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Effects of Episodic Exposure to High-pH Water on Survival of Atlantic Salmon Eggs and Juveniles: Results from Laboratory and Field Studies

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Abstract: Although effects of acidification on salmonid fish are well studied and documented, effects of episodic high pH have rarely received attention. In the present study, we investigated effects of high-pH events on Atlantic salmon (*Salmo salar*) using both field and laboratory data. Effects of an episodic high-pH event on juvenile densities in a Norwegian river were studied using data from several electrofishing surveys conducted both before and after the event. Effects of high pH on survival of eggs were studied by exposing eggs to a range of high-pH treatments for different durations. Juvenile densities from the field study showed that the high-pH event had little or no effect on the cohort that had been exposed to pH 9.7–10.3 during the egg stage. This finding was in accordance with the laboratory experiment that showed no excess mortality on eggs until pH was >12. The high-pH event occurred in March during low winter flows, and densities of older juveniles in May were significantly lower in the affected area compared to controls upstream. In June and September the difference was not significant, but there was a clear spatial trend indicating that the event had a negative effect on densities of older juvenile salmon. *Environ Toxicol Chem* 2022;41:771–780. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Ammonia; Aquatic toxicology; Freshwater toxicology; Toxic effects; Industrial effluents

INTRODUCTION

Several diadromous fish species in Europe, in particular European eel (Aarestrup et al., 2009) and Atlantic salmon (Chaput, 2012; International Council for the Exploration of the Sea, 2020), have had a significant decline since the 1990s. A status review of Atlantic salmon populations in Europe and North America showed a general decline in the entire distribution area of the species (World Wildlife Fund, 2001). Atlantic salmon populations in Norway face a wide range of anthropogenic threats, such as effects from fish farming, hydropower development, habitat alteration, and pollution (Forseth et al., 2017). Effects of acidification on fish populations have been well documented since the 1970s (Rosseland, 2021), and acidification has led to extinctions or seriously reduced Atlantic populations in 40–45 Norwegian rivers (Hesthagen et al., 2011; Rosseland & Skogheim, 1984). Episodic mortality events and extinctions due to acid rain led to much scientific and public attention through the 1980s and 1990s (Grennfelt et al., 2020). Effects of episodic exposure to elevated pH, however, have hardly received attention.

Most European rivers have pH levels far below 8.0, although some English chalk streams have high natural alkalinity and pH >8.0 (Mann et al., 1989). Experimental exposure of 14 species of freshwater fish to naturally alkaline lakes (pH 8.5–10.8) in Nebraska (USA) showed that at pH levels >9.5 none of the species could survive for more than 24 days and most of them only 4–22 h (McCarraher, 1971). Episodes of highly basic water, defined as pH levels >9.5, rarely but unfortunately occur; and knowledge on the effects such events have on fish populations is important for both assessing consequences and preventing negative effects of such events. Severely elevated pH levels in rivers are mainly caused by anthropogenic disturbances, such as effluents from the textile industry (Sarayu & Sandhya, 2012), release of tunneling wastewater (Vikan & Meland, 2013), and washing of tunnels (Rathnaweera et al., 2019). Untreated wastewater from tunneling where cement-based packing and sprayed concrete are used will commonly reach pH levels of 11–12. pH values at these levels are harmful to aquatic organisms

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(Vikan & Meland, 2013). Whereas acidification is mostly a large-scale phenomenon due to long-distance transport of pollutants to large geographic areas, severely elevated pH levels normally occur at much smaller spatial scales, that is, in parts of streams and rivers.

Exposure to high pH can have direct effects on fish by causing corrosive damage to tissue and gills (Daye & Garside, 1976) and/or by disrupting sodium balance and inhibiting ammonia excretion (Scott et al., 2005; Scott & Wilson, 2007). High pH can also affect the toxicity of other elements and chemicals. For aquatic systems, runoff from tunnel construction poses a special concern because it often has both high pH and high levels of ammonia due to unexploded remains of ammonium nitrate slurry (NH_4NO_3) explosives (Bækken, 2014). The high pH in tunneling water in combination with these ammonium remains can contribute to high levels of ammonia because the ammonium/ammonia (NH_4^+/NH_3) equilibrium at high pH is shifted toward the toxic NH_3 (Randall & Tsui, 2002). Results reported in Knoph (1992) indicate that Atlantic salmon is one of the most sensitive fish species to ammonia toxicity. Exposure of fish to elevated ammonia levels can cause gill damage, reduction in blood oxygen-carrying capacity, disruptions in the osmoregulatory system (US Environmental Protection Agency [USEPA], 2013), and effects on the central nervous system (Randall & Tsui, 2002).

Different freshwater life stages (egg, fry, parr, smolt) of Atlantic salmon may differ in tolerance to the effects of elevated pH levels and certainly in ability to avoid these. Avoidance could be done by moving to tributaries or water inlets that are not affected, which is a known strategy when fish are exposed to acidic water both in laboratory environments (Åtland & Barlaup, 1996) and in toxic mixing zones in field studies (Åtland & Barlaup, 1995). Previous research on elevated pH levels in Atlantic salmon has shown mainly sublethal effects from long-term exposure (from fertilization to fry) at pH 9.5 (Daye & Garside, 1980). Physiological responses on increased blood glucose, hematocrit, and plasma chloride concentrations in blood were found after a 3-week exposure of Atlantic salmon to water with pH >9.5 (Poléo & Hytterød, 2003). To our knowledge, no other studies have been conducted on the effect of shorter-duration exposure to higher pH levels in laboratory or field settings.

