

# Low turbidity in recirculating aquaculture systems (RAS) reduces feeding behavior and increases stress-related physiological parameters in pikeperch (*Sander lucioperca*) during grow-out

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**ABSTRACT:** There is a tendency to farm fish in low turbidity water when production takes place in the land-based recirculating aquaculture systems (RAS). However, the effect of water turbidity on stress and performance is unknown for many species cultured in RAS. The effect of different turbidity treatments as Formazine Attenuation Units (0 FAU, 15 FAU, and 38 FAU) on feed intake performance (latency, total feeding time, and total feed intake) and physiological blood stress parameters (cortisol, lactate, and glucose) in medium-sized pikeperch (*Sander lucioperca*) ( $n = 27$ , undetermined sex and age) of initial body weights of  $508.13 \text{ g} \pm 83 \text{ g}$  (at FAU 0, 15, and 38, respectively) was investigated. The rearing system consisted of 9 rectangular tanks (200 L per tank). Fish were housed individually ( $n = 1$ , per tank,  $n$  replicates per treatment = 9). All tanks were connected

to a recirculation system equipped with a moving bed biofilter. Feed intake in pikeperch kept at low turbidity (0 FAU) was 25% lower than pikeperch kept at high turbidity (38 FAU) ( $P < 0.01$ ) and also significantly (10.5%) lower compared to feed intake in pikeperch kept at intermediate turbidity (15 FAU) ( $P < 0.01$  for 0 FAU vs. 15 FAU, feed intake sign. Value as the main effect is  $P < 0.01$ ). Pikeperch kept at low turbidity showed significantly slower feeding response (latency time) towards pellets entering the tank, shorter feeding times (both  $P < 0.05$ ), and higher glucose blood concentration (73%) in contrast to pikeperch kept at highest turbidity. A reduction of 25% feed intake has obvious economic consequences for any fish farm and present data strongly emphasize the importance of considering the species-specific biology in future RAS farming.

**Key words:** feed intake, glucose, latency, RAS, stress, turbidity

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## INTRODUCTION

Feed intake in fish is influenced by numerous abiotic and biotic factors such as water temperature,

O<sub>2</sub>, CO<sub>2</sub>, pH, ionic composition and hardness, ammonia, nitrite, and nitrate levels, water flow rate, photoperiod, rearing density, genetic manipulation, exercise or feed composition (Thorarensen and Farrell, 2011; Saravanan et al., 2012a; Saravanan et al., 2012b; Ende et al., 2016; Afonso, 2020; Bayley et al., 2020; Colombo, 2020; Devlin et al.,

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2020; Kristiansen et al., 2020). The impacts of the above-mentioned factors are comprehensively studied for at least several aquaculture species and tailored to the species-specific requirements. In a natural environment, however, there are multiple other factors that control feed intake. These factors are prey (or feed item) size, shape and smell of prey (or feed item), abundance and distribution of prey, predatory avoidance, and habitat complexity (Holling, 1965; Werner, 1974; Bence and Murdoch, 1986; Chesson, 1989; Ebersole and Kennedy, 1995; Zwarts et al., 1996; Mattila, 1997; Mattila and Bonsdorff, 1998; Nilsson and Brönmark, 2000; Cogni et al., 2002; Gawlik, 2002; Brown, 2003; Smallegange and Van Der Meer, 2003; Aljetlawi et al., 2004; Barata et al., 2009; Sun et al., 2010; Fossette et al., 2012; Ende et al., 2018a, 2018b).

