



Physiological and behavioral responses of Atlantic Salmon smolts to jet injector flow for downstream guidance

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Abstract Downstream guidance of migrating fish is a major conservation challenge in fragmented riverine systems, where orientation depends on fine-scale environmental cues and physiological readiness. Flow manipulation is increasingly explored to improve behavioral guidance at hydropower facilities, particularly for salmonids during the smolt stage. We investigated the effects of laterally injected jet flow on stress physiology and behavior of Atlantic salmon (*Salmo salar*) smolts in a large-scale flume. Fish were exposed to three treatments: jet flow from

the left (Left Jet), from the right (Right Jet), and no flow (Control). Physiological analysis revealed that the Right Jet treatment significantly elevated malondialdehyde (MDA) levels and increased the activity of the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GPx) in erythrocytes, indicating the presence of oxidative stress. No significant effects were detected for cortisol (plasma), hemoglobin and lactate (whole blood), or advanced oxidation protein products (AOPP, erythrocytes). Behaviorally, smolts showed a consistent left-side preference and swam predominantly near the bottom, regardless of treatment. Nevertheless, jet flow did not significantly alter lateral or vertical swimming distribution. Together, these results demonstrate that sublethal physiological strain can occur without overt behavioral change,

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underscoring the value of integrative, non-lethal approaches for evaluating fish responses to hydrodynamic conditions in guidance systems.

Keywords Atlantic salmon · Behavioral guidance · Downstream migration · Oxidative stress · Stress analysis · Spatial behavior

Introduction

River connectivity is a fundamental feature of rivers and is crucial to maintaining the vitality of riverine ecosystems (Feio et al., 2023). The fragmentation of habitats poses a significant challenge, obstructing the natural pathways of migrating fish and disrupting the intricate balance of aquatic systems (Barbarossa et al., 2020). Disruptions to these pathways can lead to reduced population sizes, genetic isolation, and even the extirpation of species from specific areas (Pavlova et al., 2017; Coleman et al., 2018). Efforts to restore river connectivity and mitigate the effects of habitat fragmentation are thus imperative for maintaining the health and functionality of riverine ecosystems (Sun et al., 2023).

In this context, systems for guiding downstream migrating fish into bypasses past obstacles like dams are crucial for successful passage. Behavioral guidance systems, which rely on non-physical cues such as light, sound, or flow manipulation, aim to redirect fish toward safe passage routes, but their effectiveness remains variable across species and settings (Stoilova et al., 2025). Even when downstream protection measures, such as bypasses, are implemented, they do not always work well because the solution does not adequately consider that the fish often follow the river's main flow (Coutant & Whitney, 2000). When insufficient flow is redirected toward the fish pass, the fish may pass through the turbines, often resulting in severe injury or death and hence hindering their successful migration (Ben Ammar et al., 2020).

Altered flow can support downstream migration by creating favorable guidance conditions and cues that migrating fish naturally follow (Zöschg et al., 2023). While much attention has been given to the engineering and behavioral aspects of guidance systems, understanding the physiological impact of such systems is equally important. As such, physiological stress responses serve as key indicators of fish

welfare and behavioral state (Schumann et al., 2024). Physiological stress responses also provide valuable insight into how fish interact with their environment (Piva et al., 2022; Pacchini et al., 2023). Understanding these responses is crucial for anticipating how fish react to environmental stressors and for designing migration systems that support welfare. Since stress can affect key life-history traits such as growth, reproduction, and survival (Carragher et al., 1989; Balasch & Tort, 2019; Pfalzgraff et al., 2022), it is essential to include physiological indicators when evaluating the impact of fish guidance systems.

Altered hydrodynamics and increased swimming activity can release stress hormones such as cortisol and induce the formation of oxidative stress generated by reactive oxygen species (ROS) (Farrell et al., 1998; Amérand et al., 2017). In fish, stress arises from environmental challenges (stressors) that activate neuroendocrine pathways such as the hypothalamic–pituitary–interrenal (HPI) axis (Best et al., 2023). This cascade influences metabolic, immune, and oxidative processes, ultimately affecting energy allocation, cellular function, and behavioral flexibility. While such responses promote short-term survival, chronic activation can impose physiological costs that impair growth, reproduction, and welfare (Borba et al., 2022; Pfalzgraff et al., 2022; Best et al., 2023).

ROS can initiate lipid peroxidation, particularly in polyunsaturated fatty acids (PUFAs) that are abundant in fish cell membranes. Unlike mammalian erythrocytes, fish red blood cells contain mitochondria and a high PUFA content (Birnie-Gauvin et al., 2017), which increases the risk of structural instability and functional impairment of the cell membrane (Conrad et al., 2018). The peroxidation of membrane lipids not only compromises membrane fluidity and integrity but also generates reactive aldehydes, such as malondialdehyde (MDA), which can form covalent adducts with proteins and contribute to oxidative protein damage (Witko-Sarsat et al., 1998). To counteract this, fish rely on antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidases (GPx), catalase (CAT), and peroxiredoxins. These enzymes neutralize ROS and limit cellular damage, with oxidative status assessable via established biomarkers such as MDA and advanced oxidation protein products (Khare et al., 2019; Chatzidimitriou et al., 2020; Piva et al., 2022; Pacchini et al., 2023). Accordingly, the use of whole blood, including both plasma and red

blood cells, is an established but still underutilized minimally invasive strategy for small fish such as smolts, where sample volumes are limited and non-lethal sampling is desirable. This approach enables the simultaneous assessment of hormonal and oxidative stress markers, thereby maximizing physiological insight while adhering to welfare-conscious practices.

