Final report

Study on the selective effects of intensive culture environment on different pikeperch stocks

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The recent project aims to investigate the alterations in the behaviour forms, adaptive characteristics and genetic background of their correlations (called “personality types”). Regarding it, the establishing of the intensively cultured and selected stock and the determination of individual personality types were planned in the first year. The stress tolerance of pellet feeder individuals was investigated by the measurement of post-stress blood cortisol levels. Moreover, a production test was performed with selected individuals from the pellet feeders to determine growth rate, feed consumption and feed conversation. Genetic analysis has started with the investigation of genetic structure of different pikeperch groups formed on the basis of the applied propagation method.

Investigation on the correlation between exploratory behaviour and stress tolerance.
After individual marking, groups were entered into a novel object and environment tests performed by the method described by Jones and Godin (2010).The measured parameters in the novel object test were minimum distance, minimum time, time of activity, frequency of trials, while in the novel environment test these were time of entrance, time of activity, size of explored area. Based on the tests the personality scores were determined by PCA analysis and the individuals were clustered to explorative and retractile groups. Stress tolerance was measured by individual post-stress cortisol level determination using the method published by Ruiz-Gomez et al. (2008). The elevated cortisol level was generated by crowding and handling stress. Blood samples were taken from tail vein. In our preliminary experiment, the two behaviour types showed significantly different distribution pattern according to the time of the first pellet feeding. Early feeder individuals were less explorative similarly to cannibal individuals compared to late and non-feeder individuals. In the present study significant (p=0.009) differences were found between average cortisol levels in the two exploration groups (209.5 ± 81.1 ng/ml and 282.6 ± 111.2 ng/ml; in explorative and retractile groups, respectively). Comparing the cortisol values in the groups formed by the time of first pellet feeding, significant decrease was found (278.6 ± 80.9 ng/ml, 217.4 ± 96.8 ng/ml and 194.7 ± 93.5 ng/ml in early feeder, normal and late feeder groups, respectively). Low but significant correlation (r=0.308, p=0.022) was determined between individual personality type scores and cortisol levels indicating the potential presence of behavioural types (correlating behaviours in different situations). Since early feeder group showed higher body weight at the same age (21.2±3.0 g vs. 12.5± 2.8 g and 10.4±3.2 g in the normal and late groups, respectively), the selection on growth parameters could result the fixation of retractile and sensitive behaviour forms in the intensively reared populations.

Investigation of the correlation between behaviour (personality) type and individual production traits
As a continuation of the stress experiment, our next question was whether the almost doubled body weight of the early groups is the result of a better growth performance or not? It was presumed, that different personality types provide variable production under intensive culture conditions, and the retractile and sensitive early feeder group has better production capacity. The individuals with known personality type from the three pellet feeder groups (early, normal and late feeders) were entered to a six week production test. Beside the determination of individual growth rate (SGR), feed intake and feed utilisation (FCR), the categorizing of the feed consumption method (based on the categories published by Ruiz-Gomez et al, 2008) were accomplished. The feeding trial resulted marginally significant (p=0.071) differences in the feed intake; the highest value was found in the early group (13.2±6.6 g/individual) followed by the late group (11.3±5.5 g/individual) and finally the normal group (9.5±2.5 g/individual). The tendency in the weight gain was different as significantly higher weight gain was found in the early group (17.3±5.8g) compared to the other two groups, which did not differ (9.1±3.5g and 8.8±4.1g, in normal and late groups, respectively). In case of the FCR the late feeder group showed significantly higher value (0.87±0.6g/g, 0.97±0.2g/g, and 1.57±0.9g/g, in early normal and late groups, respectively). These parameters show that early group has a higher feed intake and better FCR, since late group could not achieve this weight gain beside the similar feed intake due to the worse FCR. However, the better performance of the early group will be only apparent if the standardized growth parameter the SGR is examined. This parameter was almost the same in all groups (1.44±0.4, 1.30±0.3, and 1.43±0.3 %/day, in early normal and late groups, respectively), indicating that the higher weight gain and feed intake is a result of the higher body weight. The higher initial weight could originate in the earlier consumption of the pellet (these individuals were

consuming pellet when the others were starving during the transition period). This new hypothesis was confirmed by the not significant differences found in the performance of explorative and retractile groups (feed consumption: 10.8±5.4 and 12.7±5.9 g/individual, FCR: 1.11±0.8 and 1.11±0.6 /g, SGR: 1.43 ±0.4 and 1.33± 0.3%/day). Individual exploration score showed no significant correlation with any of the production traits, however, individual cortisol level had a significant, negative correlation with the FCR (r=-0.317, p=0.018). Based on the results of the two trials it could be concluded, that a selection on body weight in intensively reared pikeperch populations results only in elimination of the explorative and more stress resistant behaviour type, but not necessarily provide a progeny with better growth performance.

