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Effects of repeated short episodes of environmental acidification on Atlantic salmon (*Salmo salar*) from a landlocked population

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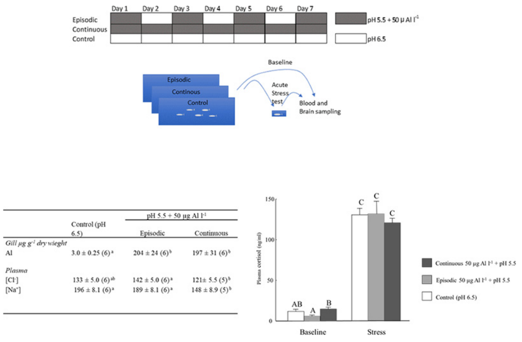
HIGHLIGHTS

- Short repeated episodes of environmental acidification still occur in Southern Norway
- Effects of repeated episodic and continuous environmental acidification on a landlocked population of Atlantic salmon were analyzed
- Continues acidification had a higher impact on homeostasis of the Bleke salmon
- The level of aluminium attached to the gills dis not differ between treatments
- There were no treatment effects on stress coping ability

GRAPHICAL ABSTRACT



	Control (pH 6.5)	pH 5.5 + 50 µg Al l⁻¹	
		Episodic	Continuous
Gill µg g⁻¹ dry wieght			
Al	3.0 ± 0.25 (6) [a]	204 ± 24 (6) [b]	197 ± 31 (6) [b]
Plasma			
[Cl⁻]	133 ± 5.0 (6) [ab]	142 ± 5.0 (6) [a]	121 ± 5.5 (5) [b]
[Na⁺]	196 ± 8.1 (6) [a]	189 ± 8.1 (6) [a]	148 ± 8.9 (5) [b]

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ABSTRACT

Chronic or repeated exposure to environmental contaminants may result in allostatic overload, a physiological situation in which the costs of coping affect long-term survival and reproductive output. Continuous measurements in Otra, the largest river in southern Norway, show the occurrence of repeated 24–48 h episodes of acidification. This work investigates the impact of repeated short acidification episodes on a unique land-locked population of normally anadromous Atlantic salmon ("Bleke"). This was done by recording physiological measures of stress and allostatic load in fish exposed for 7 days to continuous or repeated episodes of simulated environmental acidification or untreated Otra water (controls). A standardized acute stress test was performed after these different exposure regimes, with brain and blood samples taken before (baseline) or after the stress test. Treatment effects on stress coping ability were assessed by neuroendocrine indicators, including telencephalic serotonergic activity and plasma cortisol. Continuous exposure to acidification resulted in increased baseline plasma Cl^- and Na^+ and elevated baseline plasma cortisol compared to episodic exposed fish. However, both episodic and continuous acidification resulted in similar increase in gill Al, indicating similar impact on gill permeability of these two exposures. This suggests a lower impact on the electrolyte homeostasis in episodic compared to continuous exposure and that this effect is not directly related to the effects of Al complexes binding to the gills. Furthermore, there were no treatment induced differences on stress coping ability, suggesting that episodic exposure to the sublethal concentrations of Al in pH 5.5 in the present study do not result in higher allostatic load than in control or continuous exposed Bleke.

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1. Introduction

Generally, major reductions in sulfur and nitrogen depositions have resulted in considerably improved chemistry of previously acidified freshwater bodies in Europe (Garmo et al., 2014; Hesthagen et al., 2016; Tørseth et al., 2017). However, Southern Norway remains susceptible to acid deposition due to weathering-resistant siliceous bedrock and thin and patchy organic-rich soils (Wright and Henriksen, 1978) resulting in that episodic environmental acidification still occurs in this region. For example, continuous pH logging in Otra, the largest river of the region, has shown how heavy rainfall in a hydroelectric powerplant catchment area with low buffering capacity can lead to several local acidification (pH 5.0–5.5) episodes within a week (Barlaup, 2018). Generally, episodic acidification, lasting from a few hours to a few weeks, has negative effects on behavior, survival, density, and biomass of fish (Baker et al., 1996; Hesthagen et al., 1999; Wigington Jr et al., 1996). Still, potential negative impacts on fish populations of highly frequent short acidification episodes, as reported in the Otra river system (Barlaup, 2018), remains to be investigated.

