

Turkish Journal of Fisheries and Aquatic Sciences 16: 891-897 (2016)

## **RESEARCH PAPER**

# The Pike Perch (*Sander lucioperca*) Background Color First Choice in the Recirculating Aquaculture Systems

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E-mail: rtcristina@vahoo.com	Accepted 17 June 2016
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#### Abstract

A study on Sander lucioperca age classes to assess preference for background color was imagined. Two groups: G1 (92 days old, average weight:  $6.2 \pm 1.6$  g) and G2 (461 days and  $101.7 \pm 27.1$  g), were continuously observed for 24 hours after one day of acclimation, using 90 fish / group (30 fish / replication / 24 hours), their behavior being recorded with cam-recorders. For the study, the walls and bottom of a rectangular research tank were colored equally in: grey, light and dark blue and green. During the study, were made 216 observations / group (72 observations / replication) and were statistically analyzed using Duncan test, with a confidence level of P< 0.05 or lower. Results revealed that a significant number from G1 was located on the green space, in order, preferred background color being: green, grey, dark and light blue. The G2 group expressed also high affinity for the green color (P< 0.001) concluding that, green background can give a safety feeling to the pikeperch, thus, rearing or transport of pikeperch categories in green tanks could have the benefit of stress decreasing and added benefit for this fish species in the aim of advancing the Recirculating Aquaculture Systems.

Keywords: RAS, color preference, behavior, pikeperch.

# Introduction

Pikeperch (Sander lucioperca) is a valuable fish species identified as an important candidate for aquaculture in Recirculating Aquaculture Systems (RAS) in all Europe (Philipsen, 2008; FAO, 2014).

So far, significant scientific work was focused particularly on pikeperch feeding this influencing directly their growth performance. At the moment, the RAS rearing technology is not entirely optimized, several technological aspects remaining partly studied (Özyurt et al. 2012; Zakęś et al., 2003; Nyina-Wamwiza et al., 2005; Ronyai and Csengeri, 2008; Wang et al., 2009; Kowalska et al., 2010).

Though, there is already identified the influence of some physical parameters on pikeperch growing, such as: temperature variability (Ronyai and Csengeri, 2008; Frisk et al., 2012) or light intensity (Luchiari et al., 2006), information approaching other parameters remains sparse. In their study, researchers have confirmed that, in certain ontogenetic stages, the environment background color could indirectly influence the increase or decrease of aggression levels in the fish species. Therefore, for rearing in closed systems, changing the color could represent a simple, but an efficient way to ethologically manipulate the fish (Damsgård and Huntingford, 2012).

Also it was observed that, in some fish species, a darker body and / or eye color, can suppress other fish attacks, signaling their submission. This signifies that the aggression could be stimulated by pale colored fish; even when, this feature is a result of the applied husbandry practices, fact yet demonstrated in the salmon fish (Höglund et al., 2002 and Volpato et al., 2003).

All these pertinent observations reflect the importance of background color also on RAS fish farming systems, the information regarding its influence in pikeperch farming systems being studied in some initial experiments also by our group (Draşovean, 2013).

In the aim of completing the available information and to bring additional data for the RAS farming of this important fish species, it was designed a 24 hour color preference behavior study on two different pikeperch (Sander lucioperca) age classes, to ascertain the favorite background color.

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#### **Materials and Methods**

The study was performed in compliance with good laboratory practice, in accordance to all the European Convention principles for the protection of vertebrate animals used in experimental and other scientific purposes and with the approval of the Scientific Ethics Committee of the Faculty of Veterinary Medicine Timişoara.

# **Experimental Fish**

The pikeperch individuals for the study were obtained and reared in Recirculating Aquaculture System (RAS) from the authorized Aquaculture Research Laboratory at the Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania. The individuals used in experiments were obtained by natural-controlled reproduction, both fish batches being first generation RAS produced.

Both RAS (for breeding and experiments) were placed in the same hall under the same environmental conditions. Starting with the age of 21 days, when the weaning was completed, all juveniles were fed exclusively with dry food (Coppens International, Holland), corresponding to their age and category until the start of the study. In rearing conditions, to feed the fish, a belt-feeder was used.

