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Impacts of ash-induced environmental alkalinization on fish physiology, and their implications to wildfire-scarred watersheds

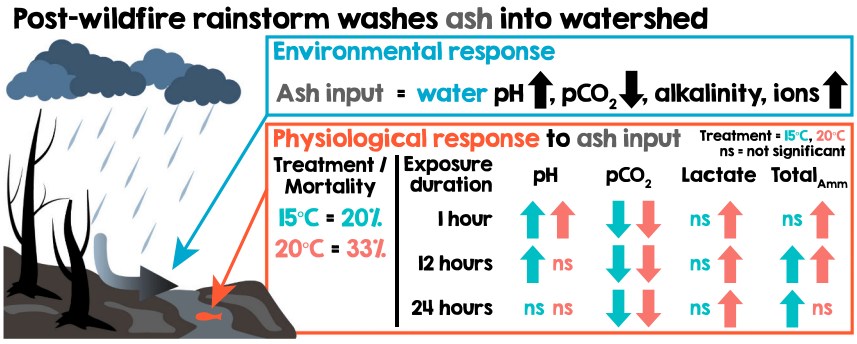
Garfield T. Kwan [a,*], Trystan Sanders [b], Sammuel Huang [a], Kristen Kilaghbian [a], Cameron Sam [a], Junhan Wang [a], Kelly Weihrauch [a], Rod W. Wilson [b], Nann A. Fangue [a]

[a] Department of Wildlife, Fish and Conservation Biology, University of California Davis, Davis, CA, United States
[b] Biosciences Department, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom

HIGHLIGHTS

- Post-wildfire ash-input rapidly alters carbonate chemistry of aquatic systems.
- Ash input increased water pH and reduced pCO_2, causing environmental alkalosis.
- 15 °C and 20 °C salmon experienced 20 and 33 % mortality within 12 h, respectively.
- Salmon regulated pH within 12–24 h, while pCO_2 remained low throughout exposure.
- Nitrogenous waste increased after exposure, but warmer temp expedited recovery.

GRAPHICAL ABSTRACT



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ABSTRACT

Changes in land use, a warming climate and increased drought have amplified wildfire frequency and magnitude globally. Subsequent rainfall in wildfire-scarred watersheds washes ash into aquatic systems, increasing water pH and exposing organisms to environmental alkalinization. In this study, 15 or 20 °C-acclimated Chinook salmon (*Oncorhynchus tshawytscha*) yearlings were exposed to an environmentally-relevant ash concentration (0.25 % *w/v*), increasing water pH from ~8.1 to ~9.2. Salmon experienced significant disturbance to blood plasma pH (pH_e) and red blood cell intracellular pH (RBC pH_i) within 1 h, but recovered within 24 h. Impacts on plasma ion concentrations were relatively mild, and plasma glucose increased by 2- to 4-fold at both temperatures. Temperature-specific differences were observed: 20 °C salmon recovered their pH_e more rapidly, perhaps facilitated by higher basal metabolism and anaerobic metabolic H^+ production. Additionally, 20 °C salmon experienced dramatically greater spikes in plasma total ammonia, [NH_3] and [NH_4^+] after 1 h of exposure that decreased over time, whereas 15 °C salmon experienced a gradual nitrogenous waste accumulation. Despite pH_e and RBC pH_i recovery and non-lethal nitrogenous waste levels, we observed 20 % and 33 % mortality in 15 and 20 °C treatments within 12 h of exposure, respectively. The mortalities cannot be explained by high water pH alone, nor was it likely to be singularly attributable to a heavy metal or organic compound released from ash input. This demonstrates post-wildfire ash input can induce lethal yet previously unexplored physiological

* Corresponding author.

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disturbances in fish, and further highlights the complex interaction with warmer temperatures typical of wildfire-scarred landscapes.

1. Introduction

Wildfires (controlled, cultural, and natural) are known to aid in pest removal and native plant reproduction (Pausas and Keeley, 2019), and are essential for healthy Mediterranean climate ecosystems (Syphard et al., 2007). The frequency and magnitude of wildfires across the world have increased, and this is attributed to anthropogenic factors including changes in land use, a warming climate, and increased droughts (Westerling et al., 2006; Huang et al., 2015; Pausas and Keeley, 2021). In the aftermath of wildfires, subsequent precipitation (snowmelt, rainstorms) can move ash across the charred watershed, increasing the concentration of sediments, trace elements, pollutants, and fire retardants in aquatic systems (Adams and Simmons, 1999; Giménez et al., 2004; Costa et al., 2014; Burton et al., 2016; Emelko et al., 2016; Raoelison et al., 2023; Whiterod et al., 2023). Adverse impacts to diverse aquatic organisms have been reported to slow development, alter behavior, shift food web and trophic transfer efficiency, and induce mass mortality events (Earl and Blinn, 2003; Spencer et al., 2003; Wells et al., 2004; Beganyi and Batzer, 2011; Nunes et al., 2017; Gonino et al., 2019; Cramp et al., 2021; Gomez Isaza et al., 2022; Lawrence et al., 2022; Muñiz González et al., 2023; Wall et al., 2024).

Wildfires are also known to alter the pH of freshwater systems. The ash-alkaline hypothesis proposes post-wildfire ash input releases alkaline elements (anions) into aquatic systems, which in principle should increase both pH and alkalinity levels (Bayley and Schindler, 1991). This increased pH and alkalinity have been documented in both wildfire mesocosm experiments (Silva et al., 2016; Cramp et al., 2021; Wall et al., 2024) and numerous ecological observations (Paul et al., 2022). For instance, one month after the 2012 High Park fire near Fort Collins (CO, United States), the pH of Cache la Poudre River increased from ~7.9 to ~8.5 (Son et al., 2015). In another case, two years after the 2007 Angora Fire at Lake Tahoe (CA, United States), Angora Creek pH in the unburned forest was ~6.25, whereas the pH at the site of the wildfire and downstream remained elevated at ~7.0 (Oliver et al., 2012). Moreover, algal growth in freshwater systems is stimulated by greater seasonal light availability, and when combined with increased nutrients from ash input (Spencer et al., 2003; Robson et al., 2018) can result in dramatic diurnal swings in water pH (Sherson et al., 2015). Other factors (e.g. baseline water pH, rainfall, water flow rate, soil pH, wildfire burn intensity) also influence these dynamic aquatic systems, and together these dramatic changes in water pH would undoubtedly challenge the acid-base regulatory capacities of aquatic organisms.

Current United States Environmental Protection Agency's Water Quality Criteria deems freshwater pH between 6.5 and 9.0 to be suitable for aquatic life (USEPA, 2013). Although the examples shared above did not exceed 9.0, many aquatic systems globally have naturally high pH (Pinheiro et al., 2021) averaging around ~8.0 (e.g. Putah Creek near Davis CA; Roaring Fork River near Glenwood Springs, CO; Snake River near Twin Falls, ID; Mississippi River near Fulton, IL), ~8.5 (e.g. Green River, UT; Lake Mattamuskeet, NC), and ~9.0 (e.g. Upper Klamath River, OR; Truckee River and Pyramid Lake, NV) (U.S. Geological Survey, 2016; Pyramid Lake Paiute Tribe, 2019) that are near to or periodically exceeds the upper limit of the recommended pH range (USEPA, 2013). These systems are not only more vulnerable to the alkalinizing impacts of post-wildfire ash-input, but they are also home to a variety of threatened and endangered species and could complicate their conservation management. Some of these fishes include the endangered coho salmon (*Oncorhynchus kisutch*) and threatened steelhead trout (*Oncorhynchus mykiss*) at Scott Creek, CA, the threatened Chinook salmon (*Oncorhynchus tshawytscha*) at Putah Creek, CA, the threatened green sturgeon (*Acipenser medirostris*) at Klamath River, OR, and the threatened Lahontan cutthroat trout (*Oncorhychus clarki henshawi*) at Truckee River and Pyramid Lake, NV. Moreover, anadromous fishes with fixed reproductive timelines such as salmon species and steelhead trout (many of which are also threatened or endangered) may be among the most vulnerable as the ionic, osmotic, and acid-base (IOA-B) regulation required to mitigate ash-induced alkalinization could further aggravate the spawning adults returning from the ocean and/or compromise their eggs and larval offspring by challenging their internal acid-base and nitrogenous waste regulation. Furthermore, organisms living in low alkalinity systems (Stoddard, 1987; Eilers et al., 1990; Catalan and Camarero, 1993; Clow et al., 1996) are also more susceptible to pH changes such as ash-induced alkalization. While this study focused primarily on the United States, other areas such as Canada (Reavie and Smol, 2001), Kenya (Eugster, 1980), Turkey (Danulat and Kempe, 1992; Danulat and Selcuk, 1992) and China (Wang et al., 2003) also have high pH systems that could be influenced by wildfire-ash input.

Despite over a century of investigation and many excellent reviews detailing IOA-B in teleost fish (Claiborne and Heisler, 1984; Cameron, 1989; Claiborne et al., 2002; Evans et al., 2005; Marshall and Grosell, 2006; Tresguerres et al., 2023; Kovac and Goss, 2024), only a handful of studies have investigated blood pH and acid-base response to respiratory alkalosis, which is induced by a sharp decrease in partial pressure of CO_2 (pCO_2) in the environment (Wilkie and Wood, 1991; Hemming and Hanson, 1992; Wilkie et al., 1993; McGeer and Eddy, 1998; Scott et al., 2005; Mcgeer et al., 2011). Teleosts exposed to high pH conditions experience an immediate increase in blood pH (pH_e) (Wilkie and Wood, 1991; Wilkie et al., 1993). In response, fish compensate for respiratory alkalosis by accumulating H^+ in their blood and expelling HCO_3^- into the water, primarily through their gill ionocytes and to a lesser extent renal transport processes (Cameron, 1978; Evans et al., 2005; Marshall and Grosell, 2006; Tresguerres et al., 2023). Rapid pH_e recovery is critical to survival: rainbow trout (*Oncorhynchus mykiss*) exposed to pH 9.5 water were able to stabilize their pH_e within 8 to 24 h of exposure (Wilkie and Wood, 1991), and the mortalities observed were associated with cannulation procedures rather than the acid-base stress. In contrast, Lahontan cutthroat trout (which naturally reside in pH 9.4 at Pyramid Lake (Nevada, United States)) were exposed to pH 10 but were unable to recover their pH_e, and >50 % died after 72 h of exposure (Wilkie et al., 1993). The inability to restore pH_e would impede nitrogenous waste excretion as gaseous NH_3 accumulates within the organism and excretion of ionic NH_4^+ is reduced, which can lead to ammonia toxicity and mortality (Wilkie and Wood, 1991, 1996; Wilkie et al., 1993). In response, organisms typically increase their metabolic activity to regulate the acid-base disturbance, resulting in elevated glucose and lactate levels (Wilkie and Wood, 1991, 1996; Wilkie et al., 1993).

Higher ambient temperature typical of aquatic systems following wildfires (Paul et al., 2022; Warren et al., 2022) may further intensify the acid-base and ammonia challenge. Warmer temperature inevitably elevates basal metabolic demands, promotes faster ammonia production, and decreases the energy budget available for mitigating the IOA-B disturbance (Gomez Isaza et al., 2022). However, the increased metabolism elevates internal pCO_2 levels, which may allow for greater H^+ generation to recover blood pH_e - though no prior studies have examined the interactions between temperature and respiratory alkalosis. As such, post wildfire ash-input represents a significant IOA-B challenge requiring greater research and consideration. To the best of our knowledge, no study has ever examined teleost IOA-B response to ash-induced respiratory alkalosis, nor their concurrent response to warmer conditions.

The goals of this study were to quantify how environmentally-

relevant levels of ash input alters water quality, and to assess the fish's initial IOA-B response to respiratory alkalinization. Given the natural pH and alkalinity range of water bodies and the wide array of biotic and abiotic factors that could influence water quality, it is important to pair the organism with an appropriate water source and ash derived from native vegetation. In this study, adult Chinook salmon were selected as our model organism as their natural spawning and rearing habitats overlap with wildfire-prone habitats (Westerling et al., 2006; Bryant and Westerling, 2014; Kramer et al., 2019), which includes across the San Francisco Bay Delta Estuary (e.g. the LNU Lightning Complex Fire in 2020 burned the upper Putah Creek watershed and areas surrounding Lake Berryessa). Moreover, the riparian habitat in the Putah Creek watershed (which includes oak and redwood trees) is regularly cleared with controlled fires. Using the ash collected from these controlled fires, we characterized the impacts of ash concentration to determine the appropriate ash concentration for a 1 pH unit increase as seen in past environmental observations (Paul et al., 2022). Next, we sought to determine the initial and short-term biological response to an environmentally-relevant level of ash-induced respiratory alkalosis. Chinook salmon were acclimated to 15 or 20 °C, and these temperatures were selected to represent downstream or at the site of the burn, respectively. After a two-week acclimation period, Chinook salmon blood was sampled without ash exposure or after 1, 12, or 24 h of ash exposure (0.25 % *w/v*) for a suite of ionic and acid-base parameters.

Given the responses of fish characterized in past acid-base studies, we hypothesized that ash-input would challenge Chinook salmon with respiratory alkalosis, which would manifest as an increase in pH_e and intracellular red blood cell pH (RBC pH_i) as well as a simultaneous decrease in pCO_2 and plasma bicarbonate concentration ($[HCO_3^-]$) within 1 h of exposure. We expected Chinook salmon to recover their pH_e and pH_i within 12 h of exposure by accumulating $[H^+]$ in their blood, which would in turn reduce the counterions $[HCO_3^-]$ and $[Cl^-]$. Given that fish metabolic rates (and thereby elevated $[HCO_3^-]$) are higher at elevated temperatures, we hypothesized that 20 °C salmon would more quickly recover their pH_e than 15 °C salmon. Moreover, the stress response and impairment to nitrogenous waste excretion should be reflected by elevated glucose, lactate, total ammonia, $[NH_3]$ and $[NH_4^+]$ levels until pH_e is recovered. Finally, we did not anticipate fish mortalities given the Chinook salmon were exposed to less alkaline pH levels (~9.2) than those used in past studies (Wilkie and Wood, 1991: pH 9.5; Wilkie et al., 1993: pH 10.0).