A novel passage strategy currently under investigation involves using high-capacity pumps or jets to manipulate surface flow direction and guide fish away from turbines and toward bypasses. This strategy aims to leverage natural flow-following behavior to enhance fish passage, but its physiological implications remain poorly understood. While tests of this method's effectiveness are ongoing, it also raises questions about its effect on the physiology of the fish. Smolts of the Atlantic salmon (*Salmo salar* Linnaeus, 1758) are particularly relevant in this context, as they possess strong downstream migratory instincts and exhibit surface-oriented behaviors that make them vulnerable to hydrodynamic disturbances and ineffective bypass cues (Nyqvist et al., 2017). For this reason, smolts are also a primary target for safe downstream passage solutions. Because flow asymmetries can alter turbulence and velocity gradients, we expected that jet orientation would generate distinct hydrodynamic conditions and potentially elicit side-specific physiological or behavioral responses.

In this study, we tested whether and to what extent jet flow injection affects salmon smolts, both in terms of behavior and stress generation. To pursue this aim, we analyzed direct changes in the physiological response, including lactate and hemoglobin blood concentration. Variations in hemoglobin impact oxygen transport and, as a result, swimming stamina, and elevated lactate levels suggest high muscular activity and energy usage (Farrell et al., 1998; Weber et al., 2016; Amérand et al., 2017). To assess whether behavioral changes correlated with physiological effects, we quantified individual swimming trajectories using video tracking, focusing on lateral position and depth occupancy across different jet flow conditions. These behavioral observations were evaluated alongside established stress-related physiological parameters, such as blood cortisol levels, two oxidative damage markers (MDA, AOPP), and the activity of specific antioxidants (SOD, GPx, CAT) in erythrocytes. The idea was to take a holistic approach by integrating physiological and behavioral data to

understand how fish acclimate to dynamic aquatic environments and ultimately to use this knowledge to support safer downstream migration.

Materials and methods

Experimental setup

The experiment was conducted in the Laxeleratorn, a recirculating ethohydraulic race-track flume at the Vattenfall Research and Development Center in Älvkarleby, Sweden, in May 2022. The flume consisted of two sections, each measuring 24 m in length, 4 m in width, and 1.7 m in depth (Fig. 1). The sections were interconnected at the ends, enabling flow to circulate in the flume at a rate up to $16 \text{ m}^3 \text{ s}^{-1}$. For the experiments, the flume was blocked to prevent the fish from passing through both at the start and in the catch region, thereby increasing the probability of catching the fish after the trial and preventing possible entrapment of the smolts in the pump openings during the trial.

Three treatments were tested: one with injector flow on the left side of the flume (Left Jet), one with injector jet flow on the right side of the flume (Right Jet), and a control with no injector flow (Control). The flume's main recirculating current was clockwise, producing higher bulk velocities along the outer (right-hand) bend. Consequently, the Left Jet represented the inner curve and the Right Jet the outer curve, where local acceleration and turbulence intensity were expected to be greater. This design allowed us to test whether smolts responded to jet orientation, turbulence asymmetry, or merely to the presence of an additional flow cue.

All conditions were replicated six times using novel fish for each trial. For the jet flow injectors, non-protruding nozzles were installed on each lateral side of the flume about 5 m from the upstream end. Each injector jet extended vertically over the full water column ($\sim 1.5 \text{ m}$) and was oriented horizontally to generate a lateral acceleration zone along the wall. Flow visualization during calibration indicated that jet momentum dissipated within approximately 2–3 m downstream, merging smoothly with the background current without inducing large-scale recirculation or surface disturbances. The size of each nozzle was 0.077 m^2 , producing a flow rate of $\sim 115 \text{ L}$