The main detrimental effect of environmental acidification on fish populations is closely linked to aluminum ions [Al^{3+}] being mobilized from surrounding soils. Dissolved in water, Al^{3+} forms complexes with water molecules which binds to fish gills at moderately low pH, affecting membrane permeability by inducing mucus production and cell swelling. This, in turn, reduces ion uptake, gas exchange and increase ion effluxes in freshwater fish (for references, see the review by Gensemer and Playle, 1999). Atlantic salmon (*Salmo salar*) has been reported to be especially sensitive to acidified fresh water (Poléo et al., 1997). A unique landlocked Atlantic salmon) population, “Bleke”, lives in Lake Byglandsfjorden in the watershed of the Otra river system. This landlocked population faced near extinction due to a combination of acidification and hydropower expansion (Wright et al., 2017). However, during the last two decades this population's size has increased under a restoration program that included making spawning areas and fish passages available in addition to elevating the water pH by liming (adding $CaCO_3$). Still, mortality together with slightly elevated gill Al in Bleke exposed to ambient Otra water have been reported (Barlaup, 2018). However, the gill Al level in the latter study was less than gill Al values that have been reported to be associated with mortality in smolts from anadromous populations (for references, see; Kroglund et al., 2007). This made the authors suggest that Al ions in combinations with other stressors, such as gas supersaturation downstream powerplants, might underly this unexpected high mortality in Bleke (Barlaup, 2018). Considering that several 24 h episodes of acidification can be present under a week in the Otra system (Barlaup, 2018), additional stress of an unstable water chemistry might also contribute to such unforeseen mortality.

In a recent study, Höglund et al. (2020) demonstrated that sublethal effects of environmental acidification could be detected with neuroendocrine indicators of allostatic overload in fish. Allostatic overload is a physiological state when unpredictable/uncontrollable chronic or repeated stress impose deficits in biological coping mechanisms, for instance the ability to respond to additional stressors (McEwen, 2000; McEwen, 2007; Schreck, 2000; Vindas et al., 2016). This is well in line with the recent study performed by Höglund et al. (2020), showing that the normal positive relationship between stress intensity, brain serotonergic turnover rate and the magnitude of the cortisol response vanished in Bleke which had been exposed to Otra water supplemented with Al < 35 μg l^{-1} in moderately acidified water (pH 5.5). Furthermore, in addition to the intensity of a stressor the frequency of stressful episodes also contributes to the allostatic load of an individual (Korte et al., 2005). However, if the high frequency of environmental acidification episodes observed at some locations at Bygeladsfjorden (Barlaup, 2018) can impose allostatic overload on fish in general, and in Bleke in particular, is presently unknown.

The aim of the current study was to investigate the impact of repeated short acidification episodes, previously documented in the watershed of the Byglandsfjord (Barlaup, 2018), on fish from the Bleke population. This was done by comparing physiological measures of stress and allostatic load in groups of Bleke exposed to either; 1) Repeated short episodes of environmental acidification, consisting of four 24 h simulated acidification episodes with three 24 h periods with exposure to untreated Otra water (pH 6.5) in between, 2) continuous exposure to the simulated acidification for seven days, 3) untreated Otra water for seven days. A standardized stress responsiveness test was employed to reveal possible effects on coping ability, using basal and post stress values of 5-HT and plasma cortisol as pertinent neuroendocrine indicators (Höglund et al., 2020). In addition, treatment effects on gill Al in plasma electrolytes were measured to investigate the physiological impact on ion balance in the fish. We hypothesized that high frequency water acidification episodes would result in allostatic overload, compromising the fish's ability to respond to further stressors.

2. Materiel and methods

2.1. Experimental animals

1+ year Atlantic salmon originating from the Bleke land-locked population in Otra, hatched and reared at the Syrtveit hatchery and weighing 103 ± 29 g (mean ± standard deviation) were used in the experiment. Before experimentation, all fish were kept in water from the Otra River, with its pH regulated to 6.5, and at a natural water temperature. The experiment was performed in Oct-Nov. Due to seasonally low water temperatures (ranging from 2 to 4 °C), the fish were fed at a minimal rate (approximately 0.1% of body weight day^{-1}) before this study and remained unfed during the experiment. Moreover, fish were kept in continuous dim light by covering half of the rearing tanks before and during experimentation, according to the standard rearing conditions for Bleke.

