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# All species matter: Comprehensive analysis of polyculture consequences on pikeperch (*Sander lucioperca*), sterlet (*Acipenser ruthenus*) and tench (*Tinca tinca*) in recirculated aquaculture system

Marielle Thomas <sup>a b\*</sup>, Nellya Amoussou <sup>a b</sup>, Mohammed El Manfaloti <sup>a</sup>, Clémentine Fleck <sup>a</sup>, Yannick Ledoré <sup>a</sup>, Alain Pasquet <sup>a</sup>, Thomas Lecocq <sup>a b</sup>  
<sup>a</sup>: URAFPA, University of Lorraine-INRAe, 54500 Vandœuvre, France

<sup>b</sup>: LTSER France, Zone Atelier du Bassin de la Moselle, 54506 Vandœuvre-lès-Nancy, France

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## Highlights

- Polyculture in RAS could be a relevant alternative for future aquaculture.
- Pikeperch, sterlet and tench respond differently to polyculture farming conditions.
- In polyculture, all species must be considered according to a multi-criteria approach.

## Abstract

Aquaculture is expected to be one of the most prominent source of animal proteins for humans in the next decades. Its importance for human food security has triggered many academic debates to improve its sustainability. Among potential solutions, polyculture of fish has previously been pointed out as a relevant alternative to monoculture in recirculated aquaculture system (RAS). However, previous studies assessed potential impacts of polyculture on a target species, and disregard the polyculture consequences on other taxa combined with this species. Yet, it cannot be ruled out that polyculture results in beneficial consequences for one species at the detriment of other taxa. Therefore, we developed a RAS polyculture design associating three fish species: pikeperch (*Sander lucioperca*), sterlet (*Acipenser ruthenus*), and tench (*Tinca tinca*). A total of 756 fishes was reared during 60 days and comprehensive analysis of polyculture consequences was performed for each of these three species by considering survival, growth, and behavioural parameters. All studied species were impacted by polyculture in our experimental design. However, species were not equally affected by polyculture: sterlet was more impacted in terms of survival, growth, and behaviour than pikeperch and tench. Therefore, our results suggest that RAS polyculture could result in beneficial or detrimental impacts on fish production depending on the species. Overall, this study underlines the importance of considering all species and a multi-criterion assessment framework to evaluate RAS polyculture.

## 1. Introduction

During the last decades, the exponential increase of human population has drastically pulled up the food demand (Foley et al., 2011). In this context, aquaculture will have a key role to ensure human food security and nutrition, especially as wild fisheries fail to meet the demand for aquatic products (FAO, 2020). In industrial western countries, monoculture (i.e. production of a single species in cages, recirculated aquaculture systems [RASs], or even ponds) has been increasingly favoured to develop aquaculture production, most

often in an intensive way (Bostock et al., 2010; Diana et al., 2013). However, intensive monoculture has been criticised from human food security and economic viewpoints, as it might have a lower ability to withstand competition and pest attacks (e.g. Dahlberg, 1979), as well as a low potential to adapt to changes in environmental and socio-economic contexts or in consumer expectations (Medeiros et al., 2017).

Given the monoculture limitations, polyculture is increasingly seen as one of the solutions that could

improve aquaculture sustainability. Existing for millennia, this practice encompasses all farming practices in which two or more species are farmed together in a same production environment at the same time (Stickney, 2013; Thomas et al., 2021). In contrast to monoculture, polyculture can enhance farming efficiency by improving use of resources that are naturally present or supplied to the agricultural environment (Nhan et al., 2007) and/or by recycling nutrients from farmed biomass (Chopin et al., 2001; Flickinger et al., 2020). Furthermore, it can decrease environmental impacts (Medeiros et al., 2017) and increase the farming system resilience (Dumont et al., 2020). However, polycultures are complex farming systems, which can result in species resource sharing as well as in interspecific competition and animal welfare issues (Thomas et al., 2021). Potential polyculture benefits can be reaped provided that species compatibility (i.e. species can live in the same production system while minimising detrimental interactions [i.e. amensalism, predation, parasitism] or competition for resources [e.g. trophic, spatial]) or, even better, species complementarity (i.e. co-farmed species can use different portions of available resources or display commensal/mutualistic interactions) occurring among co-farmed taxa.

In fish production, polyculture is widely applied in ponds, but very little in RASs (see review in Thomas et al., 2021; Thomas et al., 2022). The few existing studies underlined benefits of RAS polyculture of fish, such as improved production performance (Thomas et al., 2020) or decrease in labour in relation to the trophic behaviour of farmed species (Henne et al., 2007; Kozłowski et al.,

2014), without detrimental consequences (see review in Thomas et al., 2021). However, previous studies focus on a target species (e.g. the development of *Labeo rohita* production under various polyculture conditions, Reddy et al., 2002), but disregard the polyculture consequences on other taxa combined with this species. Yet, it cannot be ruled out that polyculture results in beneficial consequences for one species at the detriment of other taxa. This would raise fish welfare and production concerns.