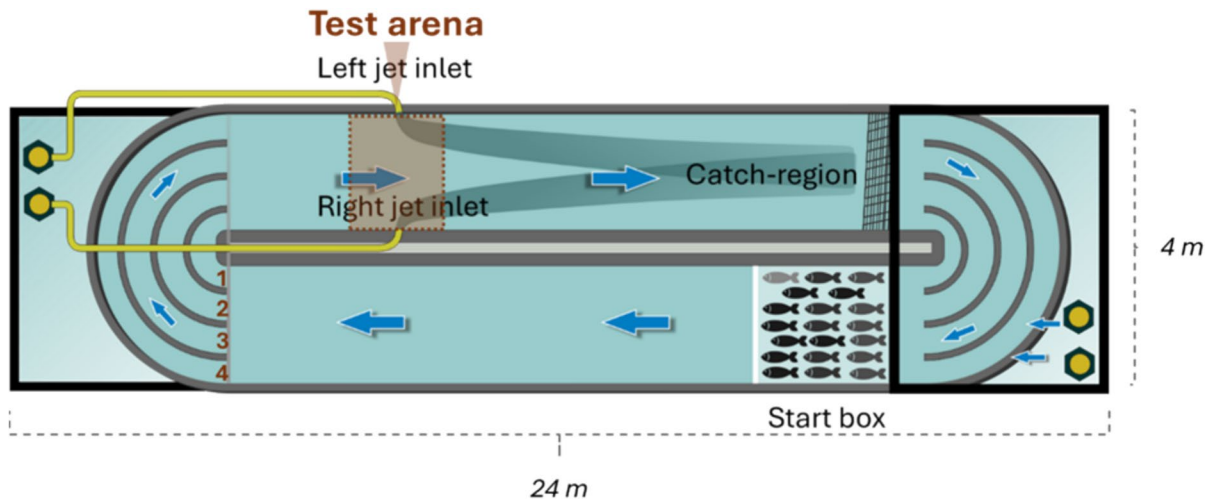


Fig. 1 Schematic drawing of the experimental arena. The flume consisted of a U-shaped, semi-loop structure with a designated test arena, located immediately downstream of the lateral jet outlets, where behavioral observations were made. Fish were released from a centrally positioned start box and entered the test arena via one of four parallel entry channels (numbered

1–4, visible in the schematic), before continuing downstream through the jet flow region and into the catch zone. The four hexagonal symbols depict the pumps for the lateral jet injectors (far left) and the main flow of the flume (far right). Arrows indicate the direction of water flow

s^{-1} (2 m s^{-1}). These jet velocities are within the range typically used in behavioral guidance systems or surface attraction flows at hydropower bypass entrances ($1\text{--}3 \text{ m s}^{-1}$; Schwevers & Adam, 2020; Calles et al., 2021). While they are lower than turbine velocities, the aim of the experiment was to simulate flow cues that could potentially be used in a behavioral solution to steer fish away from turbine intakes. In relative terms, the injected jet represented approximately 15–20% of the flume cross-sectional flow, creating a lateral velocity gradient without exceeding the swimming capacity of Atlantic salmon smolts (~ 2.5 body lengths s^{-1} ; Peake et al., 1997). This ensured that fish experienced hydraulically realistic but sublethal flow conditions suitable for behavioral and physiological assessment.

Fish were released from a centrally positioned start box at the beginning of each trial.

Jet flow

Flow fields generated by the injector jets were quantified using a three-dimensional acoustic Doppler velocimeter (ADV) operated by Vattenfall AB. Measurements were conducted in the 2 m-deep, 4 m-wide test section to obtain detailed velocity profiles across

the experimental area. In accordance with the experimental protocol, only one jet nozzle operated during each measurement series, and baseline conditions were recorded with the jet deactivated. The flume's main recirculation system maintained a uniform background flow of 0.5 m s^{-1} , while the jet was operated at 300 rpm ($Q = 115 \text{ L s}^{-1}$). Velocity data were collected at three longitudinal stations (0, 1, and 2 m downstream from the nozzle) and across nine lateral positions spaced at 0.5-m intervals along the 4 m channel width. At each lateral location, vertical profiles were sampled at four depths (0.1, 0.6, 1.1, and 1.4 m).

Animals and experimental procedure

Salmo salar smolts, with an average length of $15.4 \pm 1.6 \text{ cm}$ (SD), were sourced from the River Dalälven's wild progenitors and procured at the Västanå hatchery in Älvkarleby, Sweden. These smolts underwent a rigorous acclimation period spanning three weeks within segregated tanks measuring 3.5 m^3 . The tanks were supplied with water from the River Dalälven, the same water used in our flume experiment. Water quality was continuously monitored both in the flume and the holding tanks. In both, the water temperature averaged $10 \text{ }^\circ\text{C}$, consistent

with natural river conditions. Dissolved oxygen levels were not directly measured, but flume conditions ensured complete aeration through recirculation and turbulence, and no fish exhibited signs of hypoxia. pH and total nitrogen were recorded by facility instrumentation but were not retained for analysis.

For each trial, 20 smolts were gently netted under low-light conditions from the holding tanks and transferred in a covered container to a start box inside the flume. Fish were placed in a starting box and acclimated for 15 min before the experiments began. Each fish was only used once during the study. Trials lasted 60 min and were repeated 6 times per treatment over four continuous days. The order in which these three treatments were conducted was randomized. For each time point and treatment group, three of the 20 fish were caught immediately after each trial and anesthetized using benzocaine at a concentration of 60 mg/L (Sigma-Aldrich, Saint Louis, MO, USA). The first three individuals reaching the downstream catch zone at the end of each trial were selected for blood sampling. As all fish had traversed the entire test arena before being captured, the sampled individuals had necessarily passed through the jet region at least once to ensure consistency and avoid handling artifacts, with blood collected within less than three minutes of retrieval.

