modified the flow. The pikeperch most likely reacted to the presence of cover and pipes according to their preferred habitat [48]. Sterlet also changed their space occupation; they did not use the cover and were more numerous in the part of the tank without enrichment (zone 2). The spatial distribution of the two species was controlled by both the application of enrichment causing a low luminosity (zone 1) and the placement of the feeders in this shaded area. We did not observe aggressiveness between the fish of the two species.

In monoculture, several studies showed that physical enrichment of the rearing environment can affect the fish behaviour. In general, environmental enrichment promotes behavioural flexibility in animals reared under captive environments and can positively affect neural plasticity and cognitive abilities [17,54]; for example, Zebrafish in bare tanks can be more exploratory (cognitive items) [55]. Some results on the effect of environmental enrichment on aggressive behaviours are more contested [29]. For example, structural enrichment reduced aggression levels in zebrafish [18,29,31] and in salmon [56], but another study [57] reported that zebrafish in enriched environments were more aggressive (in the mirror test) than control.

For structural enrichment, the presence of pipes suspended vertically in the tank modified the water flow in the tank. Different studies showed that such devices can affect fish development. This has been the case for juveniles of the rainbow trout for which the feed conversion ratio, individual length and fish weight were improved in the enriched tanks [7,8,58,59], but vertical pipes did not increase growth in rainbow trout [60]. The situation is more complex when this device is associated with another as was the case in our study. While pikeperch preferred shaded areas as shown here, this was also the case for other species. For example, providing covers enhanced the use of particular areas and promoted aggregation of groups, reducing competition and aggression level in catfish [61]. We found that such enrichment with vertical pipes had no effect on the growth of the two fish species (pikeperch and sterlet). The first reason is perhaps the short duration of the experiment (90 days), which would be not sufficient to see an effect on fish growth for these species. The second reason is the fact that the two species have different speed of growth; pikeperch is a fish with a slow development compared to sterlet. One bias of our experimental design was that the belt feeders were placed just near the cover; thus, the pikeperch that occupied the place under the cover were just under the feeders. They had direct access to the food but not the sterlet. The absence of specific growth for the sterlet could be due to their exclusion out of the feeding place by the pikeperch as a consequence of their presence under the cover.

There is evidence that environmental enrichment enhances welfare in animal captivity [62]. This is the case for fish where structural complexity can alter fish behaviour, have positive effects on welfare and decrease avoidance and anxiety responses [63]. However, the benefit of an enriched environment is lost for fish exposed to painful stimuli [62]. Fish welfare can be assessed through behavioural indicators; abnormal behaviours and stereotypies are indicators of the welfare issues of animals. In our study, abnormal behaviours were observed only on the sterlet. We did not find that these behaviours were less frequent in the tanks with physical enrichment. In our study, sterlet exhibited these behaviours near the water surface and mainly along the walls of the tanks, and they were not influenced by the presence of physical enrichments in the tanks. These behaviours might be linked to the rearing conditions in closed tanks and are similar to escape behaviours. Such behaviours have been noted in this species and can be compared with those of mammals under very restrictive conditions (e.g., small enclosures).

## 5. Conclusions

The structural enrichment used in this experiment had an impact on the pikeperch and sterlet. The effect of these enrichments affected the space occupation behaviour of the pikeperch without positive consequences on their development. The enrichments did not modify the frequency of abnormal behaviours in the sterlet, suggesting that they are not adapted to the biology of this species.

To practice enrichment in a polyculture system, some guidelines must be considered: the enrichments must be adapted to traits (growth, behaviour and interspecific relationships) that are favoured or not, and the choice of physical (or structural) enrichments must consider the habitat preferences of all the species.

Since there is an increasing trend to promote enriched environments for both farmed and laboratory animals in regulation, information on enrichment approaches is essential to consider whether they influence a large diversity of traits.

**Author Contributions:** Conceptualisation, M.T., A.P. and T.L.; funding acquisition, M.T.; methodology, M.T., J.-G.R., Y.L., A.P. and T.L.; project administration, M.T.; writing—original draft, M.T., J.-G.R., Y.L., A.P. and T.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Metaprogram EcoSerV2 of the French National Research Institute for Agriculture, Food and Environment (INRAE).

**Institutional Review Board Statement:** This research was carried out in accordance with the regulations concerning the use of animals for scientific purposes.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors would like to thank Anthony Bled for formatting the figures.