2.2. Experimental protocol

The fish were exposed to the following treatments for seven days:

1. Episodic acidification: Four episodes with 24 h exposure to pH 5.5 + 50 μg Al l^{-1} (Otra River's acidified with H_2SO_4 to pH 5.5 supplemented with 50 μg Al l^{-1}) with 24 h rearing in Otra River water (pH 6.5) in between.
2. Continuous acidification: Exposure to pH 5.5 + 50 μg Al l^{-1}.
3. Control: Continuous rearing in Otra River water (pH 6.5).

The added Al concentration of 50 μg Al l^{-1} was chosen based on a previous study, showing that reduced coping ability became evident in Bleke exposed to Otra water supplemented with Al at a range between 35 and 70 μg l^{-1} (Höglund et al., 2020). The frequency and duration of the acidification pulses in episodic exposure were based on data from previous pH logging of Otra (Barlaup, 2018). Fish were exposed to these treatment in duplicate 50 l exposure tanks.

The acidified water pH was set to 5.5 and controlled by proportional integration (Pi) regulation of a peristaltic pump, which added H_2SO_4 to the inlet of a 1.6-m-long mixing tube with a pH sensor (Hamilton Polylite +) positioned at the outlet. The inflow to the mixing tube consisted of regular water from the hatchery facility (pH 6.5) at a flow rate of 30 l min^{-1}. The acidified water was collected in a tank with overflow, whereupon it was delivered to two 50 l mixing tanks. Inflow to the mixing tanks was 6 l min^{-1} plus any minor overflow. A stock solution with concentration of 0.45 g Al l^{-1} was delivered into each mixing tank via peristaltic pumps at a flow rate of 3 ml min^{-1}. This resulted in a nominal Al supplementation of 50 μg l^{-1}in the mixing tanks. Each mixing tank had two outlets linked to two 50 l exposure tanks. In the two tanks where fish were exposed to repeated acidification, the pump delivering Al was turned off and the inlet from the mixing tank was switched from acidified water to regular pH 6.5 Otra River's water every second day at 8:00 am.

After seven days of treatment, baseline stress levels were sampled by swiftly netting three fish from each exposure tank and directly anesthetizing each in MS 222 at a concentration of 0.5 g l^{-1}. In addition, four individuals from each exposure tank were subjected to confinement stress, by keeping each individual fish in a 0.4 m × 0.3 m × 0.2 m (length × width × depth) aquarium, with the water surface just above the dorsal fin for 30 min. This acute stress test principle followed previous studies methodology for detecting both heritable and environmentally induced differences in the stress-coping ability of teleost fishes (e.g., Basic et al., 2013; Johansen et al., 2012; Vindas et al., 2016; Øverli et al., 2004). Fish were confinement-stressed in the same water that they had previously been exposed to. After their confinement, fish were netted and anesthetized in MS 222 (0.5 g l^{-1}). This resulted in samples sizes of $n = 6$ for the baseline and $n = 8$ for the confinement-stressed condition in each of the three treatments. Blood (approximately 1 ml) was collected from the caudal vasculature of anesthetized fish using a syringe pre-treated with heparin, whereupon the fish were immediately killed by decapitation and the telencephalon dissected out from each brain. The dissected telencephalons were wrapped individually in aluminum foil, frozen on dry ice, and stored at −80 °C. Whole blood samples were rapidly transferred to Eppendorf tubes and centrifuged at 1500g for 10 min at 4 °C. Following centrifugation, blood plasma samples were frozen on dry ice and stored at −80 °C. In addition, three fish from each tank were sampled and killed by a blow to the head, whereupon their gills were dissected and removed and frozen on dry ice.

The experiment was conducted in accordance with the Guidelines of the European Union Council (86/609/EU) and Norwegian legislation for the use of laboratory animals. The experimental protocol was approved by the ethics committee of the Norwegian food safety authority (permit number 14193).

2.3. Analysis of gill Al levels

Gill tissue was freeze-dried, weighed, and then digested in concentrated, trace metal-grade nitric acid (HNO_3) overnight at 50 °C. Samples were then diluted to 10% HNO_3 and trace elements measured on an Agilent 7700 Q-ICP-MS. For quality control, we concurrently ran certified reference materials: DORM-4 (fish protein); and DOLT-5 (dogfish liver), both from the National Research Council of Canada, and IAEA-436 (tuna fish flesh homogenate) from the International Atomic Energy Agency. Results are expressed as μg Al per g of gill dry weight.