Blood samples were taken with a syringe from the caudal vein, a maximum of 3 min after the catchment from the flume, and transferred to heparin tubes (BD Vacutainer, Sarstedt, Nümbrecht, Germany). Lactate (Lactate Pro 2, ARKRAY, Koka-Shi, Japan), hematocrit, and hemoglobin (InSight HCT Meter, Woodley Equipment, Bolton, UK) were measured directly in whole blood, as described by the manufacturer of the equipment used. After centrifugation of the heparin tubes at $10,000\times g$ for 10 min (Eppendorf 5415D, Hamburg, Germany) to separate blood cells from plasma, the samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Cortisol analysis

Plasma samples were analyzed using a specific micro-titer radioimmunoassay (RIA), following the protocol described by Bertotto et al. (2010). Briefly, 25 μL of plasma was extracted using diethyl ether, dried, and reconstituted in phosphate buffer before being assayed using specific cortisol antibodies and radio-labeled

tracer. Radiation was measured with a beta-counter (Top-Count NXT, Perkin Elmer, USA). This RIA method is fully validated and routinely applied in our laboratory for *Salmo salar*, as established by Bortolotti et al. (2022), with demonstrated accuracy and reproducibility for small-volume plasma samples.

Oxidative stress and antioxidant enzyme assays

To assess lipid peroxidation, 42 mM sulfuric acid and phosphotungstic acid solution (Sigma-Aldrich) were utilized according to the manufacturer's instructions (Lipid Peroxidation (MDA) Assay Kit, Sigma-Aldrich, Merck, Darmstadt, Germany). For the following analysis described below, red blood cells were homogenized with a pestle using a 1:4 w/v ratio in 10 mM Tris HCl buffer (pH 7.6), supplemented with 0.5 M sucrose, 0.15 M KCl, and 1 mM DTT, while maintained at $4\text{ }^{\circ}\text{C}$. Samples were centrifuged for 30 min at 24,000 g (Eppendorf 5415D, Hamburg, Germany). The resulting cell-free extract was used for the analysis of Advanced Oxidation Proteins (AOPP) and the enzymatic activities of SOD, GPx, and CAT.

AOPP was measured in 25 μL of tissue homogenate in duplicate, following the protocol described by Witko-Sarsat et al. (1998). The absorption was measured at 340 nm using a chloramine-T standard (SpectraCount AS10000, Packard, Netherlands).

SOD activities were evaluated based on the method established by Beauchamp and Fridovich (1971), employing a 50% reduction in the NBT reduction rate to define one unit of SOD activity. GPx Se-dependent activity was assessed according to the approach described by Livingstone et al. (1992), involving monitoring GSSG formation within a coupled enzyme system utilizing glutathione reductase. The assay was conducted at 340 nm, with one unit of activity corresponding to the oxidation of 1 μmol of NADPH per minute. The activity measurement utilized H_2O_2 as a substrate. CAT was determined using the method of Livingstone et al. (1992), focusing on the reduction in H_2O_2 at 240 nm. For quantification, one unit of activity was defined as the amount of enzyme catalyzing the dismutation of 1 μmol of H_2O_2 per minute.

All analyses were normalized with the protein concentration measured with the Thermo Scientific™ Pierce BCA Protein Assay Kit (Waltham, MA, USA).

Behavioral analysis

Smolt behavior was analyzed to assess whether exposure to different jet flow configurations influenced spatial swimming patterns. Observations were confined to the test arena (Fig. 1), defined as the area immediately downstream of the jet outlets, where visibility was sufficient for reliable behavioral scoring. Due to bubble interference in the far field, behavioral data were limited to the moment fish encountered and entered the jets, but not to their movement further downstream. For the video analysis, 12 camera angles were preselected, and video footage was analyzed using Behavioural Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). Camera angles included top-down and lateral views focused on the region where fish entered the test arena. To facilitate position-based scoring, the flume floor was painted white and marked with four black transverse lines, providing spatial references in both the lateral and vertical planes.

Fish were recorded continuously for 60 min per trial. Videos were anonymized with respect to treatment group (Control, Left Jet, Right Jet), and all annotations were conducted blind by a trained observer using BORIS (Behavioural Observation Research Interactive Software; Friard & Gamba, 2016). Each behavioral event was defined as a discrete spatial observation lasting at least one second. The two main spatial descriptors used in this study were (a) lateral swimming position, categorized as left, middle, or right, based on the fish's observed position across the flume width at the moment of entering the jet region. This scoring was guided by the floor markings and fixed spatial references in the video frame; and (b) vertical swimming position: categorized as surface, mid-water, or bottom, corresponding to thirds of the water column, visually estimated based on camera perspective and structural references.

Fish were typically observed swimming close to structural boundaries (bottom or side walls), allowing deviations into “middle” categories to be distinguished with confidence. In cases where multiple fish were visible, group size was also recorded (1–5 individuals).