Investigation of genetic structure of different pikeperch groups formed on the basis of the applied propagation method

It is hypothesised that artificial propagation (due to the selection of “superior” individuals as brood stock) could act as selection factor and alter the genetic structure of the intensively reared stock. The allele size and frequency distribution between the two groups (artificially hormone-induced group (HI) and natural spawning group (NS)) was compared to the original Balaton (B) population to deduce the genetic changes in the propagated populations. Neither of the propagation techniques has resulted inbreeding in the populations. Unbiased expected heterozigozity (uHe) was 0.69±0.04, 0.58±0.06, 0.70±0.03 and fixation index (F) was 0.045±0.04, -0.062±0.07 and 0.001±0.02 in the population B, HI and NS, respectively. A low-moderate differentiation was found among the three populations (F_{st} =0.099, between HI - NS populations but F_{st} =0.098 and F_{st} =0.137 between B - NS and B – HI populations, respectively, p=0.001), 11.33% of the total genetic variance was explained by the differentiation among populations. The Nei’s genetic distance data showed a similar pattern (0.227, 0.331, 0.346 among HI-NS, B-NS, B-HI populations, respectively). Based on the results of population assignment test, 90% of the individuals could be assigned correctly to their original population. The effect of the different propagation was not significant, however a marked genetic loss was found in both pond populations compared to the original stock. Average allele number (N_A) and allelic richness was significantly higher in the B population (N_A =10.7±3.5 and Ar=9.8±3.1) compared to the other two populations, (N_A = 5.6±1.2 and 6.9±1.9; Ar=5.3±1.1 and 6.8±1.9 in the HI and NS populations, respectively) the same was found in case of the average private allelic richness too (Ar_p=3.3±1.6, 0.3±0.3 and 0.4±0.6 in B, HI and NS groups, respectively). However, the N_A, Ar and Ar_p were slightly higher in the NS population, the difference was not significant. Our results show, that using lower broodstock size (traditional pond culture) independently from the propagation method could result in lower genetic diversity compared to natural populations without inbreeding depression.

Investigation of genetic structure of different pikeperch groups formed on the basis of the behaviour (personality type).

It is hypothesised that a possible selection based on the behaviour could alter the genetic structure of the intensively reared stock. Genetic structure of the 150 individuals was planned to be investigated with microsatellite method and the possible correlation between behaviour groups and allele distribution is explored. The genetic structure of 154 individuals was investigated by 21 microsatellites. The individuals were grouped by the foraging behaviour and ability to 3 groups: pellet feeder (PF, n=70), cannibalistic (C, n=14) and pellet non-feeder (PNF, n=70). The groups were formed from a homogenous stock consisting of same-aged individuals. Significant genetic differences were found between the subgroups. The PF and C subgroups were separated from PNF by PCoA based on the genetic distances (eigen value: 1.819) and the 7% of the genetic variance was between the populations by the AMOVA results. Two genetic clusters were separated by the STRUCTURE analysis: the first contained homogenously PF individuals, while the second cluster consisted of PNF, and C subgroup individuals. The pairwise Fst value was highest between PF and PNF groups (0.097) but in case of C group the values were lower (0.031 and 0.034 with PF and PNF groups, respectively). These results confirm that selection for the ability of foraging behaviour on pellet results in genetic separation as well. This was also supported by the diversity data determined in groups PNF, C, PF and original stock respectively: NA=5.89±1.52, 4.78±1.31, 5.57±1.50, 6.52±1.64; Ar=4.59±0.85, 4.75±1.29, 4.19±0.94, 6.51±1.63; Arp=0.50±0.58, 0.15±0.17, 0.20±0.28, 0. PF group showed significantly lower diversity values compared to the original stock and also to the PNF group.

Investigation the correlation between personality type and foraging behaviour on live prey fish.

