

Article

Disruption of Osmotic Balance and Metabolic Shifting in *Oncorhynchus kisutch* Under Hypoxic Stress: Implications for Salmon Aquaculture Climate Resilience

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Abstract

As climate changes worldwide, aquaculture is increasingly exposed to stressors such as warming, altered ocean chemistry, increased CO₂ “ocean acidification”, and reduced dissolved oxygen (DO) (i.e., hypoxia). Hypoxic events may also occur naturally during upwelling, when nutrient-rich but low-oxygen deep waters reach the surface, a process that may intensify with climate change along coastal areas such as the U.S. West Coast and Chile. The aim of this study was to determine the effects of hypoxia on several osmoregulatory organs and tissues of *Oncorhynchus kisutch*, including gills, kidney, intestine (foregut, midgut, and hindgut), muscle, red blood cells, and brain. Fish were exposed for 28 days to four hypoxic conditions (60, 50, 35, and 25% DO) and a normoxic control. Plasma chloride increased significantly at 25% DO, while plasma pH decreased significantly only under this condition. Plasma osmolality and calcium showed only non-significant tendencies. NKA activity decreased significantly in gills at 50, 35, and 25% DO and in kidney under all hypoxic conditions; however, kidney H⁺-ATPase remained unchanged. Gill H⁺-ATPase decreased significantly only at 35 and 25% DO. Intestinal responses varied among segments, whereas muscle and red blood cells showed higher NKA activity at 35% DO, with no significant changes in H⁺-ATPase activity. Overall, prolonged hypoxia affected osmotic regulation in a tissue-specific way, with reduced NKA activity in key osmoregulatory organs and compensatory NKA responses in peripheral tissues, which may indicate a shift in energy use toward oxygen transport under severe hypoxia.

Keywords: hypoxia; osmoregulation; plasma chloride; coho salmon; aquaculture

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Key Contribution: Hypoxia is an aquaculture problem every day more intensively. Hypoxia altered the osmoregulatory processes. The metabolism is affected by hypoxia. The hypoxia changed the ions levels.

1. Introduction

Climate change is changing many places worldwide. It is altering ocean chemistry by warming temperatures, increasing CO₂ “ocean acidification,” and reducing dissolved oxygen (DO) (i.e., hypoxia) [1]. In addition to occurring naturally, hypoxic events are associated with upwelling, surges of deep water rich in nutrients but low in oxygen. This phenomenon can increase with climate change, for example, along the U.S. West Coast and Chile [2–4]. This drop in oxygen can stress many marine organisms and lead to high mortality [5].

In Chile, salmon farming occurs in fjords during the growth stage, where oxygen levels are often below 3 mL L⁻¹ (47% saturation) [6]. Reports indicate that dissolved oxygen (DO) levels can range from 1.4 to 1.6 mL L⁻¹, corresponding to 20% to 25% saturation. Given the high density of fish farms, crowding increases oxygen demand.

In addition, SERNAPESCA, the agency responsible for monitoring salmonid production in Chile, reported that mortality associated with environmental factors accounted for 14.4% of total coho salmon mortality in 2024 [7]. Recent evidence also indicates that deoxygenation in northern Patagonian fjords is connected to large-scale oceanographic processes. Linford et al. [8] showed that low-oxygen Equatorial Subsurface Water can be transported southward by the Peru–Chile Undercurrent as far as the Patagonian fjords, contributing to low-oxygen conditions in oceanic-fjord areas and inside the fjords. From 2016 to 2022, on-site measurements indicated a reduction in dissolved oxygen by −21.66 μmol L⁻¹ over a span of seven years near Guafo Mouth. In contrast, a more pronounced decrease of −88.6 μmol L⁻¹ was observed within the Puyuhuapi fjord system over a three-year period.

In the Chilean fjord, Harmful Algal Blooms (HABs) have increased, rapidly altering oxygen availability. In 2016, an outbreak of raphidophyte alga *Pseudochattonella* cf. *verruculosa* killed nearly 12% of Chilean salmon production, causing stress and injury to many fish [4]. More recently, in 2021, a massive die-off of farmed salmon occurred in the Patagonian fjords, affecting approximately 2.2 million kilos of fish [5].

Stress in fish can be characterized by three levels of response, depending on the duration and intensity of the stressor: acute (an intense, short-duration stressor), acute-chronic (a stressor that is both intense and persistent), and chronic (a low-to-medium-intensity, persistent stressor). These stress levels play a crucial role in the health and welfare of the animals and are vital for the viability of fish farming.