The initial color of the tanks where fish were reared until our study started was light blue and then, for acclimation, fish were maintained in the multicolored experimental tank for 24 hours / each group and replication before the experiment started. For testing, two pikeperch of different age classes were selected:

a). juveniles, 92 days of age (Group 1 = G1); with an average weight of  $6.2 \pm 1.6$  g; and

b). fingerlings, 461 days of age (Group 2 = G2), with an average weight of  $101.7 \pm 27.1$  g, were used. ), were continuously observed for 24 hours after one day of acclimation.

For the 24 hours observation period in the multicolor tank, both fish categories used for experiment didn't receive any food and to avoid any linked influence, no feeder was placed above the research tank.

The fish from the experimental groups (90 fish / group; three replications; 30 fish / replication / 24 hours) were successively introduced for a 24 hour experimental period in the multi-colored tank and their behavior was recorded, using two cam recorders CCTV CC9622BIR (Panasonic), connected to a PC unit through a capture card (NV5000 Security DVR Card (AVerMedia). Based on the images captured and visualized on the computer's monitor, snapshots at every 20 minutes, fish positioned (with more than 51% of their body) on tank's different colors were counted to be statistically analyzed. For this study were done a total of 216 observations / 24 hours / each replication.

#### **Experimental Model**

#### The Assessment Tank

The study was carried out into a rectangular shape with rounded corners recirculating aquaculture system (RAS)  $(1.5 \times 1.5 \times 0.7 \text{ m})$ , with a total capacity of 2 cubic meters and one cubic meter utile water volume. The rectangular container was specially designed for this behavior investigation, tank walls and it's bottom being equally painted in four different colors as follows: grey, light blue, green and dark blue. The tank's drainage was covered by a ruptured stainless steel sheet with an area of about 625 cm2 ( $25 \times 25$  cm) and thus, we considered and analyzed also the time spent by fish staying here, as an additional color, and therefore, we considered that in our tank we had actually five colors, the fifth one being the stainless steel metallic grey color.

In Figure 1 is presented the experimental colored tank and the fish at the very first moment of when



Figure 1. The experimental RAS tank used for the behavior study and the fish at the very first moment of when they were passed in the colored experimental tank.

they were passed from the rearing blue tanks into the experimental one.

#### Water Physical and Chemical Parameters

The main physical and chemical parameters were monitored during the entire experimental period, three times, at: 08:00; 15:00 and 22:00 hours, using a multi-parameter HI 9829 instrument (Hanna Instruments, USA). Water surface light intensity at the rearing tank was monitored at the same time, using a RO-1332 lux-meter (Roline, Germany), the measured light intensity at water surface ensuing: 26.68 ±3.77 and respectively 10.88 ±2.84 lx. The average temperature during all experimental period (3 days / experimental group 1 and 3 days / experimental group 2), was of: 21.80 ±0.79 °C and respectively 22.11 ±0.75 °C and the measured dissolved oxygen was between: 8.26  $\pm$ 0.75 mg L-1 and 9.33  $\pm$ 2.14 mg L-1.

#### Statistical Analysis

Collected data was statistically analyzed using Statistica 8 Software (StatSoft, Inc., USA), the values being presented as mean $\pm$  standard error of the mean (SEM). To assess the significance of differences among fish positioned on different background colors, Duncan test was used with a confidence level of P< 0.05 or lower (Hill and Lewicki, 2007).

# Results

## Group 1 (93-95 Days of Age)

Many individuals from Group 1, were observed on the green zone. The greatest number of fish staying on green color space, were observed in the first hours from the beginning of experiment, in all three replication (R), between: 25 (R1) and 29 fish (R2 and R3). Also a high number of individuals  $(16.66 \pm 0.51)$  located on green color was observed for the whole 24 hour experimental period. Consequently, it was ascertained that, fish had shown a very high affinity for the green color, in the stressful conditions generated by the introducing and acclimation into a new tank.

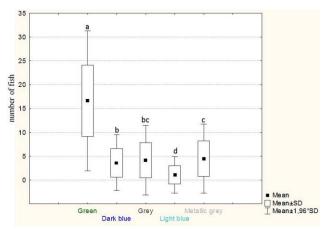
We observed also that these fish didn't react the same way for none of the other colors. Moreover, the number of fish located on other color areas didn't exceed the average, five fish:  $1.03 \pm 0.12$  fish on light blue;  $3.64 \pm 0.20$  fish on dark blue;  $4.19 \pm 0.25$  fish on grey and respectively  $4.50 \pm 0.25$  on metallic grey. The light blue background color had the worst acceptability by the pikeperch, the number of fish located on this color being significantly lower (P< 0.05) than for other colors.