Certainly, not all of the above-mentioned factors that affect intake in a natural environment apply to an artificial culture environment. However, the absence of some conditions in an artificial culture environment may also affect intake. For example, turbidity is one striking parameter that differs significantly between a culture environment and a natural environment. In land-based RAS farming, there is a tendency to process water obtaining very low turbidity (pers. Comm. B. Wecker, Förde Garnele GmbH), despite the fact that many farmed fish species have naturally adapted to medium or even high turbidities. The small body of literature suggests that water of low turbidity imposes stress on such animals; For example, delta smelt (*Hypomesus transpacificus*) showed increased swimming activity at low turbidity, and fish were seen more often swimming near the surface in the water of low turbidity. Similarly, larval rainbow smelt (*Osmerus mordax*) had lower energetic expenditures related to feeding at higher turbidities compared to energy expenditures measured at lower turbidity (Sirois and Dodson, 2000) suggesting reduced feeding-related activities. Such behavioral observations and energy balances associated with turbidity are also reflected in plasma physiological responses. Cortisol levels in tilapia kept at low suspended solids concentrations (low turbidity water) were almost doubled compared to a mean cortisol concentration observed in tilapia kept at moderate suspended solids concentrations (Martins et al., 2009).

Pikeperch is considered one of the most promising freshwater fish species in Europe and an attractive alternative for land-based RAS aquaculture species (Pyanov et al., 2014). Pikeperch have a high growth potential compared to other farmed fish species, exhibit high-quality flesh and high market

acceptance (Hilge and Steffens, 1996; Barry and Malison, 2004; Philippsen and Van der Kraak, 2008; Wang et al., 2009; FAO, 2012; Dalsgaard et al., 2013). However, many attempts at commercial production have failed in the past years. Reduced feed intake and low growth rates, in particular, have been observed during grow-out in individuals between 400 g and 1000 g (pers. Comm. M. Schmidt, Druchhorner Premium Fisch GmbH & Co. Kg).

The objective of the present study was to determine the effect of turbidity (low = 0 FAU, intermediate = 15 FAU, and high = 38 FAU) on feed intake performance (latency, total feeding time, and total feed intake) and physiological blood stress parameters (cortisol, lactate, and glucose) in medium-sized (>400 g) pikeperch (*Sander lucioperca*). Pikeperch occurs naturally in shallow eutrophic lakes with water turbidity (Sonesten, 1991; Craig, 2008; Vinni et al., 2009). It is therefore hypothesized that feed intake and feeding behavior are impaired when pikeperch fish are kept in RAS using water of low turbidity.

## MATERIAL AND METHODS

All institutional guidelines for the care and use of animals were followed by the authors and experiments were conducted by specially trained and experienced staff.

### *Fish and Rearing Conditions*

Pikeperch ( $n = 27$ , undetermined sex and age) were adapted to individual tank-holding (one fish per tank) for 1 week prior to the experimental periods and were fed at maintenance level (at approximately 0.3% average body weight) during this time. The initial body weight of pikeperch was  $436.5 \text{ g} \pm 60.9 \text{ (SD)}$ ,  $486.3 \text{ g} \pm 68.2 \text{ (SD)}$ , and  $601.6 \text{ g} \pm 120.5 \text{ (SD)}$  (for fish used at FAU 0, 15, and 38, respectively). The differences in mean weights of the three treatments are explained by the different times, at which treatments were conducted. Treatments were tested consecutively in the order of FAU 0, FAU 15, and FAU 38. Each experimental period lasts for several weeks when adaptation periods are also included. Animals kept in holding facilities in parallel for upcoming experimental periods were fed rations well above maintenance to keep all fish experimental in well-fed condition before the start of adaption (1 week of maintenance and experimental period).

The rearing system consisted of nine rectangular tanks (200 L per tank). Fish were

housed individually ( $n = 1$ , per tank,  $n$  replicates per treatment = 9). All tanks were connected to a recirculation system equipped with a moving bed biofilter. The system did not include ozone or UV treatment. Natural clay particles (Baulehm CONLUTO trocken von NaturBauHof, Neustadt (Dosse)) were used to create various turbidity treatments. Clay is traditionally used to simulate turbidity in ecological or aquaculture-related studies and has shown to have no negative effects with the concentrations that have also been used in the current study (Ardjosoediro and Ramnarine, 2002; Pekcan-Hekim and Lappalainen, 2006).

