Aquaculture Reports 32 (2023) 101702

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effect of single-generation domestication of pikeperch on the performance of the offspring in conventional and pond recirculation aquaculture system

Géza Péter [a], Jovanka Lukić [b], Zsuzsanna Brlás-Molnár [a], László Ardó [a], Zoltán Horváth [c], András Rónyai [a,1], Péter Bársony [d], Uroš Ljubobratović [a,*]

[a] Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-Liget Str. 35, H-5540 Szarvas, Hungary
[b] Laboratory for Molecular Microbiology (LMM), Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade, Serbia
[c] H&H Carpió Halászati Kft., Kossuth u. 7, H-7814 Ócsárd, Hungary
[d] Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Nature Conservation, University of Debrecen, Debrecen, Hungary

ARTICLE INFO

Keywords:
Domestication
Dry feed adaptation
Stress
Immune response
Grow-out

ABSTRACT

Pikeperch aquaculture technologies have significantly improved, yet knowledge regarding the adaptation of pikeperch to captivity is very scarce. This study aimed to evaluate the survival, growth, stress response, and immune system function of the F2 generation of pikeperch subjected to pond nursing – Recirculating Aquaculture System (RAS) dry feed adaptation, with either RAS or pond grow-out. F2 generation in this research originated from the broodstock reared in the pond system during grow-out, while F1 originated from wild breeders. Critical points in fish growth were analyzed, including transport of pond-nursed juveniles to RAS at 42 DPH, dry feed habituation (42–52 DPH) and post-habituation (52–64 DPH) phase, as well as the on-grow/grow-out (64–154 DPH) phase. Our results showed better growth and survival of the F2 generation in comparison to F1 in pond grow-out. However, the F1 generation was superior in conventional RAS grow-out. Nevertheless, during RAS dry feed habituation, F1 fish were inferior, both in terms of growth and survival, in comparison to F2 fish. Stress and immunological marker analysis revealed higher stress sensitivity, accompanied by stronger immune system activation, in F2 generation in comparison to F1. This was manifested as higher cortisol and immunoglobulin response after moving fish from one system to another. Hypothetically, stronger stress and immune response might have induced better dry feed adaptation during the habituation phase in RAS, and better control of microbial growth in the grow-out environment with a higher microbial load, such as pond. On the other hand, according to the same hypothesis, in a cleaner grow-out environment such as RAS, fish with a weaker cortisol and immunological response would be at an advantage, since these fish are expected to have a richer intestinal microbiota that would eventually support better food digestion and growth. Assumed selection points shaping pikeperch response to farming have been discussed.

1. Introduction

Aquaculture started to develop intensively only in the second half of the 20th century, giving a significant lag in fish domestication success. In addition, the domestication process may be slowed by the fear of making changes in the wild fish populations due to the risk of farmed fish escaping to the wild (Hutchings and Fraser, 2008; Teletchea and Fontaine, 2014). According to the classification provided by Teletchea and Fontaine, 2014, five levels of domestication of fish have been defined, the first two including acclimatization of fish to captivity and rearing fish for only part of their life cycle, level three - the closure of life cycle in captivity but with use of the broodstock from the wild, while level four and five imply complete independence of the wild inputs and artificial breeding programs, respectively. The final level of domestication offers numerous benefits, particularly in terms of increased productivity and reproduction success (Milla et al., 2021).

* Correspondence to: Research Institute for Fisheries and Aquaculture NARIC HAKI, Anna Liget 8, 5540 Szarvas, Hungary.
E-mail address: ljubobratovic.uros@uni-mate.hu (U. Ljubobratović).
[1] Deceased April 15, 2019

https://doi.org/10.1016/j.aqrep.2023.101702
Received 22 January 2023; Received in revised form 3 August 2023; Accepted 21 August 2023

Pikeperch (*Sander lucioperca*) is a freshwater fish native to eastern Europe but cultured in many European countries (Zakęś, 2022). As for many other fish species, little success has been achieved in the domestication of the species. Pikeperch farming is curently at the level four of domestication, though the low reproductive success of F1 fish significantly lowers the production yields (Nynca et al., 2020; Teletchea and Fontaine, 2014). Several research groups addressed the adaptation of pikeperch to captivity (Ljubobratović et al., 2018; Molnár et al., 2018; Nynca et al., 2020). The most feasible rearing procedure for pikeperch, commonly applied in Eastern European countries, involves pond nursing, then moving the juveniles to RAS for dry feed adaptation and further RAS grow-out (Policar et al., 2013; Ljubobratović et al., 2018). However, the development of intensive pond rearing technologies that implement the recirculation system principles is a recently implemented grow-out method for carnivorous fish (Brown et al., 2011; Wang et al., 2020). Of interest are split-ponds that use pond units for intensive rearing and pond units for water treatment (Brown et al., 2016); and in-pond systems that are using tanks (instead of ponds) for intensive rearing, implemented in pond units used for both extensive fish production and water treatment (Masser, 2012). Indeed, pioneer studies performed in these systems, showed that ponds utilized in this way can be equally or even more sustainable for percids in comparison to RAS, both in economic and ecological terms (Nagy et al., 2022; Stejskal et al., 2022). Considering already existing infrastructure in terms of extensive pond area in Central and East European countries, the usage of tanks as intensive units appear to be a more feasible solution due to straightforward implementation without high infrastructural investments. Nevertheless, unfavorable outdoor conditions (e.g. higher or lower temperatures) may exceptionally demand the use of RAS grow-out. Therefore, knowing the performance of domesticated pike-perch in both systems would provide plasticity to fish farming and guide the breeding programs for this species.

Previous research by Ljubobratović et al. (2018), has demonstrated superior performance during RAS grow-out of the offspring (F2) of F1 breeders grown-out in the same system (RAS), in comparison to the offspring (F1) originating from the wild breeders. However, during the RAS habituation phase after larval nursing, the same fish (F2) were inferior to F1 fish. This seems paradoxical, since their parents (F1 breeders) were habituated in the same system. The response of F2 fish to various rearing conditions to which their parents were not exposed needs to be evaluated in order to clarify the processes behind this selection. Therefore, the present study aimed to evaluate the survival, growth, stress, and immunological response of the offspring of pond grown-out F1 generation, during RAS dry feed habituation and both, conventional RAS and pond grow-out. Obtained results reveal new information on the roles of stress and immune response in the adaptation of pikeperch to farming.

2. Materials and methods

The research methodology, husbandry practices, and sampling procedures used in the present study were approved (license number 97–1/2017) by the Animal Ethical Panel of the Research Institute for Fisheries and Aquaculture (NAIK HAKI) established on the principles of Hungarian State law (10/1999. I. 27).