In the present study we explored the effects of episodic exposure to high-pH water on Atlantic salmon by combining field data on densities of juvenile Atlantic salmon before and after an episodic high-pH event with laboratory data on survival of eggs after experimental exposure to alkaline water.

METHODS

Field data

Area and event. On March 7, 2018, untreated tunneling wastewater was released into the river Sokna (N79879, W98797; Figure 1), a major tributary of the Gaula watercourse in central Norway. The Sokna drains the southwestern areas of the watercourse and has a total catchment of 586 km^2. A total river stretch of 20 km including the tributary Hauka is accessible for anadromous salmonids, and the main stem has an average slope of approximately 1%. The Gaula watercourse has populations of Atlantic salmon and brown trout (*Salmo trutta*), but whereas the salmon population most years is classified to be above the spawning target (Norwegian Scientific Advisory Committee for Atlantic Salmon, 2021), the anadromous brown trout population is at critically low levels (Solem et al., 2021).

The untreated wastewater was released approximately 2.6 km below the migration barrier for anadromous fish. The release was due to failure of addition of CO_2 to the wastewater, resulting in approximately 30 m^3 untreated wastewater with pH of up to 12.5 being released into the river over a 3.5-h period, causing severely elevated pH levels in downstream sections of the river (Figure 2). Monitoring of pH was conducted by a consultant firm (SWECO) prior to and during the episode, with continuous pH logging at two sites: one site immediately downstream from the point of release and another site 5.4 km farther downstream (Figure 1). The highest recording at the upper logging site was pH 10.26, and the pH level stayed >10 for a period of approximately 80 min. The lower site recordings had a maximum pH of 9.68, and the levels were >9.5 for a period of 90 min.

Water samples were not collected during the event, but analysis of water samples collected by SWECO approximately monthly during the tunneling operation (2017–2019) were used to assess the likely levels of ammonia during the high-pH event. The last sample before the event was collected on January 8, and the first sample after the event was collected on March 21. Results of all water chemical analyses are openly available (see Data Availability Statement). Because the ammonium/ammonia equilibrium depends on both pH and temperature, we calculated the fraction of the total ammonia–nitrogen (NH_4^+ and NH_3) present in the form of NH_3 for water temperatures of 0 °C and 1 °C and pH 10.26 (Canadian Council of Ministers of the Environment CCME, 2010). Further, total ammonia nitrogen was compared to hourly criterion maximum concentrations (CMCs) for freshwater where salmonid fish are present (USEPA, 1999). At the time of the incident, the Sokna had a minimum winter discharge (1 $m^3\,s^{-1}$), and direct and immediate assessment of the effect on Atlantic salmon juveniles was not feasible because of surface ice. In the spring of 2018, the ice breakup period was immediately followed by significant increases in water discharge during the snowmelt period, and electrofishing surveying could not be conducted until the start of May 2018. An additional and more comprehensive electrofishing survey was conducted at the end of June 2018. In addition to the electrofishing surveys in May and June 2018, the Sokna tributary has been a part of a larger surveillance program since 2013, with annual electrofishing surveys in late August and early September.

Juvenile densities in May 2018. On May 2, juvenile densities at six locations (Figure 1) were sampled using backpack electrofishing equipment. Sampling was conducted by two experienced persons but at higher flows than ideal. Electrofishing was done using a TERIK FA-50 model (Terik Technology), a