The mechanical filtration step was removed so that the clay particles that were used for turbidity stayed within the system. Water qualities were kept at constant levels and maintained as follows: dissolved oxygen (DO) 96%; temperature  $22.6 \pm 0.59$  °C; The pH was kept above 7.28 averaged over all treatments. Water quality was measured twice per week and were for FAU 0; mean  $\pm$  SD) TAN-N was  $0.06 \pm 0.06$  mg L<sup>-1</sup>, NO<sub>2</sub>-N was  $0.10 \pm 0.09$  mg L<sup>-1</sup> and NO<sub>3</sub>-N was  $47.32 \pm 49.69$  mg L<sup>-1</sup>; FAU 15 was TAN-N was  $0.05 \pm 0.00$  mg L<sup>-1</sup>, NO<sub>2</sub>-N was  $0.05 \pm 0.01$  mg L<sup>-1</sup> and NO<sub>3</sub>-N was  $25.54 \pm 2.44$  mg L<sup>-1</sup>; FAU 38 was TAN-N was  $0.03 \pm 0.01$  mg L<sup>-1</sup>, NO<sub>2</sub>-N was  $0.05 \pm 0.01$  mg L<sup>-1</sup> and NO<sub>3</sub>-N was  $17.25 \pm 21.94$  mg L<sup>-1</sup>.

Turbidity was measured daily according to ISO 7027 (Formazine Attenuation Units, FAU). Clay concentrations were adapted over time to meet predetermined turbidity values. FAU were  $4.2 \text{ FAU} \pm 0.47$  (low turbidity),  $37.7 \text{ FAU} \pm 0.92$  (high turbidity), and  $15.2 \text{ FAU} \pm 0.85$  (medium turbidity). The light cycle was kept constant at 12:12 L:D. Turbidity was obtained by adding natural clay (CONLUTO, dry NaturBauHof Neustadt, Dosse) to the RAS water. Present FAU values were selected within a range previously tested for RAS in relation to the effectiveness of ultraviolet (UV) light for sterilization (Gullian et al., 2012). The system was compensated for evaporated water, which was about 5 L per day. Clay partially settled in the sump and turbidity was measured daily and compensated if required to respective values.

Food intake was observed from above each tank. Individually housed fish were kept visually isolated from one another front side of each tank comprises a window, to allow daily observations of the fish. Individual housing was preferred in this study to be able to observe individual feeding behavior while reducing disturbance of fish by the observer.

## Experimental Design

Pikeperch adapted to single housing conditions and feed were individually weighed to the nearest 0.1 g, and re-assigned to the same tank. A commercial floating pelleted feed formulated for pikeperch (Biomar Efico sigma 870 f in 4.5 mm Pellets) was fed. From day 1 of the experimental period, fish were fed ad libitum twice a day (08:00 a.m. and 04:00 p.m.) by hand. The feeding procedure consisted of providing sequential portions of five pellets to each fish, randomly. New pellets were added only after the first portion was completely consumed. When feeding activity terminated, leftover pellets were removed by siphon and subsequently counted. Each experimental period was terminated at 9-d. At 9-d pikeperch were removed from tanks, sacrificed within 1 min by an overdose (500 mg L<sup>-1</sup>) of anesthetic (MS 222, Sigma–Aldrich Co. LLC., Germany, MS 222 has proven to be a good fish anesthetic, and, depending for killing fish), weighed, and measured to the nearest of 0.1 g. Blood was taken from the caudal vein. Lactate was measured in the fresh blood using lactate measurement device Lactate Scout (EKF Diagnostics, Germany).

## Sampling Procedures and Calculations

**Latency, total feeding time, and total feed intake.** Feed intake behavior was measured as latency (LAT, min) and total feeding time per feeding (TFT, min). Feed intake was measured as total feed intake per feeding (TFI). All three were measured directly using a stopwatch. Latency was defined as the time difference between the first pellet offered and the first pellet consumed by the fish. Total feeding time was defined as the time between the first and the last pellet being consumed (Martins et al., 2006). TFI was calculated as the total amount of pellets consumed per feeding. Unconsumed pellets were removed after each feeding session, dried according to ISO 1442 (ISO, 1997), and dry weights were deducted from calculations.