2. Methods

2.1. Water quality measurements within experimental condition

Experimental water temperature, dissolved O_2 (DO), pH and salinity were measured daily using YSI 556 MPS (Yellow Springs, Ohio, USA). Discrete water samples were taken for alkalinity following manufacturer instructions (HACH digital titrator, HACH; Loveland, CO, USA), and an end point (pH 4.3) was detected using a pH microelectrode (HI1083B, Hanna Instruments, Woonsocket, RI, United States) and meter (HI8424, Hanna Instruments). Turbidity was measured (HACH 2100Q Handheld Turbidity Meter) following manufacturer instructions. Alkalinity, pH, salinity, and temperature values were used to calculate pCO_2 using CO2SYS (version 1.05; Lewis and Wallace, 1998). The parameters pH, alkalinity, pCO_2, salinity, turbidity and DO were measured in water mixed with controlled fire ash at concentrations ranging from 0.1 to 3 % (*w/v*) to determine a level most relevant for environmental comparison.

Filtered experimental water (0.45 μm) was collected before and after ash-dosing for elemental analysis, mixed at a 13:1 ratio with 1 % nitric acid (Certified ACS Plus), stored in a sterile plastic container at room temperature, then analyzed by the Interdisciplinary Center for Plasma Mass Spectrometry at the University of California at Davis (additional details on website: ICPMS.UCDavis.edu) using an Agilent 8900 ICP-MS Triple Quad instrument (Agilent Technologies, Santa Clara, CA 95051).

Ash treatments were repeated in retrospect in March 2024 without fish, at the University of Exeter (United Kingdom) to determine ash-related release of ammonia, nitrate, nitrite and several cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and anions (Cl^-, PO_4^{3-}, SO_4^{2-}). Well water (2 × 500 mL, one for each temperature) and ash were mixed (0.25 % *w/v*) using a magnetic stirrer for 30 min and held in a water bath (15 °C or 20 °C). Water was sampled by gently swirling bottles and sampling from the surface (<5 cm depth) at 0 h (pre-ash), 1 h and 24 h, syringe filtered (0.22 μm) and stored for later analyses. Total ammonia ($NH_3 + NH_4^+$) was measured using a modified indophenol blue colorimetric assay (Ma et al., 2018) and all other ions including nitrate and nitrite were measured by ion chromatography using a 930 compact IC flex (Metrohm, Switzerland).

2.2. Water quality of field condition

Spot measurements of several aquatic systems local to Davis (California, United States) were used to determine the relevant experimental treatment (explained below). These include fresh rainwater (collected in plastic buckets and measured in the rain) and mud puddles around CABA, river water at the nearby Putah Creek, and lake water at Lake Berryessa (collected at UC Davis Putah Creek Facility) measured between February to April 2023.

2.3. Assessing ash impact on well and deionized water

Leachate tests were performed on well and deionized (DI) water to illustrate the mixing of ash and alkalinity. Briefly, sieved ash (pore size = 0.841 mm) were mixed with CABA well water (0, 0.1, 0.25, 0.5, 1, 3 %) or DI water (0, 0.25 %) and stirred with a magnetic stirrer for 5 min. Next, their temperature, DO, pH, salinity, alkalinity, and turbidity were measured as previously described. As a reference, leachate methods detailed in USGS Field sampling guide and Burton et al. (2016) use a 5 % w/v mixture.

2.4. Fish husbandry condition

This experiment was conducted in April and May 2023 in accordance with protocol no. 23316 in compliance with the Institutional Animal Care and Use Committee (IACUC) at the Center for Aquatic Biology and Aquaculture (CABA) at University of California Davis (UCD). Fall-run Chinook salmon hatched on December 4th 2021 at the Feather River Hatchery (Oroville, CA, United States) were transferred to CABA on January 31st 2022, and reared in a flow-through well-water system at 13–15 °C and fed dry pellets at 4 % body mass per day to achieve ad libitum. The walls of individual treatment tanks were insulated, and the tanks were housed within an outdoor structure at CABA. Treatment temperatures of 15 and 20 °C were achieved by mixing ambient and heated well-water, and inflow and tank temperatures were checked daily. Salmon were acclimated to their respective temperatures for at least 2 weeks to establish thermal acclimation (Fangue et al., 2006, 2008; McKenzie et al., 2021) before experimentation between March to May 2023. Fish (fork length: 17.5 ± 0.2 cm, body mass: 61.0 ± 2.2 g) were starved for 24 h prior to experimentation.

2.5. Experimental condition

The ash used in this experiment was derived from a combination of local oak trees burnt in a furnace and local control burning of pomegranate, oak and redwood trees. An experimental exposure of 0.25 % ash was achieved by mixing 250 g of ash into a 100 L tank. Each tank was stocked at a density of 6–8 fish per tank, with 2–3 replicate tanks per temperature treatment and per timepoint. Water quality parameters were measured and collected prior to ash mixing, and at the end of each respective exposure period. To minimize disturbance, ash was mixed with water in an external bucket, then transferred into the experimental

chambers using aquarium submersible pumps. CABA well water inflow was halted during the experimental ash exposure. Air bubbling and a second submersible pump within the experimental tanks assisted with water mixing. Ash input increased the pH of 15 and 20 °C treatment water from 8.13 and 8.06 to 9.27 and 9.17, respectively (Table 1). Temperature remained relatively consistent throughout exposure, though the 15 °C treatment warmed slightly over time due to the warmer air temperature (Table 1). Ash input increased pH, alkalinity, and salinity, but decreased pCO_2 (Table 1). Sample size at each timepoint were as followed: 0 h (no ash exposure; 15 °C: $n = 9$; 20 °C: $n = 10$), 1 h (15 °C: n = 9; 20 °C: $n = 5$), 12 h (15 °C: n = 9; 20 °C: $n = 11$), 24 h (15 °C: $n = 12$; 20 °C: $n = 14$).

2.6. Blood sampling and analysis

All blood sampling took place between 8:00 and 13:00. We sampled blood without the use of cannulation via a gill irrigation technique described in past studies (Harter et al., 2021; Montgomery et al., 2022; Kwan and Tresguerres, 2022; Davison et al., 2023). After ash exposure reached the designated timepoint, the entire tank of fish was anesthetized by pouring a benzocaine stock solution through an extended tube that is out of view of the fish to minimize disturbance to achieve a concentration of 75 mg/L benzocaine in the tank. After loss of equilibrium (~3 min), fish were moved to a surgery table where their gills were irrigated with aerated treatment water with maintenance anesthetic (benzocaine, 30 mg/L). Blood (~500–1000 μL) was drawn from a caudal vessel using a heparinized syringe (21 gage needle; 100 IU lithium heparin) typically within 5 min. Blood pH_e was measured using a micro pH electrode (HI1083B, Hanna Instruments) at the treatment temperature by buoying the microcentrifuge tube within the respective experimental water. Samples were then transported on ice, and a portion of the blood (65 μL per run; 1–3 sample per fish) was analyzed using the ABL90 Flex Plus (Radiometer, Copenhagen, Denmark) within 5 min of sampling to measure blood pCO_2 and the concentration of Na^+, K^+, Cl^-, Ca^{2+}, glucose and lactate. The remainder of the sample was spun for 2 min on a tabletop centrifuge, and the separated RBC and plasma fractions were flash frozen with liquid N_2 for later intracellular pH, total ammonia and total CO_2 (TCO_2) measurements. RBC pH_i was measured using the freeze-thaw technique (Zeidler and Kim, 1977). Following best practices (Baker et al., 2009a), pH_i was measured with a pH microelectrode (HI1083B, Hanna Instruments) within two weeks after the conclusion of the experiment to limit pH change.

ABL90-derived pCO_2 values were temperature-corrected using the following equation (Siggaard-Andersen, 1974).

$$pCO_2(T) = pCO_2(37)*10^{[0.021*(T-37)]}$$

In addition, ABL90-derived blood pCO_2 measurements were validated by TCO_2 measurements analyzed with a carbon dioxide analyzer (Corning 965, Ciba Corning Diagnostic, Halstead, Essex, United Kingdom). Blood pH_e and pCO_2 values were used to calculate $[HCO_3^-]$ using the Henderson-Hasselbalch equation. The solubility coefficient of CO_2 (0.0578 mmol l^{-1} $Torr^{-1}$), ionic strength (0.15 M), and pK_1 (6.20) were based upon Boutilier et al. (1984). Plasma total ammonia ($[T_{Amm}]$ i.e. $[NH_3 + NH_4^+]$) was determined spectrophotometrically in 25 μL aliquots of flash frozen plasma by enzymatic ammonia assay (Sigma-Aldrich, USA, Catalog number: AA0100). $[T_{Amm}]$ was calculated from the delta absorbance at 340 nm wavelength before and after the addition of the enzyme L-glutamate dehydrogenase. Absorbance was measured in Greiner UV-star® 96-well plates using a microplate reader (Infinite® M200 PRO, Tecan, Switzerland). Finally, pNH_3 and $[NH_4^+]$ were calculated with the Henderson-Hasselbalch equation using the solubility coefficients and pK_{Amm} values from Cameron and Heisler (1983).

Table 1
Water quality before (Pre-Ash Exposure) and after (Post-Ash Exposure) ash was added to experimental tanks. Values are mean ± SEM.

	15 °C		20 °C	
	Pre-ash exposure	Post-ash exposure	Pre-ash exposure	Post-ash exposure
pH	8.13 ± 0.01	9.27 ± 0.09	8.06 ± 0.01	9.17 ± 0.09
DO (mg/L)	9.98 ± 0.15	9.89 ± 0.10	8.76 ± 0.15	8.00 ± 0.13
Temperature (°C)	15.6 ± 0.1	16.5 ± 0.3	20.0 ± 0.2	19.9 ± 0.1
Alkalinity (μmol/kg)	3954 ± 157	4694 ± 342	3787 ± 245	4732 ± 394
pCO_2 (μatm)	1378 ± 65	84 ± 22	1680 ± 114	95 ± 29
Salinity (ppt)	0.44 ± 0.002	0.54 ± 0.015	0.45 ± 0.001	0.54 ± 0.009

2.7. Statistical analysis

Statistical analyses were performed using *R* (version 4.0.3) (R Development Core Team, 2013). Water quality parameters were analyzed with two-tailed Student's *t*-test, one-way Analysis of Variance (ANOVA), Analysis of Covariance (ANCOVA) and linear regressions with water source and ash input as covarying factors. All figures were generated using the package *ggplot2* (Wickham, 2009). The regression plots for water pH, alkalinity and pCO_2 with 95 % confidence intervals were estimated using *geom_smooth* (*method* = "*log*(*x* + *0.00001*)"). The regression plots for water salinity, turbidity and DO with 95 % confidence intervals were estimated using *geom_smooth* (*method* = "*lm*"). Differences in slopes were assessed with the interaction variable of ANCOVA, with alkalinity (CABA well water or DI water) and ash input (0 or 0.25 % *w/v*) as co-variables. Blood and plasma variables were analyzed with linear mixed-effects models using the package *nlme* (Pinheiro et al., 2014), with temperature and ash exposure duration as fixed factors and tanks and individuals as random factors. Posthoc tests were conducted using least-square means with the package *emmeans* (Lenth, 2021). Normality and homogeneity of residuals were assessed through visual inspection of QQ plots and residual boxplots, respectively. An alpha level of 0.05 was used for significance in all statistical tests. Unless noted otherwise, results were reported as mean ± SEM.

3. Results

3.1. Part 1: water quality impact from ash input

Water quality parameters were measured from fresh rain and mud puddles, DI water, CABA well water, Putah Creek, and Lake Berryessa (Supplemental Fig. 1). Fresh rainwater (pH ~6.2, alkalinity ~40 μmol/kg) was more acidic and less buffered than the adjacent mud puddles (pH ~7.3, alkalinity ~300 μmol/kg; Supplemental Fig. 1A, B) reflected the slightly alkaline soil reported around Davis, CA (Walkinshaw et al., 2022). DI water (pH ~7.0, alkalinity ~40 μmol/kg) was neutral in pH, but had a similar level of alkalinity as rainwater (Supplemental Fig. 1A, B). In contrast, natural overland water bodies such as Putah Creek and Lake Berryessa have pH ~8.3 and alkalinity ~1800 μmol/kg, and these elevated values were within nominal range of natural freshwater systems around the world (Pinheiro et al., 2021) and were likely attributed to mineral absorption from soil and rock erosion over time. Well water had similar pH (~8.1) to that of overland water, but its alkalinity (~3700 μmol/kg) was more than double that of Putah Creek and Lake Berryessa (Supplemental Fig. 1A, B) likely due to its prolonged interaction with minerals as it permeated through the groundwater system. This also explains the elevated pCO_2 levels as it could not equilibrate with the atmosphere. Turbidity was greatest in the fresh mud puddles, followed by Putah Creek and Lake Berryessa. In contrast, fresh rainwater, DI water and CABA well water had virtually no detectable turbidity (Supplemental Fig. 1C). Lastly, salinity was highest in CABA well water at 0.5 ppt, followed by Putah Creek and Lake Berryessa. In contrast, fresh rainwater, mud puddles and DI water had virtually no detectable salinity (Supplemental Fig. 1D).