The hypothesis is that the individuals adapted to the pelleted food differently, have variable ability to forage on live prey. Live prey feeding tests were performed by the method described by Turreson and Brönmark (2004). The measured parameters were the followings: number of attacks before the first success (Na), applied tactic, success of foraging, time between the entering of prey and start of foraging (Lw), the time between the start of foraging and the first attack (Lp), and type of prey manipulation. The foraging behaviour of pellet feeder (PF) (early (E), normal (N) and late (L) groups) and non-feeder (PNF) individuals was compared by the data of the first five foraging. Rosy Barb was used as prey fish. The foraging success was different between the four groups (9.3%, 18.7%, 31.4% and 33.3% in the E, N, L and PNF groups, respectively). The differences were significant only between the E and PNF groups after Bonferroni corrections. However, the foraging success of total PF group (20.2%) was also lower compared to PNF group. According to the successful foraging events, the difference between the groups in the above mentioned parameters was not significant. However, the time of prey manipulation and Lp were changed with the repetition of the foraging. Significant differences were found in the Lp between the first and fifth capture, since prey manipulation time was different between the first and 4th and 5th occasions. The later occasions did not differ significantly. In the applied foraging tactic and the type of prey manipulation no detectable tendency was shown. In case of the number of attacks, decreasing tendency. In the first occasion the average number of attacks per successful foraging was 4.1, which decreased to 2.6, 2.3, 2.6 and 1.6 in the consecutive occasions.

Exploration of candidate genes for behavioural variation described in other species.

The hypothesis of the original work plan was that personality types could be characterized with testing of possible polymorphism in “candidate genes” described in other species including DRD1, DRD4, CRH, PKG, AR) identified in pikeperch by the PCR amplification of a synthetic strand of DNA (cDNA) transcribed from mRNA. Present status of this point is different in methodology with the agreement of the National Research, Development and Innovation Office. Comparative transcriptome analysis was performed by NGS sequencing (on Illumina Hiseq4000 platform). Pond reared pikeperch fingerlings (N=500) were introduced to the weaning process at the age of 35 days post hatching. The fingerlings were weaned to pelleted food using Tubifex feeding in gradually decreasing amount. In the control group only Tubifex feeding was used. Five individual samples were taken from the control and treated groups on the day 13 of the weaning period. Total RNA of the brain and eye samples was isolated using Direct-zol™ RNA MiniPrep Kit (Zymo Research) following the manufacturer’s protocols. Finally, eight samples were used to prepare separate Illumina sequencing libraries (four-four biological replicates for the control and treated group, excluding RNA samples with worst quality from each group). The preparation of cDNA libraries and the subsequent Illumina sequencing were conducted in collaboration with Enviroinvest Ltd, (Pécs Hungary). Differential expression analysis between two experimental groups was performed using the DESeq R package (1.14.1). GO enrichment analysis of the differentially expressed genes was performed using KOBAS 3.0 software. A total of 202,770,045 and 166,742,479 clean reads was obtained for control and treated groups, respectively. The reads produced were used for de novo assembly. A total of 257475 unigenes were identified and altogether 5.9% was differentially expressed (adjusted P value < 0.05) between the two groups (4.8% up regulated and 1.1% down regulated). The top 50 up and down regulated unigenes were enriched in GO terms. The enrichment resulted that up regulated genes were related to antigen processing and immune response indicating that weaning to pelleted food results suboptimal conditions and higher capability to infections compared to the control group. More than the half (39) of the top 50 regulated unigens were not identifiable, but the remaining could be linked to behaviour. Moreover, the consensus sequence of the five candidate genes (DRD1, DRD4, CRH, PKG, AR) highlighted in the work plan was determined, which supports designing further behavioural examinations with pikeperch. These genes however, showed no significant expression differences between the control and pellet feeding groups. Unfortunately, testing 13 microsatellite markers (Pbbig

1-13) described by Laine et al. (2012) in nine-spined stickleback (*Pungitius pungitius*) located nearby candidate genes was not successful. Only two of the microsatellites (Pbbig1 and Pbbig3) were able to amplify in the pikeperch species, but these two markers were proved to be monomorphic.

Conclusions

The aim of the three years long study was to explore the possible effects of the intensive culture conditions, especially the weaning to pelleted food on the behaviour and its genetic background of the pikeperch. Our results indicate that weaning to pelleted food modifies all examined aspects of the pikeperch population. The intensively reared stock will be selected for stress sensitive and less explorative individuals. This selection happens spontaneously due to the weaning process and results in genetic selection too. Also, hormone induced propagation even under pond conditions has significant effect on the population structure and lowers genetic diversity. The intensively reared individuals have low motivation on foraging on live prey, and they need minimum three to five successful foraging to improve their technique. Weaning to pelleted food generates suboptimal conditions which raise the risk of infections. This generates an increase in the immune response and possibly acts as another selection factor during the intensive rearing.

References

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