2.4. Analysis of 5-HT brain neurochemistry

The frozen telencephalon samples were homogenized in 4% (*w*/*v*) ice-cold perchloric acid (PCA) containing 0.2% EDTA and 94.2 ng ml^{-1} of 3,4-dihydroxybenzyl amine hydrobromide deoxyepinephrine (the internal standard), by using an MSE 100 W ultrasonic disintegrator. Prior to analysis, each sample was thawed on ice, and centrifuged at 17,000 rpm for 5 min. Then its supernatant was removed, from which 5-HT and its principal catabolite, 5-Hydroxyindolacetic acid (5-HIAA), were quantified using high-performance liquid chromatography (HPLC) with electrochemical detection. Generally, the ratio between monoamine catabolite and parent monoamine have been related to> release and production and thus have been used as proxy for monoaminergic activity (Shannon and Gunnet, 1986). In the present study serotonergic activity was quantified by the [5-HIAA]/[5-HT] ratio. The HPLC system consisted of a solvent-delivery system (Shimadzu, LC-10AD), an auto injector (Famos, Spark), a reverse phase column (4 × 150 mm, C18, ReproSil-Pur 120 C18 5 μm Dr. Maisch) and an ESA Coulochem II detector (ESA, Bedford, MA, USA) with two electrodes, at −40 and + 320 mV. A conditioning electrode (ESA 5020), with a potential of +400 mV, was employed before the analytical electrodes, to oxidize any possible contaminants present. The mobile phase consisted of 86.25 mM l^{-1} of sodium phosphate, 1.4 mM l^{-1} of sodium octyl sulfate and 12.26 μM l^{-1} of EDTA in deionized (resistance 18.2 MW) water containing 7% acetonitrile brought to a pH of 3.1 with phosphoric acid. Samples were quantified by comparison with standard solutions of known concentrations and corrected for recovery of the internal standard using the HPLC software (CSW, DataApex Ltd., Czech Republic).

2.5. Analysis of plasma electrolytes and cortisol

The blood plasma electrolytes were analyzed by a Convergys© R ISE comfort Electrolyte Analyzer". Cortisol in plasma was analyzed using a commercially available DetectX® cortisol enzyme immunoassay kit (Arbor Assays, Ann Arbor, MI, USA) following the manufacturers protocol. The absorbance of the prepared ELISA plate was read in a plate reader at 450 nm and the concentrations were calculated using the four-parameter logistics curve.

2.6. Statistics

All values are presented as means ± standard error of mean. Effects of the different exposure regimes on plasma cortisol and 5-HT activity were investigated by two-way analysis of variance (ANOVAs) with acute stress (confinement or base line) and water treatments as independent variables. The cortisol values were log-transformed to obtain a normal distribution. The effects of the water treatments on gill-deposited Al and plasma electrolytes were investigated by separate one-way ANOVAs. Differences between treatment groups were investigated by conducting Tukey HSD post hoc tests. All statistical analyses were performed in Statistica v13 (Tibco software).

3. Results

3.1. Gil aluminum

The different treatments affected gill aluminum significantly (ANOVA: $F_{(2,\ 15)=}25$, p<0001), resulting in higher values in continuous and episodic acidification exposures compared to control (pH 6.5) treatment ($P < 0.001$). However, there was no difference between continuous and episodic acidification ($P < 0.97$), Table 1.

3.2. Plasma electrolytes

There where a significant treatment effect on plasma [Cl^-] (ANOVA; $F_{(2,\ 14)} = 4.1, P < 0.05$). Exposure to continuous acidification resulted in

Table 1
Gill Aluminum and plasma electrolytes concentrations in Atlantic salmon (*Salmo salar*) originating from a landlocked population in the Otra River. Fish were exposed to either repeated acidification episodes (four 24 h episodes of exposure to Otra River's water acidified with H_2SO_4 to pH 5.5 and supplemented with 50 μg Al l^{-1}, with three 24 h exposure periods to control Otra water, pH 6.5, in between), continuous acidification (exposure to Otra River's water acidified with H_2SO_4 to pH 5.5 and supplemented with 50 μg Al l^{-1}) for seven days or control (untreated Otra water, pH 6.5) for seven days. Values are mean ± S.E. (n); different letters on the same row indicate significant differences (P < 0.05).