The relationship between ash input and pH, alkalinity, pCO_2, salinity and turbidity across concentration was examined by mixing CABA well water with 0, 0.1, 0.25, 0.5, 1, and 3 % (*w/v*) locally burned ash. As expected, ash input induced a logarithmic increase in water pH ($p = 0.0001$; Fig. 1A) and alkalinity ($p < 0.0001$; Fig. 1B), and a logarithmic decrease in pCO_2 ($p = 0.0590$; Fig. 1C). In contrast, ash input linearly increased salinity ($p < 0.0001$; Fig. 1D) and turbidity ($p = 0.0054$; Fig. 1E), and did not affect DO ($p = 0.7330$; Fig. 1F). The magnitude of pH change induced by ash input was dependent on the starting alkalinity of the water. To demonstrate this, the responses of DI and well water to 0.25 % (w/v) ash input were compared. The rate of pH increase ($p < 0.0001$; Fig. 2A) and pCO_2 decrease ($p = 0.0041$; Fig. 2C) were different between CABA well water and DI water during ash exposure. In contrast, the rate of ash-induced changes in alkalinity ($p = 0.1131$; Fig. 2B), salinity ($p = 0.4780$; Fig. 2D), and turbidity ($p = 0.1360$; Fig. 2E) were relatively similar. DO slightly but significantly increased as the DI water slowly equilibrated with the atmosphere ($p = 0.0465$; Fig. 2F).

ICP-MS analysis of treatment water before or following 1, 12, or 24 h of ash input identified 25 of 31 elements within detectable range (Supplemental Figs. 2–4). None of the 25 elements was affected by temperature, nor was there significant decline in their concentrations after ash input. Instead, ash input significantly increased the concentration of 13 elements: Al, B, Ba, Cr, K, Li, Mo, Mn, Ni, P, Sb, Se, and Sn (Supplemental Figs. 2–4).

Subsequent experiment examining ion release from ash input revealed significant increases in cations (Na^+, K^+, Mg^{2+}, Ca^{2+}: all $p < 0.0001$), anions (Cl^- $p = 0.0012$; NO_3^-, PO_4^{3-}, SO_4^{2-}: $p < 0.0001$) and total ammonia levels ($p < 0.0001$) after 1 and 24 h (Supplemental Figs. 5, 6). In contrast, $[NO_2^-]$ was below the detection limits of the ion-chromatography column (<0.5 μM).

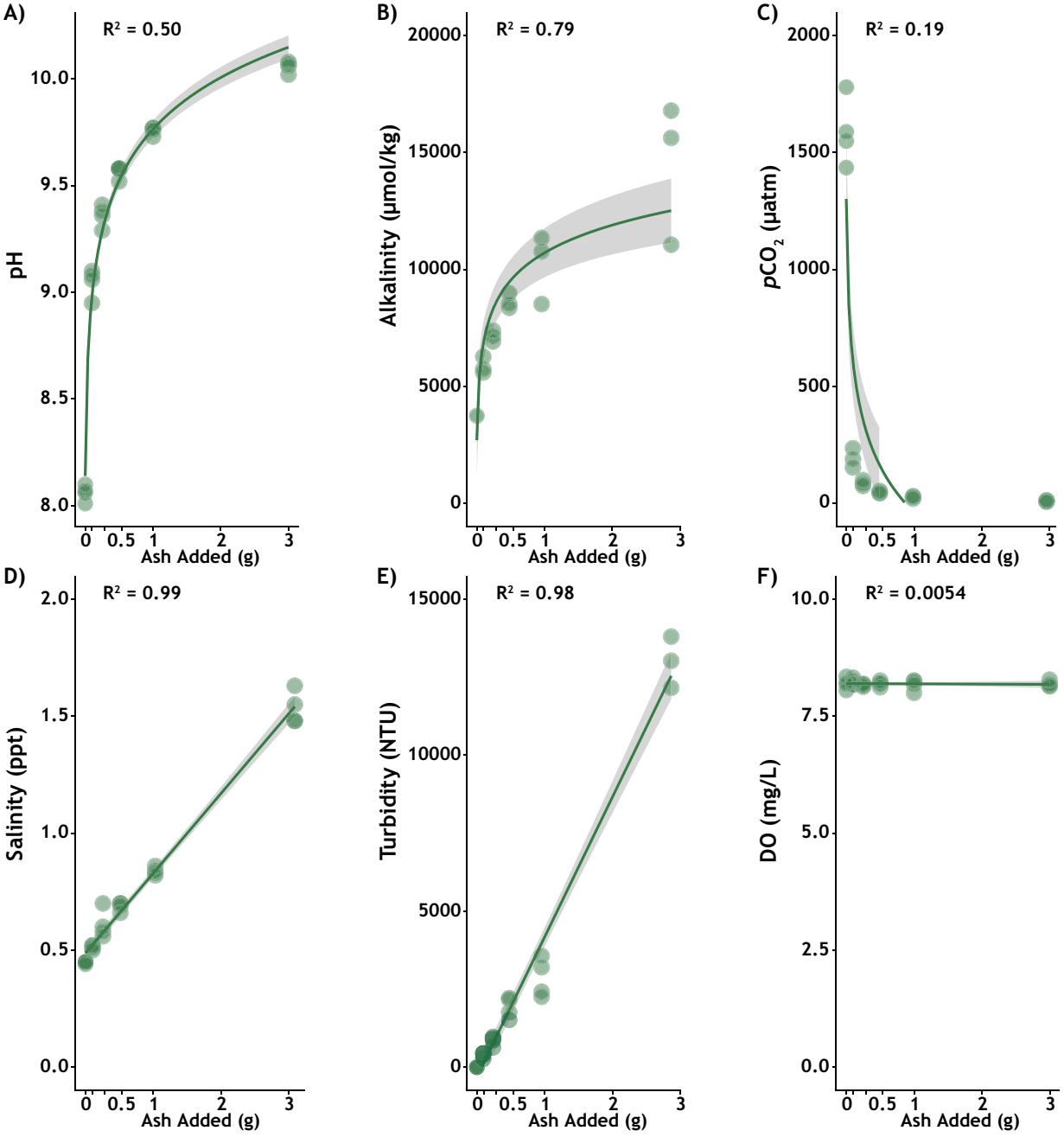


Fig. 1. Center for Aquatic Biology and Aquaculture (CABA) well water and ash dosing curve. The impact of adding 0.1, 0.25, 0.5, 1, and 3 % ash (*w/v*) on CABA well water A) pH, B) alkalinity, C) pCO_2, D) salinity, E) turbidity, and F) dissolved oxygen (DO) levels after 5 min of mixing. Shaded area denotes 95 % confidence interval.

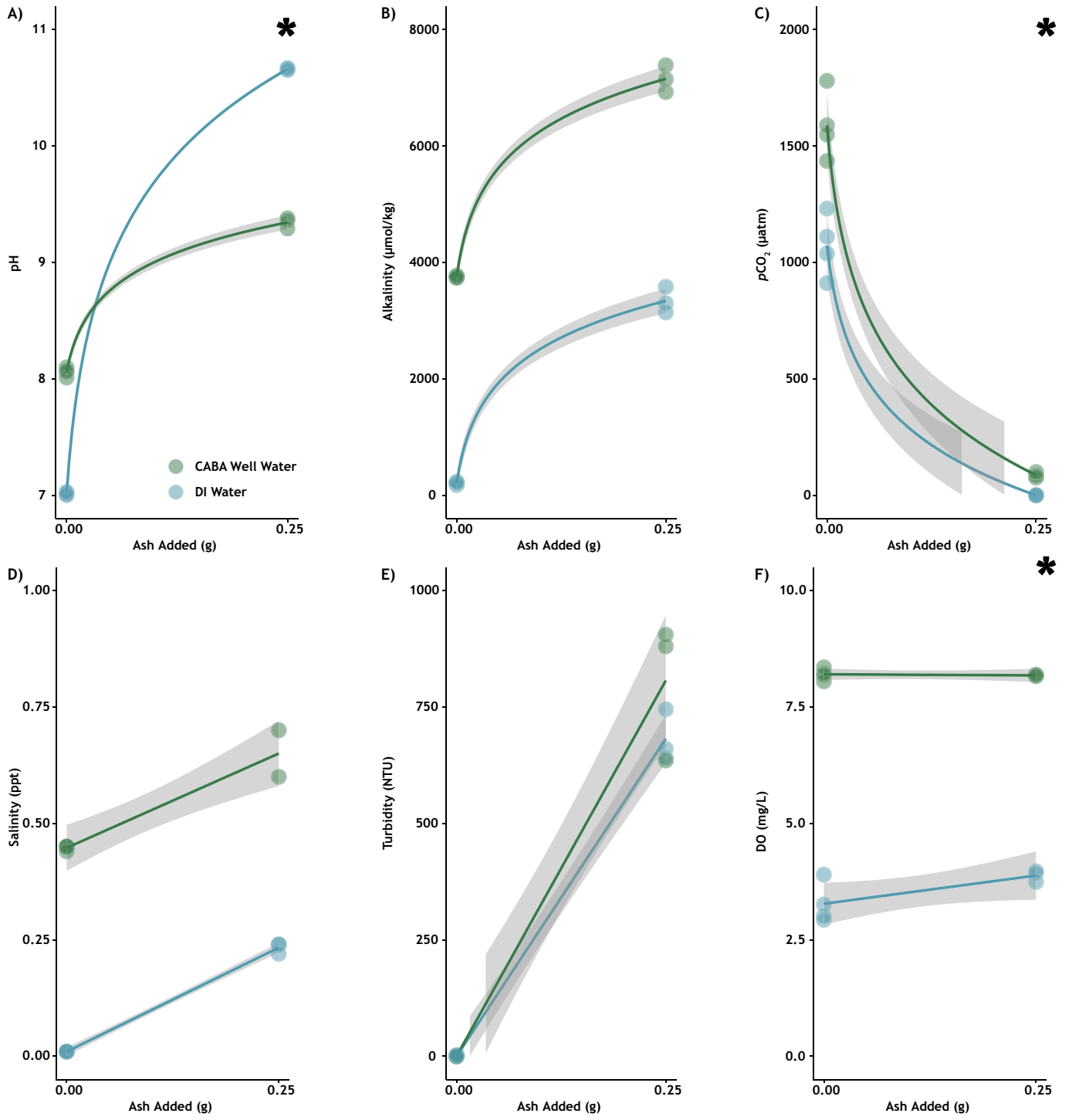


Fig. 2. Influence of alkalinity on ash-induced water chemistry change. Comparison of deionized water (DI; blue) and well water at the Center for Aquatic Biology and Aquaculture (CABA; green) at University of California Davis with and without 0.25 % (w/v) ash input on A) pH, B) alkalinity, C) pCO_2, D) salinity, E) turbidity, and F) dissolved oxygen (DO) levels after 5 min of mixing. Shaded area denotes 95 % confidence interval. Asterisk indicates significance ($\alpha = 0.05$) between treatments.

3.2. Part 2: blood response to ash exposure in 15 and 20 °C acclimated Fish

In this experiment, the mixing of CABA water and ash input of 0.25 % (w/v) alkalinized water pH from ~8.1 to ~9.2 – a level slightly higher than the EPA recommended upper limit of ~9.0 (USEPA, 2013) yet demonstrated to be within tolerable range by the related rainbow trout (Wilkie and Wood, 1991). Here, Chinook salmon yearlings acclimated to 15 or 20 °C were exposed to 0, 1, 12, or 24 h of 0.25 % (w/v) ash. In total, we observed 20 % (6 of 30) and 33.3 % (10 of 30) mortalities in the 15 °C and 20 °C treatments, respectively, all of which occurred between 1 and 12 h of ash exposure. In contrast, control salmon were reared in adjacent experimental tanks (~6–8 fish per tank; 2 tanks per temperature treatment) and a holding tank (~300 fish; ~15 °C) were not exposed to ash, and they experienced no mortalities throughout the duration of the experiment.

Prior to ash exposure, the pH_e of salmon reared at 15 °C (7.81 ± 0.02) were slightly but not significantly lower than those reared at 20 °C

(7.92 ± 0.02; $p = 0.4229$; Fig. 3A). In contrast, RBC pH_i (15 °C: 7.55 ± 0.02, 20 °C: 7.56 ± 0.02) were not significantly different between treatments ($p = 1.0000$; Fig. 3B). Salmon acclimated to 20 °C had significantly higher pCO_2 (4615 ± 269 μatm; $p = 0.0206$) than 15 °C acclimated fish (3583 ± 251 μatm; Fig. 3C). Warmer temperature also significantly elevated baseline plasma [HCO_3^-] ($p < 0.0001$) (15 °C: 4.75 ± 0.13 mM; 20 °C: 7.06 ± 0.16 mM; Fig. 3D).