To summarize spatial swimming preferences, we computed a Swimming Path Index (SPI) per trial, defined as:

$$\text{SPI} = \frac{(N_{\text{Left}} \times 1 + N_{\text{Middle}} \times 2 + N_{\text{Right}} \times 3)N}{(N_{\text{Left}} + N_{\text{Middle}} + N_{\text{Right}})}$$

where N_{Left} , N_{Middle} , and N_{Right} are the number of observations per lateral category. SPI values range from 1 (exclusive left-side use) to 3 (exclusive right-side use), with 2 indicating no lateral bias.

Additionally, the entry channel used by each fish to access the test arena was recorded as a secondary proxy for lateral preference prior to jet exposure. Four parallel side channels connected the upstream start box to the test arena, numbered 1 (innermost) to 4 (outermost, following the flume's left curve). Entry was defined as the moment a fish exited a channel into the test arena, as determined from side camera footage. Both initial entries and subsequent re-entries were included in the analysis.

Statistical analysis

All statistical analyses were performed using R software version 4.1.0 (2021-05-18) and IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA). Data normality and homogeneity of variances were assessed using Shapiro–Wilk and Levene's tests, respectively. Outliers were identified using Tukey's method, with values exceeding 1.5 times the interquartile range excluded from analysis. For physiological parameters, one-way ANOVA was applied in R to assess the effect of treatment (Control, Left Jet, Right Jet), followed by Tukey's Honest Significant Difference (HSD) test for post hoc comparisons. For behavioral data, individual Swimming Path Index (SPI) and Depth Index (DI) values were calculated for each trial using weighted position scores (1 = Left, 2 = Middle, 3 = Right). Since each trial involved a different group of smolts, repeated-measures analysis was not appropriate. Instead, treatment effects on SPI and DI were tested using a univariate General Linear Model (ANOVA) in SPSS. In all analyses, p values < 0.05 were considered statistically significant. Data are presented as mean \pm standard error of the mean (SEM), unless otherwise stated.

Results

Jet flow

ADV measurements indicated that the wall-mounted jet produced a narrow, surface-intensified plume that dissipated within approximately 2 to 3 m downstream. Near the jet outlet (~ 0 m downstream), the flow formed a defined, surface-oriented streak adjacent to the wall (0–0.5 m across) with pronounced vertical shear within the upper 0.6 m of the water column. Surface velocities in this zone exceeded the background flow, reaching a maximum of ~ 0.69 m s $^{-1}$, while conditions below 1 m depth were already near ambient. One meter downstream, the plume persisted as a localized velocity band with a mid-depth maximum of ~ 0.65 m s $^{-1}$, whereas velocities near the opposite wall (3.5–4 m across) and along the bottom closely matched the background current of 0.5 m s $^{-1}$. At 2 m downstream, lateral velocity profiles had largely flattened, with velocities across all depths converging toward ambient levels. Overall, the jet's influence remained confined to the near-surface layer and the wall-adjacent region, extending roughly 1.5 m laterally and dissipating completely within 3 m downstream, leaving deeper layers and the opposite side of the flume largely unaffected.

Traditional stress marker

Contrary to expectations, traditional stress markers such as cortisol, hemoglobin, and lactate showed no significant variations across different hydrodynamic conditions (Online Resource 1). Mean cortisol concentrations ranged between 70.9 ± 40.6 and 80.9 ± 44.9 ng mL $^{-1}$, hemoglobin between 6.9 and 7.6 ± 1.0 g dL $^{-1}$, and lactate between 2.7 and 3.5 ± 1.3 mmol L $^{-1}$.

Oxidative stress

Statistical analyses revealed significant treatment effects on antioxidant enzyme activities. GPx levels were significantly higher in the Right Jet group compared with the Left Jet group (ANOVA: $F(2, 15) = 5.788$, $p < 0.05$; Tukey's test: $p < 0.05$; Fig. 2). Similarly, CAT activity differed significantly among treatments (ANOVA: $F(2, 15) = 3.934$, $p < 0.05$), with higher levels in the Right Jet group compared to the Left Jet group (Tukey's test: $p < 0.05$; Fig. 2). In contrast, no significant SOD (182 ± 50 to 197 ± 44 U mg $^{-1}$ protein) was observed across treatments (Online Resource 1).

Further analysis revealed a significant effect of treatment on malondialdehyde (MDA) levels (ANOVA: $F(2, 15) = 9.695$, $p < 0.01$). Post