Here, we developed a RAS polyculture design associating three fish species: pikeperch (*Sander lucioperca*, Percidae), sterlet (*Acipenser ruthenus*, Acipenseridae), and tench (*Tinca tinca*, Tincidae). These species have been chosen because (i) they have similar abiotic requirements (i.e. pH, temperature, salinity, dissolved oxygen, and light parameters, Bayrami et al., 2017; Baekelandt et al., 2018; Pula et al., 2018), (ii) these species feed during the diurnal phase in different areas of their living environments (i.e. compatibility in feeding habits, see Thomas et al., 2020 and data from TOFF database, Lecocq et al., 2019), and (iii) the combination of pikeperch polyculture with sterlet and/or tench are already studied, but only impact on pikeperch was considered to date (Kozłowski et al., 2014; Thomas et al., 2020). Therefore, we performed a comprehensive analysis of polyculture consequences for each of these three species by considering survival, growth, and behavioural parameters. Ultimately, we aimed at assessing that polyculture can be beneficial for all co-farmed species of these fish combinations.

## 2. Material and Methods

The study was carried out at the Platform of Experimental Aquaculture (PEA) of the Faculty of Sciences and Technologies (University of Lorraine, Nancy, France) (registration number for animal experimentation D54–547-18). We followed the guidelines of the Council of European Communities (2010/63/UE) and the French Animal Care Guidelines. Before all manipulations, fish were previously anaesthetised with a Tricaine solution (MS-222 [150 mg. L<sup>-1</sup>]) to minimise the stress.

### 2.1. Survival rate

We used juveniles of pikeperch (initial mean weight: 58 ± 10 g), sterlet (initial mean weight: 17 ± 4 g), and tench (initial mean weight: 40 ± 6 g). The mean weights of each of the three species allow them to be reared in polyculture without risk of predation during the whole experiment

(Turesson et al., 2002). Pikeperch were bred at the PEA while sterlet and tench were sourced from fisheries cooperative Györi “Előre” Htsz (Györi, Hungary). Before bioassays, we performed a one-week acclimatisation in three RASs (2 m<sup>3</sup> tanks, one for each species) at 20.4 ± 0.7 °C, with white light (20 Lux; light/night: 10 h:14 h). Species were kept separated during this time. During acclimatisation, fishes were fed ad libitum with Sturgeon Grower, semi-floating, 3 mm (Le Gouessant, France).

### 2.2. Experimental environment and treatments

The experiment was carried out in 280 L independent experimental units (i.e. glass aquariums; 100 cm × 60 cm × 50 cm) during 60 days. The temperature and light conditions were

the same than during the acclimatisation. The temperature, dissolved oxygen, pH, ammonia, and nitrites of the water of each experimental unit were monitored three times a week throughout the experiment (i.e. mean values: temperature:  $20.5 \pm 0.8$  °C; dissolved oxygen:  $6.08 \pm 0.92$  mg.L<sup>-1</sup>; pH:  $7.46 \pm 0.25$ ; ammonia:  $0.17 \pm 0.18$  mg.L<sup>-1</sup>; nitrites:  $0.11 \pm 0.10$  mg.L<sup>-1</sup>). Fishes were still fed manually with Sturgeon Grower pellets three times per day (at 9:00 a.m., 12:00 a.m., and 3:00 p.m.) with a standardised food ration (1.5% of the total fish biomass of each aquarium). The rations were readjusted every two weeks based on total biomass of each experimental unit by weighing all the individuals. The food was distributed along the front window of each experimental unit. Aquariums were cleaned once a week in the morning.

Overall, we considered seven treatments: three monocultures (pikeperch, tench, and sterlet, referred as P, T, and S respectively); three polycultures with two species (pikeperch-tench, pikeperch-sterlet, and sterlet-tench, referred as PT, PS, and ST respectively); one polyculture with the three species (pikeperch-sterlet-tench, referred as PST). Three replicates were done for each treatment for a total of 21 experimental units. For each replicate, the initial stocking density in each aquarium was 36 fishes; the fish number by species was adjusted to the combination: 36 specimens when species were alone, 18/18 when two species were associated, and 12/12/12 for the combination of the three species.

### 2.3. Fish survival and growth

We recorded daily fish mortality and assessed the survival rate per experimental unit and for each fish species at the end of the experiment. For ethical reasons, fish that were not feeding were excluded from the protocol because of their weight loss. Weight loss is a limit point that is established by visual observation and confirmed by the value of the Fulton Coefficient Factor (FCF) (i.e. limit point for values below 0.6, 1.2 and 0.3 respectively for pikeperch, tench, and sterlet as established by the PEA animal welfare structure). This was done during growth controls (every two weeks) where all the fish of each experimental unit were measured and weighted.