Within minutes of ash input, water pH rapidly increased from ~8.1 to ~9.2 while pCO_2 simultaneously decreased from 1400–1700 to ~100 μatm (Table 1), challenging the salmon with acute respiratory alkalosis and hypocapnia. This study showed 1 h was not sufficient time for the salmon to mitigate the acid-base disturbance: both pH_e (15 °C: $p <$ 0.0001, 20 °C: $p = 0.0166$) and RBC pH_i (15 °C: $p = 0.0005$, 20 °C: $p =$ 0.0275) were significantly elevated compared to their respective baselines (Fig. 3A, B). Instead, salmon acclimated to 20 °C returned to baseline pH_e levels within 12 h ($p = 1.0000$), whereas salmon acclimated to 15 °C needed 24 h to no longer be significantly different from baseline ($p = 0.2595$). Salmon RBC pH_i for both treatments recovered within 12 h of exposure (15 °C: $p = 0.5672$, 20 °C: $p = 1.0000$). In contrast, pCO_2 (15 °C: $p = 0.0044$, 20 °C: $p = 0.0004$) significantly decreased after 1 h of ash exposure and remained significantly lower than control levels regardless of if it was measured with the Corning CO_2 analyzer (Fig. 3C) or with ABL90-derived measurements (Supplemental Fig. 7A, B). Moreover, the ABL90 and Corning analyzer-derived pCO_2 measurements formed a significantly linear relationship ($p < 0.0001$; $r^2 = 0.7321$; Supplemental Fig. 7A). With the exception that the decrease

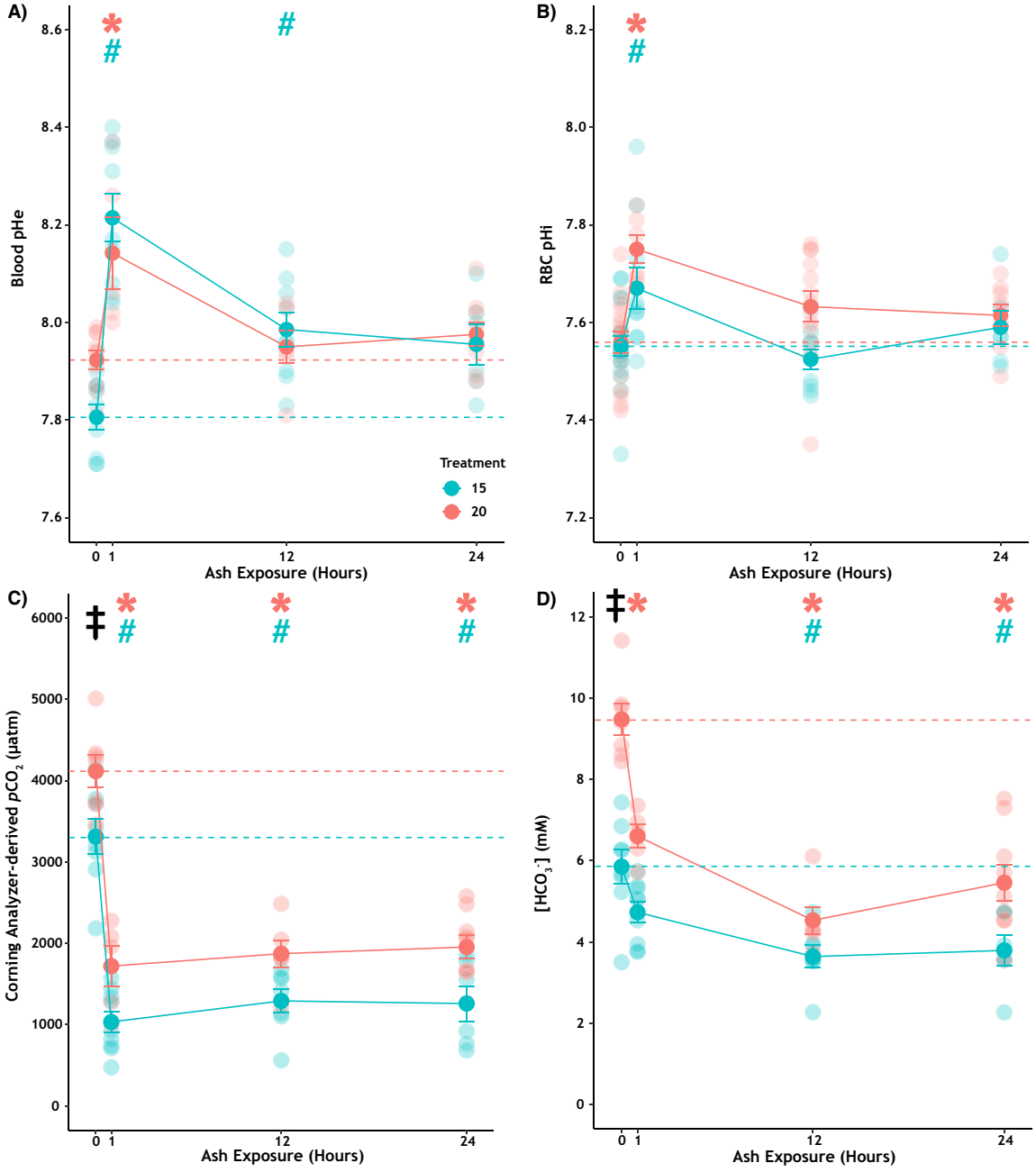


Fig. 3. Blood acid-base response to ash-induced pH ~9.2 exposure at 15 (teal) and 20 °C (red). Salmon blood A) pH_e, B) red blood cell (RBC) pH_i, C) pCO_2 and D) [HCO_3^-] over the 0, 1, 12, and 24 h of ash exposure. Values are mean ± SEM. Dotted lines represent the 0 h control levels. Black diesis (double dagger) indicates significance (α = 0.05) between the 15 and 20 °C 0 h control levels. Significance from their 0 h control levels are indicated with a teal # for 15 °C and red * for 20 °C salmon, respectively.

between 0 and 1 h within 15 °C fish was not statistically significant, plasma [HCO_3^-] (15 °C and 20 °C: $p < 0.0001$) calculated with the Corning CO_2 analyzer significantly decreased with ash exposure (Fig. 3D). Plasma [HCO_3^-] calculated with the ABL90 also significantly decreased after 1 h of ash exposure, and further declined with prolonged exposure (Supplemental Fig. 7C).

Comparison between temperature treatments prior to ash exposure revealed plasma [Na^+] ($p = 0.0326$) and [Cl^-] ($p = 0.0025$) were significantly higher in 15 °C-acclimated salmon, but no difference was detected in plasma [K^+] ($p = 1.0000$) and [Ca^{2+}] ($p = 0.9867$; Fig. 4). In

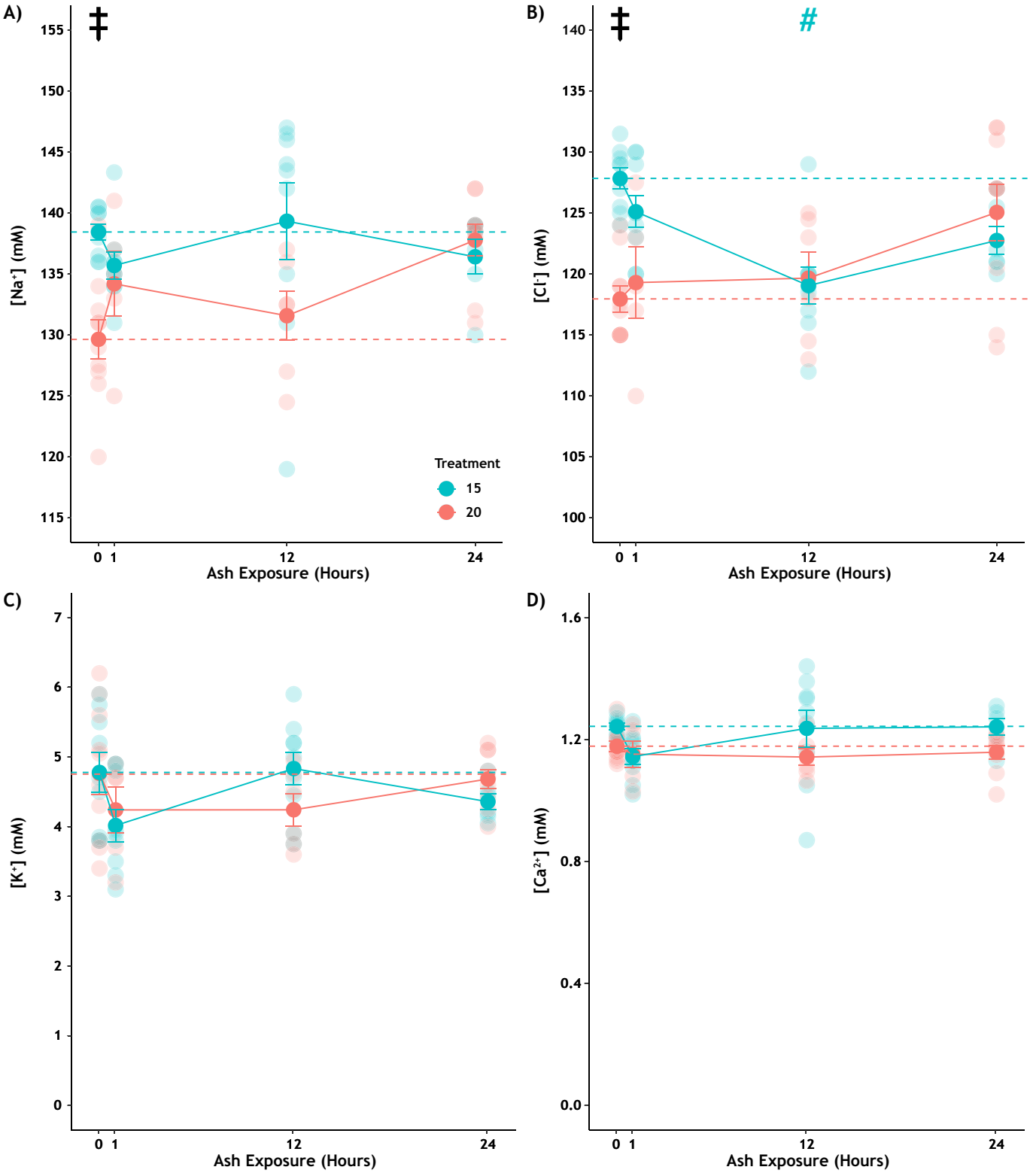


Fig. 4. Plasma ion response to ash-induced pH ~9.2 exposure at 15 (teal) and 20 °C (red). Salmon plasma A) [Na^+], B) [Cl^-], C) [K^+] and D) [Ca^{2+}] over the 0, 1, 12, and 24 h of ash exposure. Values are mean ± SEM. Dotted lines represent the 0 h control levels. Black diesis (double dagger) indicates significance ($\alpha = 0.05$) between the 15 and 20 °C 0 h control levels. Significance from their 0 h control levels are indicated with a teal # for 15 °C salmon.

both temperature treatments, ash exposure did not significantly affect plasma [Na^+], [K^+] and [Ca^{2+}] (Fig. 4A, C–D). In contrast, plasma [Cl^-] after 12 h of exposure in 15 °C salmon was significantly lower than 0 h controls ($p = 0.0110$), but this pattern was not observed in 20 °C salmon ($p = 1.0000$; Fig. 4B). Plasma glucose in 15 °C fish significantly increased by 24 h of ash exposure ($p < 0.0001$) and in 20 °C salmon after 12 h ($p = 0.0054$) and 24 h of exposure ($p = 0.0029$; Fig. 5A). Plasma lactate in both temperature treatments spiked at 1 h (15 °C: $p = 0.1240$, 20 °C: $p = 0.0004$) and decreased over the next 12 (15 °C: $p = 0.9679$, 20 °C: $p = 0.0294$) and 24 h (15 °C: $p = 0.9503$, 20 °C: $p = 0.0421$), but only those of 20 °C salmon were statistically significant (Fig. 5B).

Plasma total ammonia generally increased with ash exposure (Fig. 6A), but the two temperature treatments exhibited different response patterns: plasma total ammonia in 15 °C salmon gradually increased until it was significantly higher than baseline levels at 12 ($p = 0.0481$) and 24 h of exposure ($p = 0.0029$), whereas 20 °C salmon experienced a 7.5-fold increase after 1 h of exposure ($p < 0.0001$), remained significantly ~3-fold elevated at 12 h of exposure ($p = 0.0055$), and finally tapered down to non-significant levels at 24 h of exposure ($p = 0.1914$; Fig. 6A). [NH_3] in 15 °C salmon was not significantly elevated relative to control levels regardless of ash exposure duration (Fig. 6B). In contrast, [NH_3] in 20 °C salmon was significantly elevated at 1 h ($p < 0.0001$), but returned to control levels by 12 ($p = 0.0558$) and 24 h of exposure ($p = 0.3397$; Fig. 6B). Finally, plasma [NH_4^+] followed a similar pattern: [NH_4^+] in 15 °C salmon was significantly elevated after 12 ($p = 0.0450$) and 24 h of ash exposure (p = 0.0025), whereas [NH_4^+] in 20 °C salmon was significantly elevated after 1 ($p < 0.0001$) and 12 h of exposure ($p = 0.0052$), but returning back to control levels by 24 h ($p = 0.1900$; Fig. 6C).

4. Discussion

As predicted, ash input into DI water (~0 alkalinity/buffering capacity) resulted in a significantly greater pH increase than when ash is added into highly alkaline well water. This suggests organisms inhabiting aquatic systems with naturally low alkalinity and/or supplied with rainfall or snowmelt such as in mid- to high-elevation streams (Stoddard, 1987; Eilers et al., 1990; Catalan and Camarero, 1993; Clow et al., 1996) would be more affected by ash-induced acid-base disturbance. Conversely, CABA well water has relatively high pCO_2 and alkalinity levels (Fig. 1), which would not only dampen ash-induced pH increase (Fig. 2) but also provide more counterions for IOA-B regulation (Fig. 1D; Supplemental Figs. 2–5). As such, the results of the present study may represent an underestimation of fish blood responses.

As we had hypothesized and in agreement with past alkalosis studies, Chinook salmon exposed to pH ~9.3 initially experienced elevated blood pH_e and RBC pH_i and decreased plasma pCO_2 and [HCO_3^-]. Most (but not all) salmon recovered their pH_e and pH_i from the ash-induced respiratory alkalinization within 12 h of exposure as we observed a 20 % and 33.3 % mortality rate in Chinook salmon reared at 15 °C and 20 °C, respectively. These mortalities occurred between 1 and 12 h of exposure, which suggests their cause of death could have been attributed to failure to quickly regulate their pH_e. The greater mortality rate at the warmer temperature could not be explained by the greater acid-base disturbance (in fact it was less disturbed in the warmer fish) nor by their LC_{50} (which tends to increase with warmer temperatures; see Reinbold and Pescitelli, 1982). Instead, the greater mortality may correlate better with the rapid and substantial increase in plasma total ammonia. Although not directly comparable due to differences in experimental parameter and species, mortality was more acutely apparent when water alkalization was induced with ash input (this study) rather than with NaOH (Wilkie and Wood, 1991) or with naturally alkaline lake water (Wilkie et al., 1993). Moreover, Wilkie and Wood (1991) reported one rainbow trout perished during 72 h of exposure to pH 9.5, and in a subsequent experiment reported in the same study no rainbow trout mortality during a 5-week exposure to pH 9.5.