The highest statistically significant difference ( $\Delta = 15.63$ ; P< 0.001) has been ascertained between the number of fish on green and light blue color and the lowest non-significant difference ( $\Delta = 0.31$ ; P≥ 0.05) it was observed between the fish on grey and metallic grey background color. No significant difference ( $\Delta = 0.55$ ; P≥ 0.05), was observed between the fish on grey and dark blue background (Figure 2).

The tank's background color chosen by pikeperch to stay on was ascertained in the following order: green, metallic grey, grey, dark and light blue.

The number of the fish varied in the first 24 hours after they were stocked for acclimation into the experimental tank, but gradually they started to spread and occupy different tank areas in the 24 hour experimental time, but in all cases the period they spent on green color area was much longer comparing with all the other colors.

For the fish from G1, the preference for green color was maintained throughout the experiment, even if the number of fish located on this color area slowly decreased. Noteworthy is that, more than half of the total fish spent the first 14 hours on green color area (Figure 3).



**Figure 2.** Pikeperch from G1 (93-95 days), occupying different tank background color, 24 hours after their acclimating. Different letters indicate significant differences (P < 0.05) between number of fish on different background color (Box & Whisker plot).

#### Group 2 (462-464 Days of Age)

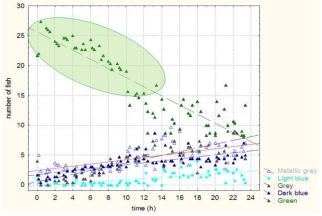
The fish from Group 2, have shown equivalent behavioral pattern alike the younger fish from G1, the majority of individuals being observed on the green area. The maximum number of fish observed on this color varied between 22 (R3), 26 (R2) and 29 (R1). The pikeperch fingerlings from G2 expressed a very high affinity for the green color, as the younger fish did. Furthermore, the G2 fish showed a longer period for accommodation after their stocking into the new tank, because the majority of these (18.08  $\pm 0.27$ ) chose to stay a longer period of time on the green color than on the other colors. The number of the pikeperch fingerlings located on other colors was very low (0.88  $\pm$ 0.05 fish on metallic grey; 2.33  $\pm$ 0.15 fish on grey;  $3.55 \pm 0.25$  fish on dark blue and respectively  $5.16 \pm 0.24$  on light blue) (Figure 4).

It seems that in this case, the drainage metallic grey had the worst acceptability by pikeperch fingerlings, the number of fish located on this color being lower and statistically significant (P < 0.001)

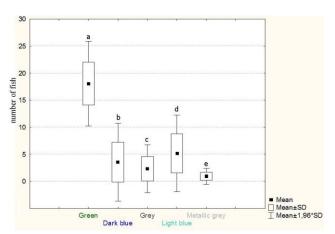
than on all other colors. The highest difference ( $\Delta = 17.20$ ; P< 0.001), it was ascertained between the number of fish on green and metallic grey color, and the lowest ( $\Delta = 1.22$ ; P< 0.001), between the fish on grey and dark blue background color.

Significant differences (P< 0.001) were found between the numbers of fish from all background colors, the background color preferred by pikeperch fingerlings being in the order: green, light blue, dark blue, grey and metallic grey. The number of pikeperch fingerlings observed in different color areas varied, as the time passed, and the fingerlings from G2 have spread gradually into the experimental tank, but more than a half of them preferred to remain on the green area for the entire period they were monitored (Figure 5).

Comparing the studied G1 and G2 pikeperch age groups there were found significant differences (P< 0.001) in the number of fish occupying: green ( $\Delta =$ 1.42), grey ( $\Delta =$  1.86), light blue ( $\Delta =$  4.13) and metallic grey ( $\Delta =$  3.58) background spaces but no significant difference (P $\geq$  0.05) it was ascertained for



**Figure 3.** Regression curves showing the movement of the pike perch from G1 (93-95 days) on different background colors in 24 hours after their acclimating into the poly chrome tank.