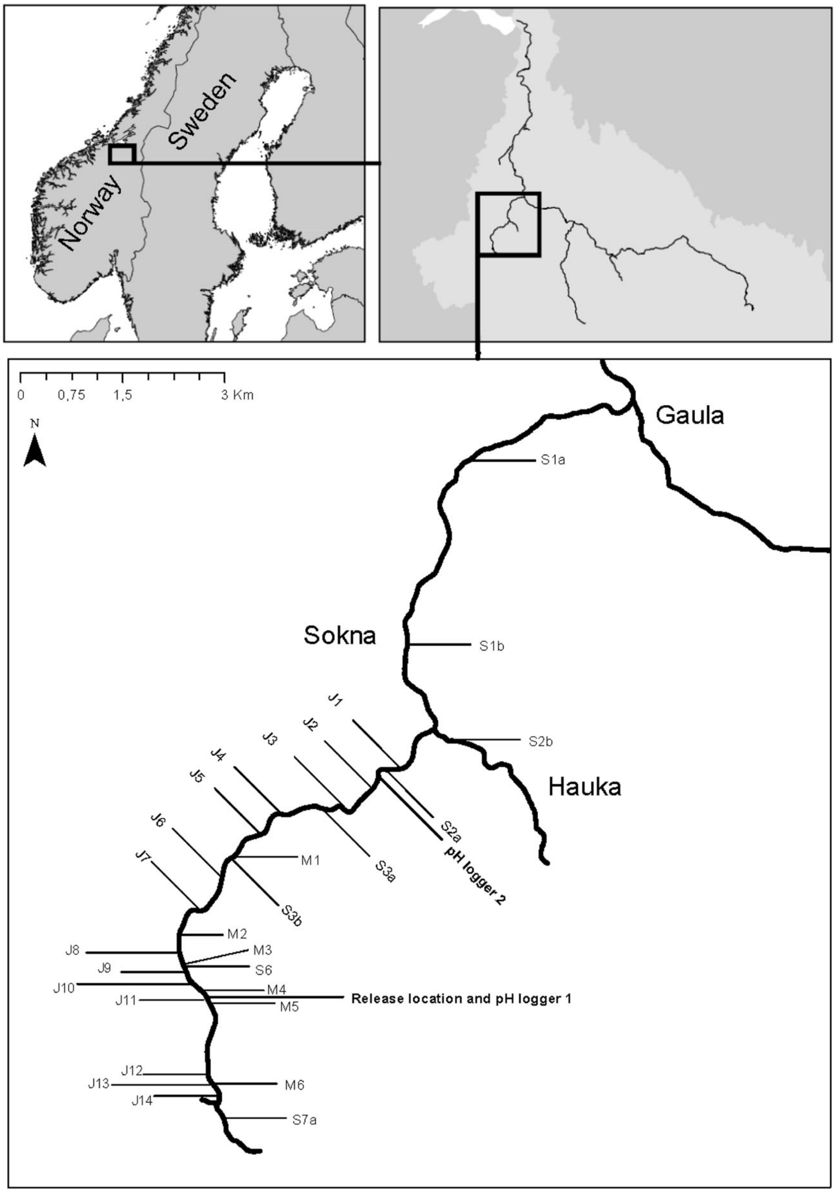

FIGURE 1: The two top panels indicate the location of the Sokna. The lower panel shows the release location of the high-pH water, the locations of the two logging stations, and the locations sampled during the three surveys. Prefixes "M" and "J" indicate locations sampled in May and June 2018, respectively. Prefix "S" indicates locations sampled in September in the years 2013–2020.

pulse direct current generator model which adjusts the voltage applied to the water conductivity to minimize the conductivity-induced bias, while maintaining a voltage level low enough to minimize damage to the fish. Voltage varied between 700 and 1050 V, depending on the water conductivity of the site under investigation. Electrofishing was carried out by an experienced two-person team wading upstream through the river in a zigzag path, one of whom operated the electrofishing gear while the other assisted and took care of captured juveniles. During the sampling in May only single-pass electrofishing was conducted. All captured juveniles were registered, classified by species, and measured for length. Two locations, one upstream of the affected section of river and one on the opposite bank of the point of the outlet, were sampled as controls; and four locations at various distances (240–2600 m) downstream were sampled to assess effects of high-pH exposure. During sampling only juvenile Atlantic salmon and brown trout were caught, and all fish were identified to species and measured to the nearest millimeter. Fish were assigned to one of two age groups based on total length: underyearlings and parr. The actual age of fish classified as underyearlings was very close to 1 year, and estimated hatching time for this cohort using calculations from Crisp (1981) was approximately May 10. This estimate is based on water temperatures in the Gaula watershed in 2017–2018 and an expected peak spawning time of approximately October 20 (Heggberget, 1988; personal observations). The number of caught fish was divided by the area of the locations to give densities of fish per 100 m^2. Because of low total numbers of fish per location and possible differences between species in tolerance to high-pH exposure, only the most numerous group

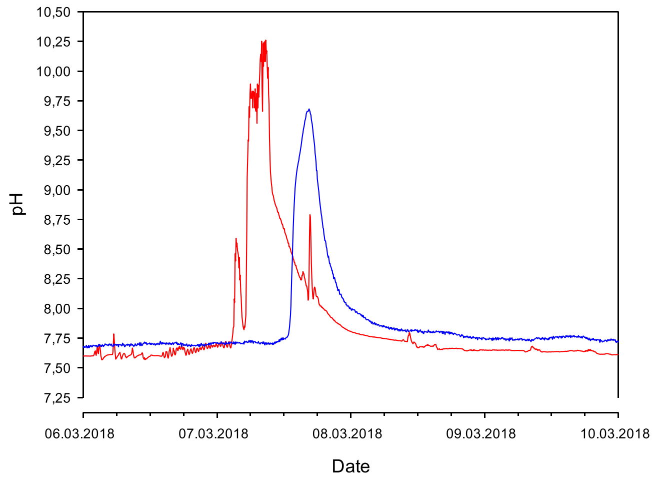

FIGURE 2: Plot of pH at two logging stations from March 5 to 11, 2018. Red line indicates values from the logging station located directly downstream the release of high-pH water; blue line indicates values from a logging station 5.4 km downstream.

(Atlantic salmon underyearlings; Table 1) was included in the statistical analysis. Analysis was conducted using the statistical software RStudio 1.3 (RStudio Team, 2020). A one-tailed two-sample *t* test was conducted to assess if densities in the six exposed locations were lower than in the two controls.

Juvenile densities in June 2018. On June 27 and 29, 2018, a total of 14 locations were sampled using electrofishing (Figure 1), with the same field protocol as for the May survey. Three locations were sampled upstream of the affected area as controls, and 11 were located in the affected section of the Sokna from 120 to 7300 m downstream from the point of the outlet. During sampling young-of-the-year were not collected. The number of caught fish was divided by the area of the locations to give densities of fish per 100 m^2, and a two-sample *t* test on densities above and below the outlet was conducted.