**Blood sampling.** Lactate was measured in the fresh blood using Lactate Scout (EKF Diagnostics, Germany). The remaining part was centrifuged ( $2,000 \times g$ ) at 4 °C for 15 min to obtain blood plasma for glucose and cortisol determination. Plasma glucose measurement was performed with Fuji Dry Chem NX500i blood autoanalyzer using Fuji Dri-Chem Slides GLU-PIII (Fujifilm Europe). An enzyme-linked immunosorbent assay (ELISA) (RE52611, IBL International, Germany, ELISA assay is used to measure antigen or antibody

concentration) was used to measure the plasma cortisol level, as already performed by Reiser et al. (2010), Fuchs et al. (2017), and Bögner et al. (2018). Briefly, 100  $\mu\text{L}$  plasma samples were prepared by denaturation at 80 °C for 1 h, resuspended in 0.05 M PBS, vortexed for 20 s and centrifuged at  $13,000 \times g$  for 20 min. For competitive ELISA, 50  $\mu\text{L}$  supernatant of the sample was used. 30 min after TMB incubation the activity of the horseradish peroxidase was stopped plate was measured at 450 nm with a microtiter plate reader (Tecan Infinite 200 SN, Switzerland). The standard curve and sample value were calculated with the Cubic Spline method (SRS1 Cubic Spline for Excel, version 2.5.1.0) for quantifying plasma cortisol. Weighing and measuring took place after blood sampling. Fish were weighed and measured to the nearest of 0.1 g and 0.1 cm.

### Statistical Analysis

All statistical analyses were performed using the open-source software R version 4.0.3 (R Core Team, 2021). After testing the data for normal distribution and outliers (Shapiro Test and qqplots), we used the Kruskal–Wallis test to check for differences in mean values. Where the null hypothesis was rejected, pairwise comparisons were conducted using Wilcoxon rank sum post-hoc test (implemented in the psych package in R) to identify the level of significance between the treatments. We used the “bonferroni” function to adjust for p-values. The significance level was set at  $P < 0.05$ .

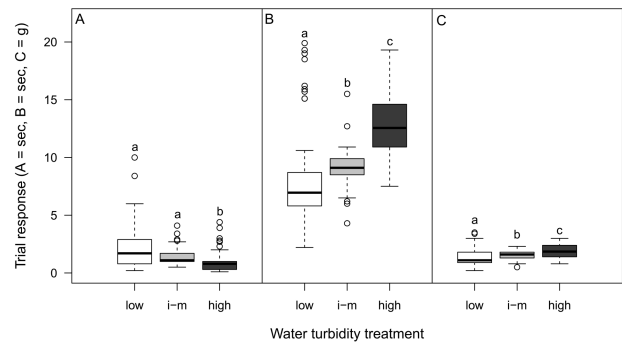
## RESULTS

Latency (LAT 1A, in sec) was shortest in pikeperch kept at high turbidity compared to pikeperch kept at intermediate and low turbidity ( $P < 0.05$ , Fig. 1). Pikeperch kept at the highest turbidity responded nearly two times faster compared to pikeperch at other turbidities, i.e. when mean LAT values of intermediate and no turbidity were combined. Total feeding time (TFT 1B, in min.) was the longest in pikeperch kept at high turbidity compared to pikeperch kept at intermediate and low turbidity ( $P < 0.05$ ). In pikeperch kept at the high turbidity TFT was 38% longer compared to TFT in pikeperch at no turbidity and still 29% higher compared to pikeperch kept at intermediate turbidity. Total feed intake (TFI 1C, in g) was positively correlated with turbidity, differed significantly across turbidity treatments ( $P < 0.05$ ) and was highest in