**Conflicts of Interest:** There was no conflict of interest among authors.

## References

1. FAO. *The State of World Fisheries and Aquaculture 2020: Sustainability in Action Rome*; FAO: Rome, Italy, 2020; 401p. [\[CrossRef\]](#)
2. Ashley, P.J. Fish welfare: Current issues in aquaculture. *Appl. Anim. Behav. Sci.* **2007**, *104*, 199–235. [\[CrossRef\]](#)
3. Huntingford, F.A.; Adams, C.; Braithwaite, V.A.; Kadri, S.; Pottinger, T.G.; Sandøe, P.; Turnbull, J.F. Current issues in fish welfare. *J. Fish Biol.* **2006**, *68*, 332–372. [\[CrossRef\]](#)
4. Arechavala-Lopez, P.; Cabrera-Alvarez, M.; Maia, C.M.; Saraiva, J.L. Environmental enrichment in fish aquaculture: A review of fundamental and practical aspects. *Rev. Aquac.* **2021**, *14*, 704–728. [\[CrossRef\]](#)
5. Näslund, J.; Johnsson, J.I. Environmental enrichment for fish in captive environments: Effects of physical structures and substrates. *Fish Fish.* **2016**, *17*, 1–30. [\[CrossRef\]](#)
6. Kientz, J.L.; Barnes, M.E. Structural complexity improves the rearing performance of rainbow trout in circular tanks. *N. Am. J. Aquac.* **2016**, *78*, 203–207. [\[CrossRef\]](#)
7. Kientz, J.L.; Crank, K.M.; Barnes, M.E. Enrichment of circular tanks with vertically suspended strings of colored balls improves rainbow trout rearing performance. *N. Am. J. Aquac.* **2018**, *80*, 162–167. [\[CrossRef\]](#)
8. Crank, K.M.; Kientz, J.L.; Barnes, M.E. An evaluation of vertically suspended environmental structures during rainbow trout rearing. *N. Am. J. Aquac.* **2019**, *81*, 94–100. [\[CrossRef\]](#)
9. Rosburg, A.J.; Fletcher, B.L.; Barnes, M.E.; Treft, C.E.; Bursell, B.R. Vertically suspended environmental enrichment structures improve the growth of juvenile landlocked fall Chinook salmon. *Int. J. Innov. Stu. Aquat. Biol. Fish.* **2019**, *5*, 17–24. [\[CrossRef\]](#)
10. White, S.C.; Barnes, M.E.; Krebs, E.; Huysman, N.; Voorhees, J.M. Addition of vertical enrichment structures does not improve growth of three salmon species during hatchery rearing. *J. Marine Biol. Aquac.* **2018**, *4*, 48–52.
11. Ebbesson, L.O.E.; Braithwaite, V.A. Environmental effects on fish neural plasticity and cognition. *J. Fish Biol.* **2012**, *81*, 2151–2174. [\[CrossRef\]](#)
12. Berejikian, B.A.; Tezak, E.R.; Flagg, T.A.; LaRae, A.L.; Kummerow, E.; Mahnken, C.V.W. Social dominance, growth and habitat use of Age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. *C. J. Fish Aquat. Sci.* **2000**, *57*, 628–636. [\[CrossRef\]](#)
13. Brown, C.; Davidson, T.; Laland, K. Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *J. Fish Biol.* **2003**, *63*, 187–196. [\[CrossRef\]](#)
14. Braithwaite, V.A.; Salvanes, A.G.V. Environmental variability in the early rearing environment generates behaviourally flexible cod: Implications for reability wild populations. *Proc. R. Soc. B* **2005**, *272*, 1107–1113. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Ahlbeck-Bergendahl, I.; Salvanes, A.G.V.; Braithwaite, V.A. Determining the effects of duration and recency of exposure environmental enrichment. *Appl. Anim. Behav. Sci.* **2016**, *176*, 163–169. [\[CrossRef\]](#)
16. Ullah, I.; Zuberi, A.; Khan, K.U.; Ahmad, S.; Thornquist, P.; Winberg, S. Effects of enrichment of the development of behaviour of an endangered fish masheer (*Tor putitora*). *Appl. Anim. Behav. Sci.* **2017**, *186*, 93–100. [\[CrossRef\]](#)
17. Salvanes, A.G.; Moberg, O.; Ebbesson, L.O.; Nilsen, T.O.; Jensen, K.H.; Braithwaite, V.A. Environmental enrichment promotes neural plasticity and cognitive abilities in fish. *Proc. Biol. Sci. Royal Soc.* **2013**, *280*, 20131331. [\[CrossRef\]](#)
18. Carfagnini, A.G.; Rodd, F.H.; Jeffers, K.B.; Bruce, A.E.E. The effects of habitat complexity on aggression and fecundity in zebrafish (*Danio rerio*). *Environ. Biol. Fishes* **2009**, *86*, 403–409. [\[CrossRef\]](#)
19. Mikheev, V.N.; Pasternak, A.F.; Tischler, G.; Wanzenbock, J. Contestable shelters provoke aggression among 0+ perch, *Perca fluviatilis*. *Environ. Biol. Fishes* **2005**, *73*, 227–231. [\[CrossRef\]](#)