Juvenile densities in September 2018. Densities of juvenile salmon and brown trout in the river Sokna have been monitored yearly at seven sampling sites since 2013 (Figure 1). In addition, one electrofishing site in the tributary Hauka has been sampled yearly from 2014. The densities have been surveyed using the same equipment previously described. Except from 2015 and 2018, two electrofishing locations have been surveyed using three-pass electrofishing, while the six others were surveyed by single-pass electrofishing. In 2018 six locations were fished three-pass and one single-pass. In 2015 three locations were fished three-pass and the rest with single-pass. Results from the locations that were fished three times were used to calculate densities of juveniles according to Zippin (1958) and Bohlin et al. (1989). The mean catchability from these locations was used to calculate the densities of juveniles at one-pass locations. Fish were identified by species, and total length was measured to the nearest millimeter; at each location scale samples from a subset of individuals were taken for age determination.

TABLE 1: Densities of Atlantic salmon underyearlings and parr per 100 m^2 at six locations in Sokna surveyed on May 2, 2018[a]

Location	Underyearlings	Parr	Trout
M1	0.69	0.69	0.00
M2	2.38	0.00	0.79
M3	4.05	0.00	1.35
M4	1.48	0.00	0.00
M5	4.52	2.58	1.94
M6	6.15	1.54	0.77

[a]Locations M1–M4 are in the area affected by elevated pH levels, and M5 and M6 are upstream.

Details regarding stations and sampling can be found in Solem et al. (2021). To assess if the exposure to high-pH water had influenced densities of juvenile Atlantic salmon, densities of young-of-the-year and older parr for all stations and all years were plotted using the boxplot function in R. This was used to visually identify potential outliers, which then were tested for significance using the Dixon outlier test. For older parr, densities are likely to be strongly dependent on the young-of-the-year densities in the previous year; and to account for this affecting the analysis, the same outlier analysis was performed on densities of older parr divided by densities of young-of-the-year in the previous year.

Laboratory experiment. The experiment was carried out at Haukvik gene bank station for wild Atlantic salmon, central Norway. For the experiment 6300 eyed eggs of Atlantic salmon from the Rauma gene bank stock were counted in batches of 100 and placed in 63 Whitlock Viber boxes (Whitlock, 1977). In addition, one box with eggs was kept for reference. Eggs were at a developmental stage of 60% from fertilization to hatching based on calculations in Crisp (1981).

Nine different treatments with regard to pH were created in 10-L buckets, using untraded water from the gene bank station. The pH was adjusted using 0.1 M HCl and 0.1 and 1 M NaOH. Seven Whitlock Viber boxes were placed in each of the nine treatments. One box from each bucket was removed after 5, 15, 45, 90, 180, 360, and 1180 min. Boxes were briefly dipped in a large tank of untreated water and placed in hatching trays (CompHatch). The pH levels were measured at the same intervals during the experiment, using an OxyGuard handy pH meter, which was calibrated between each round of measuring. During the length of the experiment the nine treatments had pH levels of (average ± standard deviation): 2.16 (±0.08), 2.78 (±0.06), 3.91 (±0.05), 5.56 (±0.12), 6.37 (±0.73), 8.47 (±0.13), 9.70 (±0.24), 11.34 (±0.11), and 12.23 (±0.07). Temperature and oxygen saturation (percentage) were measured at the same intervals as pH using a HACH HQ 30D-Multi meter. During the exposure to treated water, temperatures and oxygen saturation ranged between 1.0 °C and 1.9 °C and 98.7% and 101.6%. Effects of treatments were measured as the number of dead eggs left in the boxes after all fish were expected to have hatched based on water temperature using calculations in Crisp (1988). Untreated water was collected for chemical analysis.

RESULTS

Field data

Juvenile densities in May 2018. At all six locations sampled on May 2, 2018, juvenile densities of both salmon and brown trout were low compared to the other samplings (Table 1). The overall low density estimates were probably due to high river discharge resulting in low catchabilities and are not directly comparable to densities found in June and September. Differences in densities between the two control locations and the four exposed locations was analyzed using densities of underyearling salmon with a one-tailed two-sample *t* test. Shapiro-Wilk normality tests of densities of underyearlings in the exposed stations and densities for all locations combined showed no significant difference from a normal distribution ($p = 0.83$ and $p = 0.85$). The *F* test for homogeneity in variances showed no differences between the control and exposed locations ($p = 0.96$). The *t* test showed that densities of salmon underyearlings in the control locations were significantly higher than those in the exposed locations ($t = 2.67$, $df = 4$, $p = 0.03$).

Juvenile densities in June 2018. Densities of parr (age >0+) tended to increase with distance downstream (Figure 3). The three control locations upstream (nos. 12–14) had lower densities than the two stations directly downstream from where the untreated tunnel water was released into the river (nos. 10 and 11). Location 9 had the lowest density, and densities increased gradually at locations downstream, to the maximum density at location no. 1. Shapiro-Wilk normality tests of densities of yearlings in the exposed locations and densities for all locations combined showed a significant difference from a normal distribution (both $p < 0.002$). Wilcoxon's rank sum test showed no significant difference between densities of older salmon between the three control locations and the 11 exposed locations ($p = 0.17$).