Temperature and salinity are widely recognized stress factors in fish, with a multitude of studies emphasizing their impacts. Nonetheless, dissolved oxygen (DO) has garnered growing interest because of its substantial influence on growth in aquaculture, particularly in the case of *Oreochromis niloticus* [9,10], where it plays a role in altering feeding behavior and metabolic activities [11]. In *Oncorhynchus mykiss*, long-term hypoxia exposure alters cardiorespiratory physiology [12], while in *Sparus aurata*, low dissolved oxygen levels cause structural changes, such as gill lesions, and physiological changes, including alterations in hematocrit [13]. In *Salmo salar*, hypoxia induces changes in plasma cortisol, lactate, and osmolality [14]. Additionally, in *Seriola lalandi*, both temperature and dissolved oxygen levels have been shown to affect growth and digestive enzyme activity [15]. Hypoxia should be understood not only as a respiratory stressor but also as a metabolic challenge that requires rapid reorganization of energy use and

physiological priorities, as in rainbow trout (*Oncorhynchus mykiss*). In these typically hypoxia-sensitive species, the decline in available oxygen can threaten to decouple cellular energy supply from metabolic demand, potentially leading to exhaustion of ATP reserves [16].

Chilean aquaculture is mainly characterized by salmonid culture, with coho salmon "*Oncorhynchus kisutch*" production being the highest in the world [17]. Its culture is affected by several factors, including pathogens and abiotic variables such as hypoxia, which induce stress and affect growth as a tertiary response.

Teleost fish are strict osmoregulators. They actively maintain internal ionic balance despite environmental fluctuations in osmotic pressure or electrolyte concentrations. Changes in water salinity beyond their optimal range can lead to osmoregulatory failure, as can deviations from the optimal thermal range; both can modify the osmotic response [18–26]. Key organs involved in this homeostasis include the gills, kidneys, and intestines, which express the major ion-transport proteins necessary for sodium and chloride balance in seawater [22,27–32]. This study aims to investigate how hypoxic episodes, common in marine environments and aquaculture systems, affect these osmoregulatory organs in *Oncorhynchus kisutch*. We assessed changes in $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{H}^+\text{-ATPase}$ activities, plasma osmolality, and calcium and chloride levels, alongside metabolic responses, to monitor osmoregulatory effects in the gills, kidneys, brain, muscle, red cells, and three intestinal sections: foregut, midgut, and hindgut. Overall, this study identifies how hypoxia affects osmoregulatory function in *Oncorhynchus kisutch*.

2. Materials and Methods

The research adhered to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals provided by the National Commission of Science and Technology (CONICYT, Chile) and the Universidad Austral de Chile. Approval for the Ethics protocol was granted by the Committee on Ethics for Animal Experimentation at Universidad Austral de Chile, according to Memorandum No. 261/2016.

2.1. Fish

Osmoregulation was evaluated using the same specimens and experimental procedures as those of Martínez et al. [33] and Oyarzún-Salazar et al. [34]. Juvenile coho salmon, scientifically known as *Oncorhynchus kisutch*, with an average body weight of 26.96 ± 2.73 g, were sourced from the Spring Water fish farm (Ventisqueros S.A., Puerto Montt, Chile) (CAM: 542019112425) and transported to the Crustacean Ecophysiology Laboratory (LECOFIC) at Universidad Austral de Chile. These fish underwent a 30-day acclimation period in a flow-through tank with a capacity of 5 m^3 , maintaining a stocking density of approximately 3 kg per m^3 . The tank system utilized seawater that was filtered to $1 \mu\text{m}$ and sterilized with UV light, ensuring controlled conditions of salinity at 30 PSU, a temperature of $10 \pm 2 \text{ }^\circ\text{C}$, and a photoperiod comprising 12 h of light followed by 12 h of darkness. The specimens were fed to satiation once daily using a commercially balanced diet [34].

2.2. Experimental Protocol

The fish were assigned at random to five different experimental conditions, with each condition having five fish, one per tank. The treatments involved varying levels of dissolved oxygen (DO), measured as a percentage of air saturation, with the conditions set at 100% (serving as the control), 60%, 50%, 35%, and 25%. Oxygen levels were initially established and monitored as a percentage of oxygen saturation (% O_2 saturation). To improve methodological comparability with other hypoxia studies in fish physiology,

oxygen saturation values were additionally expressed as approximate dissolved oxygen concentrations ($\text{mg O}_2 \text{ L}^{-1}$) and oxygen partial pressure values (pO_2). These values were estimated considering the experimental conditions of seawater temperature (10°C), salinity (30 PSU), and atmospheric pressure near sea level in Puerto Montt. Under these conditions, the experimental treatments corresponded approximately to: 100% saturation ($8.0 \text{ mg O}_2 \text{ L}^{-1}$; 159 mmHg; 21.2 kPa), 60% saturation ($4.8 \text{ mg O}_2 \text{ L}^{-1}$; 95 mmHg; 12.7 kPa), 50% saturation ($4.0 \text{ mg O}_2 \text{ L}^{-1}$; 80 mmHg; 10.6 kPa), 35% saturation ($2.8 \text{ mg O}_2 \text{ L}^{-1}$; 56 mmHg; 7.4 kPa), and 25% saturation ($2.0 \text{ mg O}_2 \text{ L}^{-1}$; 40 mmHg; 5.3 kPa).