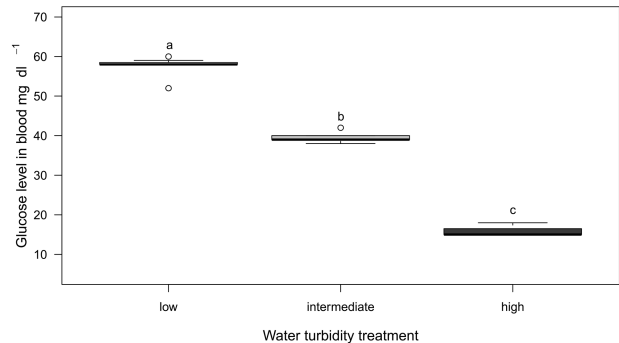
pikeperch kept at high turbidity compared to pikeperch kept at intermediate and low turbidity. Total feed intake in pikeperch kept at the high turbidity was 25% higher compared to TFI in pikeperch at low turbidity and still 16% higher compared to pikeperch kept at intermediate turbidity (Fig. 1).

Plasma glucose concentrations (Fig. 2) were affected by turbidity ( $P < 0.05$ ) resulting in the lowest concentration in fish kept at high turbidity compared to fish kept at intermediate and low turbidity. Glucose blood concentration in pikeperch kept at the high turbidity was 73% lower compared to Glucose blood concentration in pikeperch kept at low turbidity and still 60% lower compared to pikeperch kept at intermediate turbidity.

Lactate blood concentrations (Fig. 3A, in  $\mu\text{mL}^{-1}$ ) were affected by turbidity ( $P < 0.05$ ) and were lowest in pikeperch kept at high turbidity compared to pikeperch kept at intermediate turbidity though not different to mean concentrations measured at



**Figure 1.** Feeding response in pikeperch held at three different turbidities (1: 0 FAU; 2: 15 FAU; 3: 38 FAU): 1A Latency time (LAT in sec.); 1B total feeding time (TFT in min.); 1C total feed intake (TFI in g). (1: low FAU; intermediate: 2 FAU, high: 3 FAU). Numbers of pikeperch per treatment ( $n = 9$ ). Pikeperch were held in individual tanks. Means with different superscripts are significantly different. The significance level was set at 0.05.

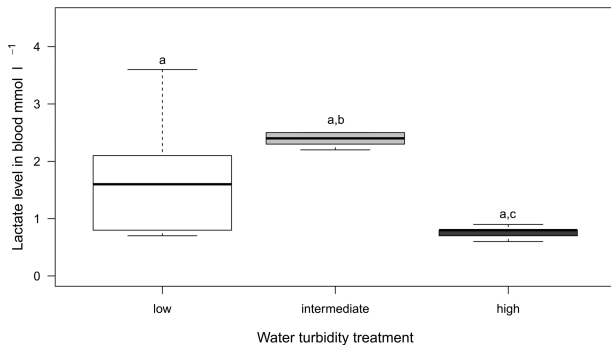


**Figure 2.** Glucose concentrations (in  $\text{mg dL}^{-1}$ ) in the blood of pikeperch held at different water turbidity conditions (low: 0 FAU; intermediate: 15 FAU high: 38 FAU). Superscripts with different letters indicate significant differences. Significance level was set at 0.05. Numbers of pikeperch per treatment ( $n = 7$  for 0 FAU,  $n = 7$  for 15 FAU, and  $n = 8$  for 38 FAU, respectively). Pikeperch were held in individual tanks.

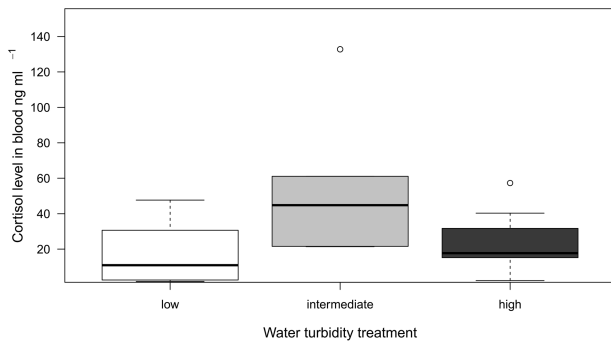


low turbidity. Nonetheless, in terms of percentages lactate blood concentration in pikeperch kept at the highest turbidity was 55% lower compared to lactate blood concentration in pikeperch kept at low turbidity and even 65% lower compared to pikeperch kept at intermediate turbidity.