Juvenile densities in September 2018. Densities of young-of-the-year salmon in 2018 did not diverge significantly from densities found in the other years (Figure 4A). The only potentially significant outliers are the high densities of young-of-the-year salmon in 2019 (Figure 5A), and no further analysis on densities of young-of-the-year was conducted. Densities of older parr were overall low at all locations in 2018 (Figure 4A). Ranked densities showed that seven of eight locations had the lowest or second lowest densities recorded in the period 2013–2020; this included the control locations upstream of the event and in the Hauka tributary. Box plots of densities for each station only indicated the low density at location S6 in 2018 as a potential outlier (Figure 5B). This location is directly below where the untreated water was released. Dixon's test for outliers showed that this low value was not significantly different from densities at this location in the other years ($Q = 0.55$, $p = 0.10$). Densities of older parr divided by young-of-the-year the previous year in general were low in 2018 compared to other years (Figure 4C), indicating that the low densities of older parr are not the result of a low number of young-of-the-year in 2017. Ranked values showed that six of eight locations had the lowest or second lowest value in the period 2014–2020. Box plots indicated the low value at location S3b as a potential outlier (Figure 5C). Dixon's test for outliers showed that this low value was not significantly different form values at this location in the other years ($Q = 0.58$, $p = 0.08$).

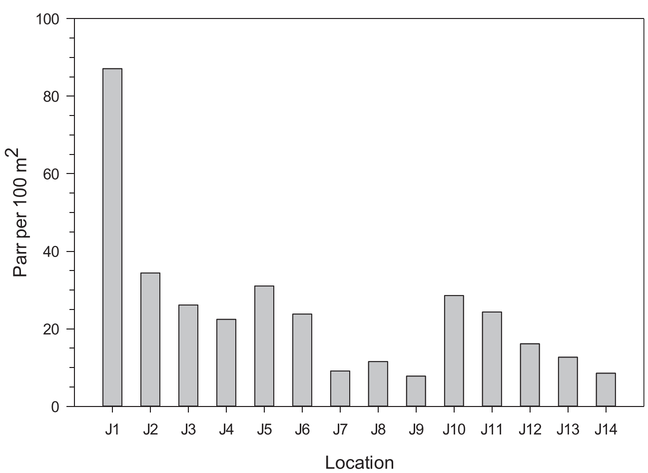

FIGURE 3: Densities of salmon parr per 100 m^2 at 14 locations in the Sokna during the electrofishing survey conducted June 27–29, 2018. Locations J12–J14 are upstream of the release site of the high-pH water.

Water chemistry. Water chemical parameters analyzed using inductively coupled plasma–sector field mass spectrometry (ALS Laboratory Group Norway) from samples collected directly below the release site from 33 unique sampling days in the period October 2017 to September 2018 were consistently below the limit values defined to give acute toxic effects at short-term exposures for arsenic (As), cadmium, chrome (Cr), copper, nickel (Ni), lead, and zinc (Norwegian Environment Agency, 2016). At two sampling dates some values were in the range defined to give chronic effects at long-term exposure (Norwegian Environment Agency, 2016): April 3, 2018, As 0.958 µg/L, Cr 7.2 µg/L, and Ni 8.36 µg/L; April 24, 2018, As 1.34 µg/L. The results of all water chemical analyses are openly available (see Data Availability Statement). Because no water samples were collected during the high-pH event, we used the total ammonia–nitrogen (NH_4^+ and NH_3) from 54 samplings in 2017–2019 to calculate the corresponding NH_3 concentrations given pH of 10.26 and water temperatures of 0–1 °C. Average NH_3 concentrations (NS 4733; ALS Laboratory Group Norway) from these samples calculated for conditions with pH 10.26 and 0 °C were 38.48 µg/L (minimum 2.40, maximum 390.48) and for 1 °C 39.76 µg/L (minimum 2.48, maximum 403.44).

Laboratory experiment. For the eggs exposed to untreated water (pH 6.37) and slightly basic (pH 8.47) and slightly acidic (5.56) water, the number of dead eggs in each treatment of 100 eggs ranged from 0 to 6 (mean 2), and mortality showed no apparent increase with exposure time (Table 2). In the box kept for reference there were two mortalities. Using these numbers to calculate background mortality and assuming this mortality to be either a binomic or Poisson process makes 5 the smallest integer *x* such that $p\ (X \leq x) \geq 0.95$. Of the remaining groups