For a duration of 28 days, the fish were subjected to various treatments within an open-circulation system that maintained a steady flow of water. The entire volume of 550 L was refreshed five times each day. In this setup, seawater that had been filtered was pumped into a composed column and then directed to the aquaria with a 25% Dissolved Oxygen Water Supply (WS). The column includes tubes with diameters of 50, 75, 110, and 150 mm, and the configuration of columns and rings for gas diffusion provides two counter-current sections to enhance gas exchange. The gas that accumulates at the top of the outer tube is pre-mixed with incoming seawater using four nozzles. Nitrogen gas is supplied at a steady, pre-calibrated flow rate throughout the duration of the experiment. Water from WS was directed into each of the five 8 L experimental aquariums according to their specific treatments (see Supplementary Figure S1). The overflow from the WS helps to reoxygenate the remaining water before it moves to the subsequent WS aquarium. This setup relies on a constant flow of seawater, maintained by a pump, alongside a steady flow of nitrogen, which is regulated using a nitrogen flowmeter. When necessary, adjustments were made by modifying the nitrogen flow. Oxygen saturation levels were checked at least three times a day using an optic sensor linked to a temperature-compensated Microx MX3 AOT oxygen meter (PreSens Precision Sensing GmbH, Regensburg, Germany), which was calibrated with fully saturated seawater (100%) and a 5% sodium sulfite solution (0%). The control treatment was defined as normoxia, with 100% dissolved oxygen (DO). The other hypoxic conditions were established based on temporary records of coastal environments in the Chilean Patagonia, which are subject to commercial fishing and aquaculture activities [6,35–38].

2.3. Sampling

On the 28th day following the hypoxic challenge, the fish underwent a 24 h fasting period before sampling. The fish were gently caught using nets and moved to another container with identical water conditions. They were then exposed to lethal doses of 2-phenoxyethanol (1 mL per liter of seawater) and further subjected to spinal transection before tissue extraction, as described in references [20,22,39]. The fish were weighed, measured, and sampled.

Blood was collected from the caudal peduncle into a 1 mL ammonium heparinized syringe, spun at 2000 g for 5 min at 4°C , and plasma was aliquoted and stored at -80°C .

Four to six gill filaments, and pieces of posterior kidney and intestine portions (foregut, midgut, and hindgut), brain, muscle and red cells were placed in $100 \mu\text{L}$ of ice-cold sucrose-EDTA-imidazole (SEI) buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, $\text{pH } 7.3$) and frozen at -80°C for measurement of NKA and H^+ activity, as was described by Vargas-Chacoff et al. [22]. The foregut, midgut, and hindgut were separated based on visible anatomical features, primarily differences in the coloration and appearance of the intestinal muscular tissue. The foregut was identified as the intestinal region immediately connected to the pyloric caeca, while the midgut and hindgut were defined sequentially along the remaining intestinal tract based on their relative anatomical position and tissue appearance. The intestinal contents were gently removed during sam-

pling. When necessary, the intestinal lumen was carefully rinsed with pre-cooled physiological saline to remove remaining contents while minimizing manipulation of the mucosal surface.

2.4. Plasma Levels

The plasma osmolality ($\text{mOsm KgH}_2\text{O}^{-1}$) was measured with a freezing-point depression micro-osmometer (Model 3320; Advanced Instruments, Inc., Norwood, MA USA). Calcium and Chloride in plasma were measured using commercial kits from Spinreact S.A.U. (Sant Esteve de Bas, Girona, Spain; refs. 1001065 and 1002310) adapted to 96-well microplates. All assays were performed with a Multiscan Go Microplate Reader (Thermo Fisher Scientific Oy, Vantaa, Finland) using the ScanIt v3.2 software. Plasma pH was determined using a pH meter equipped with a micro pH electrode (Orion™ 9863BN; Thermo Fisher Scientific Inc., Beverly, MA USA), allowing measurements in plasma samples as small as 20 μL . The electrode was calibrated with standard buffers before use, and measurements were performed immediately after plasma collection.

2.5. NKA and H^+ ATPase Activity in Different Tissues

NKA activity in the gills, posterior kidney, foregut, midgut and hindgut, brain, muscle, and red cells was determined using the micro-assay method described by McCormick [40]. Ouabain-sensitive ATPase activity ($\mu\text{mol ADP/mg protein/h}$) was detected through the indirect enzymatic coupling of ATP dephosphorylation to NADH oxidation. The H^+ -ATPase (HA) activity was measured in the same manner as NKA using Bafilomycin A1 as a specific inhibitor of the V-type H^+ -ATPase [41,42]. Total protein contents were measured in undiluted samples in triplicate using a PierceBCA Protein Assay (Cat. No. 23225; Pierce Biotechnology, Rockford, IL, USA). Both assays were run on a MultiscanGo Microplate Reader (Thermo Fisher Scientific Oy, Vantaa, Finland) using the ScanIt v3.2 software.

