New species for EU aquaculture

Deliverable Report

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Objective: Effect of pikeperch domestication level and geographical origin on growth and stress sensitivity

Description: Due to recent intensive culture, pikeperch (*Sander lucioperca*) populations displaying various domestication levels are reared so far (Teletchea & Fontaine, 2012). Domestication highly affects the stress and immune status of fish in farm conditions (Verbeek et al., 2008; Zuberi et al., 2008; Douxfils et al., 2011). The effects of domestication process (wild vs domesticated strains) and geographical origin (freshwater vs brackish water strains) are investigated in the present deliverable. Different batches of juveniles (3 or 4 geographical origins, 1 or 2 populations of the same geographical origin with 2 levels of domestication) were produced from larval stage in similar conditions. Then, using the optimal combination of factors identified on Task 22.1, fish were reared and examined for physiological stress responses, immune competence and global resistance to infectious diseases. The analytical approaches are similar to the ones presented for Task 22.1. This deliverable will specify the effect of domestication level and geographical origin on pikeperch growth and stress sensitivity, allowing the further selection of a pikeperch strain according to the rearing conditions of commercial fish farms.

SUMMARY

This report is targeted on the study of the effects of the domestication level and geographical origin on physiological stress response and immune status of pikeperch. Selection of the experimental pikeperch populations was based on the Deliverable 4.2, which distinguished two main genetically differentiated groups that are actually available in European pikeperch farms, namely the northern European population and the central European population. Three batches of pikeperch larvae were transferred to the URAFPA facilities at the University of Lorraine, France, in order to test juveniles acclimatized to the same rearing conditions. These 3 batches included a wild French F0 strain (Lindre River, France) and two Czech strains with a wild (F0) and a domesticated (F4) batch.

Results indicated that basal physiological stress status did not differ between wild Czech and French populations F0. Moreover, the two wild pikeperch juvenile populations exhibited a comparable high stress response to the application of stress (net chasing) in terms of plasma cortisol level. This finding indicates that there are no marked differences in stress responsiveness between the Northern and Central pikeperch populations despite the differences in their geographical origin or genetic differences. While basal stress level did not differ between the Czech F0 and F4 populations, the response to the net chasing stress was higher in the Czech F4 juveniles than the Czech and French F0 juveniles, indicating that domestication level increased stress response to the net chasing stress in pikeperch. Moreover, the highest stress sensitivity of F4 populations observed in the current study was associated to the best immune status as evidenced by immune bactericidal markers.

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1. Objectives

Effects of pikeperch domestication level and geographical origin on growth, stress sensitivity and immune status.

2. Background

While it is known that percid fish are highly sensitive to frequent manipulations, which significantly depress their growth rate (Jentoft et al, 2005, Strand et al, 2007), grading manipulations are still unavoidable in pikeperch farming during its first developmental stages. Such grading manipulations are often followed by high mortalities and disturbances in growth rate that may be related to stress allostatic charge. It is well known that domestication process highly affects the stress and immune status of fish in farm conditions (Verbeek *et al*., 2008; Zuberi *et al*., 2008). In salmonids, high cortisol response was associated to low growth rate and low immune status, and cortisolemia-induced stress was shown to be heritable (Pottinger and Carrick, 1999; Felvoden et al, 2002). But such decrease in stress responsiveness through the domestication selection may be species-related since it was demonstrated that the heritability of cortisol response to stress was low in European sea bass (Volckaert et al, 2012). Moreover, differential physiological and immune response was reported in Eurasian perch juveniles depending on the type of aquaculture stressor (Douxfils et al, 2011; 2012; 2014).

Although it is hypothesized that domestication process can impact positively fish stress responsiveness and immune system, the effect of the intrinsic genetic pool of pikeperch broodstock cannot also be ruled out. Indeed, various domesticated populations, including strains from different geographic origin and wild populations are actually used in the recent pikeperch culture (Teletchea and Fontaine, 2014). The stress sensitivity of these strain populations is not yet characterized but it has been reported that geographic origin has a marked impact on growth rate of percid fish, such as Eurasian perch *Perca fluviatilis* and yellow perch *Perca flavescens* (Mandiki et al, 2004; Rosauer et al, 2011). For the Eurasian perch, early juvenile populations originated from the Northern European countries, and reared in the same rearing conditions in Belgium, displayed higher growth rate than those from the central countries. However, their stress responsiveness and immune status were not determined.