20. Gwak, W.S. Effects of shelter on growth and survival in age-0 black sea bass, *Centropristis striata* (L.). *Aquac. Res.* **2003**, *34*, 1387–1390. [[CrossRef](#)]
21. Christou, P.; Savin, R.; Costa-Pierce, B.A.; Misztal, I.; Whitelaw, C.B.A. *Sustainable Food Production*; Springer: Berlin/Heidelberg, Germany, 2013.
22. Kozłowski, M.; Szczepkowski, M.; Wunderlich, K.; Szczepkowska, B.; Piotrowska, I. Polyculture of Juveniles pikeperch (*Sander lucioperca* (L.)) and sterlet (*Acipenser ruthenus* L.) in a recirculating system. *Arch. Polish Fish* **2014**, *22*, 237–242. [[CrossRef](#)]
23. Thomas, M.; Lecocq, T.; Abregal, C.; Nahon, S.; Aubin, J.; Jaeger, C.; Wilfart, A.; Schaeffer, L.; Ledoré, Y.; Puillet, L.; et al. The effects of polyculture on behaviour and production of pikeperch in recirculation systems. *Aquac. Rep.* **2020**, *17*, 100333. [[CrossRef](#)]
24. Martins, C.I.M.; Galhardo, L.; Noble, C.; Damsgård, B.; Spedicato, M.T.; Zupa, W.; Beauchaud, M.; Kulczykowska, E.; Massabuau, J.C.; Carter, T.; et al. Behavioural indicators of welfare in farmed fish. *Fish Physiol. Biochem.* **2012**, *38*, 17–41. [[CrossRef](#)] [[PubMed](#)]
25. Näslund, J.; Rosengren, M.; Del Villar, D.; Gansel, L.; Norrgard, J.R.; Persson, L.; Winkowski, J.J.; Kvingedal, E. Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic Salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **2013**, *70*, 585–590. [[CrossRef](#)]
26. Zhang, Z.; Zhang, X.; Li, Z.; Zhang, X. Effects of different level of environmental enrichment on the sheltering behaviors, brain development and cortisol levels of black rockfish *Sebastes schlegelli*. *Appl. Anim. Behav. Sci.* **2019**, *218*, 104825. [[CrossRef](#)]
27. Zhang, Z.; Bai, Q.; Xu, X.; Guo, H.; Zhang, X. Effects of environmental enrichment on the welfare of juvenile black rockfish *Sebastes schlegelli*. Growth, behaviour and physiology. *Aquaculture* **2020**, *518*, 734782. [[CrossRef](#)]
28. Zhang, Z.; Xu, X.; Wang, Y.; Zhang, X. Effects of environmental enrichment on growth performance, aggressive behaviour and stress-induced changes in cortisol release and neurogenesis of black rockfish *Sebastes schlegelli*. *Aquaculture* **2020**, *528*, 735483. [[CrossRef](#)]
29. Zhang, Z.; Fu, Y.; Zhang, Z.; Zhang, X.; Chen, S. A comparative study on two territorial fishes: The influence of physical enrichment an aggressive behaviour. *Anim. Open Access J.* **2021**, *11*, 1868. [[CrossRef](#)]
30. Mork, O.I.; Bjerkeng, B.; Rye, M. Aggressive interactions in pure and mixed groups of juveniles farms and hatchery-reared wild Atlantic salmon *Salmo salar* L. in relation to tank substrate. *Aquac. Res.* **1999**, *30*, 571–578. [[CrossRef](#)]
31. Basquill, S.P.; Grant, J.W.A. An increase in habitat complexity reduces aggression and monopolization of food by zebra fish (*Danio rerio*). *Can. J. Fish Aquat. Sci.* **1998**, *76*, 770–772. [[CrossRef](#)]
32. Wilkes, L.; Owen, S.F.; Readman, G.D.; Sloman, K.A.; Wilson, R.W. Does structural enrichment for toxicology studies improve zebrafish welfare. *Appl. Anim. Behav. Sci.* **2012**, *139*, 143–150. [[CrossRef](#)]