For fish growth, all fishes were measured (total length with a precision of 0.1 cm) and weighted (precision of 1 g) at the beginning ( $n = 756$ ) and at the end ( $n = 698$ ) of the experiment. We assessed fish growth by the final weight per experimental unit and species. Moreover, from individual measures (weight and total length), we calculated the FCF

per individual. At the level of each experimental unit and per species, we calculated the Coefficient of Variation (CV) for the weight and the Specific Growth Rate (SGR, %·day<sup>-1</sup>) (Thomas et al., 2020). These parameters were compared for each species between treatments (monoculture or polyculture).

### 2.4. Space occupation and group structure

Video recordings were done during two feeding times (12:00 a.m. and 3:00 p.m.) between day 32 and day 35. The 9.00 a.m. feeding was not considered because it occurred shortly after the start of the photophase. A total of 14 videos were recorded during four consecutive days; two replicates were done per treatment with two cameras (Sony Handycam DCR-SR-72E) used simultaneously and placed 0.80 cm in front of the aquariums. Each day, recorded treatment was chosen at random. Each video lasted 80 min: 30 min before and 50 min after feeding.

To analyse the data for the three species, only the observable fish within a 15 cm depth of field from the front window were considered. This observed water volume was chosen because it is a strategic place, where the feed was distributed. The space occupation and the group structure were assessed through the fish positions in this volume. These positions were determined with ImaJ (Schneider et al., 2012). For that, 10 pictures were extracted during the two periods (30 min before and 30 min after feeding). On each picture, all visible fish were pointed out using the extremity of the snout as landmark. These individual data were reported on a two-dimensional Cartesian coordinate system. From these picture analyses, two types of data were extracted: (i) all the distances between each fish on a picture and (ii) the y coordinates for each fish representing the height of the fish position in the water column. Then, we combined the data of the ten pictures for each fish species and modality and period.

For space occupation, we considered only the height of the fish in the water column (Y component for each individual). To complete the space occupation study, we calculated for each picture the percentage of fish per species seen on this picture relatively to the number of conspecifics in the aquarium (i.e. percentage of fish in the strategic place). Group structure was based on three types of inter-individual distance measurements (see Buske and Gerlai, 2011): the minimal distance between two conspecific fish, the mean distance for each fish of the group to all of its conspecifics, and the variance of these latter distances. These

measures were characteristic of (i) the group aggregation, (ii) the group cohesion and (iii) the group homogeneity (Buske and Gerlai, 2011). These distance measures were obtained from each picture; for one replica and one treatment, the mean of the measures of the ten pictures was used by combining the replicas. The statistical comparisons between the treatments was done on these means.

## 2.5. Statistical analyses

The survival rate was compared for each species between the four treatments using a Chi-square test (Statistica 10.0). The other characteristics (growth, space occupation, inter-individual distances, and percentage of fish in the strategic place) were compared for each species by using

ANOVA (R-package *stats*, R Core Team, version 3.6.1, 2019), considering all the survival fish in the different treatments. We assessed that parametric statistical analyses (i.e. normal distribution with Shapiro Wilk test and variance homogeneity with Levene test) are appropriate for data. Comparisons between treatments inside a given species were done with a Fisher Protected Least Significance Difference test. When data did not fit parametric statistical needs, we used a Kruskal - Wallis test for global analyses followed by Dunn test for paired comparisons; it was the case for the mean percentage (after a square root transformation) of fish seen on the successive pictures. These latter statistical analyses were done with R software (R-package *stats*, R Core Team version 3.6.1, 2019) and the significance thresholds were set at  $p < 0.05$  when it was between 0.05 and 0.01, and  $p < 0.01$  when it was less than 0.01.

## 3. Results

### 3.1. Survival rate

The survival rates were always very high for pikeperch and tench (between 95% and 100% for both species) (Fig. 1). The sterlet survival varies significantly between treatments ( $\chi^2 = 8.03$ ,  $df = 3$ ,  $p < 0.05$ ) ranging from 93% (modality ST) to 73% in S (Fig. 1). The survival rate was lower in S (73%) than in ST (93%) ( $p < 0.01$ ), and a trend ( $p = 0.06$ ) was observed with PST (80%); there was no difference with PS (76%) (Fig. 1). Eleven sterlet fish were removed from S because they have reached a limit point (lean fish with a very low FCF, less than 0.27) and were not included in the above comparisons.

### 3.2. Fish growth

At the beginning of the experiment, there was no statistical weight difference of fish of the treatments regardless the species (Weight: pikeperch,  $F_{(6,251)} = 1.2$ , sterlet,  $F_{(6,251)} = 0.7$  and tench,  $F_{(6,251)} = 0.3$ ) ( $p > 0.05$ ).

For pikeperch, among the different variables used to describe the growth, only the final weight showed a significant difference between the treatments (ANOVA  $F_{(3,249)} = 4.3$ ,  $p < 0.05$ ) (Fig. 1). The final weight of pikeperch was significantly lower in P than in PST ( $p < 0.01$ ) (Fig. 1).