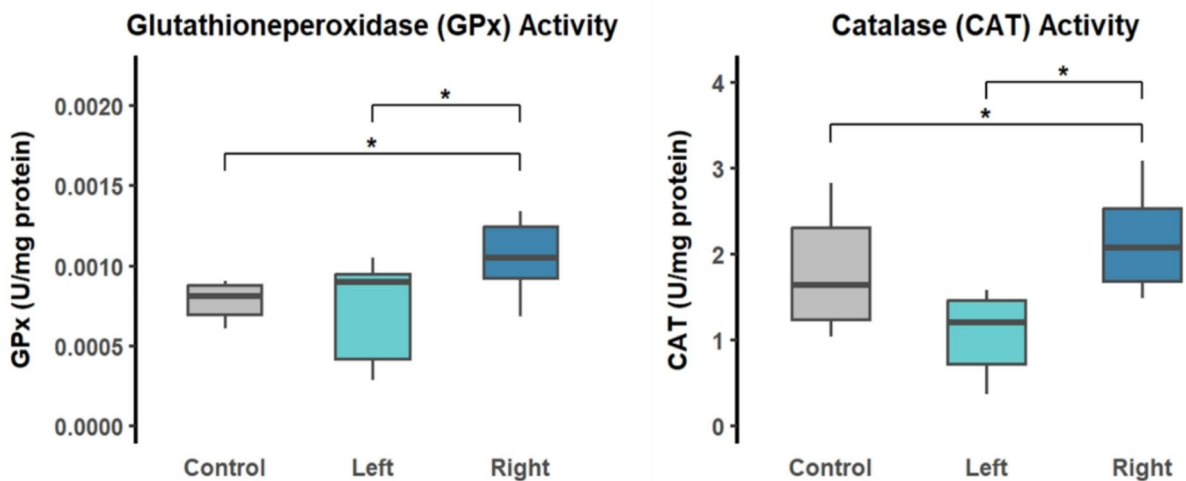


Fig. 2 GPx and CAT levels in fish erythrocytes exposed to the three treatments, normalized by the sample's protein content. Bars represent mean \pm SEM ($n = 6$ fish per treatment). Sig-

nificant differences between groups are indicated by asterisk ($p < 0.05$ for *; one-way ANOVA with Tukey's post hoc test)

hoc testing indicated that MDA levels were significantly higher in the Right Jet group compared to both the Control ($p < 0.01$) and Left Jet ($p < 0.05$) groups, indicating increased lipid peroxidation under this hydrodynamic condition (Fig. 3). In contrast, no significant differences were found in AOPP between treatments (0.35 ± 0.15

to 0.39 ± 0.11 nmol mL⁻¹ mg⁻¹ protein; Online Resource 1).

Behavioral analysis

Analysis of lateral swimming distribution revealed no significant treatment effect. However, smolts exhibited a consistent preference for the left side of the flume across all treatments. The Side Preference Index (SPI) averaged 1.56 ± 0.07 , with no trial exceeding an SPI of 2.0, indicating a stable left-side bias independent of jet direction (Fig. 4).

Vertical swimming distribution also did not differ significantly between treatments. Across all treatments, smolts predominantly occupied the bottom third of the water column ($n = 560$ observations), compared to the mid-water layer ($n = 285$) and the surface layer ($n = 31$) (Fig. 5).

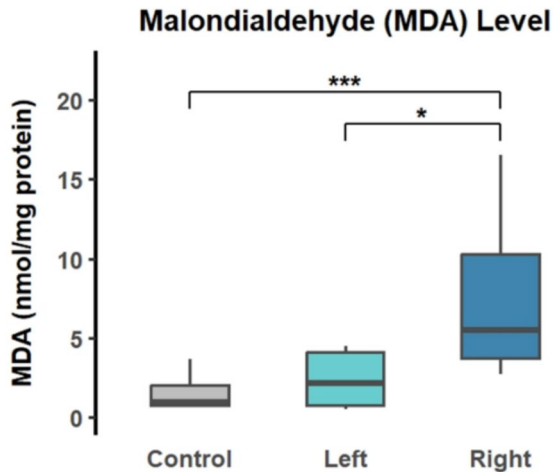


Fig. 3 Erythrocyte Malondialdehyde (MDA) levels in smolts across treatments. MDA concentrations were normalized to total protein content. Bars represent mean \pm SEM ($n = 6$ per group). Asterisks indicate significant differences between groups ($p < 0.05$ for *, $p < 0.001$ for ***; one-way ANOVA with Tukey's post hoc test)

Discussion

This study provides novel evidence that jet-induced flow can elicit sublethal physiological responses in Atlantic salmon smolts without producing overt behavioral changes. While traditional stress markers such as cortisol, hemoglobin, and lactate remained unchanged across treatments (Tab. S1), erythrocyte-based oxidative stress indicators revealed