Cortisol blood concentrations (Fig. 3B, in  $\text{ng mL}^{-1}$ ) were not affected by turbidity ( $P < 0.05$ ). Cortisol was highest in pikeperch kept at intermediate turbidity compared to pikeperch kept at high and low turbidity though not significantly different to mean concentrations measured at highest turbidity. In terms of percentages, cortisol blood concentration in pikeperch kept at the intermediate turbidity was 27% higher compared to cortisol blood concentration in pikeperch kept at high turbidity and 34% lower compared to pikeperch kept at low turbidity (Fig. 4).



**Figure 3.** Lactate concentration (in  $\text{mmol L}^{-1}$ ) in the blood of pikeperch held at different water turbidity conditions (low: 0 FAU; intermediate: 15 FAU, high: 38 FAU). Numbers of pikeperch per treatment ( $n = 9$  for 0 FAU,  $n = 6$  for 15 FAU, and  $n = 9$  for 38 FAU, respectively). Superscripts with different letters indicate significant differences. Significance level was set at 0.05. Pikeperch were held in individual tanks.



**Figure 4.** Cortisol concentrations (in  $\text{ng mL}^{-1}$ ) in the plasma of pikeperch held at different water turbidity conditions (low: 0 FAU; intermediate: 15 FAU, high: 38 FAU). Numbers of pikeperch per treatment ( $n = 9$  for 0 FAU,  $n = 7$  for 15 FAU, and  $n = 8$  for 38 FAU, respectively). Superscripts with different letters indicate significant differences. Significance level was set at 0.05. Pikeperch were held in individual tanks.

## DISCUSSION

This study revealed that feed intake and feed intake behavior in adult on-growing pikeperch was significantly positively affected by increased water turbidity across treatments. Water quality parameters were far below any critical limits reported to negatively affect fish performance across all treatments, and are not discussed as the cause of increased stress level. Hence, the observed reduced performance and increased stress level in pikeperch will be discussed for turbidity mainly, but also other possible factors.

Feed intake was significantly lower at low turbidities in the current study, and reduced feed intake in water at low turbidity has been reported for fish species such as chinook salmon (*Oncorhynchus tshawytscha*) where foraging rates were low in the water of low turbidity and highest at intermediate turbidity levels (35–150 Nephelometric Turbidity unit NTU) (Gregory and Northcote, 1993). Also, feed intake in Inanga (*Galaxias maculatus*), increased linearly with increasing turbidity until a plateau was reached (Rowe et al., 2002). In smelt (*Retropinna retropinna*), feed intake showed a slight increase with increasing turbidity with levels ranging between 0 and 160 NTU (Rowe et al., 2002). Both species are migratory species living in both turbid and water of low turbidity and are considered less sensitive to turbidity alterations (Rowe and Dean, 1998). In rainbow trout (*Oncorhynchus mykiss*), turbidity levels of 15 NTU reduced the reactive distance to feed by 45%, whereas at 30 NTU reactive distance was reduced by 80% (Barrett et al., 1992). A reduction in feed intake in the water of low turbidity probably reflects adaption to reduce the potential risk to predators (Gregory and Northcote, 1993). In contrast, a high feed intake in water of low turbidity is especially observed in species that highly rely on vision and use other means to avoid predation. However, pikeperch is a nocturnal piscivore that prefers to hunt in dim light and is less dependent on sight than pike or perch (Popova and Sytina, 1977). This may be a key explanation why pikeperch successfully forage in turbid water and use turbidity as a means of predatory avoidance.