33. Bosakowski, T.; Wagner, E.J. Experimental use of cobble substrates in concrete raceways for improving fin condition of cutthroat (*Oncorhynchus clarki*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **1995**, *130*, 159–165. [[CrossRef](#)]
34. Wagner, E.I.; Intelmann, S.S.; Routledge, M.D. The effect of fry rearing density on hatchery performance, fin condition, and agonistic behaviour of rainbow trout *Oncorhynchus mykiss*. *J. World. Aquac. Soc.* **1996**, *27*, 264–274. [[CrossRef](#)]
35. Arndt, R.E.; Routledge, D.; Wagner, E.J.; Mellenthin, R.F. Influence of raceway substrate and design on fin erosion and hatchery performance of rainbow trout. *N. Am. J. Aquac.* **2001**, *63*, 312–320. [[CrossRef](#)]
36. Berejikian, B.A.; Tezak, E.P. Rearing in enriched hatchery tanks improves dorsal fin quality of juvenile steelhead. *N. Am. J. Aquac.* **2005**, *65*, 289–293. [[CrossRef](#)]
37. Coulibaly, A.; Ouattara, I.N.; Koné, T.; Douba, V.N.; Snoeks, J.; Gooré Bi, G.; Kouamélian, E.P. First results of floating cage culture of the African catfish *Heterobranchius longifilis* Valenciennes 1840: Effect of stocking density on survival and growth rates. *Aquaculture* **2007**, *263*, 61–67. [[CrossRef](#)]
38. Gesto, M.; Jokumsen, A. Effects of shelter on growth performance and welfare of rainbow trout juveniles. *Aquaculture* **2022**, *551*, 737930. [[CrossRef](#)]
39. Barcellos, L.J.G.; Kretz, L.C.; Quevedo, R.M.; Da Rosa, J.G.S.; Koakoski, G.; Centenaro, L.; Pottker, E. Influence of color background and shelter availability on jundia (*Rhamdia quelen*) stress response. *Aquaculture* **2009**, *288*, 51–56. [[CrossRef](#)]
40. Millidine, K.J.; Armstrong, J.D.; Metcalfe, N.B. Presence of shelter reduces maintenance metabolism of juvenile salmon. *Funct. Ecol.* **2006**, *20*, 839–845. [[CrossRef](#)]
41. Hecht, T.; Appelbaum, S. Observations on intraspecific aggression and coeval sibling cannibalism by larval and juvenile *Clarias gariepinus* (Clariidae: Pisces) under controlled conditions. *J. Zool.* **1988**, *214*, 21–44. [[CrossRef](#)]
42. Rahmah, S.; Kato, K.; Yamamoto, S.; Takii, K.; Murata, O.; Senoo, S. Improved survival and growth performances with stocking density manipulation and shelter availability in bagrid catfish *Mystus nemurus* (Cuvier & Valenciennes 1840) larvae. *Aquac. Res.* **2014**, *45*, 2000–2009. [[CrossRef](#)]
43. Mihailov, S.A.; Mihoc, N.A.; Lalescu, D.; Grozea, A. Polyculture of the pikeperch (*Sander lucioperca*) fingerlings into recirculating aquaculture system, with sterlet (*Acipenser ruthenus*) or european catfish (*Silurus glanis*). A preliminary study. *Res. J. Agri. Sci.* **2017**, *49*, 193–198.
44. Prokes, M.; Barus, V.; Mares, J.; Penaz, M.; Baranek, V. Growth of sterlet *Acipenser ruthenus* under experimental and farm conditions of the Czech republic with remarks on other sturgeons. *Acta Univ. Agric. Sylv. Mendel. Brun.* **2011**, *59*, 81–290.
45. Baekelandt, S.; Redivo, B.; Mandiki, S.N.M.; Bournonville, T.; Houndji, A.; Bernard, B.; El Kertaoul, N.; Schmitz, M.; Fontaine, P.; Gardeur, J.N.; et al. Multifactorial analysis revealed optimal aquaculture modalities improving husbandry fitness without clear effect on stress and immune status of pikeperch *Sander lucioperca*. *Gen. Comp. Endocrinol.* **2018**, *258*, 194–204. [[CrossRef](#)] [[PubMed](#)]