	Control (pH 6.5)	pH 5.5 + 50 μg Al l^{-1}	
		Episodic	Continuous
Gill μg g^{-1} dry wieght			
Al	3.0 ± 0.25 (6) ^{a}	204 ± 24 (6) ^{b}	197 ± 31 (6) ^{b}
Plasma			
[Cl^-]	133 ± 5.0 (6) ^{ab}	142 ± 5.0 (6) ^{a}	121 ± 5.5 (5) ^{b}
[Na^+]	196 ± 8.1 (6) ^{a}	189 ± 8.1 (6) ^{a}	148 ± 8.9 (5) ^{b}
[Ca^{2+}]	1.21 ± 0.06 (6) ^{a}	1.15 ± 0.06 (6) ^{a}	1.11 ± 0.06 (5) ^{a}
[K^+]	2.8 ± 0.22 (6) ^{a}	3.1 ± 0.22 (6) ^{a}	3.4 ± 0.24 (5) ^{a}

decreased plasma [Cl^-] compared to episodic acidification ($P < 0.05$). There were no significant differences between controls (pH 6.5) and exposure to continuous or episodic acidification ($P < 0.32$ and $P < 0.41$, respectively), Table 1. Plasma [Na^+] was also affected by treatment (ANOVA; $F_{(2, 14)} = 9.0, P < 0.005$), resulting in significantly lower values in fish exposed to continuous acidification compared to control water and episodic acidification ($P < 0.005$ and $P < 0.05$, respectively), Table 1. However, there were no significant differences between control treatment (pH 6.5) and episodic acidification ($P < 0.79$), Table 1. Moreover, there was no significant effect of water treatment on plasma [$Ca2^+$] (ANOVA: $F_{(2, 14)} = 0.77, P < 0.48$) or [$K^+$] (ANOVA: $F_{(2, 14)} =$ 2,0, $P < 0.17$)

3.3. Plasma cortisol

There was a significant interaction effect between the acute stress test and treatment (two-way ANOVA: $F_{(2,33)} = 7.3$, $p < 0.005$) on plasma cortisol. Generally, the acute stress test generated higher values than baseline conditions ($P < 0.001$), Fig. 1. However, there were no significant differences in plasma cortisol between fishes exposed to the different treatments in acutely stressed fish ($P < 0.99$). During baseline conditions, fish exposed to continuous acidification showed elevated values compared to episodic acidification ($P < 0.05$). However, plasma levels of cortisol in fish exposed to continuous or episodic acidification did not differ significantly from control (pH 6.5) treatment ($P < 0.99$ and $P < 0.053$, respectively).

3.4. Serotonergic activity

The acute stress test resulted in a general elevation of the [5-HIAA]/[5-HT] ratio compared to baseline values (ANOVA; $F_{(1, 29)} =$ 5.3, $P < 0.028$), Fig. 2. However, water treatment, or an interaction between water treatment and stress, did not affect the [5-HIAA]/[5-HT] ratio significantly, (ANOVA; $F_{(2, 29)} = 2.2$, $P < 0.13$ and two-way ANOVA; $F_{(2, 29)} =$ 2,7, $P < 0.08$, respectively).

4. Discussion

This study shows that 7 days of continuous exposure to acidified Otra river's water (pH 5.5) supplemented with 50 μg Al l^{-1} had higher impact on plasma electrolyte homeostasis and resulted in higher baseline cortisol compared to episodic exposure (four 24 h episodes over 7 days) to similar water chemistry. However, these discrepancies between continuous and episodic exposure were not reflected in gill Al, since both treatments resulted in gill Al of about 200 μg g^{-1} gill dry weight.