In the present study, plasma glucose was highest in pikeperch kept at low turbidity, i.e. at 0 FAU. However, high plasma glucose levels in pikeperch kept at 0 FAU may still be considered at basal levels. Reference values in pikeperch of similar weight (approximately 300 g) reported for unstressed control groups of pikeperch (ranging between 60 and 80  $\text{mg dL}^{-1}$  for plasma glucose (Falahatkar and

Poursaeid, 2014) are very close to present plasma glucose values ( $57.57 \text{ mg dL}^{-1} \pm 2.57$  at 0 FAU). Nevertheless, in the present study, glucose concentration was clearly affected by turbidity changes and differences to literature values could have numerous reasons.

Significant differences in glucose may have been caused by different perceptions of photoperiod. The daily rhythm of glucose is linked to photoperiodic (Cerdá-Reverter et al., 1998). This could possibly explain the very low values at 15 and especially at 38 FAU. In sea bass (*Dicentrarchus labrax*) glucose concentrations significantly progressively increased during the dark period and decreased during the photophase (Cerdá-Reverter et al., 1998). Glycemia showed large changes in trout when subjected to stressors such as hypoxia or high housing density which may also reflect habitat oxygen availability or even differences in the level of domestication (Polakof et al., 2012). Similarly, high plasma glucose concentrations in pikeperch kept in low turbidity RAS may be related to stress (by environmental disturbance) that change the metabolism of glucose in fish. At the time of stress, insulin inactivation causes the use of glucose by stopping cells, resulting in increased glucose in the bloodstream (Malini et al., 2018). Similar plasma glucose increases as in the present study, i.e. a 3-fold increase in plasma glucose between pikeperch kept at high turbid conditions to those kept in the water of low turbidity, have been reported for a number of species studied in response to environmental stress; For example, *Rastrelliger kanagurta*, *Lactarius lactarius*, *Scomberomorus Plumieri*, *Carangoides praeustus*, *Trichiurus lepturus*, and *Lutjanus rivulatus* (20, 20, 160, 146, 136,3 and 94  $\text{mg dL}^{-1}$ , respectively) when exposed to environmental stressor conditions (water temperature, DO levels and carbon dioxide concentrations) (Malini et al., 2018). Basal blood glucose level of fish range between 40 and 90  $\text{mg dL}^{-1}$  (Patriche, 2009).

High plasma glucose concentrations result in a negative feedback signal interrupting further feed intake (Mayer, 1991; Wilson, 1994). Reduced feed intake in response to high plasma glucose has previously been reported in glucose-intolerant fish species of higher trophic levels (Hemre et al., 2002; Geurden et al., 2007). Hence, reduced feed intake in pikeperch kept at 0 FAU in the present study may be explained by reduced glucose uptake through the gut and the feedback signal to stop further feed intake.

### **Pikeperch Biological Adaptation to Turbidity**

Pikeperch are predatory fish species occurring both natively and as an intentionally introduced species in fresh and brackish waters in Europe and Asia (Craig, 2008; Kopp et al., 2009). Pikeperch occurs naturally in shallow eutrophic lakes with water turbidity (Sonesten, 1991; Craig, 2008; Vinni et al., 2009), being within the range of medium and high turbidities used in the present study, i.e. FAU 15 and 38 (Vinni et al., 2009). They can also flourish in oligotrophic low-turbid water lakes if they are deep enough to provide refuge during daytime (Sonesten, 1991). Especially this data indicate that pikeperch seek visual shelter in water of low turbidity at day times when the risk of own predation is high, and foraging predominantly during night-time. This may explain present results, i.e. the reduced feeding behavior and signs of high stress in pikeperch kept at low turbidity. Present pikeperch may be considered premature (<600 g individual body weight). In nature, pikeperch can reach a bodyweight of 15–20 kg (Sonesten, 1991). The recorded predators of the zander include other zanders, as well as European eels (*Anguilla anguilla*), Northern pike (*Esox lucius*), European perch (*Perca fluviatilis*), the catfish (*Silurus glanis*), and the Caspian seal (*Phoca caspia*). Especially pike, seals, and catfish prey on all life stages of pikeperch (CABI, 2019). The present pikeperch size class would still be under predatory pressure in a natural environment and would display predatory avoidance strategies in water of low turbidity. No information exists on the degree of domestication of the pikeperch used in the present study nor on the natural origin of this strain. Therefore, it is not clear to what degree present results, i.e. reduced feed intake and stress at low turbidity can be explained by any absence of adaptation or life history experiences. Present pikeperch originated from a pond culture (federal state of Thuringia, Germany). Neither Broodstock, larvae nor juveniles have been adapted to water of low turbidity. With this background and in the visual and chemical absence of any predator, visual human encounter may have caused stress for pikeperch at low turbidity in the present study. In the present study, the front side of the tanks were not covered to be able to observe feeding behavior and latency was even observed in close tank proximity from above. However, such encounter will also exist in a commercial farm situation when using water of low turbidity in RAS. Hence, if present behavioral and physiological response was truly related to human encounter the same effect will be expected in a commercial farm environment.