**Figure 4.** Pikeperch from G2 (462-464 days), occupying different tank background color, 24 hours after their acclimating. Different letters indicate significant differences (P < 0.001) between number of fish on different background color (Box & Whisker plot).

the number of fish between the two groups located on the dark blue background ( $\Delta = 0.09$ ). Besides, even if it was ascertained a small difference between groups regarding the fish located on green area, this color remained the first choice for both pikeperch age classes (Figure 6).

# Discussion

The fish welfare is defined as the internal state of a fish when it remains under conditions that were freely chosen (Volpato et al., 2007). In this respect studies accomplished, including the most important welfare indicators, ascertained that light can affect essentially the growth and survival rate in fish, not only with the light characteristics, but also, it was suggested that, the light dispersal and reflection in water due to algae presence or due to tank color, can influence these parameters (Monk et al., 2008).

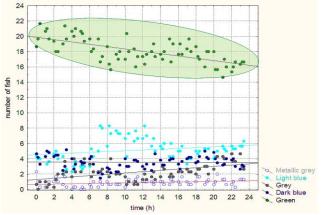
Therefore, the tank color could have an important influence on fish productive performances, fact observed until now, in studies on Eurasian perch larvae (Tamazouzt et al., 2000; Jentoft et al., 2006),

yellow perch (Hinshaw, 1985), striped bass (Martin-Robichaud and Peterson, 1998), pot-bellied seahorses (Martinez-Cardenas and Purser, 2007), grouper (Duray et al., 1996), carp's juveniles (Papoutsoglou et al., 2000) and barramundi (Ullmann et al., 2011). In addition, some previous studies affirmed that tank color can contribute significantly to fish stress level (Rotllant et al., 2003; Papoutsoglou et al., 2005).

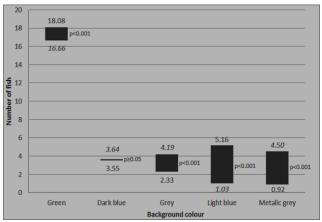
For example, it was demonstrated that in jundiá (Rhamdia quelen) exposed to blue light, cortisol level following stress was reduced compared to the fish exposed to white light (Barcellos et al., 2006).

In rainbow trout (Oncorhynchus mykiis), eel (Anguilla anguilla) (Baker and Rance, 1981) and carp (Cyprinus carpio) (Papoutsoglou et al., 2000), higher cortisol levels were established, if these were kept in darker tanks.

In this respect, no result about pikeperch was found, our study joining this large research group and ascertaining that green color on the tank background could give to pikeperch a safety feeling. It seems that green color gave pikeperch fingerlings from G2 a safety feeling in the stress conditions, due to



**Figure 5.** Regression curves showing the movement of the pikeperch from G2 (462-464 days) on different background colors in 24 hours, after their acclimating into the poly chrome tank.



**Figure 6.** Comparison of tank background color occupancy by fish from the two groups. Data represent mean (G1 with italic digit; G2 with regular digit) of fish on background color. The boxes represent the differences between groups ( $\Delta$ ), and on their right the significance of these differences.

introducing into the new tank, same observation being made similarly for the younger pikeperch from G1 (but it was observed that these fish have spread sooner into the tank). Hence, it could be affirmed that the younger pikeperch accommodate faster to the new conditions, passing more rapidly over the initial stress than the older fish.

We assume that pikeperch juveniles and fingerlings rearing into green tanks could be advantageous because is offering a comfort status to the fish that could lead with certainty to a better body weight growth. From our perspective, the background color should be followed to improve the culture conditions of this important species and to enlarge the knowledge to the influence of background color on pikeperch feeding behavior and / or their aggression.

In conclusion, green is the best accepted color by pikeperch age categories reared in RAS, in the stressful conditions produced by fish transfer. Rearing or transport of the pikeperch juveniles and fingerlings in green tanks could have an advantage with an expected better production performance in this species.

# Acknowledgements

This study is a part of a greater research, done in BUASVMT Timisoara, Romania and in NARIC-HAKI Szarvas, Hungary (in the frame of AQUAREDPOT, FP7-316266project, supported by the European Commission and the POSDRU project, ID:107/1.5/S/80127, founded by European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007-2013. Also many thanks to S.C. Hipocar 3 S.R.L. from Oradea, Romania for the kind supplying of the experimental tank used in this research.

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