The amount of Al bound to the gills (200 μg g^{-1} gill dry weight) in our study corresponds well to a recent study performed by Höglund et al. (2020), showing that adding 35–75 μg Al l^{-1} to the Otra river's water at pH 5.5 resulted in 200–230 μg Al g^{-1} gill dry weight. Furthermore, in our study, baseline plasma cortisol (the main stress hormone in fish) showed higher values after continuous compared to episodic exposure. A higher stress level after continuous exposure was partly supported by the serotonergic turnover rate, showing a non-significant trend for elevated values in this treatment group. Notably, Höglund et al. (2020), found a positive relationship between gill aluminum and increased telencephalic 5-HT turnover rate and plasma cortisol during baseline conditions. This lends support to the belief that the adverse effects of environmental acidification in fishes are related to aluminum binding to gills (Gensemer and Playle, 1999) and that brain 5-HT takes part in the control of cortisol release (Höglund et al., 2000; Øverli et al., 1999). However, it is important to point out that continuous and episodic acidification did not differ in gill Al in the present study, suggesting that gill Al is not directly linked to the stress response in fish. Possible processes underlying this uncoupling between gill Al and the neuroendocrine stress response are discussed below.

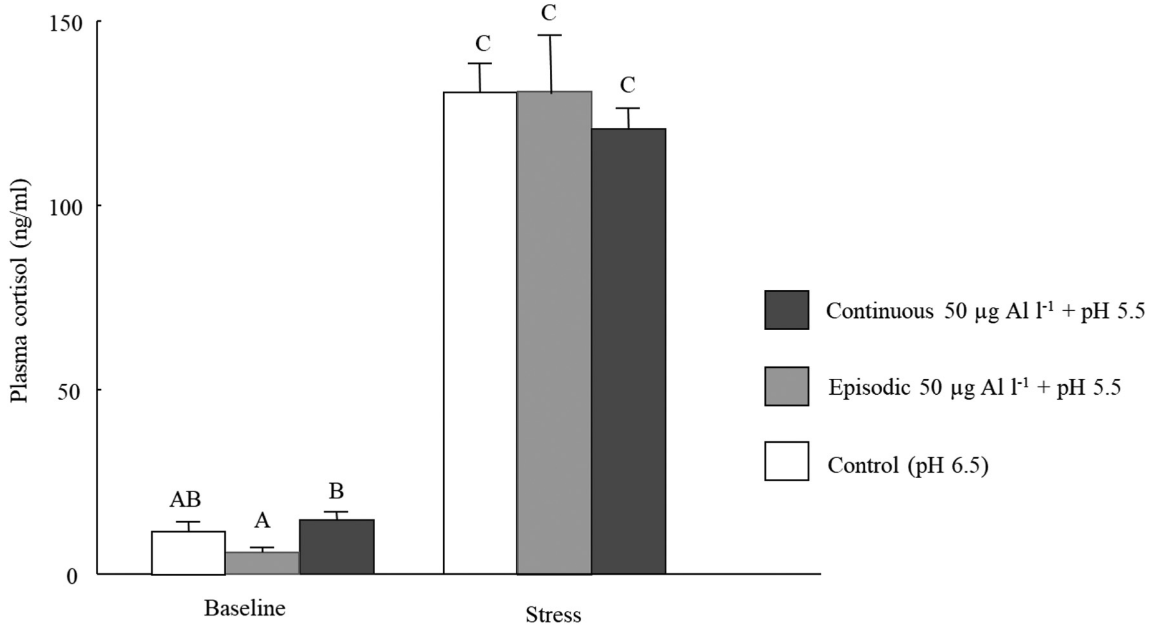

Fig. 1. Plasma levels of cortisol before (baseline) and after a standardized stress test in Atlantic salmon (*Salmo salar*) originating from a landlocked population in the Otra River. Fish were exposed to either repeated acidification episodes (four 24 h episodes of exposure to Otra River's water acidified with H_2SO_4 to pH 5.5 and supplemented with 50 μg Al l^{-1}, with three 24 h exposure periods to control Otra water, pH 6.5, in between), continuous acidification (exposure to Otra River's water acidified with H_2SO_4 to pH 5.5 and supplemented with 50 μg Al l^{-1}) for seven days or control (untreated Otra water, pH 6.5) for seven days. Results are from a two-way ANOVA with acute stress (confinement or base line) and water treatments as independent variables.. Numbers within parentheses correspond to sample sizes of each treatment group; differing letters indicate significant differences between groups ($P < 0.05$). For further statistical information, see results and material and methods.