### ***Applications for Pikeperch Aquaculture and Observations in Other Aquaculture Species***

High mortality and impairment in growth rate during pikeperch (*Sander lucioperca*) on-growing are among the major bottlenecks for its development in aquaculture. These failures may be related to high-stress responsiveness because the rearing conditions are not yet optimized for this species. Despite that pikeperch of on-growing size are the top predators and may not decide a foraging approach based on their risk of falling prey, present results demonstrate that there remains a positive effect of turbidity during feeding behavior.

In commercial RAS systems, there is a trend toward keeping process water at low turbidity to improve fish observation and system management. Previous studies mainly found that juvenile fish profit from turbidity. To the best of our knowledge, this is the first study demonstrating a positive effect of turbidity in fish during grow-out, in this case, pikeperch. The highest positive effect was observed at the highest tested turbidity. This turbidity, however, counteracts UV-disinfection efficiencies (Gullian et al., 2012). In this study, UV was unlikely to be a cost-effective way of removing heterotrophic bacteria at turbidity above 11.0 NTU.

In the present study, only one size class of pikeperch was investigated. However, there may be age-related differences in stress response as observed in other species; e.g., whole-body Total immunoreactive corticosteroid (IRC) levels in minnows (*Erimonax monachus*) and Whitetail Shiners (*Cyprinella galactura*) were highest in young fish and lowest in older fish, suggesting there may be some effect by the apparatus itself and these effects may be age-related. As IRC levels at all treatment levels increased with decreasing age it is possible that there is an inverse relationship between age and tolerance to suspended sediments (Sutherland et al., 2008).

There is a problem when comparing present results with most of the literature reported in an aquaculture-related context. Studies focused on the effect of substance accumulation on fish performance, changes in turbidity were a side effect of intensity of culture conditions (such as stocking densities and water exchange rates). Large Tilapia (*Oreochromis niloticus*) for example showed the highest growth performance in water of low turbidity (Martins et al., 2009). Considering the size class of this “large” Tilapia, this seems unlikely to be an age-related adaptation to the absence of

natural predators. It seems more likely to be related to either benefit of periphyton feeding and shelter for smaller size classes increasing their performance, or, problems of larger individuals coping with the accumulation of substances at higher intensities rather than assuming a good performance at low turbidity. Maybe larger individuals are more susceptible to the accumulation of hormones and other substances that are naturally not encountered with higher turbidities.

### **CONCLUSION**

Feed intake and feeding behavior are impaired when pikeperch fish are kept in RAS using water of low turbidity. This reduced feed intake may be related to extended high plasma glucose concentration, which may be related to stress. A reduction of 25% in feed intake has obvious negative consequences for any fish farm but present data suggest that this problem of reduced feed intake and consequently reduced growth currently observed in larger pikeperch cultured in RAS may at least partially be overcome by using turbid RAS water.

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