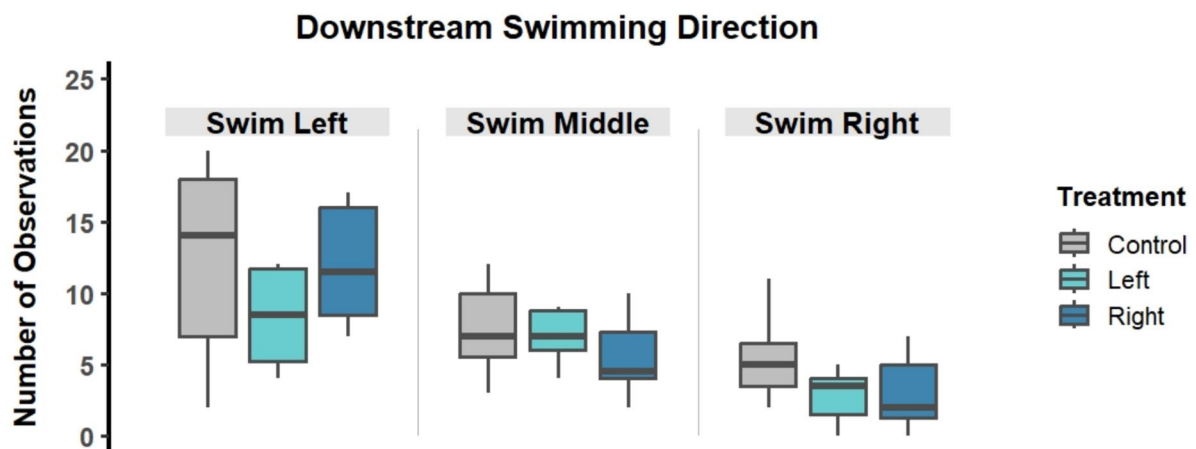


Fig. 4 Lateral distribution of downstream-swimming smolts across treatments. Bars represent the total number of behavioral observations recorded in each lateral position (Left, Middle, Right) across all trials for the Control, Left Jet, and Right

Jet treatments. Each treatment involved 6 trials with 20 fish per trial ($n = 120$ fish per treatment), and multiple behavioral observations per fish were pooled. No significant differences were detected between treatments (one-way ANOVA, $p > 0.05$)

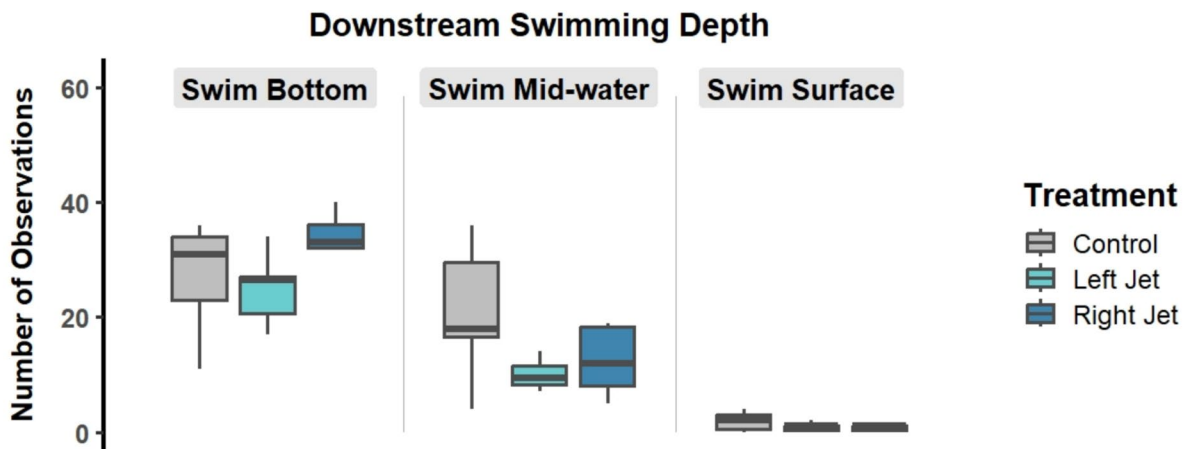


Fig. 5 Vertical distribution of downstream-swimming smolts under different treatments. Bars represent the total number of behavioral observations recorded in each depth category (Surface, Mid-water, Bottom) across all trials for the Control, Left Jet, and Right Jet treatments. Each treatment involved 6 trials

with 20 fish per trial ($n=120$ fish per treatment), and multiple behavioral observations per fish were pooled. No significant differences were detected between treatments (one-way ANOVA, $p > 0.05$)

apparent, treatment-specific effects that offer a mechanistic view of how fish interact with local hydraulic conditions.

Contrary to expectations, cortisol, a primary stress hormone in fish, showed no significant differences between treatments (Balasch & Tort, 2019; Sadoul & Geffroy, 2019). Similarly, hemoglobin and lactate, indicators of oxygen transport and anaerobic metabolism, respectively, remained stable across treatments (Rummer & Brauner, 2015; Pettinau et al., 2022). These results may indicate that the jet treatments were not perceived as strong or acute stressors (Roza e Silva et al., 2020). However, given that cortisol levels across all treatments were relatively low (70.9 ± 40.6 to 80.9 ± 44.9 ng/mL) in smolts, it is unlikely that procedural stress associated with fish transfer and sampling masked treatment-specific effects. Instead, these values support the interpretation that the jet treatments were not perceived as strong acute stressors (Cornell et al., 1994; Culbert et al., 2022).

Importantly, the purpose of this study was not to induce stress but to understand how localized hydraulic cues influence the physiology of fish under conditions relevant to downstream passage. Behavioral guidance systems are designed to redirect fish away from turbines and toward bypasses, while minimizing fear or aversive reactions. From a welfare perspective, an effective design must refine hydrodynamic cues so

that they are perceptible but not harmful. The absence of a cortisol response in our experiment, therefore, supports the view that jet-induced flow can serve as a non-invasive guidance stimulus, altering fish orientation without imposing acute stress. Such outcomes align with the ethical and regulatory principles of refinement and animal welfare embedded in modern fish passage research (Directive 2010/63/EU, European Parliament and Council, 2010). These observed baseline values are consistent with ranges reported for unstressed Atlantic salmon smolts (Bertotto et al., 2010; Bortoletti et al., 2022), which reinforces that our treatments remained within a non-stressful physiological window, with occasional outliers likely reflecting natural inter-individual variation.