2.6. Metabolic Responses

Frozen gills were homogenized by ultrasonic disruption in cold 0.6 N HClO_4 , with the volume 7.5 times the weight of the tissue. The mixture was then neutralized with an equal volume of 1 M potassium bicarbonate. The homogenates were centrifuged at 4500 g for 30 min at 4 °C (Centrifuge 5415 R; Eppendorf AG, Hamburg, Germany), and the resulting supernatants were used for metabolite analysis. Triglycerides (TAG) and lactate were quantified using (Triglycerides ref. 1001311; Lactate Ref. 1001330; Spinreact S.A.U., Sant Esteve de Bas, Spain) commercial kits adapted for 96-well microplates, as described previously by Vargas-Chacoff et al. [43]. Glycogen was quantified using the amyloglucosidase method outlined by Roehrig & Allred [44], and glucose was measured after glycogen breakdown (after subtracting free glucose levels) with a commercial kit (Glucose-HK Ref. 1001200; Spinreact S.A.U., Sant Esteve de Bas, Spain). Total α -amino acids were assessed colorimetrically using the ninhydrin method by Moore [45].

2.7. Statistical Analyses

All data provided are expressed as the mean $\pm$ standard error (SE). The experimental unit was the tank, and one fish was sampled from each tank to ensure independence among observations. Thus, each treatment was represented by five independent fish ($n = 5$), corresponding to one fish per tank, except the 25% DO, where $n = 3$ fish (mortality of two fish). Before conducting statistical analyses, the assumptions regarding normality and the consistency of variances were examined. The Shapiro–Wilk test (shapiro.test

function) was used to assess normality, while Levene's test (leveneTest function) from the car package was utilized to evaluate the homogeneity of variances. In addition, graphical diagnostic procedures, including quantile–quantile (Q–Q) plots and standardized residual plots, were examined to further assess compliance with model assumptions. Differences among treatments were initially evaluated using a conventional one-way analysis of variance (ANOVA) implemented through the aov function. Given the relatively small sample size and potential heterogeneity of variances among treatments, the robustness of the results was additionally assessed using Welch's one-way ANOVA (oneway.test function) and a permutation-based one-way ANOVA implemented through the aovperm function of the permuco package (see supplementary material). When significant differences were detected, Tukey's post hoc tests were used to identify pairwise differences among treatment groups. Statistical significance was established at $p < 0.05$. To quantify the magnitude of treatment effects, omega squared (ω^2) effect sizes and their corresponding 95% confidence intervals were calculated using the effect size package. To investigate relationships among physiological and biochemical variables, Pearson product–moment correlation analyses with associated p -values were conducted using the metan package. Variables exhibiting both statistical significance ($p < 0.05$) and a correlation coefficient ≥ 0.60 were subsequently selected for simple linear regression analyses. Regression models were fitted using the lm function, and graphical representations were generated using ggplot2 and ggarrange packages. The coefficient of determination (R^2) and regression equations were reported for all significant relationships [46–48] (Supplementary data).

3. Results

Only the group exposed to 25% DO exhibited mortalities on days 15 and 20, with 1 fish dying at each time point; the other experimental groups and the control group showed no mortality or alterations in sanitary performance.

3.1. Plasma Parameters

Osmolality and calcium levels showed a non-significant tendency to be higher at 25% DO than in some hypoxia treatments, but they did not differ significantly from the control group at 100% DO (Figure 1A,B). Plasma chloride levels increased significantly at 25% DO compared to the control and other experimental conditions (Figure 1C). The plasma pH decreased in all conditions but was only statistically significant ($p < 0.05$) at 25% DO (Figure 1D).

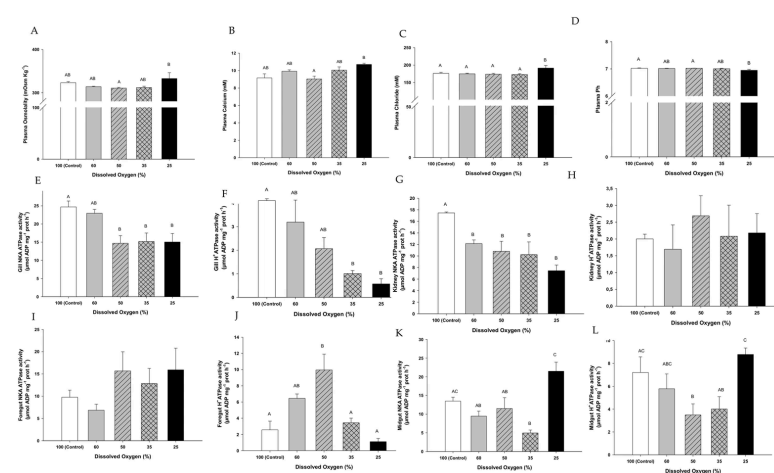


Figure 1. (A–D) Plasma levels; (E,G,I,K) NKA activity: gill, kidney, foregut and midgut (F,H,J,L) H⁺-ATPase activity: gill, kidney, foregut and midgut. Data are expressed as mean $\pm$ SEM ($n = 5$ per