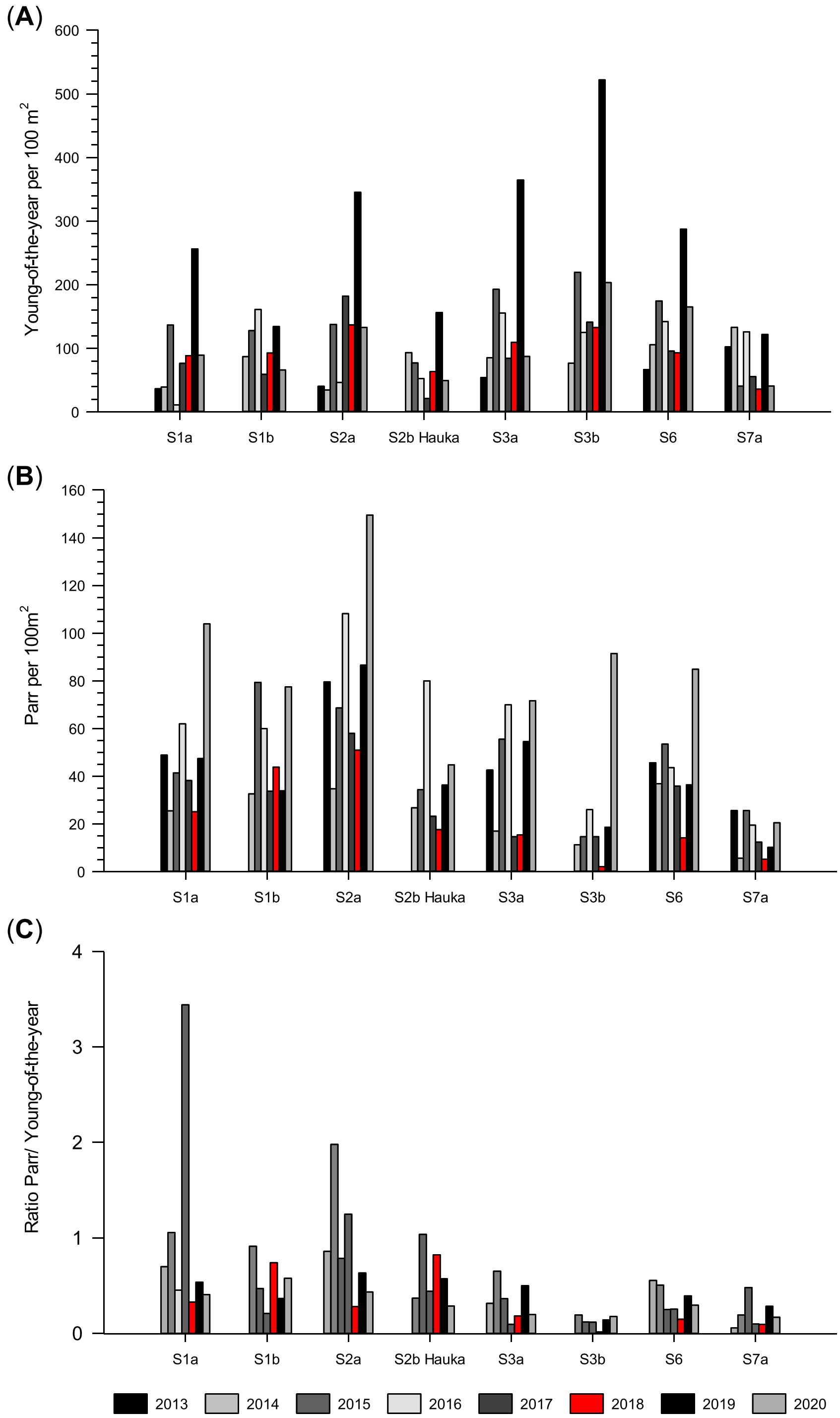

FIGURE 4: Densities of (**A**) young-of-the-year salmon, (**B**) salmon parr, and (**C**) salmon parr divided by density of young-of-the-year salmon the previous year at seven locations in the Sokna River and one in the Hauka tributary in annual surveys in 2013–2020. Location S7a is above the release site of the high-pH water, and location S2bHauka is unaffected by the release.

only three treatments had mortalities exceeding this, the two most acidic and the most basic. For the pH 2.16 treatment high mortality was seen at 90 min of exposure, and total mortality at 360 min. For the pH 2.78 treatment an increase in mortality was seen at 360 min of exposure and almost complete mortality at 1080 min of exposure. Within the range pH 3.91–11.34 no significant mortality was observed regardless of the duration of exposure. For the highest pH treatment (pH 12.23), no significant mortality was observed at 5 min of exposure but complete mortality at 15 min.

Calculation of concentrations of NH_3 for the four treatments with the highest pH (8.47–12.23) were conducted as described, using total ammonia–nitrogen from the water sample (NS-EN ISO 13395; LabTjenester), pH from the individual treatments, and a temperature of 1.61 °C. Calculated concentrations ranged between 0.21 and 9.95 μg/L.