3. Experiment

a. Materials and methods

Experimental design and rearing conditions

Selection of the experimental pikeperch populations was based on the Deliverable 4.2, which distinguished two main genetically differentiated groups that are actually available in European pikeperch farms, namely the northern European population and the central European population. Three batches of pikeperch larvae were transferred to the URAFPA facilities at the University of Lorraine, France, in order to test juveniles acclimatized to the same rearing conditions. These 3 batches included a wild French F0 strain (Lindre River, France) and two Czech strains with a wild (F0) and a domesticated (F4) batch. During the acclimation period, fish were maintained in the same rearing conditions optimized according to the results from the multifactorial experiment (Deliverable 22.1): temperature: 21-22 °C; low white light intensity: 15 lux; photoperiod: 12D:12L and fed twice daily at 2.0 % biomass with a sinking food. The same rearing conditions were maintained during the 2-month experimental comparison.

It was planned that the experimental comparison would start when fish reached 20 g body weight, but due to high variations in feeding behavior and growth between the Czech populations and the French one, fish were randomly distributed into 9 indoor 800 L tanks (3 tank units per strain) on the basis of comparable biomasses (5 kg m^{-3}), but with different initial body weight. Therefore, only parameters of stress and immune status were considered.

Samplings and output variables

To characterize the stress response of the different batches, we exposed pikeperch to chasing of 30 sec. Samples were collected both before and after the application of this stress event. Four fish were removed randomly from each tank and anesthetized with MS-222 (150 mg l^{-1}). Blood was quickly collected by caudal vein puncture with heparinized syringes within 5 min and centrifuged at 4,000 g for 5 min at 4 °C. Plasma was aliquoted and stored at -80 °C until assayed.

Plasma cortisol was assayed in duplicate using a cortisol ELISA kit (DRG, EIA-1887), following the manufacturer's instructions (BioSource, Belgium). The intra-assay coefficient of variation was 3.6 %, the assay dynamic range was between 0-800 ng ml^{-1} and the analytical sensitivity was 2.5 ng ml^{-1}. Plasma glucose, also assayed in triplicate, was determined calorimetrically based on a glucose oxidase/peroxidase method described by Trinder (1969).

Lysozyme activity was evaluated in plasma samples by the turbimetric method (Siwiki and Studnicka, 1987; Douxfils et al, 2012). Plasma samples (10 µl) were mixed with 140 µl *Micrococcus lysodeikticus* (Sigma-Aldrich) solution (0.6 g l^{-1}). This assay was performed in duplicate. Absorbance was measured at 450 nm every minute for 15 min at room temperature. Lysozyme activity (units) is defined as the amount of enzyme decreasing the turbidity of 0.001 OD per min.

The total peroxidase activity in plasma was assessed following the method described in Quade and Roth (1997). Briefly, 15 µl of plasma was diluted in 140 µl of HBSS without Ca2+ or Mg2+ and mixed with 50 µl of 20 mM 3,3',5,5'-tetramethylbenzidine hydrochloride (Sigma) and 5 mM H2O2. The reaction was stopped after 2 min by adding 50 µl of 4 M sulphuric acid and absorbance was measured at 450 nm. The peroxidase activity was determined defining as one unit the peroxidase that produces an absorbance change of 1 OD.

Statistical analyses

Physiological and immune data are expressed as the mean ± SEM. Replicate tank was considered as the statistical unit ($n = 3$). Kolmogorov and Smirnov's test were used to assess the normality of data sets ($p < 0.05$) and Levene's test was conducted to evaluate variance homogeneity ($p < 0.05$). Results were then analyzed with a two-way ANOVA ($p < 0.05$) taking the batch and the time point (before or after the stress event) as factors. When significant, means were compared according to Tuckey's HSD post-hoc test ($p < 0.05$). The results were analyzed with JMP 12.1 software (SAS Institute Inc., North Carolina, USA) and graphs were performed using R software (V3.2).