2.1. Fish origin and rearing management

2.1.1. Broodstock

Two broodstock origins were used for the present study. The first broodstock consisted of wild fish (F0) harvested from the oxbow of river Körös in late October 2015. The other broodstock (F1) originated from wild breeders from the same location, which was artificially reproduced in April 2013. Five-day post-hatch (DPH) larvae were stocked into the ponds for extensive nursing and four weeks later transported to the RAS system for habituation to the dry diet. More details on fish management are described by Ljubobratovic et al. (2016). Four weeks following the habituation to a dry diet, fish were transported to the tank supplied with pond water (tandem tank-pond system). Fish were further grown in this system and fed a dry diet exclusively. The sturgeon range feed, 1-8 mm size (Altech Coppens, Netherlands), with a daily feeding rate from 0.3% to 1%, was supplied to the fish, depending on fish size and water temperature. In late October 2015, on the day of the harvest of wild fish, eight pairs of breeders from each group (F0 and F1) were transported to the common 400 m^2 pond of the Research Center for Fisheries and Aquaculture (MATE HAKI). The mean weight in each group was 0.7 ± 0.1 kg, and 0.6 ± 0.1 kg for females and males, respectively. The pond was stocked with live prey consisting of bleaks (*Alburnus alburnus*), roach (*Rutilus rutilus*), common rudd (*Scardinius erythrophthalmus*), common bream (*Abramis brama*) and white bream (*Blicca bjoerkna*), comprising 50% of the total pikeperch biomass. This stocking rate of the prey fish was set according to our previous experience in pikeperch broodstock management in pond conditions, validated in several studies (Ljubobratović et al., 2017, 2019, 2023) and, according to the thermal schedule in our region, is within the range defined for pikeperch in RAS conditions (Malinovskyi et al., 2020, 2022). The prey fish was harvested during the same harvest when the wild breeders were captured. Since photo-thermal conditions and water quality were equal in ponds and oxbow and the stocking density of the fish in the pond was rather low (about 0.05 kg/m^3), the straightforward adaptation of wild breeders to extensive pond conditions was anticipated, as was proven earlier at our station (Rónyai, 2007). In early April 2016, breeders were harvested from the pond and transported to the indoor spawning RAS. Indoor temperature was equilibrated to outdoor at 14 °C and hormonal stimulation of fish was conducted at the time of stocking to the RAS. The immediate hormonal induction was conducted to minimize the effect of stressful adaptation of outdoor-reared fish to indoor conditions. Because four females from each group spawned in the pond before transport, only four females per group were hormonally treated, while all eight males of each group were used for fertilization. Further on, fish were artificially reproduced using the protocol described by Ljubobratović et al. (2019). In short, fish were hormonally treated with human chorionic gonadotropin in doses of 500 IU/kg and 250 IU/kg for females and males, respectively. Once reaching germinal vesicle breakdown, ovulation was evaluated every four hours, and upon noticing eggs disposal, eggs were stripped and fertilized with freshly stripped milt of two males of the common group (different two males for each female) in a total amount of 1 mL of sperm per 100 g of eggs. The eggs of each female were incubated in separate jars. In total, larvae of four females per each origin were used to form the groups - F1 and F2. The hatching rate (percent of hatched larvae from the total amount of fertilized eggs) was 45.1 ± 28.7% and 37.9 ± 16.2% for F1 and F2 groups, respectively without significant difference between the groups ($p = 0.805$, t-test).

2.1.2. Offspring

At the initiation of the hatching, 20,000 volumetrically counted live eggs per female were pooled in a hatching bowle per each group, hatched and stocked in a common group's tank (as explained by Ljubobratović et al., 2019). Thus, each group's larvae were stocked in a separate tank. At 5 DPH, larvae were stocked in the group-separated duplicated 0.15 ha ponds for extensive nursing (40,000 larvae/pond). These four used ponds were of equal dimensions, placed next to each other and assignment of the ponds for each of the groups was done randomly. Pond preparation and management were conducted as explained in Péter et al. (2023). Pond manuring was performed using 1 t/ha of manure. The pond was 50% filled with oxbow water five days before stocking the larvae and was gradually filled to 100% over the next three weeks. After 37 days of pond nursing, at 42 DPH, fish were harvested from ponds and transported to the experimental RAS consisting of twelve 250 L tanks, bead filter, moving bed bioreactor, and ozone and UV disinfection units. Three tanks were stocked with F1, and three tanks with F2 fish (1 500 fish/tank, mean weight 0.45 ± 0.06 g and 0.49 ±

0.06 g, and stocking density 2.7 kg/m^3 and 2.9 kg/m^3 in F1 and F2, respectively) placed in the common RAS. In this way, the tank was considered a replicate and each origin was evaluated in three replicates.

Upon transport to the experimental RAS, fish were subjected to the habituation to dry feed. Therefore, in the following nine days, fish were gradually weaned from natural food - frozen bloodworms, to the dry diet - Otohime (Marubeni Nisshin Feed Co. Japan) C2 (pellet size 0.9–1.4 mm, crude protein 58.3%, crude fat 12.9%, crude fiber 1.6%, crude ash: 15%, Ca: 2.7%, P: 2.5%). The initial feeding rate was 20% of the total biomass of bloodworms and was reduced by 50% every two days, finally being excluded from the diet from 48 DPH. Dry feed was first introduced at 44 DPH in a daily amount of 2.5% of total biomass and was doubled every two days, finally being stable at 10% from 48 DPH until the end of habituation. Feed was supplied manually in five equal daily meals. At 52 DPH, all the fish from each tank were harvested and counted. Starved fish and significantly larger fish (assumed cannibals) were removed. The rest of the fish were considered habituated to a dry diet and stocked back into the tank for the following two-week post-habituation period. In this period, fish were supplied dry feed only with automatic belt feeders in daily amounts of 8% of initial tank biomass. At 64 DPH, all the fish from each tank were harvested and counted, while the sample of 30 fish from each tank was individually measured for weight and total length.

After the post-habituation phase, initial RAS on-grow took place in the 64–84 DPH period, using the same RAS as above. Each group was stocked in three separate tanks with a density of 700 fish per 250 L tank (2.8 fish/L). During this period, fish were fed by automatic belt feeder at a rate of 4% of initial biomass with Coppens (Alltech Coppens, Netherlands) Top (1.5 mm pellets, crude protein: 56%, crude fat: 21%, crude fiber: 0.3%, crude ash: 9.8%, P: 1.5%). At 84 DPH, all the fish were counted and divided into two groups, 150 fish each, for a grow-out in the 84–154 DPH period, which took part in both RAS tanks and pond tanks. From each group, all fish (150) were evaluated for body weight, and 10 randomly chosen fish were evaluated for total length.

Each system consisted of six equal 250 L tanks. RAS tanks were connected to conventional RAS treatment units (described earlier) while the pond tanks were filled with pond water. Three tanks of each system were stocked with F1 fish, while the other three tanks were stocked with F2 fish for 10 weeks of rearing. Each tank was stocked with 150 fish (mean weight 8.3 ± 0.8 g and 8.4 ± 0.9 g for F1 and F2 respectively, with approximate stocking density of 5 kg/m^3). Every two weeks a probe harvest of 45 fish per tank was conducted to monitor the fish growth and to recalculate daily feeding rate. The feed supplied during the first four weeks was Coppens Star Alevin (2 mm pellets, crude protein: 54%, crude fat: 15%, crude fiber: 4.9%, crude ash: 8.4%, P: 0.9%). Further on, Biomar (BioMar Group, Denmark) Efico sigma 811 (3 mm pellets, crude protein: 46%, crude fat:14%, crude fiber: 4.9%, crude ash: 6.1%, P: 0.9%) was introduced gradually during 10 days and given until the end of the trial. Feed was administered at a daily rate of 2% of the total biomass provided by the automatic belt feeder. Disinfection (Söll Peridox preparation in a concentration of 45 g/m^3 (Németh, 2013)) of tanks was performed three times a week during the first eight weeks, and, afterwards, it was increased to seven times a week, since the rapid deterioration of water quality in the pond was observed. At the beginning and the end of the trial, individual body weights were assessed in all fish, while the total length was assessed on a sample of 30 fish.

Water temperature and oxygen percent were checked daily at the outflow of each tank. Water temperature in RAS was kept at 24.4 ± 0.3 °C, while the water oxygen saturation was 95.3 ± 5.4%. Both parameters were maintained at a stable level throughout the trial. The water temperature in pond tanks was in the range of 20.3–27.2 °C, being on average 24.3 ± 1.3 °C, and mean oxygen saturation was 82.1 ± 4.2%, with a minimum recorded value of 72.7%. Nitrogen components in the water were checked twice per week in the sample of the merged outflow water. The values of ammonium nitrogen and nitrite nitrogen were below 0.311 mg/L and 0.313 mg/L, 0.19 mg/L, and 0.026 mg/L, in RAS and pond respectively.