Sterlet was the species most affected by polyculture compared to pikeperch and tench. The final weight differed depending on the treatments (ANOVA,  $F_{(3,197)} = 9.4$ ,  $p < 0.01$ ). The final weight of the fish in S was lower than in ST, and PST (Fig. 1). Moreover, the final weight in ST was higher than

those in PS (Fig. 1). The final FCF differed between treatments (ANOVA,  $F_{(3,197)} = 6.3$ ,  $p < 0.05$ ); it was higher in ST than in S (Fig. 1).

For tench, the FCF was significantly different between the treatments (ANOVA,  $F_{(3,247)} = 3.1$ ,  $p < 0.05$ ) (Fig. 1). This FCF was significantly lower for tench in ST than for the other treatments (Fig. 1). There was no significant difference for the other parameters.

### 3.3. Space occupation

In monoculture, the three species did not occupy the water column on the same manner; tench and pikeperch were more in the lower part of aquariums as sterlet was in the upper part (Fig. 2). The combination with another species influenced this baseline position except for the pikeperch (Fig. 2). Tench increased its height in the water column in PST (ANOVA,  $F_{(3,915)} = 12.9$ ,  $p < 0.01$ ) (Fig. 2), but did not change in PT and ST. The sterlet swam more down in the water column in ST (ANOVA  $F_{(3,488)} = 6.6$ ,  $p < 0.01$ ) (Fig. 2).

The percentage of fish in the strategic place varies according to the treatments for pikeperch (KW,  $\chi^2 = 46.3$ ,  $p < 0.01$ ), for tench (KW,  $\chi^2 = 62.7$ ,  $p < 0.01$ ) and for sterlet (KW,  $\chi^2 = 9.9$ ,  $p < 0.05$ ) (Fig. 2). For pikeperch, it was lower in PT than in P, PS and PST (Fig. 2). For the sterlet, the percentage of visible fish decreased in ST. The percentage of sterlet was significantly lower in ST than in S and PS (Fig. 2). For tench, it was lower in PT and PST (Fig. 2).

### 3.4. Group structure

The distance means between fish differed only for the pikeperch (ANOVA,  $F_{(3, 141)} = 21.6$ ,  $p < 0.01$ ); it was higher in P, than when they were associated with one or two other species (Fig. 3).

The mean variances of these distances differed for pikeperch (ANOVA,  $F_{(3, 119)} = 14.9$ ,  $p < 0.01$ ) and tench (ANOVA,  $F_{(3, 105)} = 3.89$ ,  $p < 0.05$ ) but not for sterlet (Fig. 3). For pikeperch, mean variance was higher in P than in polyculture treatments (PS, PT, PST). For tench, mean variance was higher in T than in PT; there was no difference with the other associations.

## 4. Discussion

### 4.1. Species-specific consequences of polyculture

We showed that all studied species were impacted by polyculture in our experimental design. However, species are not equally affected: sterlet was more impacted in terms of survival, growth, and behaviour than pikeperch and tench. From an aquaculture viewpoint, our results suggest that polyculture could result in beneficial or detrimental impacts on fish production depending on the species.

Considering fish survival rate, growth, and FCF, our experiment shows that polyculture can result in an improvement of zootechnical performances for pikeperch and sterlet compared to their monoculture (Fig. 1). This is congruent with previous studies (see similar results in Kozłowski et al., 2014; Thomas et al., 2020), which suggest that polyculture could be a relevant fish production strategy in RAS (e.g. Papoutsoglou et al., 1992). Conversely, we recorded a decrease of FCF of tench when this species is associated with sterlet. Since the FCF is considered as a good indicator of the general fitness for fish (Bolger and Connolly, 1989), this combination between sterlet and tench seems negative for the latter, although there is no significant effect on its survival and growth.

Space occupation and group structure of studied species are also impacted by polyculture (Fig. 2, Fig. 3). We consider space occupation and group structure in monoculture as baseline behaviour under the environmental conditions applied here to each species, and when there is no constraint shaped by interspecific relationships. Therefore, modification of these behavioural traits in polyculture suggests potential competition for space or exclusion phenomenon between species (Middlemiss et al., 2019). For instance, space occupation by pikeperch is not significantly

The means of the minimal distances varied significantly among treatments for the three species. They differed for pikeperch (ANOVA,  $F_{(3, 141)} = 7.8$ ,  $p < 0.01$ ) (Fig. 3) and was lower in P than in PT and PST. For sterlet, the minimal distance differed also between treatments (ANOVA,  $F_{(3, 112)} = 6.3$ ,  $p < 0.01$ ) and was lower in S than in PS (Fig. 3). The minimal distances between treatments differed also for tench (ANOVA,  $F_{(3, 125)} = 25.63$ ,  $p < 0.01$ ) (Fig. 3). It was lower in T than in all polyculture treatments (from ST, PT and PST, Fig. 3).