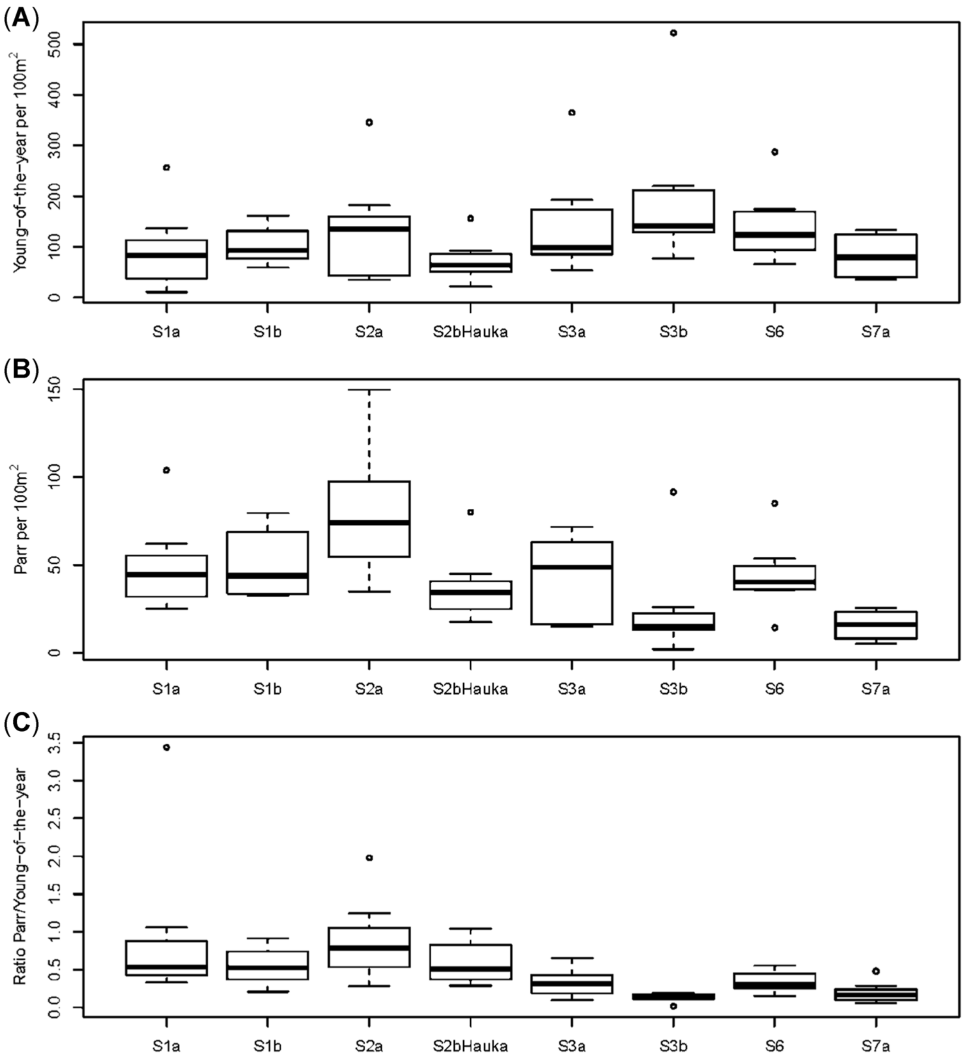

FIGURE 5: Boxplots of (**A**) young-of-the-year salmon, (**B**) salmon parr, and (**C**) salmon parr divided by density of young-of-the-year salmon the previous year at seven locations in the Sokna and one in the tributary Hauka in surveys in 2013–2020. Location S7a is above the release site of the high-pH water, and location S2bHauka is unaffected by the release. The bottom and top of the box indicate the 25th and 75th percentiles (i.e., the boxes include the middle 50% of observations). The whiskers span to the most extreme data point, which is no more than 1.5 times the interquartile range; and the bold horizontal line represents the median value.

DISCUSSION

The results of the present study indicate that the effects of exposure to high pH differ between life stages (eggs and juveniles) of Atlantic salmon. Experimental exposure of eggs to a range of high pH values showed no excess mortality for eggs exposed to pH 9.70 or pH 11.34, while total mortality was observed for eggs kept in water with pH 12.3. Field data from electrofishing surveys conducted in September the years before and after a high-pH event in the river Sokna (March 2018, maximum pH 10.26) also indicate no effect on densities of juveniles exposed to high pH as eggs (young-of-the-year in 2018). However, densities of Atlantic salmon exposed to high pH as juveniles appear to have been negatively affected by the release.

Juvenile densities from the electrofishing survey conducted in May 2018 showed significant differences in densities of juveniles above and below the release site. This survey was conducted as soon as there was no river ice and the high discharge from snowmelt had subsided. Nonetheless, conditions during this sampling were less than optimal, likely resulting in low catchabilities and density estimates that are not comparable to the surveys in June and September.

The survey of juvenile densities in June covered a larger part of the river with a higher spatial resolution, and although there was no significant difference in densities between the control locations upstream and the locations in the affected river reach, there was a clear spatial trend in juvenile densities. With the exception of locations 4 and 5 that were directly below where the untreated water entered the river, juvenile densities steadily increased with distance downstream. Stations 4 and 5 were located on the opposite bank of the tunnel construction site and might have been unaffected by the untreated water. Also, densities at the upper part of the affected area are likely to be positively influenced by dispersing juveniles from unaffected areas upstream.

TABLE 2: Number (percentage) of dead eggs from the total of 100 eggs used per treatment exposed to different pH levels (average pH ± standard deviation during the experiment) and different durations (minutes)[a]

Time (m) \ pH	Mortality 2.16 (±0.08)	2.78 (±0.06)	3.91 (±0.05)	5.56 (±0.12)	6.37 (±0.73)	8.47 (±0.13)	9.70 (±0.24)	11.34 (±0.11)	12.23 (±0.07)
5	4	1	1	2	3	3	1	0	3
15	2	1	1	1	2	1	3	1	100
45	2	3	0	0	2	3	0	0	100
90	94	2	1	1	1	5	1	5	100
180	99	1	3	4	2	6	0	2	100
360	100	13	2	2	0	2	3	4	100
1080	100	98	4	1	0	1	5	0	100

[a]Cells marked in green indicate values used for background mortality calculation; red cells indicate values exceeding the 95% quantile (binominal test to compare two proportions, Poisson lambda 2).