2.2. Fish sampling

Three production phases throughout the juvenile production were analyzed: 1) the dry feed adaptation period, including the habituation period (42–52 DPH), when live food was administered in parallel to dry feed; 2) the post-habituation period (52–64 DPH), when fish shifted to dry feed as the only nutrient source; 3) on-grow phase (64–84 DPH); and 4) grow-out phase (from 84 to 154 DPH). While the first three phases were evaluated in RAS exclusively, the last phase evaluation was conducted in both, RAS and pond. Aside from the assessment of survival and morphometric indices (length and weight), as mentioned above, biochemical parameters of stress and immunological response, including immunoglobulins (Ig) and cortisol, were analyzed in all of the above evaluation points (52, 64, 84 and 154 DPH) to provide complete information on the traits being potentially selected in F1 (Martinez-Porchas et al., 2009; Smith et al., 2019). Biochemical analyses were also performed at the moment of transport to RAS for habituation (42 DPH) and at the 1, 6, 24 and 48 h post-transfer, in order to determine acute reaction of fish to the transfer. Skin mucus was used as the matrix for the assessment of the above biochemical markers due to the non-invasive nature of the sampling procedure and still satisfactory correlation with plasma levels of analyzed parameters (Dash et al., 2018; Salinas et al., 2021; Sanahuja et al., 2019). After anesthetizing the fish using clove oil, mucus was collected by shaking fish in plastic bags. One (84 and 154 DPH) or six (42, 52, and 64 DPH) randomly chosen fish were put into plastic bags, where they were gently shaken for 5 min in 2.5 mL of 50 mM NaCl solution. The solution with the mucus was collected in 1.5 mL micro-centrifuge tubes and centrifuged for 15 min at 4 °C and 675 x *g*. Supernatants were put into new 1.5 mL tubes and stored at − 70 °C. For sampling points (42, 52, and 64 DPH) when pooled samples (six fish per sample) were used due to smaller fish size, the number of samples per group was six (giving a totally of 36 fish per group), while for sampling points when one sample equaled one fish (84 and 154 DPH), the number of samples per group was 10. Additional analyses of lysozyme and glucose levels in mucus throughout the study, as well as plasma levels of all analyzed parameters at the end of the study, were performed (see Supplement 1).

2.3. Biochemical analyses

2.3.1. Immunoglobulin assay

Determination of total immunoglobulin level was based on the estimation of total protein content before and after immunoglobulin precipitation using polyethylene glycol (PEG) (Page and Thorpe, 2002). Mucus samples (50 μL) were mixed with 50 μL of PEG in a 96-well microtiter plate. After two hours of incubation at room temperature, plates were centrifuged at 1000 x *g* for 15 min. The total protein contents of the supernatants were measured using FLUITEST Total Protein Kits (Analyticon GmbH, Germany). These values were subtracted from the total protein levels measured before PEG addition. Obtained value represents total immunoglobulin concentration of mucus. Results were expressed as g/L.

2.3.2. Cortisol assay

Cortisol levels in the mucus were measured using NovaTec Cortisol ELISA and CortisolSalivaELISA Kits (NovaTec Immundiagnostica GmbH, Germany), respectively, according to the manufacturer's instructions. Results were expressed as ng/mL.

2.4. Data analysis

Results of the study are presented in tables as mean values ± standard deviation, using tank average values as biological replicates for morphometric analysis and individual or pooled (6 individuals) fish samples from all three tanks per group as biological replicates for biochemical analyses. The number of fish used in statistical analysis at

each sampling point is provided in Table 1. Specific growth rate (SGR, %/day), feed conversion ratio (FCR), coefficient of variation (CV, %), relative condition factor (Kn), and allometric coefficient (b), were calculated as described in Péter et al. (2023):

SGR = 100 × (LN(Wf) − LN (Wi))/Δt (Wf = final weight (g), Wi = initial weight (g), Δt = duration of the analyzed period in days).

FCR = F/ΔB (F = given feed amount in g, ΔB = biomass gain in g).

CV = SD/Wa (SD = standard deviation, Wa = average weight).

Kn = Wo/Wc (W0 = observed weight, Wc—calculated weight using length - weight realtionship (LWR).

Allometric coefficient (b) was calculated from the equation:

LN (W) = a + b LN (L) (W = fish weight in grams, L = fish length in cm, a = the intercept of the regression line, b = slope of the regression line).

Comparison of two treatments (F1 vs. F2) was performed using a two-tailed Student t-test assuming equal variances if the p-value of the Levene's test was > 0.05, or unequal variances if the p-value of the Levene test was < 0.05. To evaluate the effects of transport on acute immune and stress parameters, results of Ig and cortisol at 0–48 h post-transfer to RAS at 42 DPH were estimated using two-way Analysis of Variance (ANOVA), with the timeline as an additional factor. Two-way ANOVA was also used to estimate the interaction between a conventional- and pond grow-out and fish origin. Significant results of ANOVA were checked for variance homogeneity using Levene's test. If the p-value of Levene's test was < 0.05, the results of ANOVA were not accepted. As explained in Péter et al. (2023), differences between SGR and FCR were adjusted for the potential confounding variable (ISD), using Analysis of Covariance (ANCOVA) if the effects of ISD on SGR and FCR were significant ($p < 0.05$). As with ANOVA, variance homogeneity was estimated using Levene's test and the results were accepted only if the variance homogeneity assumption was met. Outliers were not eliminated in any analysis. All statistical analyses were performed using IBM SPSS, 2012.

3. Results

3.1. Habituation

Morphometric parameters, along with SGR, b, and Kn, at the start of the habituation phase (42 DPH) are provided in Table 2. Except for the fish length, which was higher in F2 fish, no differences in other analyzed parameters were observed.

The acute reaction was analyzed in terms of changes of total mucus immunoglobulins (Ig) and cortisol values in comparison to pre-transfer (0 h) levels. Two-way interaction analysis (Table 3) revealed higher levels of Ig 6 h post-transfer and cortisol 48 h post-transfer in the F2 group. Ig levels in both groups started to decline after transfer and this decline was faster in the F1 group. Cortisol levels in the F2 group increased rapidly 1 h post-transfer, returning to baseline values at 24 h post-transfer. No elevation of cortisol levels after transfer was observed in the F1 group; however, a substantial drop of cortisol, below the baseline values, was observed in these fish at 48 h post-transfer.

Table 1
The number of samples included in statistical analysis. DPH = day post-hatching; 1 - all surviving fish in tanks were counted; 2 - a totally six pooled samples per group (two per tank) were taken but samples for which measurements failed due to technical issues were eliminated; 3 – a totally of 10 samples per group (three-four per tank) were taken but samples for which measurement failed due to technical issues were eliminated.

Sampling point (DPH)	Individual length	Weight	Mucus
42	10 per tank	10 per tank	3–6 per group[2]
52	20 per tank	20 per tank	3–6 per group[2]
64	14 per tank	30 per tank	3–6 per group[2]
84	20 per tank	300 per tank	8–10 per group[3]
98–154	30 per tank	63–130 per tank[1]	8–10 per group[3]

Table 2
Morphometric indices of F1 and F2 pikeperch pond-nursed juveniles at 42 DPH, before transfer to RAS for dry feed habituation. # indicates statistically significant ($p < 0.05$) difference in comparison to the F2 group. SGR=specific growth rate, CV=coefficient of variation, b=allometric coefficient, Kn=relative condition factor.