modified by polyculture, which seems to rule out potential species displacement due to interspecific interactions. In contrast, we speculate that higher resting position of tench in the water column when reared with the other two species could reflect a negative impact of polyculture for this taxon because this positioning displacement could be detrimental for getting food for this bottom feeder species (Giles et al., 1990). Likewise, more compact groups (i.e. mean inter-individual distances and variance of this mean were lower) of pikeperch in polyculture and less compact group of tench when it was associated with pikeperch and/or sterlet could be interpreted similarly to group structure modification induced by threat (e.g. another predator or aggressive species) (Milinski et al., 1990), and thus reflect a stressful situation for reared species.

### 4.2. Why the polyculture consequences tend to be species-specific?

The divergent consequences of polyculture between species can be explained by species-specific traits, fish density impact, or ability of some species to outperform other taxa to access to food and space resources.

First, studied species have different growth rates, which can explain the different intensity of response to polyculture between taxa. Tench has slow development (Wolnicki et al., 2006), which could result in low weight variation between the different treatments (from 12% when the tench was associated with sterlet versus 22% when it was alone). Conversely, the higher growth rate of sterlet (i.e. sturgeon species have moderate to high growth rates; Sion et al., 2011; Gerasimov and Vasuyra, 2013) most likely accelerates the divergence process between treatments and could

explain why this species is the most influenced in zootechnical traits by the treatments.

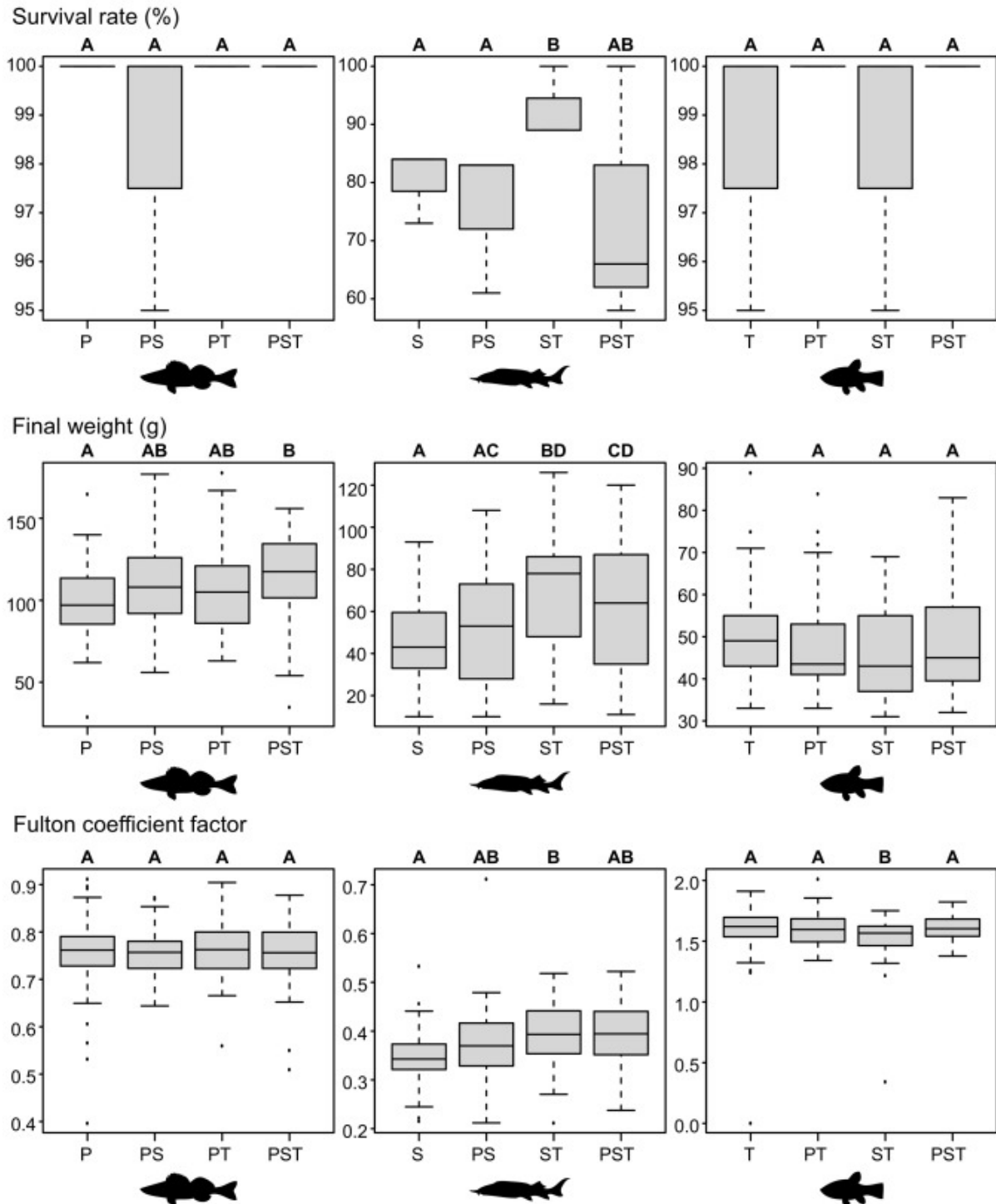
Second, density of each species is different between the treatments (i.e. lower in polyculture than in monoculture in our study). These differences can explain the enhanced growth rate observed for pikeperch and sterlet in polyculture since stocking density can impact fish development (e.g. Rafatnezhad et al., 2008; Yang et al., 2009) and food conversion efficiency (e.g. Jodun et al., 2002), notably due to a decrease of intraspecific competition. Moreover, fish behaviour (schooling behaviour, swimming activity, risk-taking responses) can also be influenced by stocking densities, as shown by previous studies (i.e. Arechavala-lopez et al., 2020; Carbonara et al., 2019). Although these studies were conducted in monoculture systems, we cannot exclude in our case that the recorded changes in tench behaviour are not partly related to differences in density between monoculture and polyculture. Thus, for the development of polyculture, regardless of the rearing system, it is necessary to carefully consider fish biomass and species ratios, beside the choice of species to associate, their initial sizes and growth rates. Third, studied species display different feeding strategies that could result in uneven ability to access feeding resources or compete against other species. Pikeperch is a piscivorous species displaying a sit-and-wait strategy to capture mobile prey (Turesson and Brönmark, 2004). In our experimental design regardless of the modality, pikeperch adopt a static position in the lower part of the water column in the food distribution area. They form a compact group waiting for the food that slowly sinks to the bottom of the tank. This enables them to exploit efficiently the distributed food ration (see similar observation in Thomas et al., 2020). In contrast, tench and sterlet are both benthic foragers, feeding largely on small invertebrates in their natural environment (Coad, 2019; Djikanovic et al., 2015; Monk and Arlinghaus, 2017). This similar feeding strategy could increase potential competition in RAS and explain the lower performance of tench when reared with sterlet. Species activity could also explain species-specific responses to polyculture. For instance, sterlet individuals continually prospect the rearing volume during the experiment, which seems to result in a better exploitation of the trophic resource by the sterlet than tench and could explain why the former outperform the latter in our polycultures. Overall, this shows that the treatments could affect the same trait but in different ways depending on the species.