group). Different letters indicate significant differences among groups ($p < 0.05$, one-way ANOVA Tukey test).

3.2. $\text{Na}^+\text{-K}^+\text{-ATPase}$ “NKA” Activity and $\text{H}^+\text{-ATPase}$ Activity

NKA activity in gills decreased in all experimental conditions, being significant at 50, 35, and 25% DO compared to the control group; meanwhile, at 60% DO, it did not reach significance. $\text{H}^+\text{-ATPase}$ activity in gills decreased in all experimental conditions, but the difference was statistically significant only at 35% and 25% DO (Figure 1E,F).

Kidney NKA activity significantly decreased in all experimental conditions, with respect to the control group; meanwhile, $\text{H}^+\text{-ATPase}$ activity was unchanged (Figure 1 G,H).

Three portions of the intestine were assessed. In the foregut, NKA activity showed a non-significant trend across the 50%, 35%, and 25% DO groups, with no statistically significant differences among treatments. However, $\text{H}^+\text{-ATPase}$ activity in the foregut increased at 50% DO, reaching 4-fold that of the control group; the other experimental groups did not differ from the control group (Figure 1I,J). The midgut showed a significant response at 35% DO, with a 2.5-fold decrease compared to the control group. In contrast, at 25% DO, the other showed an upward trend, although without statistical differences compared with the control group, and was significantly different from the other experimental groups (Figure 1K). $\text{H}^+\text{-ATPase}$ activity in the midgut decreased by 50% at 50% DO compared to the control group ($p < 0.05$), and at 25% DO showed an upward trend, although not statistically significant compared to the control group (Figure 1L). The hindgut showed high NKA activity at 25% DO, which was 2-fold higher than in the control group and statistically different (Figure 2A). The other experimental groups did not present statistical differences. $\text{H}^+\text{-ATPase}$ activity in the hindgut was lowest at 50% DO but did not differ significantly from the control group (Figure 2B).

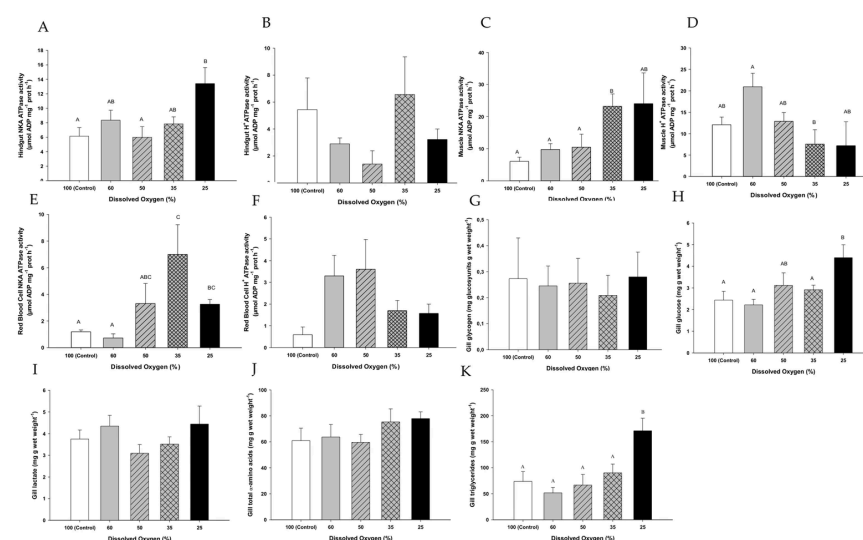


Figure 2. (A,C,E) NKA activity: hindgut, muscle, red blood cell; (B,D,F) $\text{H}^+\text{-ATPase}$ activity: hindgut, muscle, red blood cell; (G) Gill glycogen levels; (H) Gill glucose levels; (I) Gill lactate levels; (J) Gill Total α -amino acid levels; (K) Gill triglycerides levels. Data are expressed as mean $\pm$ SEM ($n = 5$ per group). Different letters indicate significant differences among groups ($p < 0.05$, one-way ANOVA Tukey test).