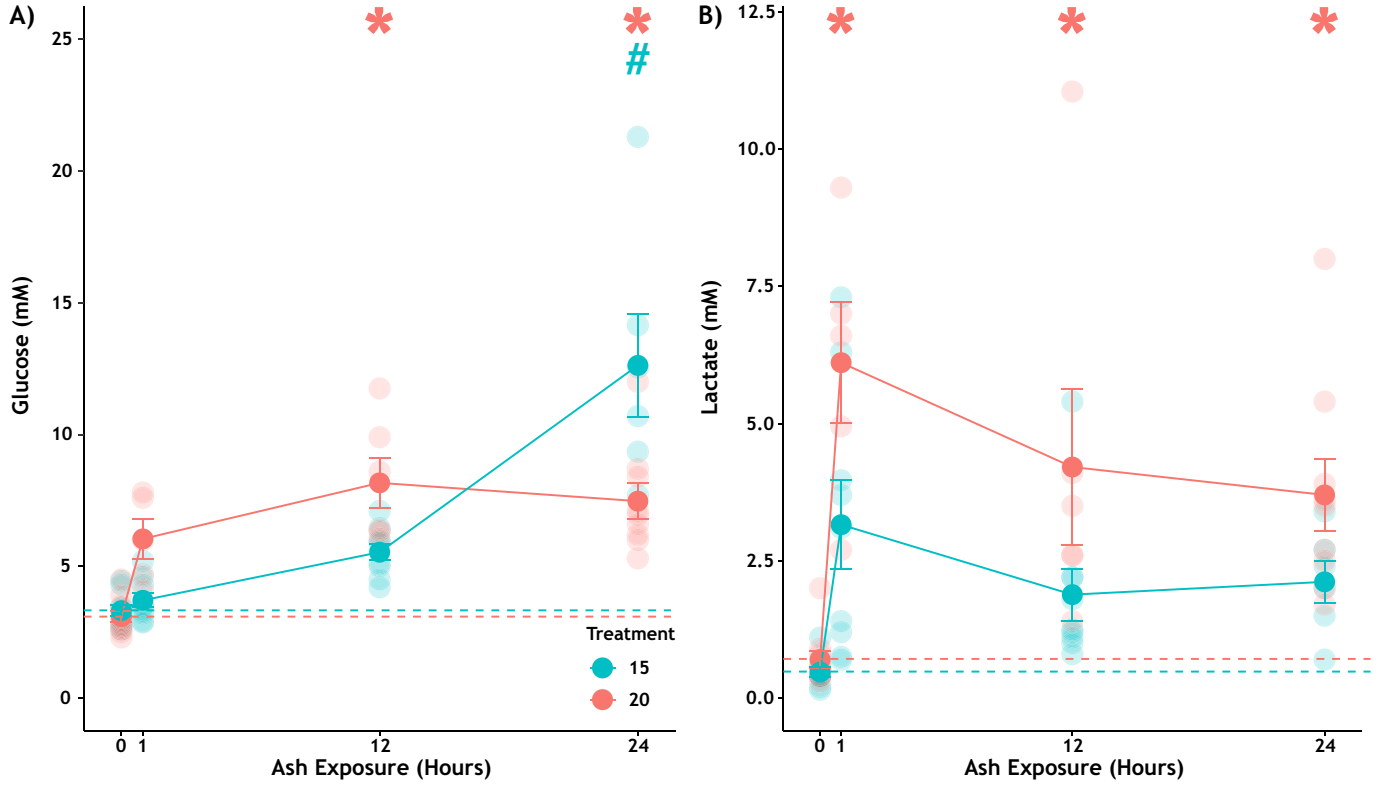


Fig. 5. Plasma glucose, lactate, and ammonia response to ash-induced pH ~9.2 exposure at 15 (teal) and 20 °C (red). Salmon plasma A) glucose and B) lactate over the 0, 1, 12, and 24 h of ash exposure. Values are mean ± SEM. Dotted lines represent the 0 h control levels. Significance from their 0 h control levels are indicated with a teal # for 15 °C and red * for 20 °C salmon, respectively.

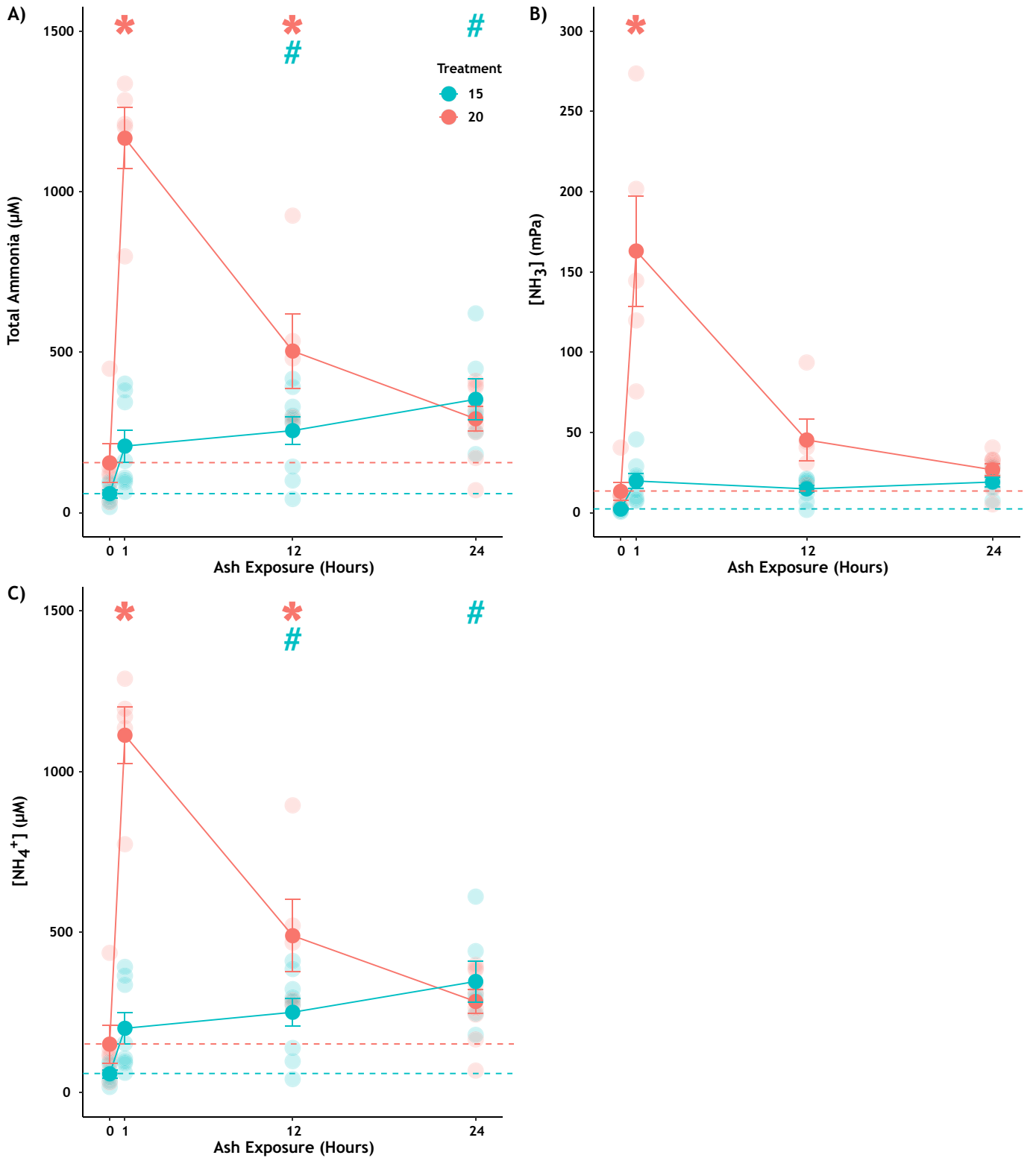


Fig. 6. Plasma total ammonia, $[NH_3]$, and $[NH_4^+]$ response to ash-induced pH ~9.2 exposure at 15 (teal) and 20 °C (red). Salmon plasma A) total ammonia, B) $[NH_3]$, and C) $[NH_4^+]$ over the 0, 1, 12, and 24 h of ash exposure. Values are mean ± SEM. Dotted lines represent the 0 h control levels. Significance from their 0 h control levels are indicated with a teal $^{\#}$ for 15 °C and red * for 20 °C salmon, respectively.

Thus, Wilkie and Wood (1991) concluded that high pH exposure did "render the fish more susceptible to other stresses" as they observed more successful recovery from surgical procedures (e.g. cannulation) when kept at a lower pH (8.15).

Salmon reared at 20 °C appeared to have recovered their pH_e more quickly and completely than those reared at 15 °C. One possibility was that salmon in the 15 °C treatment experienced greater alkalosis than those at 20 °C, though the reason for this remains unclear. Alternatively, salmon reared at 20 °C were generating CO_2 at a faster rate and could therefore generate H^+ more quickly to recover pH_e (assuming they can

excrete the HCO_3^- generated by CO_2 hydration); this may also explain the lower pH_e observed at 1 h as well as the faster and more complete recovery by 12 h. Moreover, past results showed rainbow trout reared at 15 °C (the colder temperature we used) and challenged with environmental alkalinization (pH 9.5) also could not return to their baseline pH_e of ~7.83 even after 72 h, and instead stabilized their pH_e at 7.97 (Wilkie and Wood, 1991). In and of itself, the pH 9.5 exposure did not induce mortality; instead, their inability to fully recover their pH_e is thought to have left them vulnerable to secondary stressors (Wilkie and Wood, 1991). Similarly, the inability for the 15 °C Chinook salmon to fully recover their pH_e could have led to additional mortality should the alkaline condition persisted for over 24 h. Moreover, the mortalities observed in this study may have stemmed from the combination of multiple secondary stressors including heavy metals, polycyclic aromatic hydrocarbons (PAHs), and fire retardants (discussed below). Yet from solely the pH_e perspective, our results suggest that while warmer temperature may increase mortality, their higher metabolic rate (and by extension basal pCO_2) could facilitate pH recovery by promoting H^+ synthesis.

We initially hypothesized pH_e recovery from respiratory alkalosis would result in a significant change in plasma ion concentrations, but the present study found these responses to be relatively muted. Here, the only significant change was decreased $[Cl^-]$ in 15 °C fish after 12 h of ash exposure. Past studies reported fishes exposed to high pH between 10 °C to 15 °C have decreased $[Na^+]$ and $[Cl^-]$ as well as elevated $[K^+]$ by 8 h of exposure, with these effects persisting up to 72 h of exposure (Wilkie and Wood, 1991; Hemming and Hanson, 1992; Wilkie et al., 1993; Scott et al., 2005). These changes in ionic concentrations reflect the putative mechanism for pH recovery during respiratory alkalosis: H^+ is accumulated and HCO_3^- removed through the coordinated effort of apical anion exchanger (AE), cytoplasmic carbonic anhydrase, and basolateral Na^+/H^+ Exchanger (NHE1) and electrochemically driven by basolateral vacuolar-type H^+-ATPase similar to base-secreting gill ionocytes in elasmobranchs (Tresguerres et al., 2005; Roa et al., 2014) or by basolateral Na^+/K^+-ATPase (NKA) in teleost intestinal epithelium (Grosell and Genz, 2006). Perhaps the muted plasma ion response can be attributed to the greater availability of counterions (e.g. Na^+, Cl^-) released during ash-input, which may increase the efficiency of ion-transport mechanisms. Moreover, a warmer acclimation temperature (and by extension greater metabolic rate) could have increased the fish's capacity to generate metabolic H^+ (see below), which could in turn have dampened ionic responses in the 20 °C fish.

Ash input led to a small but significant increase in total ammonia in the water, but these levels were well below reported toxic levels of ~14.7 μmol/L (0.25 mg/L) at pH 9.0 (Ip et al., 2001). Instead, elevated plasma ammonia and ammonium levels are caused by acute exposure to high water pH condition which inhibits the mechanisms of ammonia excretion across the gills (Wilkie and Wood, 1996). Although we hypothesized that elevated pH would impair nitrogenous waste excretion, we did not anticipate temperature-specific plasma total ammonia, $[NH_3]$ and $[NH_4^+]$ responses. Chinook salmon in the 15 °C treatment experienced a gradual accumulation of ammonia, with levels that were not significantly different until 24 h of exposure. This gradual accumulation over 24 h was similar to previous alkaline water exposure studies at similar temperatures and slightly higher water pH levels (e.g. Wilkie and Wood, 1991, 1994; McGeer and Eddy, 1998). In contrast, Chinook salmon in the 20 °C treatment experienced a ~7.5-fold spike at 1 h of exposure to ~1100 μM of total ammonia, which remained elevated at 12 h, and returned to baseline levels after 24 h of exposure. This suggests that higher temperature may assist in pH recovery (see above), and that the higher metabolic rate may also lead to a greater acute ammonia challenge and contribute to the higher observed mortality.

Past studies exposed fishes for longer than the present study, and they found that plasma ammonia continued to accumulate over 48 and 72 h of alkalinization exposure. Despite this, Chinook salmon after 24 h of ash exposure had lower ammonia levels than the surviving Lahontan cutthroat trout and rainbow trout in past alkalinization studies (Wilkie and Wood, 1991; Wilkie et al., 1993) despite relatively similar LC_{50} levels (Thurston et al., 1981; Erickson, 1985; Servizi and Gordon, 1990). This suggests acid-base regulation and elevated plasma $[NH_4^+]$ were unlikely to be the sole cause of the observed Chinook salmon mortalities.