#### **4.3. Guidelines for further fish polyculture development in RAS**

Polyculture is often applied in ponds (e.g. da Silva et al., 2006; Wang et al., 1998; Martinez-Porchas and Martinez-Cordova, 2012). In these farming systems, polyculture benefits arise from combination of species, which (i) exploit different ecological niches, (ii) display different feeding habits, or (iii) develop mutually beneficial interactions (Azim et al., 2002; Kestemont, 1995; Milstein, 2005; Thomas et al., 2021). Conversely, the less complex environment of RAS makes less likely that species combination results in such valuable coexistence because there is usually no spatial partitioning (i.e. all fish are in the same water volume), no physical complexity (e.g. no refuge), no food diversity (most often only one formulated food), and no opportunity of display different feeding habits (i.e. food is often distributed at the same time in only one of few specific areas of the rearing tank). Therefore, special attention should be paid of traits to which species are combined in RAS. In order to develop relevant fish combination, we here propose three main guidelines to future RAS polyculture development.

First, we recommend to consider both zootechnical data and behavioural responses of all taxa when assessing the relevance of fish combinations in RAS since our results underline species-specific consequences of polyculture. Second, functional traits (i.e. phenotypic characteristic of individual organisms that impacts [in]directly the fitness of such organisms and is relevant to their effects on ecosystem properties) can provide useful piece of information in order to minimise interspecific competition for trophic and space resources in RAS. It concerns the food requirements, but also morphological (rate of development) and behavioural traits (space occupation, group structure). Other traits (i.e. vision abilities, mouth position, foraging abilities, activity, intraspecific relationships) must be introduced for the choice of the species used in a polyculture combination. Third, developing polyculture raises the question about fish densities and species ratios that should be applied. Although further experiment will be needed to optimise this rearing condition, we underline that fish density leading to better farming performances should be applied when it has already been determined in monoculture (e.g. for pikeperch, Baekelandt et al., 2018).