NKA activity in peripheral osmotic tissue, such as muscle, was highest in conjunction with lower oxygen concentrations, being statistically different at 35% DO compared to the control group (Figure 2C); meanwhile, $\text{H}^+\text{-ATPase}$ activity showed downward

trends, presenting the lowest activities at 35 and 25% DO but without statistical differences compared to the control group (Figure 2D). The red blood cells showed a pattern similar to that of muscle, with the highest NKA activity at 35% DO (Figure 2E), being statistically different from the control group. Additionally, H⁺-ATPase activity did not show statistical differences (Figure 2F). In contrast, NKA and H⁺-ATPase activity in the brain were not statistically significant (Figure S1).

The metabolic response in gills showed only statistical differences in gill glucose and triglyceride levels, with the highest levels at 25% OD (Figure 2H,K).

3.3. Correlations

Figure 3A–D shows us the positive correlations of the variables with higher R. Only variables with an R greater than or equal to 0.6 and a statistical significance less than 0.01 (2 and 3 stars) were selected. In the upper-left corner, the linear equation and R² are displayed. The shaded area on the blue line indicates the 95% confidence interval in plasma chloride/Plasma osmolality, gills triglycerides/gills glucose, gills glucose/NKA hindgut, and NKA muscle/NKA red cell blood. Figure 3E indicates a Corr-plot of Pearson correlations, with a color scale indicating the direction of the correlation between the different variables. The negative correlation graphs of the variables with an R greater than or equal to 0.6 and a statistical significance less than 0.01 (2 and 3 stars) were selected. In the upper-left corner, the linear equation and R² are displayed. The shaded area on the blue line indicates the 95% confidence interval in NKA muscle/H⁺-ATPase gill, NKA foregut/plasma pH, plasma osmolality/plasma pH, H⁺-ATPase muscle/plasma calcium, and H⁺-ATPase/gill triglycerides (Figure 4A–E) (to see Supplementary data).

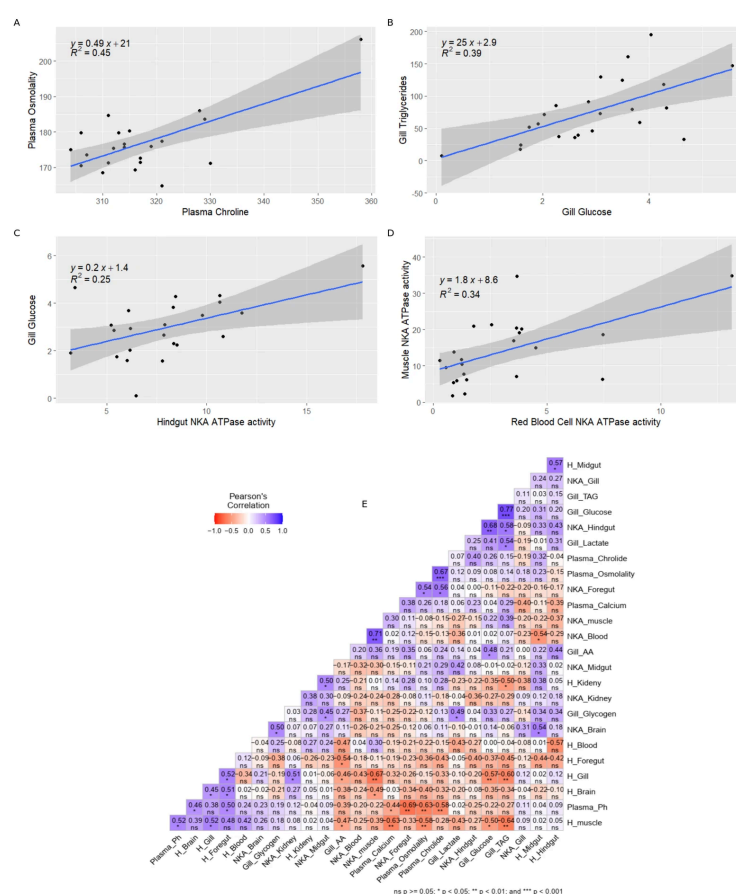


Figure 3. (A–D) Plots of positive correlations of the variables with higher R. Only variables with an R greater than or equal to 0.6 and a statistical significance less than 0.01 (2 and 3 stars) were se-

lected. In the upper left the linear equation and R^2 are presented. The shaded area on the blue line indicates the 95% confidence interval. The correlation plots were made with the ggplot2 and ggar-range package of Posit Cloud. (E) Corr-plot of Pearson correlations (R) of all variables. The colour scale indicates the direction of the correlation between the different variables, with the blue-violet colour indicating a positive correlation, while the red-pink color indicates a negative correlation, and the white colour indicates no correlation between the variables. In both colours (red and blue-violet), a greater intensity indicates a greater degree of correlation, which is also displayed with its R and its level of statistical significance (asterisks) value in each box. The plot was made with the metan package in Posit cloud.