Parameter	F2	F1
SGR (1–42 DPH) (%/day)	16.4 ± 0.12	16.17 ± 0.13
Weight, g	0.49 ± 0.25	0.45 ± 0.24
Length, c=mm	4.1 ± 0.6	**#3.9 ± 0.6**
CV (%)	11.93 ± 1.41	12.77 ± 2.22
b	2.66 ± 0.46	2.83 ± 0.3
Kn	1.001 ± 0	1.001 ± 0

Table 3
Biochemical indicators of stress and immunological response of F1 and F2 pikeperch pond-nursed juveniles at 42–44 DPH, 1–3 d post-transfer to RAS. # indicates statistically significant ($p < 0.05$) difference in comparison to the F2 group. Numbers in superscripts indicate significant differences within the same group (F1 or F2) in comparison to the earlier time-point.

Parameter		F2	F1
Ig in mucus, g/L	0 h	1.59 ± 0.47	1.75 ± 0.47
	1 h	1.8 ± 0.37	1.48 ± 0.45
	6 h	1.47 ± 1.02	**#0.69 ± 0.37**
	24 h	[0, 1, 6]0.52 ± 0.41	[0, 1]0.46 ± 0.24
	48 h	[0, 1, 6]0.58 ± 0.44	[0, 1]0.3 ± 0.25
Cortisol in mucus, ng/mL	0 h	12.35 ± 0.93	13.97 ± 1.05
	1 h	[0]17.15 ± 1.08	15.85 ± 0.77
	6 h	13.48 ± 2.35	14.98 ± 0.74
	24 h	[1]13.09 ± 1.18	[1]12.15 ± 0.65
	48 h	[1]10.48 ± 1.08	[0, 1, 6, 24] **#1.61 ± 3.94**

Assessment of survival, cannibalism, morphometry, and mucus composition of feed-habituated fish 10-day post-transfer (Table 4) showed better survival and increased length of F2 fish. There were no differences in either, number of significantly larger fish (assumed cannibals) per tank (3.9 ± 0.4% in F2 and 4.9 ± 1.7% in F1 $p = 0.480$), or between the number of obviously starved fish (11.1 ± 1.9% in F2 and 17.0 ± 3.7% in F1, $p = 0.056$, statistical trend). According to ANCOVA, there was no interference of initial stocking densities (ISD) (2.948 ± 0.151 and 2.674 ± 0.145 kg/m^3 in F2 and F1 fish, respectively) with specific growth rate (SGR) during the habituation phase.

3.2. Post-habituation

Initial stocking densities (3.619 ± 0.439 and 2.781 ± 0.186 kg/m^3 in F2 and F1 fish, respectively) significantly ($p = 0.009$) affected the SGR and FCR of fish during the post-habituation phase, so the comparisons of ISD adjusted SGR and FCR between the groups, were performed using ANCOVA. The analysis revealed higher SGR and lower FCR of F2 fish. No differences were observed in other analyzed parameters (Table 4).

3.3. On-grow

During the on-grow period (64–84 DPH), there was no significant interference of fish stocking densities with fish SGR ($p = 0.002$) and FCR. Analysis of mucus samples showed significantly higher Ig and cortisol levels in F1 in comparison to F2 fish (Table 4).

3.4. Grow-out

During the second evaluation period of the fish on-grow phase (84–154 DPH) (Table 5), after the separation of groups to RAS and pond at 84 DPH, RAS rearing was associated with higher SGR and body weight of F1 in comparison to F2 fish. On the other hand, in the pond, F2 fish had better survival than F1. In addition, RAS-reared F1 fish had better

Table 4
Morphometric and biochemical indices of F1 and F2 pikeperch pond-nursed juveniles at 52 DPH (the end of the dry feed habituation phase. 42–52 DPH), 64 DPH (the end of dry feed post-habituation phase, 52–64 DPH) and 84 DPH (the end of on-grow phase, 64–84 DPH, before the separation of RAS and pond grow-out groups). # indicates statistically significant ($p < 0.05$) difference in comparison to the F2 group. SGR=specific growth rate, FCR = feed conversion ratio, CV=coefficient of variation, b=allometric coefficient, Kn=relative condition factor; Data are presented as mean ± standard deviation.

	52 DPH		64 DPH		84 DPH	
Parameter	F2	F1	F2	F1	F2	F1
Cannibalism, %	12.27 ± 2.77	14.07 ± 0.48	7.23 ± 2.43	7.1 ± 1.62		
Survival, %	68.09 ± 2.99	**#57.78 ± 1.44**	91.47 ± 2.78	89.09 ± 1.72	93.47 ± 2.32	93.39 ± 1.14
SGR, %/day	6.51 ± 0.44	6.53 ± 1.08	11.28 ± 0.22 A	* **9.94 ± 0.22** A	4.22 ± 0.18	4.48 ± 0.17
FCR			0.31 ± 0.03 A	* **0.44 ± 0.03** A	0.18 ± 0.02	0.16 ± 0.01
Weight, g	0.88 ± 0.76	0.8 ± 0.47	3.45 ± 0.12	3.24 ± 0.13	8.36 ± 0.81	8.36 ± 0.81
Length, mm	47.1 ± 1.6	**#44.4 ± 0.4**	71.2 ± 2.2	70 ± 0.5	100.8 ±1	101 ±0.6
CV, %	32.45 ± 5.05	37.02 ± 4.03	21.75 ± 8.32	23.27 ± 3.98		
b	3.44 ± 0.31	3.75 ± 0.71	2.72 ± 0.22	3.02 ± 0.41	2.51 ± 0.41	2.69 ± 0.73
Kn	1.008 ± 0.001	1.019 ± 0.014	1.01 ± 0.008	1.005 ± 0.003	1.001 ± 0.001	1.001 ± 0
Ig in mucus, g/L	0.6 ± 0.14	0.81 ± 0.36	4.36 ± 2.75	1.65 ± 1.56	0.18 ± 0.3	* 0.9 ± 0.51
Cortisol in mucus, ng/mL	1.03 ± 1.01	1.35 ± 1.18	7.32 ± 4.23	4.88 ± 3	1.72 ± 1.51	* 7.02 ± 3.02

A = ANCOVA estimated marginal means

Table 5
Morphometric and biochemical indices of F1 and F2 pikeperch pond-nursed juveniles at 84–154 DPH, after separation to the RAS and Pond-RAS grow-out groups. * indicates a statistically significant difference in comparison to pond groups within the same origin (F1 or F2). # indicates a statistically significant ($p < 0.05$) difference in comparison to the F2 groups within the same treatment (pond or RAS). SGR=specific growth rate, FCR = feed conversion ratio, b=allometric coefficient, Kn=relative condition factor, Ig=immunoglobulin; Data are presented in a form mean ± standard deviation.