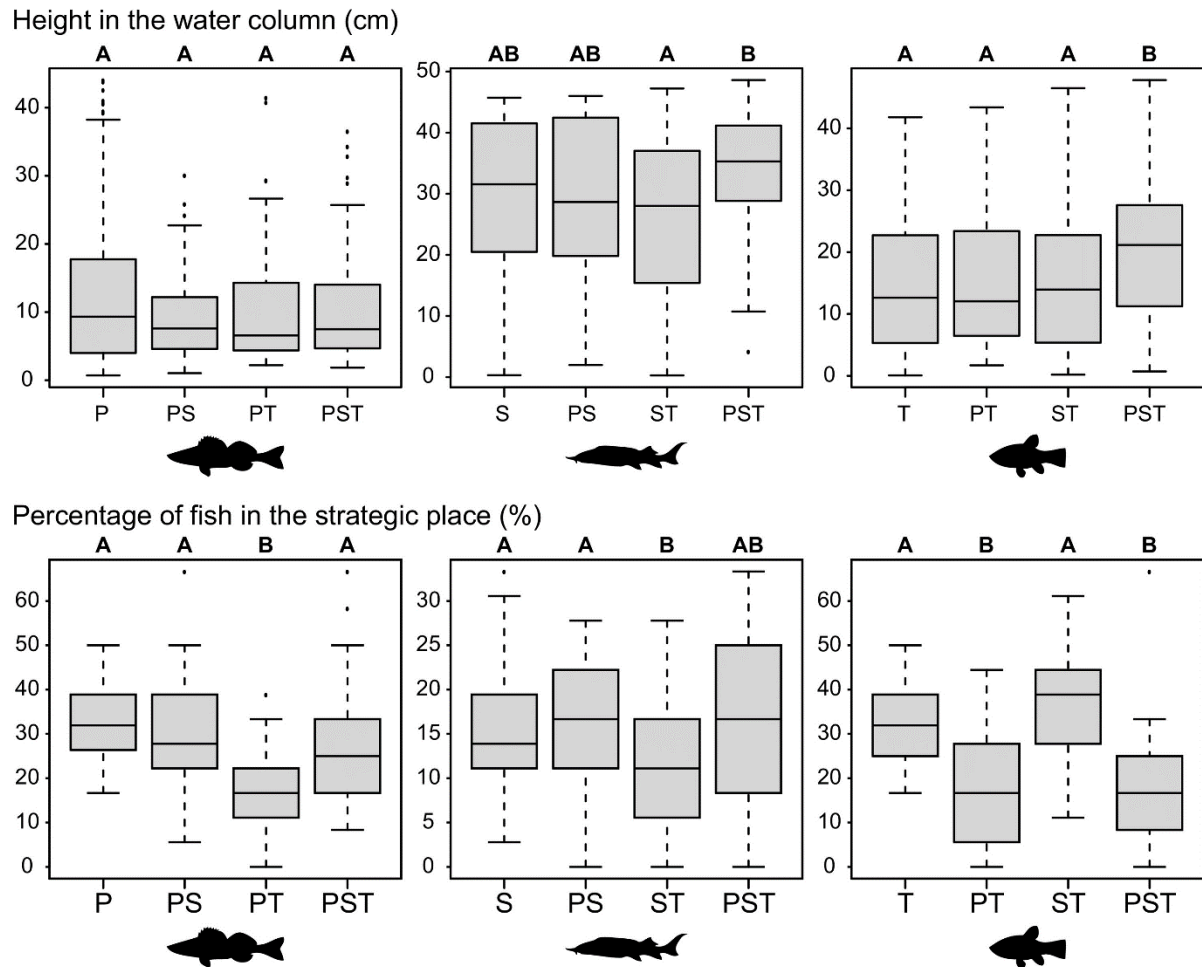
While these guidelines could pave the way for new rearing approaches in RAS, further research on intra- and inter-specific competition of fish according to size, rearing biomass and species ratios could also provide useful information for applications in other rearing systems (cages, ponds).



**Figure 1. Boxplots representing results obtained for survival rate, final weight, and Fulton coefficient factor recorded at the end of the experiment for pikeperch (left column), sterlet (central column), and tench (right column). Fish silhouettes corresponds to each species**