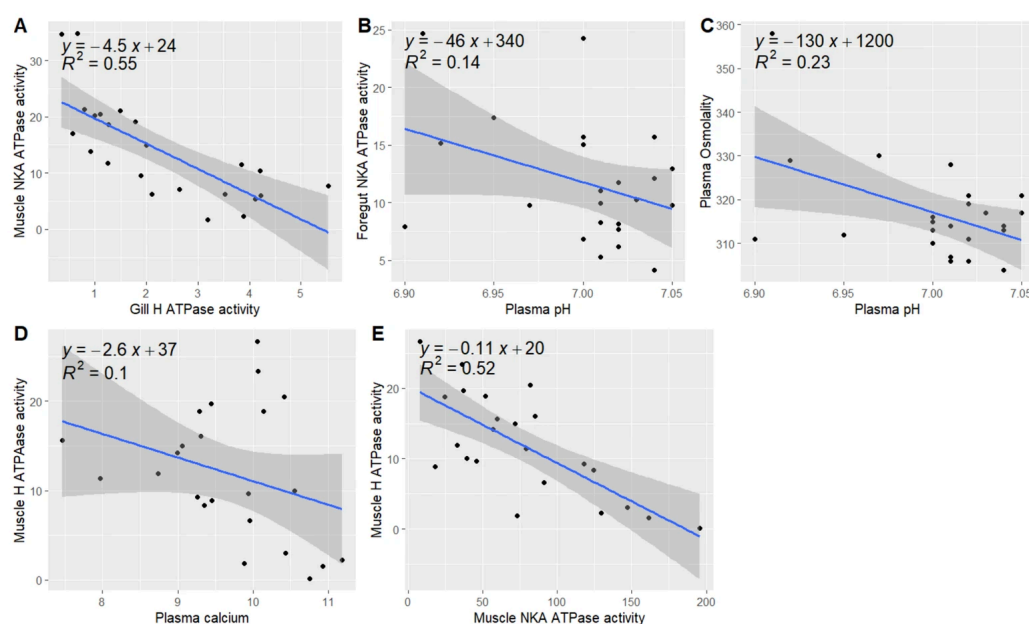


Figure 4. (A–E) Negative correlation graphs of the variables with higher R . Only variables with an R greater than or equal to 0.6 and a statistical significance less than 0.01 (2 and 3 stars) were selected. In the upper left the linear equation and R^2 are presented. The shaded area on the blue line indicates the 95% confidence interval. The correlation plots were made with the ggplot2 and ggar-range functions of Posit Cloud.

4. Discussion

In the Chilean fjords, tidal regimes can rapidly alter oxygen concentrations, triggering swift hypoxic events that can change within a few hours. Additionally, Harmful Algal Blooms (HAB) contribute to fluctuations in oxygen levels [4,5]. In a 28-day study, *Oncorhynchus kisutch* specimens subjected to different dissolved oxygen treatments exhibited mortality of less than 25% in DO conditions on days 15 and 20. Our results indicate that hypoxia acts as a stress stimulus that can trigger the secondary stress response, increasing ion levels (calcium and chloride), plasma osmolality, and affecting osmoregulatory activity across various tissues. Furthermore, as reported by Martínez et al. [33], hypoxia can elevate cortisol levels, a marker of the stress response. Although gills, intestines, and kidneys are the main osmoregulatory organs in teleost fish [27,29], muscle, brain, and red blood cells were considered here as indicators of systemic responses to hypoxia rather than as primary osmoregulatory effectors. Muscle was interpreted as a major metabolic and water-distribution compartment, red blood cells as components of oxygen transport and acid–base/ion regulation [49,50], and the brain as an integrative tissue involved in hypoxia-related physiological regulation. Therefore, changes in these tissues should be interpreted as systemic responses to hypoxia rather than as direct evi-

dence of osmoregulatory capacity.

The capacity for ion regulation in the gills and kidneys is reduced under conditions of low DO, which may be linked to the ability to elevate or sustain high ion levels when oxygen is scarce. In the gills, Na^+ , K^+ -ATPase (NKA) activity decreased at DO levels of 50%, 35%, and 25%, and in the kidneys, NKA activity decreased progressively from 60% to 25% DO. This decrease coincides with elevated ion levels—such as plasma chloride and calcium—only at 25% DO, leading to increased osmolality and compromised homeostasis, with ion transporters becoming less effective. Conversely, peripheral tissues like muscle and red blood cells showed the highest levels of NKA activity at the lowest DO levels, suggesting a compensatory mechanism to maintain homeostasis.