Parameter		F2		F1	
		Pond	RAS	Pond	RAS
Survival %		74.7 ± 2.5	83 ± 1.7	**#57.3 ± 13.9**	* **85.7 ± 1.2**
Cumulative survival %		43.4 ± 1.2	48.3 ± 2.6	38.6 ± 15.6	41.2 ± 1.9
SGR %/day		1.1 ± 0.19	1.23 ± 0.07	1.25 ± 0.13	* **#1.6 ± 0.15**
FCR		2 ± 0.44	1.25 ± 0.13	11.33 ± 16	0.73 ± 0.07
Weight, g		18.2 ± 2.3	19.8 ± 1.2	20.3 ± 0.9	* **#25.7 ± 1.8**
Length, mm		135.4 ± 4	138.9 ± 4.6	136.5 ± 3	* **145.1 ± 2.6**
b		2.3 ± 2.4	2.2 ± 1.5	2.8 ± 1.7	3.9 ± 0.2
Kn		1.036 ± 0.031	1.05 ± 0.051	1.032 ± 0.05	1.003 ± 0.001
Ig in mucus, g/L	$2w^{PS}$	0.95 ± 0.57	* **0.12 ± 0.18**	0.7 ± 0.31	**#0.72 ± 0.36**
	$4w^{PS}$	1.33 ± 1.74	0.38 ± 0.32	**#0.46 ± 0.27**	* **0.65 ± 0.34**
	$6w^{PS}$	0.65 ± 0.31	0.61 ± 0.22	**#0.26 ± 0.23**	0.46 ± 0.31
	$8w^{PS}$	0.35 ± 0.33	0.37 ± 0.39	#**0.06 ± 0.09**	#**0.04 ± 0.12**
	$10w^{PS}$	0.12 ± 0.36	0.12 ± 0.19	0.27 ± 0.45	0.026 ± 0.06
Cortisol in mucus, ng/mL	$2w^{PS}$	1.82 ± 1.727	1.52 ± 1.36	**#0.37 ± 0.83**	0.57 ± 0.84
	$4w^{PS}$	0.05 ± 0.13	0.13 ± 0.41	0.00 ± 0.01	0.13 ± 0.4
	$6w^{PS}$	0.52 ± 1.1	0.49 ± 1.22	0.8 ± 0.89	0.28 ± 0.6
	$8w^{PS}$	0.23 ± 0.35	0.15 ± 0.29	#0.84 ± 1.17	* 0.22 ± 0.39
	$10w^{PS}$	2.18 ± 1.78	* **0.78 ± 1.02**	**#0.21 ± 0.67**	0.00 ± 0.15

PS = post-separation

SGR, survival, individual length, and final body weight in comparison to pond-reared F1 fish. No significant effect of fish stocking density on SGR and FCR was seen during this growth phase. RAS-reared fish maintained the same Ig trend in mucus as before group separation (84 DPH), with F1 fish having higher Ig levels in comparison to F2 fish two weeks post-transfer, and in comparison to pond-reared F1 fish four weeks post-transfer. Transfer to the pond was associated with a significant increase of Ig levels in the mucus of F2 fish four weeks post-transfer and the same trend was also seen two weeks later, in comparison to pond-reared F1 fish.

4. Discussion

4.1. Habituation/post-habituation – intense stress response provides advantage after environmental change

The results of the present study indicated that domestication significantly affects pikeperch performance, by providing survival and growth benefits to F2 fish during dry feed habituation and post-habituation phases in RAS after pond nursing. However, during grow-out, the effects of domestication were dependent on the system which was used. Namely, F2 (descendants of broodstock cultured for one generation in pond-RAS-pond system) had superior survival in comparison to F1 (descendants of wild broodstock) fish when grown-out in the pond, but had lower growth rate when grown-out in RAS. At the physiological level, the difference between F1 and F2 fish was evident already during the transport phase: cortisol secretion after transport was higher in the F2 group, indicating stronger acute stress response. The F1 group appeared to have higher stress resilience, reflected in a fast decline of cortisol levels 48 h post-transport, even below the pre-stress levels. This is indicative of pronounced negative glucocorticoid feedback and fast attenuation of hypothalamic-pituitary-adrenal (HPA) axis activity (Kageyama et al., 2021). It has been reported that fish loses its ability to react to stressors after only one generation of domestication (Pasquet, 2018). This increases fish's ability to prey but it also decreases its ability to respond to danger, which may increase the survival of fish because of a higher feeding rate (Pasquet, 2018). However, in cannibalistic fish, such as pikeperch, this adaptation may have detrimental effects, since the cannibalism rate would increase, leading to higher mortality and slower dry feed acceptance (Molnár et al., 2018; Riesch et al., 2022). So, the increasing stress response is expected to reduce competitiveness, aggressiveness, and cannibalistic behavior in farmed cannibalistic fish. This will force fish to adapt to dry feed and increase growth rate with an increase in the proportion of dry feed in their diet (Molnár et al., 2018). In the current research, this was evident from the higher SGR and lower FCR of F2 fish in the post-habituation phase. Aside from size heterogeneity, a higher growth rate is also known to stimulate cannibalistic behavior (Kestemont et al., 2003; Folkvord, 1997), and this might have possibly canceled out the assumed decrease of type II cannibalism resulting from suppression of aggressiveness and increased danger alertness.

4.2. Grow-out - intense immunological reaction reduces fish performance in RAS

Stronger acute stress response in the F2 group was accompanied by higher Ig levels in these fish after transport to RAS at the 42 DPH. Acute stress is a strong stimulator of innate and adaptive immunity (Dhabhar, 2008). Though this may be beneficial in terms of adaptation to an unknown environment (Barton, 2002), intense stress and accompanying immunological response in the F2 group might have reduced the bacterial diversity, both through the reduction of social interactions (Levin et al., 2016) and through immune-mediated mechanisms (Deriu et al., 2016). This may eventually cause poor growth, given the important role of gut microbiota in nutrient absorption and extraction of energy from food (Nayak, 2010). Indeed, after the initial phase of faster growth of F2 fish, this trend was reversed during the grow-out phase and the F1 group gained growth and survival advantage in unchanged growth conditions (RAS grow-out). We note here that Ig levels dropped after transport to RAS in both F1 and F2 fish, presumably as the result of a higher bacterial load in the pond (10^4-10^6 colony forming units (CFU)/mL of the rearing water) in comparison to RAS (10^3-10^4 CFU), according to previous studies (Chislock et al., 2013, 2020; Ajayi and Okoh, 2014; Luigi, 2007; Schoina et al., 2022; Dahle et al., 2022; Ye and Ying, 2015).

In line with the above put hypothesis on the role of commensal microbiota in F1 fish, after the post-habituation phase, these fish experienced an increase of Ig levels. This may reflect the reaction to the expansion of commensal microbiota established after total transition to dry feed. A somewhat similar phenomenon was reported in mammals after weaning, as an immunological response to solid feed. This so-called "weaning reaction" has been attributed to an important role in the prevention of future immunological pathologies (Al Nabhani et al., 2019). In addition, cortisol, which was increased alongside Ig in F1 fish during on-grow, has been reported to be an integral part of the "weaning reaction" (Moeser et al., 2017). According to the levels of total Ig and cortisol during the post-habituation and on-grow phase, a "weaning reaction" can also be noticed in F2 fish, but it appears to have started earlier, which could be expected, given the assumed faster dry feed acceptance by this group. "Weaning reaction" in F2 fish was, however, weaker in comparison to F1 fish, in line with the assumed reduced microbial diversity.

4.3. Grow-out - strong immunological reaction improves fish performance in the outdoor rearing system

Although using RAS for fish on-grow/grow-out provided growth and survival advantage to F1 fish, movement to the pond at 84 DPH was linked to F1 fish mortality. It is possible that a stronger immunological reaction, presumably as the accompanying reaction to higher cortisol release, may be beneficial when a high load of microorganisms in a pond could act as a potential selective pressure (Van Doan et al., 2022). In line with this, Ig levels in F2 fish in the pond were elevated four and six weeks after transfer, in comparison to F1 fish. This may be the reaction to increased stocking density and presumable deterioration of water quality, as mentioned above.