Past studies have shown fishes to have elevated lactate levels during high pH exposure despite little to no change to arterial O_2 levels (Wilkie and Wood, 1991; Wilkie et al., 1993), and this suggests metabolic H^+ were being produced as another mechanism for pH regulation. While we expected respiratory alkalosis challenge would cause salmon to release stored glucose and to upregulate anaerobic respiration (as indicated by lactate levels), we did not anticipate temperature specific responses. Perhaps the greater ammonia spike observed in 20 °C salmon necessitated faster glucose release from glycogen stores and upregulated metabolic H^+ production (as evident by plasma lactate build-up) (Robergs et al., 2004) to aid in rapid pH_e recovery. This may also explain why salmon in the 15 °C treatment experienced a slower accumulation of ammonia, did not significantly upregulate their glucose until 24 h of exposure, and consistently had roughly half the lactate levels as those of 20 °C fish.

Another strategy to assist in recovery from high pH stress is to slow ventilation rates, which could accumulate greater CO_2 (and by extension the capacity to produce more H^+) in an effort to acidify their blood. However, it remains unclear whether salmon can manipulate ventilation rate and metabolic H^+ production to counteract high pH stress, or whether these responses were limited by their temperature-dependent aerobic scope (Zillig et al., 2023). Regardless, these responses were likely not evident in our current study as artificial gill ventilation manipulated by the experimenters could have masked organismal ventilation-mediated blood pCO_2 differences (Iwama et al., 1987). Therefore, future respirometry and ventilation rate studies are necessary to determine whether recovery from high pH exposure is influenced by their temperature-dependent aerobic capacity and ventilation rate.

In the wild, fishes (especially those trapped in lakes and reservoirs) would be chronically exposed to various trace metals that can produce long-lasting impacts on the fish (reviewed in Wood et al., 2012a, 2012b). Although this study found 13 of 25 elements to increase significantly following ash input (Supplemental Figs. 2–4), their abundance remains far below their respective LC_{50} (USEPA, 2013; Shuhaimi-Othman et al., 2015; Heijerick and Carey, 2017). For instance, Cr LC_{50} in freshwater fishes were between ~24,000–106,000 μg/L (Sanyal et al., 2017) and damage to DNA integrity was observed at ~5200 μg/L (Ahmad et al., 2006), all of which are several magnitudes greater than the concentrations observed in this study (<35 μg/L). In another case, Cu is known to reduce gill ion-transport capacity by disrupting NKA activity (Li et al., 1998; Lorin-Nebel et al., 2013) and could have contributed additive effects that cumulate in fish mortality. However, Cu was not detected to significantly increase with ash input despite increasing by nearly 5-fold to ~4 μg/L in our 20 °C treatment (Supplemental Fig. 2), and that level remains significantly lower than the Cu LC_{50} for salmonids of ~243 μg/L (Chakoumakos et al., 1979). However, species-specific variance in elemental sensitivity exists and could be much lower: *Rasbora sumatrana* has significantly lower at 24 h (~54 μg/L) and 96 h (~6 μg/L) Cu LC_{50} (Shuhaimi-Othman et al., 2015). As such, the released heavy metals (or at least the ones we analyzed) were unlikely to be the sole cause of the observed Chinook salmon mortalities.

Intrinsically, ash is also a pollutant that can lead to mortality, and zebrafish (*Danio rerio*) 24 and 48 h LC_{50} is at 41.1 g/L and 32 g/L, respectively (Brito et al., 2017), which is magnitudes higher than the concentration employed in this study (0.25 g/L). One major toxic component found within ash are PAHs, which are toxic organic compounds released during combustion and bioaccumulates within organisms, and ultimately inducing a wide range of negative effects (Silva et al., 2016; Brito et al., 2017; Harper et al., 2019; reviewed in Collier et al., 2013) including mortality (Heintz et al., 2000; Heintz, 2007; Almeida et al., 2012). PAH levels vary greatly in the wild: a recent

review found PAHs within 4 months post-wildfire to be between 0.1 and 20 ng/L (Raoelison et al., 2023). However, the LC_{50} of two PAHs, phenanthrene and retene, in two trout species are 1 g/L (5,610 μmol/L) and 17.7 g/L (75,600 μmol/L) (Billiard et al., 2000; Hawkins et al., 2002, respectively). Given that we tried to recreate ecologically-relevant conditions, this suggests PAHs are also unlikely to be the sole cause of the observed mortality. Even so, this study cannot rule out PAHs as a factor, and future research should quantify PAH concentration to examine whether they are responsible for fish mortality. Similarly, fire retardants (Giménez et al., 2004; Dietrich et al., 2013) could also adversely impact organisms during post-wildfire runoffs, and should be investigated in future studies alongside their interaction with temperature and IOA-B regulation.

Changes in environmental O_2 levels may also reduce organismal capacity to regulate internal acid-base and ammonia levels. In fact, wildfires and ash input are known to introduce nutrients into aquatic systems, promoting primary production and microbial activities, increasing the likelihood of algal blooms and hypoxic events and ultimately increasing O_2 variability (Gomez Isaza et al., 2022; Paul et al., 2022). In one past study, the combination of ash input and lower O_2 levels was shown to reduce the thermal tolerance of multiple fishes and crustaceans (Cramp et al., 2021). As such, future multi-stressor studies should consider exploring the impacts of sub-lethal O_2 levels on acid-base and ammonia regulation.

4.1. Future directions, environmental relevance, and potential management strategies

Over the past decades, numerous studies have focused on fish responses to high-CO_2 low-pH conditions in the context of ocean acidification and aquaculture (Ellis et al., 2016; Tresguerres and Hamilton, 2017). In contrast, aquatic organismal responses to low-CO_2 high-pH conditions remain relatively unexplored, leaving an abundance of exciting research questions that need answering. For instance, how would fish with preferential pH_i regulation (such as sturgeons (Baker et al., 2009b)) respond to acute ash input and environmental alkalosis, and would their adaptation to the naturally high pH habitats such as the Klamath River (which reaches pH ≥10 during the summer; U.S. Geological Survey, 2016) boost their tolerance for alkalinization. Moreover, what are some conservation management solutions that could reduce the impacts of post-wildfire ash-input in areas inhabited by endangered fishes and other aquatic organisms. Undoubtedly, further comparative physiology and conservation biology research are necessary as wildfires become increasingly common due to global climate change.

Many natural systems, especially those found at mid- to high-elevation and sourced with snowmelt, have very low alkalinity levels (Stoddard, 1987; Eilers et al., 1990; Catalan and Camarero, 1993; Clow et al., 1996) and are experiencing more rapid warming than lower elevation aquatic systems (Zhi et al., 2020). Thus, these systems are potentially more susceptible to more severe post-wildfire ash-induced pH changes, which could be detrimental to fishes that are endemic to remote mid- to high-elevation stream habitats like the Paiute Cutthroat Trout (*Oncorhynchus clarkii seleniris*), the California state fish golden trout (*Oncorhynchus aguabonita*), and various salmon runs returning from the ocean. As mentioned earlier in this study, organisms living in systems with naturally high pH levels (e.g. green sturgeon in Klamath River, Lahontan cutthroat trout in Pyramid Lake) and migratory species with rigid reproductive strategies (e.g. salmon) are also more susceptible to the alkalinization-linked impacts of post-wildfire ash input. Besides baseline water pH and alkalinity, there are many other variables that could influence post-wildfire pH responses including native vegetation and wildfire intensity (Santín et al., 2015; Sánchez-García et al., 2023), soil pH and composition (Marcotte et al., 2022), amount of rainfall or snowmelt (Rhoades et al., 2011), watershed size and slope (Neary et al., 2003), and algal biomass (Hohner et al., 2019). In sum, the impacts of wildfire on aquatic watersheds will likely be system-specific, and their organismal responses species-specific.

Habitats vulnerable to ash input can be identified with greater pH and alkalinity monitoring effort. Monitoring stations equipped and maintained with calibrated pH probes would provide valuable information on pre-wildfire baselines and natural pH variations, which would in turn help determine whether further management action is necessary following a wildfire. For instance, the release of dam water could help dilute the ash-induced alkalization, as well as to mitigate potential co-stressors such as high temperature, low oxygen, and the accumulation of heavy metals. Moreover, species found within areas of concern could be opportunistically transplanted during vulnerable life stages (e.g. returning adults, fertilized redds) to a non-burned watershed or aquaculture facility to safeguard the population. These two strategies are already employed for other scenarios, and with greater monitoring could be applied to help mitigate exposure to excessive ash input.

5. Conclusion

This study highlights how post-wildfire ash input can have significant physiological consequences for Chinook salmon. Following ash exposure, most Chinook salmon were able to restore pH_e balance (presumably by accumulating $[H^+]$, excreting $[HCO_3^-]$), and by extension restore nitrogenous waste removal. Warmer temperature appeared to be a double-edged sword: 20 °C salmon more quickly and completely recovered pH_e, but they experienced a significantly greater ammonia spike and a higher mortality rate than those exposed at 15 °C. Despite our best efforts, there remains no obvious sole culprit responsible for their mortality as the experimental condition had levels of pH, nitrogenous wastes, heavy metals, ash and likely PAHs that were far below the LC_{50} reported in salmonids. Yet regardless of the culprit(s), our study suggests wild aquatic organisms will be significantly and negatively challenged by acute ash exposure, and in particularly threatening those at sensitive life stages (e.g. returning spawning adults, early life stages) or endemic in geographical range. Moreover, our data suggests aquatic systems that are have high baseline pH and/or low alkalinity (such as mid- to high-elevation snow-melt/rain-fed systems) are particularly vulnerable. Future studies should continue identifying the major factor (s) responsible for fish mortality, and to safeguard the vulnerable species, life stage, and habitats as wildfire frequency and intensity continues to escalate around the world.

CRediT authorship contribution statement

Garfield T. Kwan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Trystan Sanders:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Sammuel Huang:** Writing – review & editing, Investigation, Data curation. **Kristen Kilaghbian:** Writing – review & editing, Investigation, Data curation. **Cameron Sam:** Writing – review & editing, Investigation, Data curation. **Junhan Wang:** Writing - review & editing, Investigation, Data curation. **Kelly Weihrauch:** Writing – review & editing, Investigation, Data curation. **Rod W. Wilson:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nann A. Fangue:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition.

Declaration of competing interest

No competing interests declared.

Data availability

The data that support the findings of this study are openly available in Dryad (https://doi.org/10.5061/dryad.866t1g1xf).

Reviewer sharing link: https://datadryad.org/stash/share/kCVkuCYQ327MfQ7m2-w1jU9b8JRwYGTpzB9bkpL0NeI

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176040.