Aboagye and Allen [51] observed in paddlefish that changes in blood ions (Cl^- and Na^+), pH, and osmolality were consistent with patterns described by Baldisserotto et al. [52] in Amazonian fish, where levels decreased due to rapid haemodilution, likely a result of increased gill permeability, as suggested by McDonald and Milligan [53]. In contrast, Damsgaard et al. [54] and Fang et al. [55] reported that plasma chloride ion concentrations and osmolality levels were elevated during hypoxia in coho salmon (*Oncorhynchus kisutch*), a finding echoed by Hvas and Oppedal [15] in Atlantic salmon (*Salmo salar*) and ballan wrasse (*Labrus bergylta*). These observations of elevated ionic and osmolality levels during hypoxia align with the results presented in our study. The higher RBC NKA activity under hypoxia may be partly related to catecholaminergic modulation of erythrocyte ion transport. In teleost red blood cells, catecholamines stimulate Na^+/H^+ exchange, promoting Na^+ influx and intracellular pH regulation [49,50,56,57]. This could increase the need for Na^+ extrusion through NKA, although this remains a proposed mechanism because catecholamines and Na^+/H^+ exchange were not measured in this study.

In the gills and kidneys, NKA and H^+ activity decreased, suggesting that impaired functional activity may contribute to elevated ionic and osmotic levels. Gusarova et al. [58] found that hypoxia inhibits NKA activity in alveolar type II cells by reducing the number of active Na^+ pump molecules, a finding supported by Dada et al. [59]. However, this is at odds with the observations by Ern and Esbaugh [60] in *Sciaenops ocellatus*, where hypoxia-induced hyperventilation is thought to increase active ion transport to counteract passive ion diffusion at certain salinities. In our case, ion accumulation may be due to the inactivation of main ion pumps, while the intestinal pumps (across various sections) and peripheral tissues continue to function, attempting to maintain homeostasis. This compensatory response is consistent with findings by Vargas-Chacoff et al. [61] in *Eleginops maclovinus* and Arjona et al. [62] in *Solea senegalensis* following salinity exposure.

The data indicated an increase in NKA activity in the foregut under hypoxic conditions, reinforcing the regional specialization previously documented in Atlantic salmon smolts [63]. Low oxygen availability typically restricts aerobic capacity; this unexpected upregulation appears to represent a compensatory response aimed at maintaining ionoregulatory integrity and homeostatic balance under environmental stress. Sundell et al. [64] and de Fonseka et al. [63] have shown that the anterior intestine, also known as the foregut, is a significant site of metabolism. Maintaining elevated ATP demand in a low-oxygen environment likely constitutes a short-term trade-off, which may account for the observed disruption in synchrony between intestinal and branchial mechanisms in the experimental groups.

The association between disrupted ion homeostasis and the drop in NKA and H^+ -ATPase activity indicates a metabolic trade-off in *O. kisutch* under acute hypoxia. By scaling back energy-intensive processes to conserve cellular ATP [65], the fish reaches a

point at which active osmoregulation becomes unsustainable [66]. This physiological adjustment, likely involving the HIF-1 α signaling pathway, effectively prioritizes immediate survival over secondary ion transport [67]. In the Patagonian fjords, where oxygen levels are highly unstable [37], these results underscore a physiological bottleneck for Coho salmon; their baseline hypoxia tolerance is ultimately constrained by the breakdown of osmo-metabolic coupling [54]. Giacomini et al. [68] highlighted that a key issue caused by hypoxia is maintaining metabolic energy balance. The gills are primary osmoregulatory organs, and their metabolic response is influenced by stressors. In the context of hypoxia, glucose and triglyceride levels increase, indicating a need for energy that is met through glycolysis and gluconeogenesis, but the increase in triglycerides in the gills may be due to the fact that their metabolism requires oxygen, given that lipid metabolism is strictly aerobic (requiring a lot of oxygen). When fish encounter hypoxia, they trigger hypoxia-inducible factors (HIFs), channeling all accessible resources into the anaerobic decomposition of glucose and glycogen. This process generates energy rapidly without the need for oxygen, which accounts for the rise in glucose levels observed at the most reduced oxygen concentrations. This metabolic shift reflects a reallocation of energy, as described by Vargas-Chacoff et al. [43] in *Sparus aurata*, which exhibited similar metabolic changes in response to temperature and salinity stressors.

5. Conclusions

In conclusion, our data suggest that hypoxia alters the osmotic response, with prolonged exposure potentially leading to a shutdown of the primary ion pump. Although it appears that the required energy is allocated for oxygen transport rather than the osmotic process. Additionally, peripheral tissue attempts to maintain homeostasis, but there is a gradual loss of ionic balance. Also, we demonstrated that this metabolic reorganization that supports the use of ATPases-Pumps is conditioned by the lack of oxygen, which reduces the use of triglycerides as an energy source, leading to their accumulation and non-use, and also increases the use of glucose as a fuel at these low oxygen levels.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fishes11070420/s1>. File S1: Detailed Statistical Results; Figure S1: Experimental details; Figure S2: Brain NKA and H⁺-ATPase activities.

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