4.4. Two-selective pressures hypothesis –stress & immunological response circuit

Our research suggests that the performance of the offspring

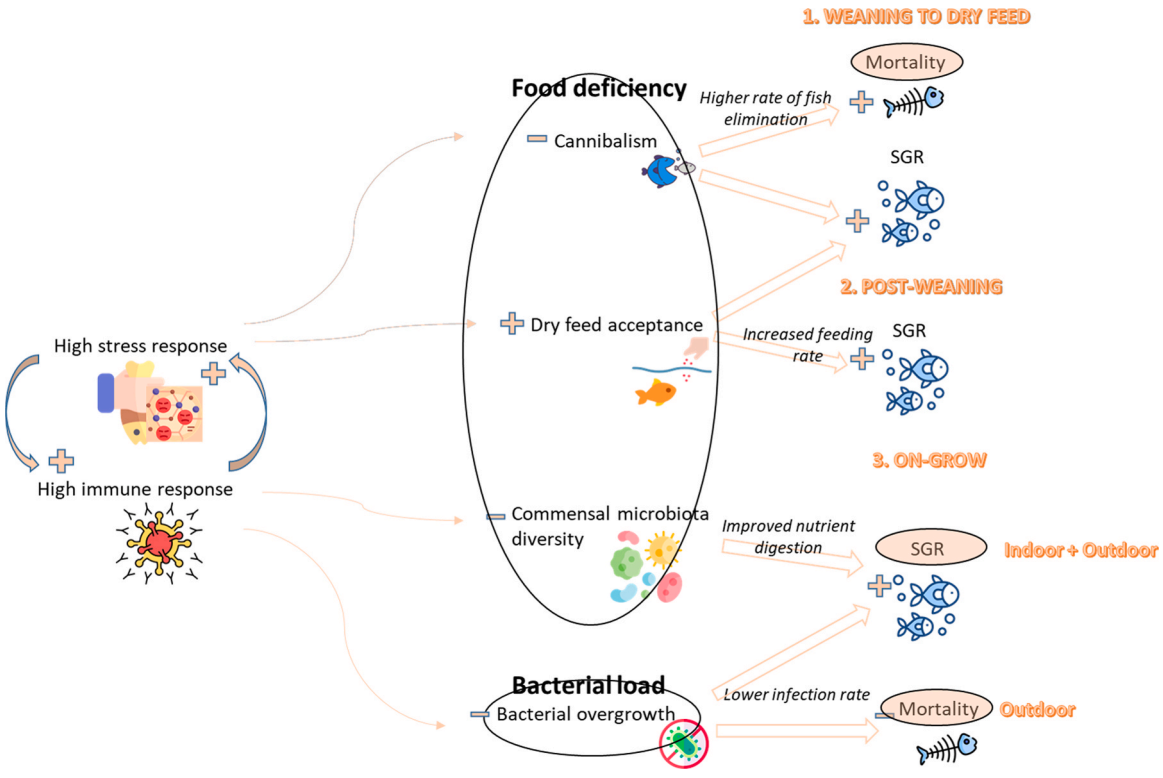

Fig. 1. Illustration depicting two-selective pressures hypothesis, explaining the adaptation of pike-perch to farming. Stress and immune response shape the performance of fish subjected to two selective pressures (highlighted in blue) acting in different phases of fish growth: food deficiency/ digestibility, during weaning/ post-weaning and grow-out, and high bacterial load in outdoor rearing system during grow-out. A high stress response is of critical importance during the weaning and post-weaning growth stages, while high immune response mostly affects grow out phase through the determination of microbiota composition. Mortality during any growth stage and SGR during on-grow could be considered the most important traits under selection during fish growth (highlighted in orange). The detailed explanation is provided in the text. SGR = specific growth rate. Icons used in the illustration have been downloaded from www.flaticon.com under free license and are used in accordance with the Terms of Use provided at https://www.freepikcompany.com/legal#nav-flaticon. Icons created by: *Freepik*, *Backwoods*, *Victoruler*, *Prashanth Rapolu 15 & Peerapak Takpho* .

originating from fish cultured in identical conditions (pond-RAS-pond system) is superior in comparison to the offspring originating from wild broodstock. We hypothesize that two selective pressures, which were present during the lifetime of pond-RAS-pond reared parents, shaped the performance of the offspring (Fig. 1). The first selective pressure was feed deficiency/low digestibility, leading to high mortality during habituation to dry feed from 42 to 64 DPH. This mortality is presumably the result of a high cannibalism rate and/or poor dry feed acceptance so this first selection was towards suppression of cannibalism and stimulation of dry feed acceptance. As assumed above, a stronger acute stress response could suppress cannibalism and improve dry feed acceptance and this could hence be the trait under selection during the dry feed adaptation period. The second selection point, relevant to the present research, is the increased mortality seen after the transfer of fish to the pond during the grow-out phase, presumably due to a sudden increase in microbial burden, which acted as the second selective pressure. Fish with stronger stress and, hence, immunological response, were superior again, providing a double selection advantage for stress-sensitive fish.

In contrast to the pond-RAS-pond reared offspring, the offspring reared in the pond-RAS system (RAS grow-out) did not have any advantages compared to the offspring of wild broodstock. Fish having weaker stress response (F1 fish in this case), gained growth advantage, assumedly due to better nutrient assimilation capacity provided by commensal microbiota. Since only large fish are commonly used for breeding, this provided selective advantage to the fast-growing fish. So, the same trait selected positively during the habituation phase seems to protect the fish against the same selective pressure (feed digestibility) during grow-out in RAS, though this pressure is not very strong during the latter growth stage. It comes out that the adaptation of fish to the RAS grow-out may be associated with improved fish welfare, since cortisol is a common indicator of compromised fish welfare (Daskalova, 2019). We note here that this selective pressure (feed digestibility) is also present in the pond during grow-out, but fish growth in the pond is additionally affected by other selective force - microbial load, thus leading to lower growth in fish with lower immune response.

In the light of obtained results, concerning evaluated grow-out systems, it is worthwhile mentioning the performance of fish in the present study. To the best of our knowledge, the only study that tested the pikeperch intensive culture in a pond-RAS environment was performed by Nagy et al. (2022), evaluating an older class of fish (year-old juveniles). Considering this, it appears that the in-pond culture of young pikeperch is characterized by lower survival. Therefore, high survival obtained in F2 group grown-out in pond in this research indicates the importance of domestication. Thus, before setting the breeding program, the environment of interest, as well as the breeding goals (growth, robustness, etc.), need to be set. For the commercial implementation of pond-connected grow-out, it seems that domestication will have a crucial role. On the other hand, the performance of both groups (F1 and F2) in RAS is in line with previously published results (reviewed by Policar et al., 2019).

4.5. Conclusions

The knowledge on individual traits affecting the adaptation to specific conditions, and relevant environmental factors, provided in this work, could aid the selection process in pikeperch, e.g. rapid reduction of live food percent during RAS habituation or an increase of microbial burden in grow-out ponds to select for fish with higher stress and/or immunological reaction. In this line, however, this study raises ethical issues, given the stress sensitivity connected with the domestication to the pond grow-out. From this perspective, aside from being climate-resilient, RAS may offer this additional advantage in comparison to outdoor farming. Overall, the differences in the performance of F1 group against F2 in various rearing conditions, seen in this research, indicate that, before starting the work on domestication, the rearing system of interest and properly set goals need to be defined.

CRediT authorship contribution statement

Géza Péter: Investigation, Methodology, Writing – review & editing. **Jovanka Lukić:** Formal analysis, Writing - original draft; **Zsuzsanna Brlás-Molnár:** Methodology, Writing – review & editing. **László Ardó:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Zoltán Horváth:** Methodology, Writing – review & editing. **András Rónyai:** Conceptualization, Funding acquisition, Investigation, Supervision. **Péter Bársony:** Conceptualization, Investigation, Writing-review & editing. **Uroš Ljubobratović:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing.

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.