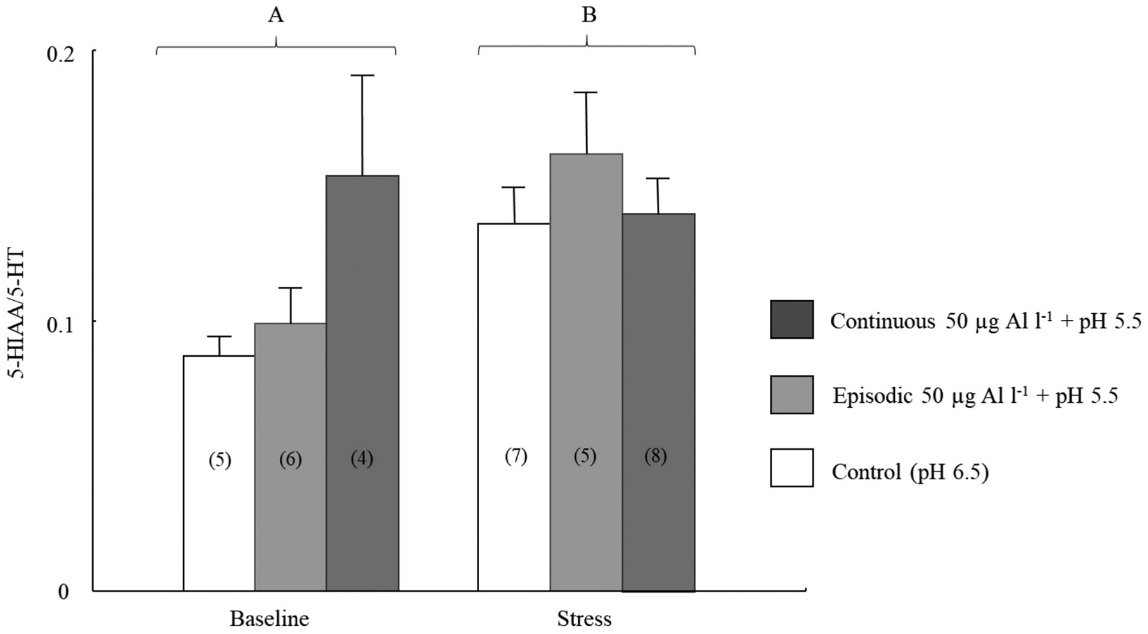

Fig. 2. Serotonergic activity (5-hydroxyindoleacetic acid (5-HIAA)/serotonin (5-HT)) in telencephalon before (baseline) and after a standardized stress test in in Atlantic salmon (*Salmo salar*) originating from a landlocked population in the Otra River. Fish were exposed to either repeated acidification episodes (four 24 h episodes of exposure to Otra River's water acidified with H_2SO_4 to pH 5.5 and supplemented with 50 μg Al l^{-1}, with three 24 h exposure periods to control Otra water, pH 6.5, in between), continuous acidification (exposure to Otra River's water acidified with H_2SO_4 to pH 5.5 and supplemented with 50 μg Al l^{-1}) for seven days or control (untreated Otra water, pH 6.5) for seven days. Numbers within parentheses correspond to sample sizes of each treatment group. Results are from a two-way ANOVA with acute stress (confinement or base line) and water treatments as independent variables. Numbers within parentheses correspond to sample sizes of each treatment group; differing letters indicate significant differences between acute stress and baseline ($P < 0.05$). For further statistical information, see results and material and methods.

Höglund et al. (2020) reported that the above positive relationship between water [Al+] concentrations, brain 5-HT activity and plasma cortisol vanished when exposed to an acute stress test. Instead a blunted cortisol response was observed in fish exposed to the highest Al concentration (resulting in 230 μg Al g^{-1} gill dry weight), while the positive relationship between water [Al] concentrations, brain 5-HT and activity persisted. Changes in the relationship between central 5-HT signaling and cortisol release together with a blunted cortisol response is in accordance with the concept of allostatic overload. A situation when the total impact of stressors imposes deficits in the way brain and other coping systems respond to additional stressors (McEwen, 2000, 2007). However, in our study, the stress test did not reveal any treatment induced differences in post stress plasma cortisol or in the relation between plasma cortisol and 5-HTergic responsiveness. Taken together with> higher baseline cortisol levels in continuous exposure, this suggests that the impact of the higher baseline stress levels in continuous exposure was not intense enough to impose a higher allostatic load than control and episodic treatments. Moreover, this implies that short episodes of moderate environmental acidification in a relatively high frequency do not have higher impact on fish stress coping ability than continuous exposure to environmental acidification.