The electrofishing survey in September 2018 is the only survey where also young-of-the-year were sampled and the only survey that has temporal replication of samples at the same locations over several years. Densities of young-of-the-year salmon in 2018 appeared to be unaffected by the high-pH event. This is in accordance with what could be expected given the results of the experimental exposure of eggs to high pH. Densities of older parr (age ≥1+) were generally low at all sampled locations in 2018 compared to the other years (Figure 4B). Density at the location closest to the release site of the high-pH water (S6) appears to be much lower than at the other locations, but the value is not low enough to be significantly considered an outlier that could be caused by the event. Because densities of older parr at a location are likely to be strongly influenced by the densities of young-of-the-year the previous year (Foldvik et al., 2012), densities of older parr were divided by young-of-the-year the previous year (Figure 4C). Lower than expected values of this ratio could indicate excess mortality or emigration from an area. Although most locations had low values of this ratio in 2018, none were significantly lower than could be expected based on the other years.

The effects of the release of untreated water are not limited to high pH alone but also interactions between high pH and other water chemical parameters. Increased pH can potentially mobilize metals present in the river and change metal speciation, toxicity, and bioavailability. Because the untreated water came from tunneling, a special concern arises from the potential combination of high pH and high levels of ammonia due to unexploded remains of ammonium nitrate slurry (NH_4NO_3) explosives (Bækken, 2014). Average NH_3 levels were calculated using values from the monitoring program in 2017, 2018, and 2019 and, using temperature and pH from the river during the release on March 7, 2018 (0–1 °C, pH 10.26), exceed concentrations previously shown to be lethal to Atlantic salmon fry (30–45 µg/L, 96-h median lethal concentration [LC50] [Knoph, 1992]). Furthermore, the levels of total ammonia–nitrogen (NH_4^+ and NH_3) exceeded the calculated CMC for freshwater with pH 10.26 where salmonid fish are present (USEPA, 1999). Although this indicates potential for toxic levels of NH_3 during the episode, it remains inconclusive; because no water samples were collected during the episode, the only published LC50 is for a much longer time period than the episode, and the pH during the episode is above the recommended maximum for calculations of CMC (pH 9).

The apparent differences between eggs and juveniles to exposure to high pH and ammonia could have several explanations. The observed densities of older parr could be the result of either mortality or avoidance response to the high pH. Whereas Atlantic salmon eggs are immobile and buried in the substrate, juveniles have the potential to actively move away from harmful environments. For such avoidance to occur for high pH and/or ammonia the fish must be able to perceive the harmful chemical property (Tierney, 2016). Although Atlantic salmon show avoidance behavior to low pH (Åtland & Barlaup, 1996), it is not known if they can perceive and avoid high pH and ammonia. Avoidance behavior to high pH (>9.5) and ammonia has been documented for some fish species (Richardson et al., 2001; Serafy & Harrell, 1993).

Concentrations of ammonia and pH that constitute toxic levels are expected to differ between eggs and juveniles. Within the eggs salmonid embryos are to a large degree protected from physical and chemical damage by membranes (reviewed in Finn, 2007), while juveniles are directly exposed to any harmful properties of the surrounding water. Corrosive damage to tissues is thus more likely to occur and have detrimental effects for juveniles than for eggs. For rainbow trout (*Oncorhynchus mykiss*), another salmonid species, eggs have been shown to tolerate significantly higher levels of ammonia than fry (Rice & Stokes, 1975). Inhibition of ammonia excretion as a consequence of high pH (Scott et al., 2005; Scott & Wilson, 2007) is likely only relevant for juvenile stages. While the gill is the primary site of nitrogen excretion in juvenile and adult stages, effective excretion of ammonia from eggs is restricted by the chorion membrane (Zimmer et al., 2017). Despite limited excretion, embryos have a metabolism based primarily on amino acids, generating a substantial ammonia load (Zimmer et al., 2017). This indicates that both the importance of ammonia excretion and tolerance to ammonia differ substantially between these life stages.

Effects of high pH and/or ammonia could potentially affect aquatic macroinvertebrates. These constitute an important type of pray for Atlantic salmon juveniles (Johansen et al., 2011), and reduced densities of macroinvertebrates could hence affect growth and/or survival. However, aquatic macroinvertebrate species important to juvenile salmon have substantially higher tolerance to ammonia than salmonids (USEPA, 1999) and appear to both tolerate higher pH and recover rapidly after disturbance (Berezina, 2001; Hull et al., 2014).

Our analysis and interpretation of findings are affected by the different strengths and limitations of the three field surveys. The sampling in May was conducted shortly after the event, making it the least affected by subsequent dispersal and redistribution of juveniles; but high discharge during sampling and lack of replication are clear limitations. The June sampling had good conditions during sampling and high spatial resolution in sampling sites within the affected area. But in retrospect, both number and spatial distributions of control locations upstream of the affected area should have been increased to allow more direct assessment of effects. The field conditions were satisfactory during the September survey, and the locations have been sampled for several years both before and after 2018. This allows assessment of changes in juvenile densities from what is expected at the different locations based on the time series data, while the spatial resolution within the affected area is quite low. Further, the only control location upstream is not ideally placed because it is located upstream of a waterfall that some years may restrict ascending adult salmon.

In the present study we used mortality and changes in juvenile densities as metrics for assessing effects. It is, however, important to keep in mind that sublethal effects may be substantial and will not be detected in our data. Combined data from field surveys and laboratory studies give important knowledge on severity of exposure for different juvenile life stages and provide information important for guiding and planning future research.

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Conflict of Interests—The authors declare that there are no conflict of interests.

Data Availability Statement—This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://osf.io/5nw8b/?view_only=0e480ae7e73d4507898d6727e17a59ae. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

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