Data Availability

Data will be uploaded to ResearchGate.

Acknowledgments

This work was funded by the European Union's Horizon 2020 research and innovation program under grant agreement No. 652831 (AQUAEXCEL^{2020}) and the European Regional and Development Fund and the Government of Hungary within the project GINOP-2.3.2-15-2016-00025, and supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under Contract No. 451-03-68/2022-14/200042. The authors would like to thank David Koljukaj (https://www.linkedin.com/in/david-koljukaj/?originalSubdomain=rs), an English speaker, holding the Common European Framework of Reference for Languages: Learning, Teaching, Assessment (CEFR) C2 certificate (issued by the Council of Europe), for proofreading the manuscript.

References

Adebami, G., Fasiku, S., Solomon, O., Benjamin, B., 2020. Physicochemical and microbial evaluations of different fish ponds' wastewaters and the antibiotics profiles of isolated bacteria. Ethiop. J. Environ. Stud. Manag. 13, 1998-0507. ⟨https://www.academia.edu/44857934/Physicochemical_and_Microbial_Evaluations_of_Different_Fish_Ponds_Wastewaters_and_the_Antibiotics_Profiles_of_Isolated_Bacteria⟩.

Ajayi, A.O., Okoh, A.I., 2014. Bacteriological study of pond water for aquaculture purposes. J. Food Agric. Environ. 12 https://doi.org/10.1234/4.2014.5325.

Al Nabhani, Z., Dulauroy, S., Marques, R., Cousu, C., Al Bounny, S., Déjardin, F., Sparwasser, T., Bérard, M., Cerf-Bensussan, N., Eberl, G., 2019. A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. e5 Immunity 50, 1276–1288. https://doi.org/10.1016/j.immuni.2019.02.014.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517–525. https://doi.org/10.1093/icb/42.3.517.

Brown, T.W., Chappell, J.A., Boyd, C.E., 2011. A commercial-scale, in-pond raceway system for Ictalurid catfish production. Aquac. Eng. 44, 72–79. https://doi.org/10.1016/j.aquaeng.2011.03.003.

Brown, T.W., Tucker, C.S., Rutland, B.L., 2016. Performance evaluation of four different methods for circulating water in commercial-scale, split-pond aquaculture systems. Aquac. Eng. 70, 33–41. https://doi.org/10.1016/j.aquaeng.2015.12.002.

Chislock, M.F., Doster, E., Zitomer, R.A., Wilson, A.E., 2013. Eutrophication: causes, consequences, and controls in aquatic ecosystems. Nat. Educ. Knowl. 4, 10. ⟨https://www.nature.com/scitable/knowledge/library/eutrophication-causes-consequences-and-controls-in-aquatic-102364466/⟩.

Dahle, S.W., Attramadal, K.J.K., Vadstein, O., Hestdahl, H.I., Bakke, I., 2022. Microbial community dynamics in a commercial RAS for production of Atlantic salmon fry (Salmo salar). Aquaculture 546, 737382. https://doi.org/10.1016/j.aquaculture.2021.737382.

Dash, S., Das, S., Samal, J., Thatoi, H., 2018. Epidermal mucus, a major determinant in fish health: a review. Iran. J. Vet. Res 19, 72–81. https://doi.org/10.22099/ijvr.2018.4849.

Daskalova, A., 2019. Farmed fish welfare: stress, post-mortem muscle metabolism, and stress-related meat quality changes. Int Aquat. Res 11, 113–124. https://doi.org/10.1007/s40071-019-0230-0.

Deriu, E., Boxx, G.M., He, X., Pan, C., Benavidez, S.D., Cen, L., Rozengurt, N., Shi, W., Cheng, G., 2016. Influenza virus affects intestinal microbiota and secondary salmonella infection in the gut through type I interferons. PLoS Pathog. 12, e1005572 https://doi.org/10.1371/journal.ppat.1005572.

Dhabhar, F.S., 2008. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection versus immunopathology. All Asth Clin. Immun. 4, 2. https://doi.org/10.1186/1710-1492-4-1-2.

Folkvord, A., 1997. Ontogeny of cannibalism in larval and juvenile fishes with special emphasis on Atlantic cod. In: Chambers, R.C., Trippel, E.A. (Eds.), Early Life History and Recruitment in Fish Populations. Chapman & Hall Fish and Fisheries Series, vol 21. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-1439-1_9.

Hutchings, J.A., Fraser, D.J., 2008. The nature of fisheries- and farming-induced evolution. Mol. Ecol. 17, 294–313. https://doi.org/10.1111/j.1365-294X.2007.03485.x.

IBM SPSS Statistics 21. IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

Kageyama, K., Iwasaki, Y., Daimon, M., 2021. Hypothalamic regulation of corticotropin-releasing factor under stress and stress resilience. Int. J. Mol. Sci. 22, 12242. https://doi.org/10.3390/ijms222212242.

Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., Baras, E., 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. Aquaculture 227 (2003), 333–356. https://doi.org/10.1016/S0044-8486(03)00513-1.

Levin, I.I., Zonana, D.M., Fosdick, B.K., Song, S.J., Knight, R., Safran, R.J., 2016. Stress response, gut microbial diversity and sexual signals correlate with social interactions. Biol. Lett. 12, 20160352. https://doi.org/10.1098/rsbl.2016.0352.

Ljubobratovic, U., Kucska, B., Sandor, Z., Peteri, A., Ronyai, A., 2016. Effects of stocking density, feeding technique and vitamin C supplementation on the habituation on dry feed of pikeperch (*Sander lucioperca*) pond reared juveniles. Iran. J. Fish. Sci. 15, 1337–1347. ⟨http://hdl.handle.net/1834/12118⟩.

Ljubobratović, U., Péter, G., Horváth, Z., Żarski, D., Ristović, T., Percze, V., Sándor, Z., Lengyel, S., Rónyai, A., 2017. Reproductive performance of indoor-reared pikeperch (*Sander lucioperca*) females after wintering in outdoor earthen ponds. Aquac. Res 48, 4851–4863. https://doi.org/10.1111/are.13305.

Ljubobratović, U., Péter, G., Horvath, Z., Ristović, T., Rónyai, A., 2018. Effect of parental origin on dry feed habituation and intensive on-growing results in pikeperch (*Sander lucioperca*) offspring. Turk. J. Fish. Aquat. Sc. 18, 425–433. https://doi.org/10.4194/1303-2712-v18_3_08.

Ljubobratović, U., Péter, G., Sándor, Z., Kugyela, N., Rónyai, A., 2019. The effect of hormonal preparation (gonadotropins vs. gonadoliberins) on pre-seasonally obtained eggs and larvae quality in pikeperch (*Sander lucioperca* L.). Aquac. Int 27, 1009–1024. https://doi.org/10.1007/s10499-019-00368-4.

Ljubobratović, U., Kitanović, N., Milla, S., Marinović, Z., Fazekas, G., Stanivuk, J., Nagy, Z., Horváth, Á., 2023. Predicting population's oocyte maturation competence and evaluating individual's latency time using in vitro oocyte maturation in pikeperch (Sander lucioperca). Aquaculture 562, 738851. https://doi.org/10.1016/j.aquaculture.2022.738851.

Luigi, M., 2007. Microbial communities of recirculating aquaculture facilities: interaction between heterotrophic and autotrophic bacteria and the system itself. PhD Thesis, Université de Montpellier II. ⟨https://archimer.ifremer.fr/doc/00000/2682/⟩.

Malinovskyi, O., Blecha, M., Křišťan, J., Policar, T., 2020. Feeding activity of pikeperch (Sander lucioperca) under winter and spring photothermal conditions of pre-spawning period. Aquac. Res. 51, 852–857. https://doi.org/10.1111/are.14401.