4. Results

Physiological stress response

Plasma cortisol level did not differ among strain populations, and values significantly increased in fish submitted to chasing stress ($p < 0.001$) whatever the strain (**Figure 4.1**). But, the magnitude increase was higher in domesticated Czech F4 than in wild F0 ($p < 0.05$). Domesticated Czech F4 also showed the highest glucose values after the chasing stress while non-significant differences were observed in wild Czech F0 and French F0 batches (**Figure 4.2**).

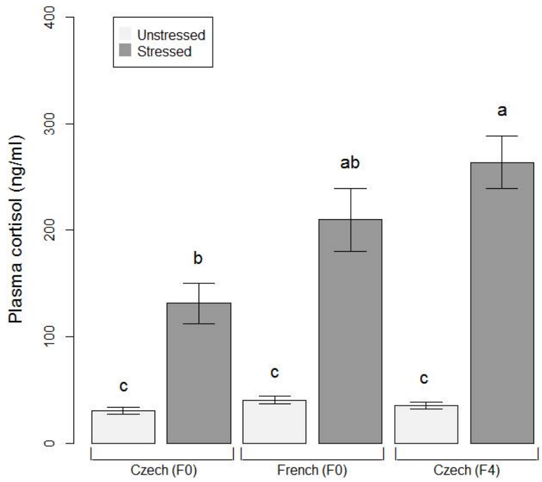

Figure 4.1: Mean (±SEM) plasma cortisol of pikeperch juveniles (n = 12) exposed or not to chasing of 30 sec. Tested batches included a wild French F0 strain and two Czech strains with wild (F0) and domesticated (F4) fishes. Significant results are indicated by different letters.

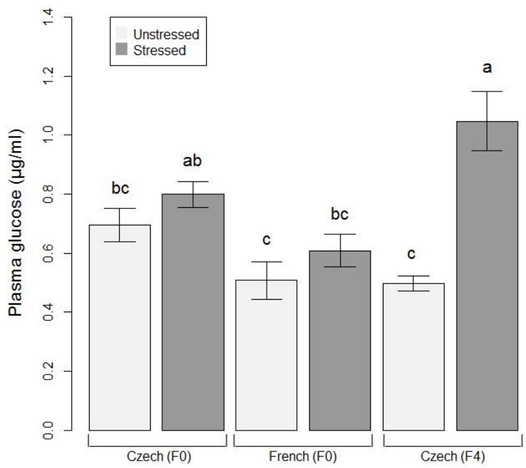

Figure 4.2: Mean (±SEM) plasma glucose of pikeperch juveniles (n = 12) exposed or not to chasing of 30 sec. Tested batches included a wild French F0 strain and two Czech strains with wild (F0) and domesticated (F4) fishes. Significant results are indicated by different letters.

Immune parameters

The domesticated Czech F4 showed significantly higher values for both lysozyme (**Figure 4.3**) and peroxidase (**Figure 4.4**) activities ($p < 0.01$) before and after stress chasing.

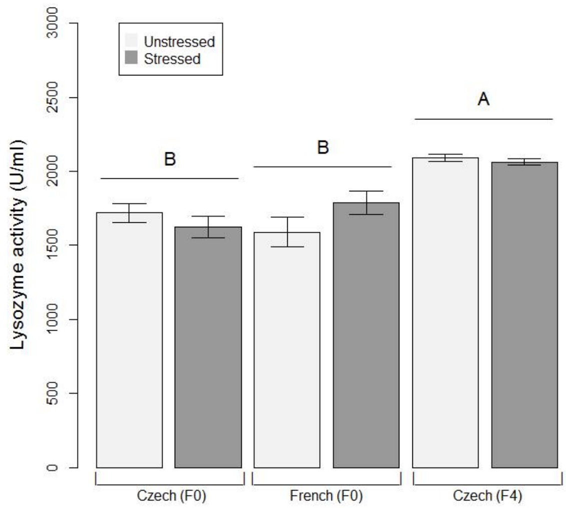

Figure 4.3: Plasma lysozyme activity (Mean ± SEM) of pikeperch juveniles (n = 12) exposed or not to chasing of 30 sec. Tested batches included a wild French F0 strain and two Czech strains with wild (F0) and domesticated (F4) fishes. Significant results are indicated by different letters.