In the present study, continuous acidification resulted in decreased plasma [Cl^-] and [Na^+] concentrations compared to episodic exposure, indicating a higher level of ion leakage in continuous exposed fish. This suggests that the 24 h periods in between acidification episodes enables fish to regain homeostasis and/or that longer acidification episodes than 24 h is needed for affect the homeostasis of the fish. Previous studies in brook trout (*Salvelinus fontinalis*) demonstrate that long term pre-exposure (10 weeks) to sublethal concentrations of Al in combination with low pH can make the fish more resistant to higher Al concentrations (Wood et al., 1988). Furthermore, McDonald and Millsgan (1988) demonstrated that the gill damaging effect, resulting in electrolyte loss and respiration deficits, was present during the initial phase (24–48 h) of exposure to sublethal Al concentrations in moderately acidic water. Adaptation to these environmental factors was a relatively slow acting process, including decreased mucus production and restored ion transport over the gills. However, interestingly, this adaptation process did not include effects on gill Al. In our study, the plasma electrolyte concentrations of [Na^+] and [Cl^-] in fish exposed to continuous acidification showed lower values compared to fish exposed to episodic acidification. Still, the gill Al values in fish exposed to continuous acidification was virtually the same as in fish exposed to episodic acidification, suggesting that the potential protective effects of previous acidification episodes, in the present study, does not include mechanisms affecting Al binding to the gills. Thus, the results from the present study emphazize the need of further studies investigating to which extent protective effects of previous acidification episodes together with time to regain homeostasis after an acidification episode, and the duration of acidification episodes, contributes to the lower impact of episodic exposure to moderate acidification in salmon from the Bleke population and in other fresh water fishes. Furthermore, this study was designed to investigate the effect of short episodes of exposure to moderately acidified water previously reported in parts of the Otra rives system holding the Bleke population. Considering the dual nature of Al toxicity, where effects on electrolyte homeostasis occurs mainly at lower pH, and respiratory effects of increased mucus production predominates at moderate acidification (pH 5–6) (Alstad et al., 2005), future studies should focus on investigating metabolic rate and swimming performance to clarify potential effects of repeated short episodes of moderate environmental acidification on salmon from the Bleke population.

The life cycles of Bleke resembles the life cycle of anadromous Atlantic salmon. They spawn in creeks and show a rudimentary smoltification process where they adapt to a pelagic life stage, and they return to their native creeks to spawn after this pelagic period (Barlaup, 2018). Studies in anadromous Atlantic salmon show that smoltifying salmon are especially sensitive to environmental acidification (Kroglund et al., 2007). Since the current study was done in smoltified Bleke, further studies investigating water quality requirements during other life stages, including smoltification, are needed for safeguarding and managing the unique Bleke population. Especially since the pH have been reported to be lower in areas in which the salmon from the Bleke population spawn and spend their early life stages (Barlaup, 2018).

5. Conclusions

Both episodic and continuous acidification resulted in similar increase in gill Al, indicating similar impact on gill permeability of these two exposures. However, plasma electrolytes in continuous and episodic acidification did not follow gill Al. On the contrary, lower plasma concentrations of Na and Cl suggests a lower impact on the electrolyte homeostasis of episodic compared to continuous acidification. A lower impact of episodic acidification is supported by lower baseline plasma cortisol in this treatment. The length and intensity of the episodes, recovery time between these episodes, together with potential protective effects of these factors, may contribute to the lower impact of the repeated moderate environmental acidification episodes observed in the present study. Furthermore, that there were no treatment induced differences in stress coping ability suggest that episodic exposure to sublethal concentrations of Al in pH 5.5 in the present study do not result in higher allostatic load than control conditions or continuous exposure in Bleke.

CRediT authorship contribution statement

Erik Höglund: Investigation, Formal analysis, Writing - original draft. **Rolf Høgberget:** Investigation, Writing - original draft. **Åse Åtland:** Data curation, Writing - original draft. **Tormod Haraldstad:** Investigation, Writing - original draft. **Øyvind Øverli:** Data curation, Writing - original draft. **Marco A. Vindas:** Data curation, 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.

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