Malinovskyi, O., Veselý, L., Yanes-Roca, C., Policar, T., 2022. The effect of water temperature, prey availability and presence of conspecifics on prey consumption of pikeperch (Sander lucioperca). Czech J. Anim. Sci. 67, 465–473. https://doi.org/10.17221/162/2022-CJAS.

Martinez-Porchas, M., Martinez-Cordova, L.R., Ramos-Enriquez, R., 2009. Cortisol and glucose: reliable indicators of fish stress? Pan-Am. J. Aquat. 4, 158–178. ⟨http://panamjas.org/pdf_artigos/panamjas_4(2)_158-178.pdf⟩.

Masser, M.P., 2012. In-pond raceways. In: Tidwell, J.H. (Ed.), Aquaculture Production Systems. John Wiley & Sons, Inc., Hoboken, New Jersey, US https://doi.org/10.1002/9781118250105.ch15.

Milla, S., Pasquet, A., El Mohajer, L., Fontaine, P., 2021. How domestication alters fish phenotypes. Rev. Aquac. 13, 388–405. https://doi.org/10.1111/raq.12480.

Moeser, A.J., Pohl, C.S., Rajput, M., 2017. Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs. Anim. Nutr. 3, 313–321. https://doi.org/10.1016/j.aninu.2017.06.003.

Molnár, T., Csuvár, A., Benedek, I., Molnár, M., Kabai, P., 2018. Domestication affects exploratory behaviour of pikeperch (*Sander lucioperca* L.) during the transition to pelleted food. PLoS ONE 13, e0196118. https://doi.org/10.1371/journal.pone.0196118.

Nagy, Z., Ardó, L., Demény, F., Gál, D., Sándor, Z.J., Ljubobratović, U., 2022. Case study on the aptness of in-pond raceways for pikeperch, *Sander lucioperca*, grow-out. Aquac. Rep. 27, 101356 https://doi.org/10.1016/j.aqrep.2022.101356.

Nayak, S.N., 2010. Role of gastrointestinal microbiota in fish. Aquac. Res 41, 1553–1573. https://doi.org/10.1111/j.1365-2109.2010.02546.x.

Németh, A., 2013. A new technology for the induced spawning and rearing of pikeperch (Sander lucioperca L.) targeting a more profitable production in south transdanubian fish ponds. Doctoral thesis, University of West Hungary, Mosonmagyaróvár, Hungary.

Nynca, J., Żarski, D., Bobe, J., Ciereszko, A., 2020. Domestication is associated with differential expression of pikeperch egg proteins involved in metabolism, immune response and protein folding. Animal 14, 2336–2350. https://doi.org/10.1017/S1751731120001184.

Page, M., Thorpe, R., 2002. Purification of IgG by precipitation with polyethylene glycol (PEG). In: Walker, J.M. (Ed.), The Protein Protocols Handbook. Springer Protocols Handbooks. Humana Press. https://doi.org/10.1385/1-59259-169-8:991.

Pasquet, A., 2018. Effects of domestication on fish behaviour. In: Teletchea, F. (Ed.), Animal Domestication [Internet]. IntechOpen, London. https://doi.org/10.5772/intechopen.78752.

Péter, G., Lukić, J., Alvestad, R., Horváth, Z., Nagy, Z., Rónyai, A., Bársony, P., Ljubobratović, U., 2023. Nursing of Pike-Perch (Sander lucioperca) in Recirculating Aquaculture System (RAS) Provides Growth Advantage in Juvenile Growth Phase. Animals 13, 347. https://doi.org/10.3390/ani13030347.

Policar, T., Stejskal, V., Kristan, J., Podhorec, P., Svinger, V., Blaha, M., 2013. The effect of fish size and stocking density on the weaning success of pond-cultured pikeperch *Sander lucioperca* L. juveniles. Aquac. Int 21, 869–882. https://doi.org/10.1007/s10499-012-9563-z.

Policar, T., Schaefer, F.J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., Żarski, D., 2019. Recent progress in European percid fish culture production technology—tackling bottlenecks. Aquac. Int. 27, 1151–1174. https://doi.org/10.1007/s10499-019-00433-y.

Riesch, R., Araújo, M.S., Bumgarner, S., Filla, C., Pennafort, L., Goins, T.R., Lucion, D., Makowicz, A.M., Martin, R.A., Pirroni, S., Langerhans, R.B., 2022. Resource competition explains rare cannibalism in the wild in livebearing fishes. Ecol. Evol. 12, e8872 https://doi.org/10.1002/ece3.8872.

Rónyai, A., 2007. Induced out-of-season and seasonal tank spawning and stripping of pike perch (*Sander lucioperca L.*). Aquac. Res 38, 1144–1151. https://doi.org/10.1111/j.1365-2109.2007.01778.x.

Salinas, I., Fernández-Montero, Á., Ding, Y., Sunyer, J.O., 2021. Mucosal immunoglobulins of teleost fish: a decade of advances. Dev. Comp. Immunol. 121, 104079 https://doi.org/10.1016/j.dci.2021.104079.

Sanahuja, I., Fernández-Alacid, L., Sánchez-Nuño, S., Ordóñez-Grande, B., Ibarz, A., 2019. Chronic cold stress alters the skin mucus interactome in a temperate fish model. Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.01916.

Schoina, E., Doulgeraki, A.I., Miliou, H., Nychas, G.-J.E., 2022. Dynamics of water and biofilm bacterial community composition in a Mediterranean recirculation aquaculture system. Aquac. J. 2, 164–179. https://doi.org/10.3390/aquacj2020008.

Smith, N.C., Rise, M.L., Christian, S.L., 2019. A comparison of the innate and adaptive immune systems in cartilaginous fish, ray-finned fish, and lobe-finned fish. Front. Immunol. 10, 2292. https://doi.org/10.3389/fimmu.2019.02292.

Stejskal, V., Paolacci, S., Toner, D., Jansen, M.A., 2022. A novel multitrophic concept for the cultivation of fish and duckweed: a technical note. J. Clean. Prod. 366, 132881 https://doi.org/10.1016/j.jclepro.2022.132881.

Teletchea, F., Fontaine, P., 2014. Levels of domestication in fish: implications for the sustainable future of aquaculture. Fish Fish. 15, 181–195. https://doi.org/10.1111/faf.12006.

Van Doan, H., Soltani, M., Leitão, A., Shafiei, S., Asadi, S., Lymbery, A.J., Ringø, E., 2022. Streptococcosis a re-emerging disease in aquaculture: significance and phytotherapy. Animals 12, 2443. https://doi.org/10.3390/ani12182443.

Wang, Y., Xie, S., Nie, Z., Li, Q., Sun, Y., Shao, N., Gao, J., Hu, J., Xu, P., Xu, G., 2020. Optimum feeding frequency of juvenile largemouth bass (*Micropterus salmoides*) reared in in-pond raceway recirculating culture system. Fish. Physiol. Biochem. 46, 2197–2212. https://doi.org/10.1007/s10695-020-00866-w.

Ye, S. and Ying, Q., 2015. Water Quality in RAS for Salmonids and Performance of MBBR - Case Study at Vik Settefisk AS. Master thesis, Norwegian University of Life Science, Ås, Norway. ⟨https://nmbu.brage.unit.no/nmbu-xmlui/handle/11250/294210⟩.

Zakęś, Z., 2022. Sander lucioperca. Cultured Aquatic Species Information Programme. In: FAO, Fisheries and Aquaculture Division [online]. Rome. ⟨https://www.fao.org/fishery/en/culturedspecies/Sander_lucioperca/en⟩.