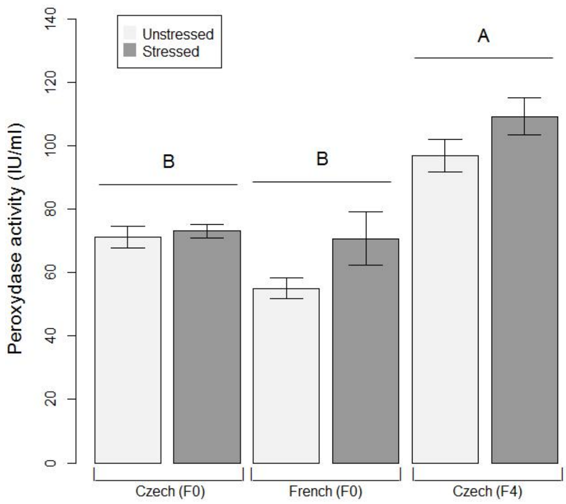

Figure 4.4: Plasma peroxidase activity (Mean ± SEM) of pikeperch juveniles (n = 12) exposed or not to chasing of 30 sec. Tested batches included a wild French F0 strain and two Czech strains with wild (F0) and domesticated (F4) fishes. Significant results are indicated by different letters.

5. Discussion

Effect of strain on stress responsiveness and immune status

The results from the current experiment confirmed the findings from the multifactorial study (see Deliverable 22.1) that rearing conditions with low light intensity sustained a basal stress physiological status for pikeperch juveniles as evidenced by basal plasma levels of cortisol and glucose. The basal physiological stress status did not differ between wild Czech and French populations F0. Moreover, the two wild pikeperch juvenile populations exhibited a comparable high stress response to the application of net chasing stress in terms of plasma cortisol level. This finding indicates that there are no marked differences in stress responsiveness between the Northern and Central pikeperch populations despite the differences in their geographical origin or genetic differences (see Deliverable 4.2). There is no information about the stress sensitivity among the strain populations of percid fish. As found in the current study, previous studies have reported a high stress response in other percid fish, such as Eurasian perch submitted to a single or repeated netting stressor for 0.5-1 minute (Milla et al, 2010; Douxfils et al, 2014). It was also shown that the latter species, as a new aquaculture species, is more sensitive to aquaculture stressors than some salmonid aquaculture species (Jentoft et al, 2005), and this may be the case for pikeperch. As for stress parameters, levels for the tested immune markers at day 60 were comparable before and after the stress application, indicating comparable immune status between the wild Czech and French populations F0. This is likely because stress sensitivity and immune status are often associated, but such relationship is not yet characterized for pikeperch populations.

Effect of domestication on stress responsiveness and immune status

While basal stress levels did not differ between Czech F0 and F4 populations, the response to the net chasing stress was higher in the Czech F4 juveniles than the Czech and French F0 juveniles, indicating that domestication level increased stress response to the net chasing stress in pikeperch. In other percid species, such as in Eurasian perch, it has been shown that the effect of domestication level on stress response varies depending to the type of aquaculture stressor. For example, when exposing Eurasian perch juveniles to single and repeated emersion or hypoxia stress manipulations, it was observed that both F1 and F5 generations displayed similar stress responsiveness (Douxfils et al, 2012; 2014), whereas chronic confinement induced an increased sensitivity of the hypothalamic-pituitary-interrenal axis and a significant cellular stress response (HSP70) in F4 individuals compared to F1 juveniles (Douxfils et al, 2011). Such differential effect of domestication regarding stress sensitivity may depend on how the induced stress intensity threatens the internal homeostasis. Indeed, stress response is first an adaptive mechanism to promote the best chance of survival towards deleterious environmental changes. So, the higher stress sensitivity of F4 populations compared to F0 populations observed in the current study may be an adaptive feature to the net chasing stressor driven along the domestication process of pikeperch.

As a matter of fact, the highest stress sensitivity of F4 populations observed in the current study was associated to the best immune status as evidenced by immune bactericidal markers. Such modulation of the association between the stress responsiveness and the immune status has been reported in other percid fish. Indeed, previous authors observed a decrease in both innate and specific immune functions in F1 exposed to various aquaculture stressors (confinement, hypoxia and emersion) fish, while a stimulation of immunity occurred in F4 populations of Eurasian perch juveniles, suggesting a rapid adjustment of the immune system to the stress event as an improvement of immune competence through the domestication process (Douxfils et al, 2011; 2012; 2014).

6. References

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