46. Akbulut, B.; Feledi, T.; Lengyel, S.; Ronyal, A. Effect of feeding rate on growth performance, food utilization and meat yield of sterlet (*Acipenser ruthenus* Linné, 1758). *J. Fish Sci.* **2013**, *7*, 216–224. [[CrossRef](#)]
47. Bayrami, A.; Noverian, H.A.; Sharif, E.A. Effects of background colour on growth indexes and stress of young sterlet (*Acipenser ruthenus*) in a closed circulated system. *Aquac. Res. Dev.* **2017**, *48*, 2004–2011. [[CrossRef](#)]
48. Luchiari, A.C.; De Morais Freire, F.A.; Koskela, J.; Pirhonen, J. Light intensity preference of juvenile pikeperch *Sander lucioperca*. *Aquac. Res.* **2006**, *37*, 1572–1577. [[CrossRef](#)]
49. Moine, J.; Barnes, M.E.; Kientz, J.; Simpson, G. Flow patterns in circular rearing tanks containing vertical structures. *J. Fish. Livest. Prod.* **2016**, *4*, 4. [[CrossRef](#)]
50. Fieszl, J.; Bogacka-Kapusta, E.; Kapusta, A.; Szymanska, U.; Martyniak, A. Feeding ecology of the sterlet *Acipenser ruthenus* L. in the Hungarian section of the Danube river. *Arch. Pol. Fish.* **2011**, *19*, 105–111. [[CrossRef](#)]
51. Vehanen, T.; Lathi, M. Movements and habitat use by pikeperch (*Stizostedion lucioperca* (L.)) in a hydropeaking reservoir. *Ecol. Freshwater Fish* **2003**, *12*, 203–215. [[CrossRef](#)]
52. Giraudoux, P. Pgirmess: Data Analysis in Ecology. R Package Version 1.5.8. 2013. Available online: <http://CRAN.R-project.org/package=pgirmess> (accessed on 17 January 2022).
53. Colchen, T.; Gisbert, E.; Ledoré, Y.; Teletchea, F.; Fontaine, P.; Pasquet, A. Is a cannibal different from its conspecifics? A behavioural, morphological, muscular and retinal structure study with pikeperch juveniles under farming conditions. *Appl. Anim. Behav.* **2020**, *224*, 104947. [[CrossRef](#)]
54. Kotrschal, A.; Taborsky, B. Environmental change enhances cognitive abilities in fish. *PLoS ONE* **2010**, *8*, e1000351. [[CrossRef](#)] [[PubMed](#)]
55. Spence, R.; Magurran, A.E.; Smith, C.L. Spatial cognition in zebrafish: The role of strain and rearing environment. *Anim. Cogni.* **2011**, *14*, 607–612. [[CrossRef](#)] [[PubMed](#)]
56. Hazegawa, K.; Maekawa, K. Potential of habitat complexity for mitigating interference competition between native and non-native salmonidae species. *Can. J. Fish Aquat. Sci.* **2008**, *86*, 386–393. [[CrossRef](#)]
57. Woodward, M.A.; Winder, L.A.; What, P.J. Enrichment increases aggression in zebrafish. *Fishes* **2019**, *4*, 22. [[CrossRef](#)]
58. Krebs, E.; Huysman, N.; Voorhes, J.M.; Barnes, M. Suspended arrays improve rainbow trout growth during hatchery rearing in circular tanks. *Int. J. Aquac. Fish. Sci.* **2018**, *4*, 27–30. [[CrossRef](#)]
59. Voorhees, J.M.; Huysman, N.; Krebs, E.; Barnes, M.E. Influence of water velocity and vertically suspended structures on rainbow trout rearing performance. *Open J. Anim. Sci.* **2020**, *10*, 152–161. [[CrossRef](#)]
60. Huysman, N.; Krebs, E.; Voorhees, J.M.; Barnes, M.E. Use of large vertically-suspended rod array in circular tanks during juvenile rainbow trout rearing. *Int. J. Mar. Biol. Res.* **2019**, *4*, 1–5.
61. Slavík, O.; Maciak, M.; Horký, P. Shelter use of familiar and unfamiliar groups of juvenile European catfish *Silurus glanis*. *Appl. Anim. Behav. Sci.* **2012**, *142*, 116–123. [[CrossRef](#)]
62. Broom, D.M.; Johnson, K.G. *Stress and Animal Welfare*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 2019.
63. Pounder, K.C.; Mitchell, J.L.; Thomson, J.S.; Pottinger, T.G.; Buckley, J.; Sneddon, L.U. Does environmental enrichment promote recovery from stress in rainbow trout? *Appl. Anim. Behav. Sci.* **2016**, *176*, 136–142. [[CrossRef](#)]