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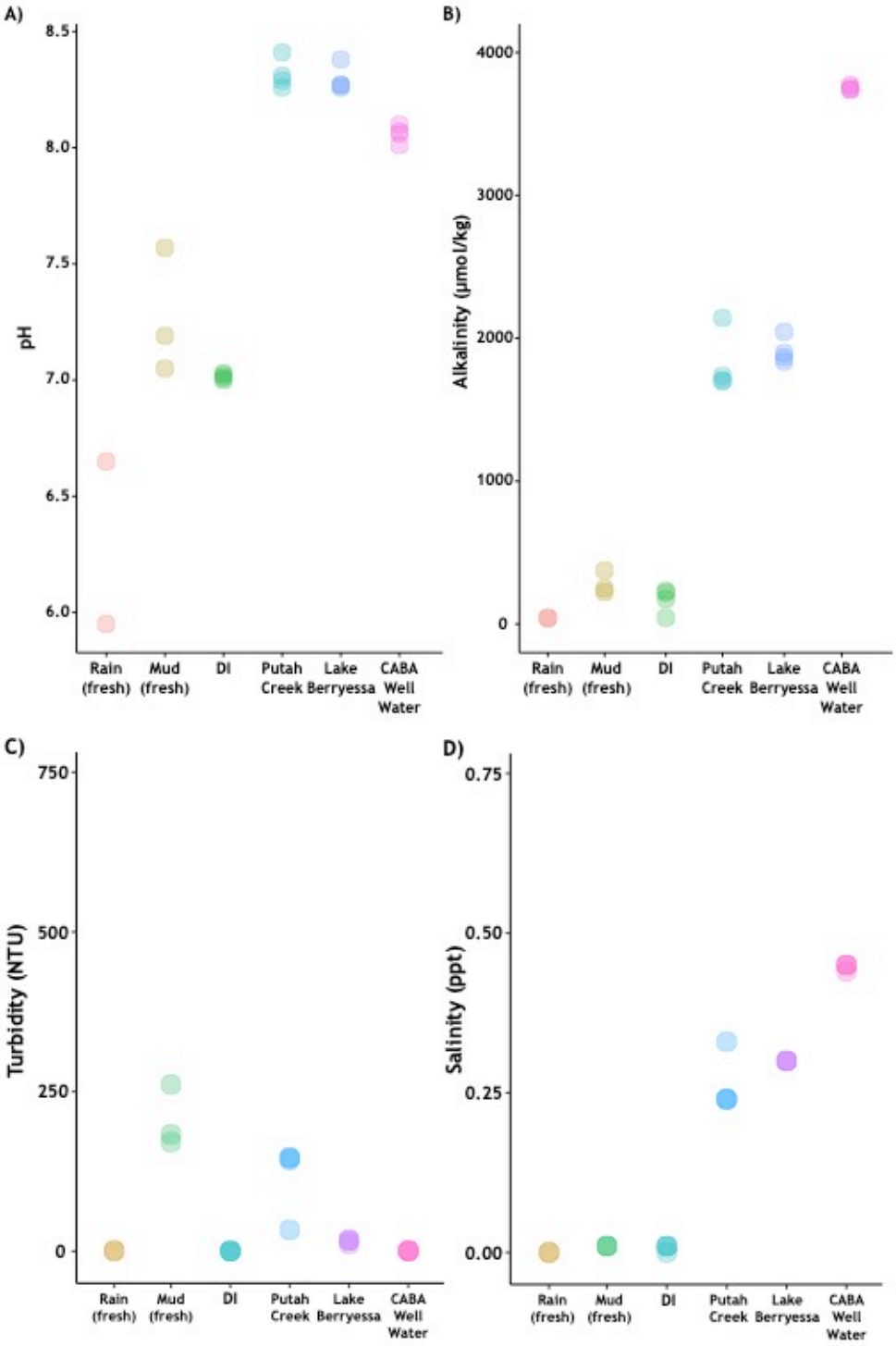
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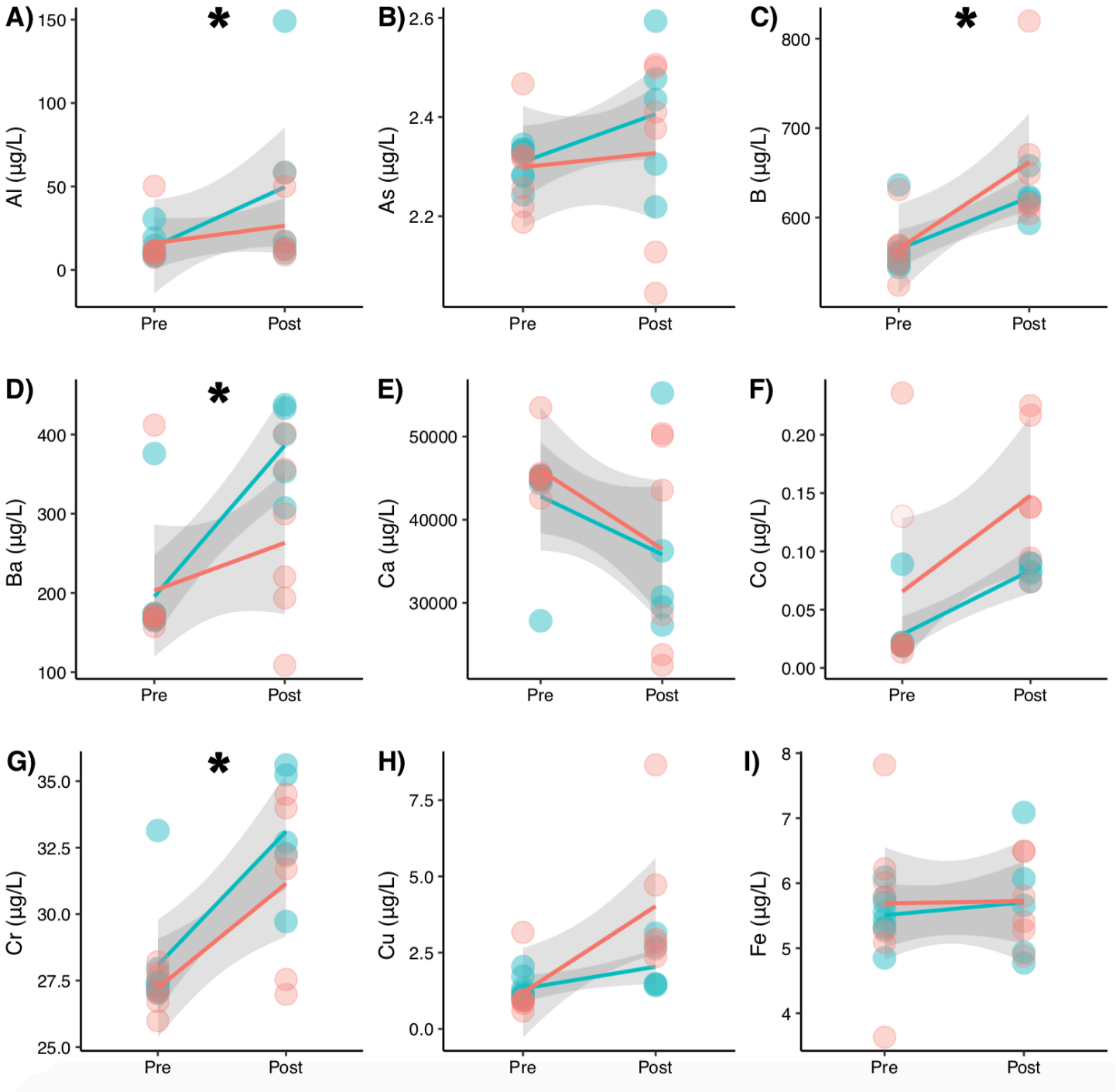
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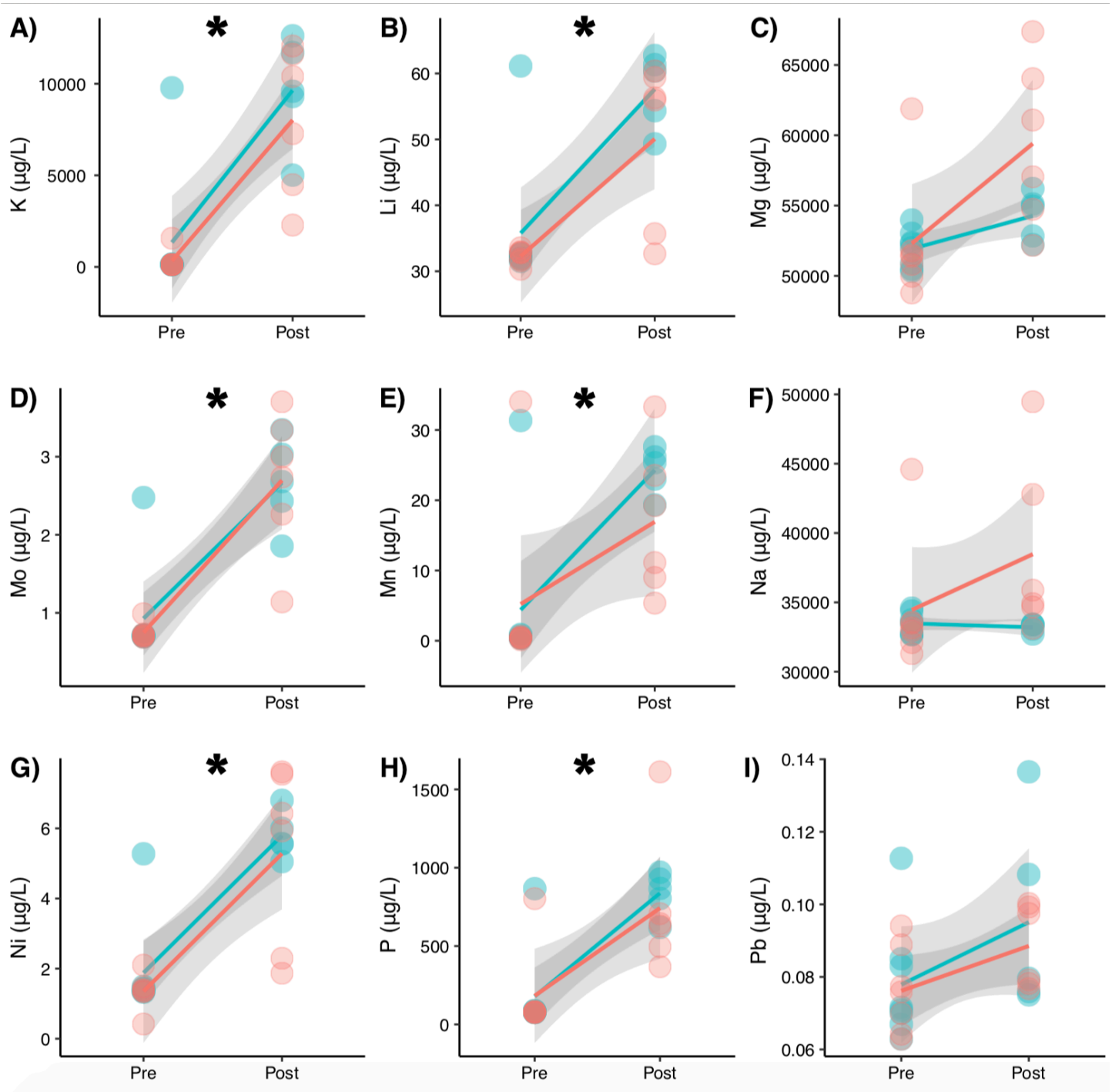
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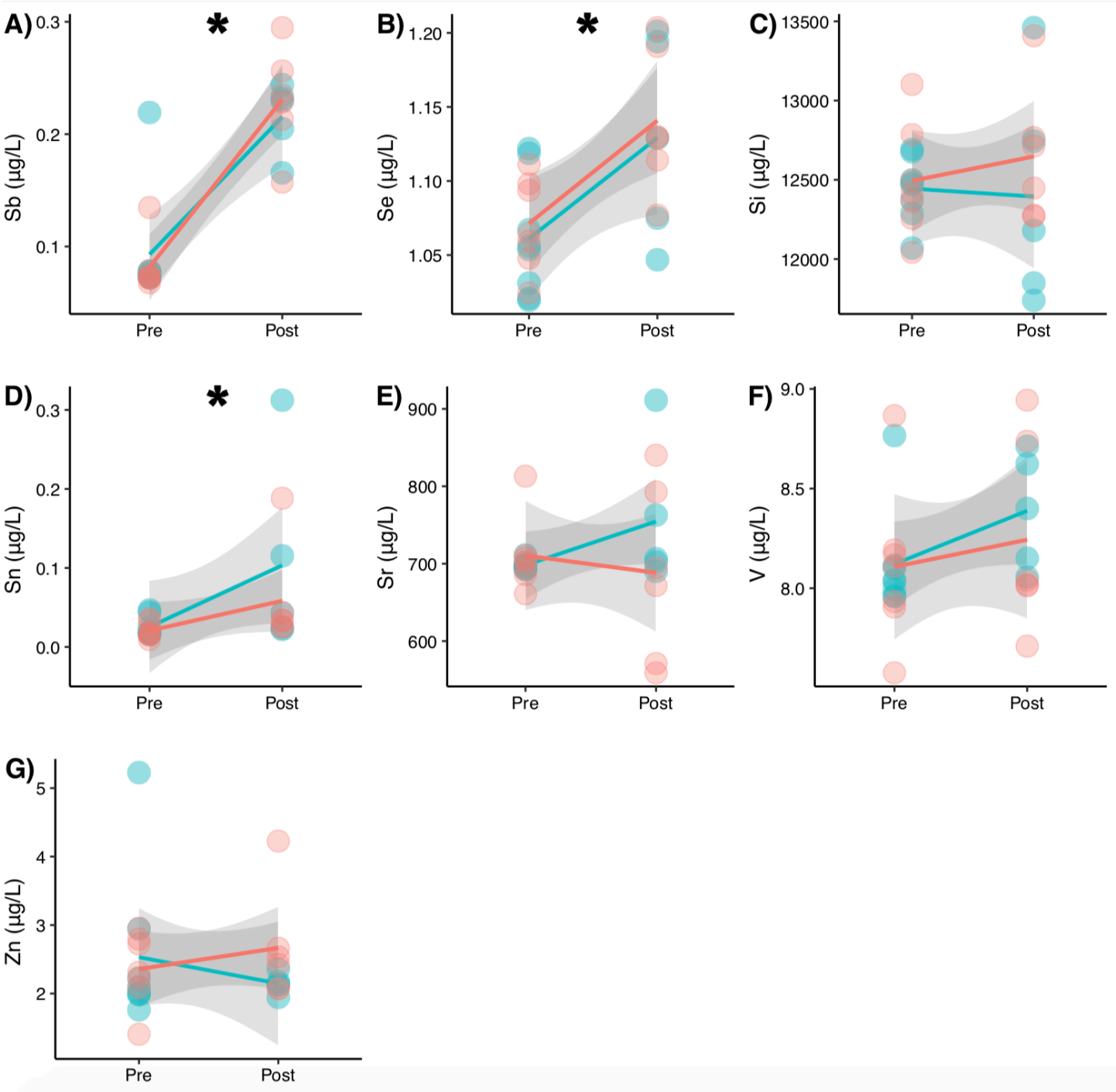
Supplemental Figure 1: Spot pH and alkalinity measurements of selected water bodies in February and March 2023. A) pH, B) alkalinity, C) turbidity, and D) salinity measurements from rain (stagnant and fresh), mud puddles (stagnant and fresh), deionized (DI) water, Putah Creek, Lake Berryessa, and well water from the Center for Aquatic Biology and Aquaculture (CABA), University of California, Davis.