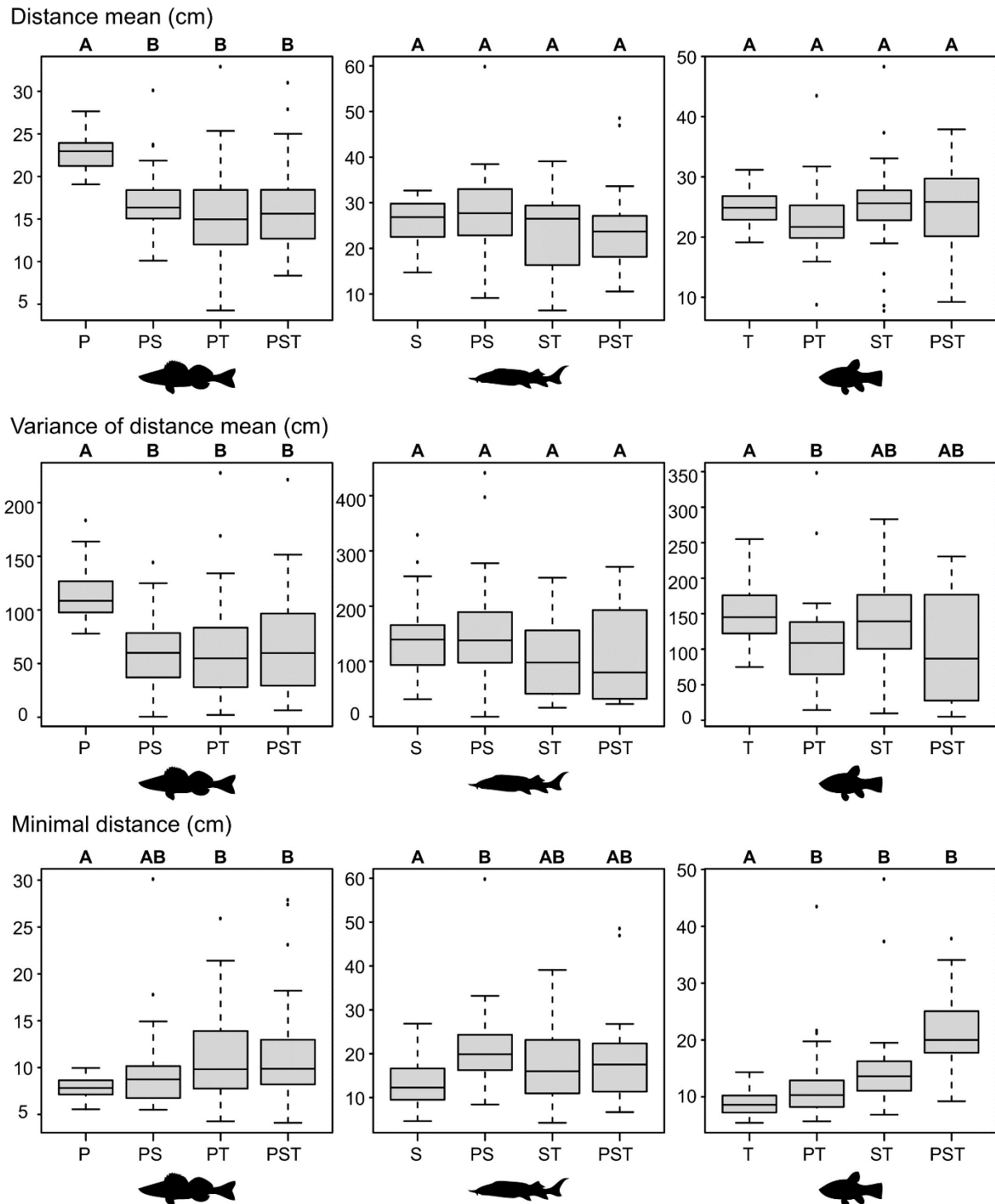
Y-axis shows values of each parameter (units are provided in the title of each line). X-axis of each graph displays treatments: P = pikeperch monoculture; S = sterlet monoculture; T = tench monoculture; PS = polyculture combining pikeperch and sterlet; PT = polyculture combining pikeperch and tench; ST = polyculture combining sterlet and tench; PST = polyculture combining pikeperch, sterlet and tench. Different letters at the top of each boxplot indicate significant differences between treatments using post-hoc tests for each parameter for a particular species..





**Figure 2. Boxplots representing results obtained for height in the water column and Percentage of fish in the strategic place for pikeperch (left column), sterlet (central column), and tench (right column). Fish silhouettes corresponds to each species.**

Y-axis shows values of each parameter (units are provided in the title of each line). X-axis of each graph displays treatments: P = pikeperch monoculture; S = sterlet monoculture; T = tench monoculture; PS = polyculture combining pikeperch and sterlet; PT = polyculture combining pikeperch and tench; ST = polyculture combining sterlet and tench; PST = polyculture combining pikeperch, sterlet and tench. Different letters at the top of each boxplot indicate significant differences between treatments using post-hoc tests for each parameter for a particular species..



**Figure 3. Boxplots representing results obtained for distance mean, variance of distance mean, and minimal distance for pikeperch (left column), sterlet (central column), and tench (right column).**

Fish silhouettes corresponds to each species. Y-axis shows values of each parameter (units are provided in the title of each line). X-axis of each graph displays treatments: P = pikeperch monoculture; S = sterlet monoculture; T = tench monoculture; PS = polyculture combining pikeperch and sterlet; PT = polyculture combining pikeperch and tench; ST = polyculture combining sterlet and tench; PST = polyculture combining pikeperch, sterlet and tench. Different letters at the top of each boxplot indicate significant differences between treatments using post-hoc tests for each parameter for a particular species.

## 5. References

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## 6. Declaration of Competing Interest

The authors declare that they have not known competing financial interest or personal relationships that could have appeared to influence this work and reported in this paper

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