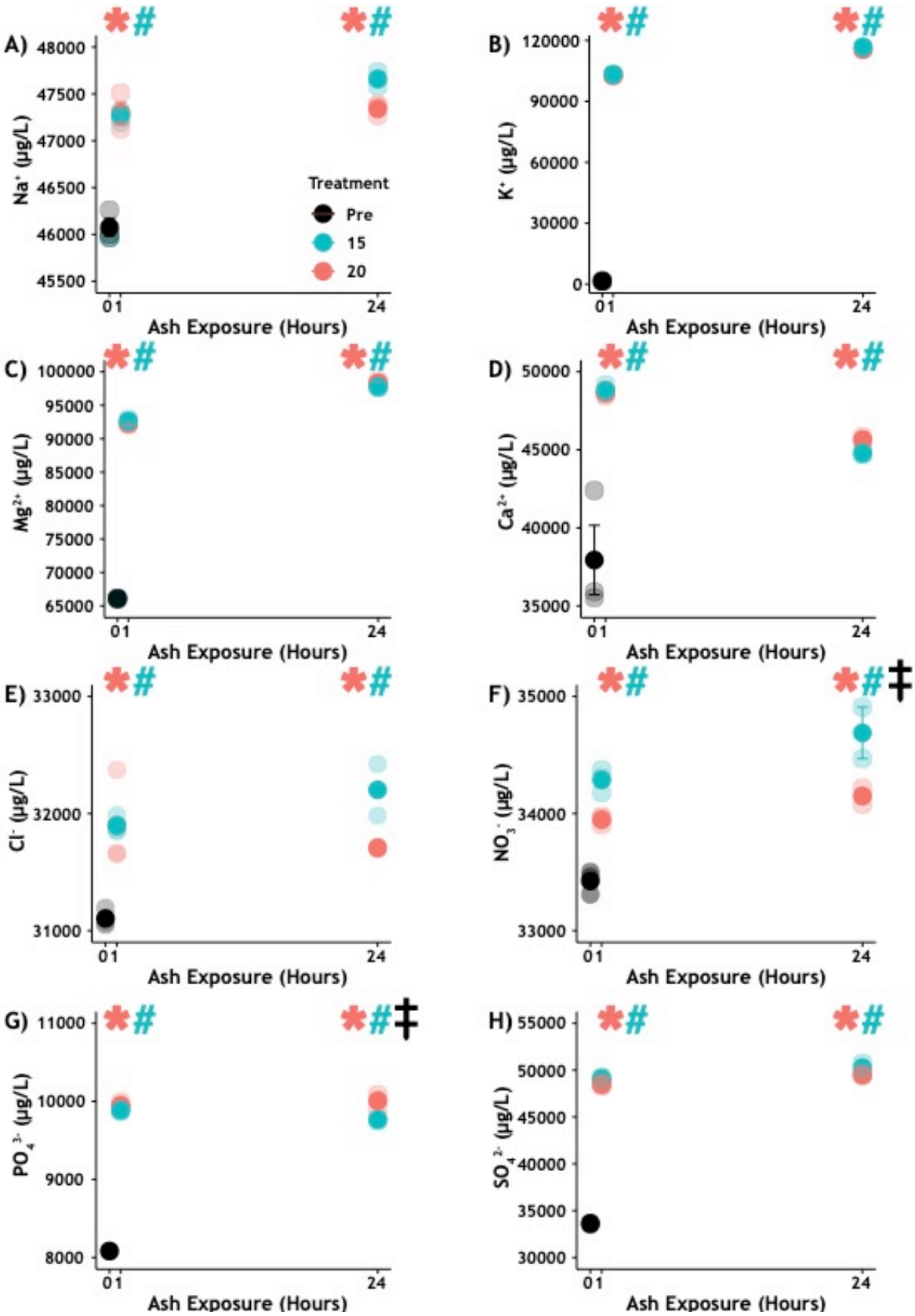
Supplemental Figure 2: Change in elemental concentration from ash input. Change in A) Al, B) As, C) B, D) Ba, E) Ca, F) Co, G) Cr, H) Cu, I) Fe concentration after ash input into experimental treatment water. No temperature difference (teal = 15°C, salmon = 20°C) was detected. Black asterisk indicates significance (α = 0.05) between pre- and post-ash input. Shaded area denotes 95% confidence interval.



Supplemental Figure 3: Change in elemental concentration from ash input. Change in A) K, B) Li, C) Mg, D) Mo, E) Mn, F) Na, G) Ni, H) P, I) Pb concentration after ash input into experimental treatment water. No temperature difference (teal = 15°C, salmon = 20°C) was detected. Black asterisk indicates significance (α = 0.05) between pre- and post-ash input. Shaded area denotes 95% confidence interval.

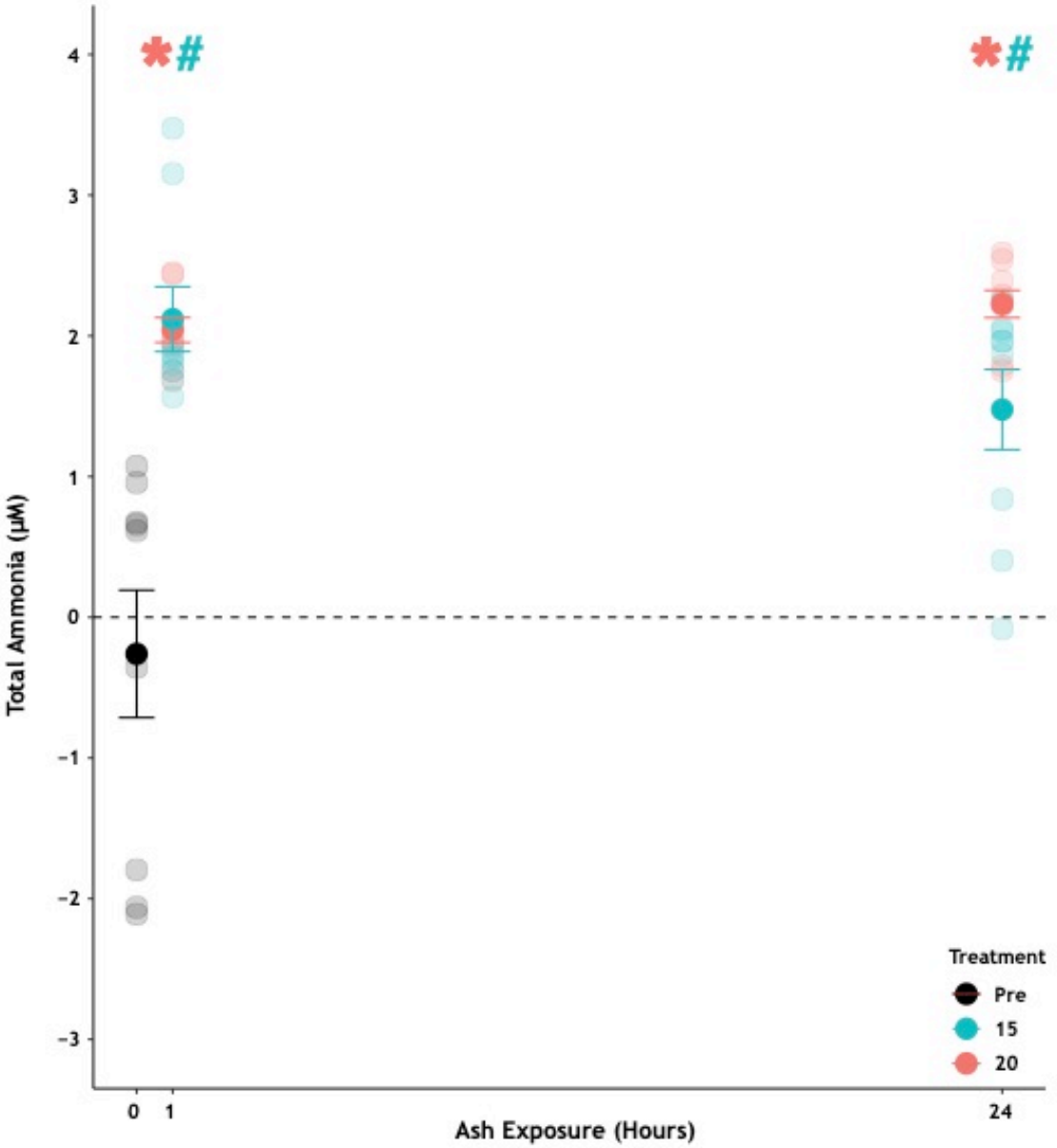


Supplemental Figure 4: Change in elemental concentration from ash input. Change in A) Sb, B) Se, C) Si, D) Sn, E) Sr, F) V and G) Zn concentration after ash input into experimental treatment water. No temperature difference (teal = 15°C, salmon = 20°C) was detected. Black asterisk indicates significance ($\alpha = 0.05$) between pre- and post-ash input. Shaded area denotes 95% confidence interval.

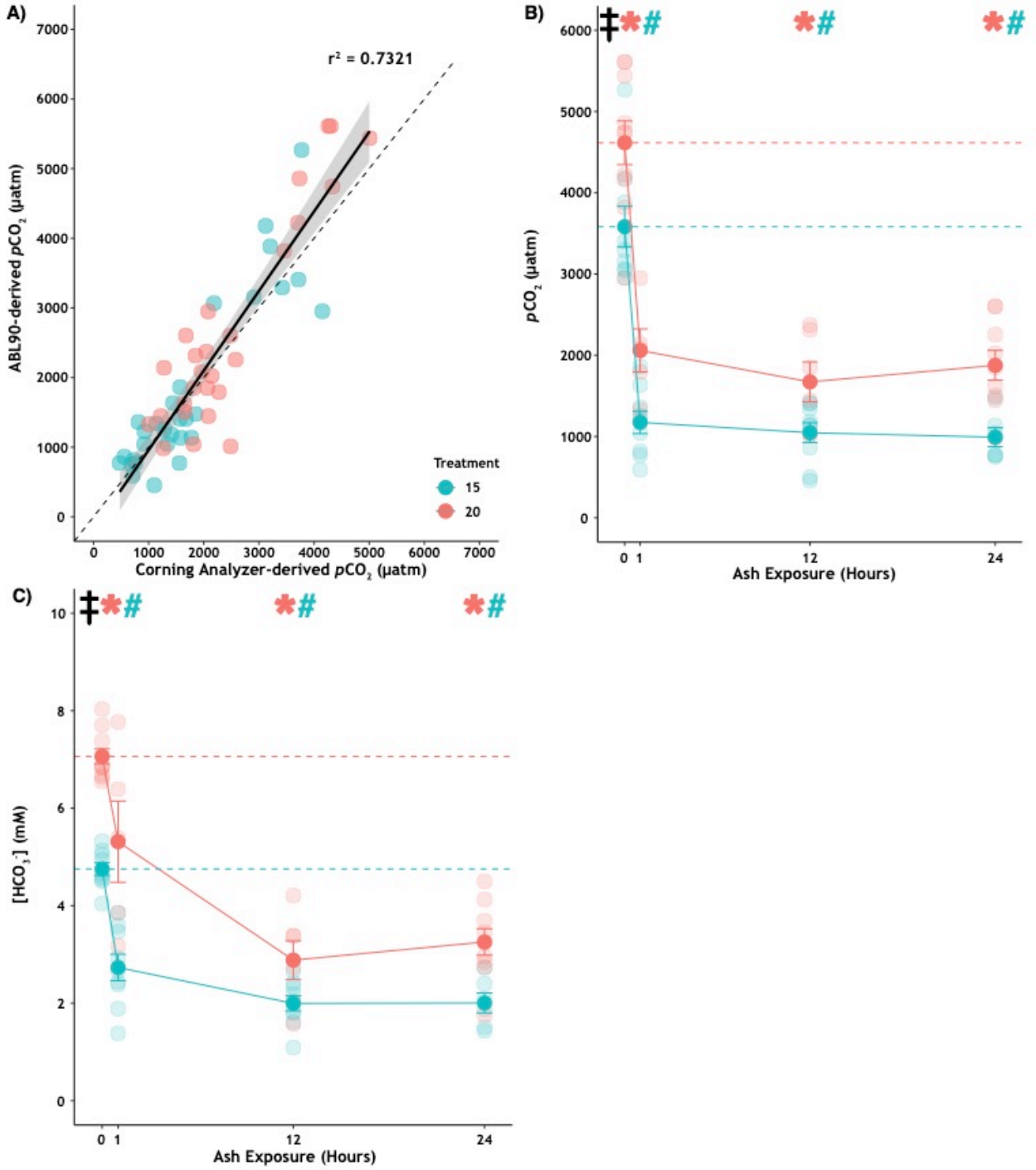


Supplemental Figure 5: Change in elemental concentration across temperatures and time following 0.25% (w/v) ash input. The mixing of well water and ash resulted in a significant increase in A) Na^+, B) K^+, C) Mg^{2+}, D) Ca^{2+}, E) Cl^-, F) NO_3^-, G) PO_4^{3-}, and H) SO_4^{2-} at both 15 and 20°C after 1 and 24 h. Significant temperature effects after 24 h of exposure were detected in NO_3^- and PO_4^{3-} levels. Black diesis (double dagger) indicates significance ($\alpha = 0.05$) between the 15 and 20°C levels. Significance from their 0 hour control levels are

indicated with a teal # for 15°C and red * for 20°C treatment, respectively. N=3 per time point and per temperature. Note: these results are from a subsequent experiment with no live animals.



Supplemental Figure 6: Change in total ammonia across temperature and time following 0.25% (w/v) ash input. Total ammonia concentration did not significantly increase after 1 and 24 h of ash input into experimental treatment water. No temperature difference (teal = 15°C, red = 20°C) was detected. Black dotted line indicates 0 h control value, which at times were below detectable limits. N=8 per time point and per temperature. Note: these results are from a subsequent experiment with no live animals. Significance from their 0 hour control levels are indicated with a teal # for 15°C and red * for 20°C salmon, respectively.



Supplemental Figure 7: ABL90-derived pCO_2 and [HCO_3^-] measurements. A) The pCO_2 values measured with the ABL90 were similar to those measured with the Corning CO_2 analyzer. Chinook salmon B) pCO_2 and C) [HCO_3^-] response to ash-induced pH ~9.2 exposure at 15 and 20°C over the 0, 1, 12, and 24 h of ash exposure. Comparison between the two datasets reveals the ABL90 may be slightly overestimating CO_2 levels, and future studies are needed to validate and potentially generate a correction equation. Values are mean ± SEM. Shaded area denotes 95% confidence interval. Dotted line represents perfect fit. Black diesis (double dagger) indicates significance (α = 0.05) between the 15 and 20°C levels. Significance from their 0 hour control levels are indicated with a teal # for 15°C and red * for 20°C salmon, respectively.