Significant differences in the activities of the antioxidant enzymes GPx and CAT were observed, with both enzymes upregulated in the Right Jet treatment. GPx showed the strongest response, consistent with enhanced ROS detoxification in the cytosol and mitochondria of erythrocytes, where metabolic ROS production predominantly occurs. CAT, in contrast, is localized to peroxisomes and becomes more effective at higher H_2O_2 concentrations (Baker et al., 2023), suggesting that the oxidative challenge remained within a range primarily managed by GPx. Since SOD levels remained stable across treatments, the primary generated ROS was likely H_2O_2 generated

by dielectronic reduction rather than superoxide radicals (O_2^-) (Case, 2017). This pattern aligns with the unique physiology of fish erythrocytes, which retain functional mitochondria, implying that hydrodynamic stress may have increased mitochondrial metabolic activity, thereby elevating H_2O_2 formation and triggering GPx-mediated detoxification as an early protective response.

The significant increase in lipid peroxidation, reflected by elevated MDA levels in the Right Jet treatment, reinforces this interpretation. ROS-driven lipid damage in erythrocyte membranes likely represents an early target of oxidative stress, as membrane phospholipids are particularly vulnerable to peroxidation. Such damage can alter bilayer fluidity, impair membrane protein function, and destabilize the cytoskeleton (Farag & Alagawany, 2018). Interestingly, protein oxidation (AOPP) remained unchanged, indicating that the oxidative challenge was moderate and primarily confined to the lipid compartment, rather than causing extensive protein damage. This selective effect is consistent with a scenario of sublethal, metabolically driven ROS production rather than acute oxidative injury. Previous studies have shown that similar oxidative processes can accelerate erythrocyte aging and induce morphological changes such as elongation (Shahjahan et al., 2018; Rizzotti et al., 2022), which, under prolonged stress exposure, could further compromise oxygen delivery capacity.

The Right Jet likely generated zones of higher turbulence or acceleration along the fish's preferred swimming path, increasing hydrodynamic resistance and, consequently, metabolic demand. ADV measurements confirmed local velocity peaks of ~ 0.69 m s^{-1} near the right-hand wall and a rapid attenuation within 2–3 m downstream. Such confined acceleration zones could elevate hydrodynamic loading without creating large-scale turbulence, explaining the sublethal but measurable oxidative response. The elevated energetic cost is consistent with the observed upregulation of GPx and CAT and the concomitant rise in MDA levels, suggesting that increased ROS production was primarily linked to mitochondrial metabolism in erythrocytes. In contrast, the Left Jet produced lower oxidative responses, possibly reflecting reduced turbulence and a lower physiological cost of navigation. These findings indicate that the physiological burden of jet-induced flow is direction-dependent and that oxidative stress markers can

reveal subtle, flow-related metabolic strains that traditional stress indicators such as cortisol fail to capture (Conrad et al., 2018).

Behavioral analysis provided complementary insights. While no significant treatment effects on lateral swimming distribution were detected, smolts consistently displayed a left-side preference across treatments, with the highest number of observations on the left side of the flume and the lowest on the right, which was also supported by the SPI value. Although behavior alone did not differentiate between treatments, its alignment with the physiological asymmetry observed in the Right Jet condition suggests that hydrodynamic features may shape both subtle physiological costs and lateral positioning tendencies.

This behavioral bias may reflect flow asymmetries in the flume, potentially reinforced by learned path-of-least-resistance behaviors (Funk et al., 2024). Although we did not quantify the velocity field or turbulence intensity within the flume, visual cues and the structural layout suggest that asymmetries in the wider outer curve may have influenced swimming trajectories. The jets were tested on separate sides of the flume to prevent overlapping flow fields and confounding effects, reflecting conditions relevant to guidance flow rather than the complex hydraulics inside a bypass. In practical applications, such cues are typically unidirectional, steering fish toward a single safe passage route.

Consistent with this, smolts most frequently used the leftmost of the four connecting channels (Channel 4) for entry and exit between the start box and test arena (Online Resource 2). Channel 4, which followed the outer curve of the flume, appeared more open and likely offered lower flow resistance than the inner channels, potentially reinforcing the observed left-side preference.

While there was no statistically significant difference in swimming depth between treatments, smolts predominantly occupied the lower third of the water column. This behavior likely reflects an energy-saving strategy, allowing fish to avoid areas of elevated turbulence or surface disturbance (Liao et al., 2003; Riley, 2007). However, the concurrent elevation of oxidative stress markers in the Right Jet treatment suggests that even in the absence of clear behavioral shifts, fish experienced a measurable physiological cost when exposed to this specific hydrodynamic

condition. This implies that smolts may mitigate, rather than fully avoid, unfavorable flow conditions by adopting subtle behavioral strategies that reduce but do not eliminate hydrodynamic stress.

The lack of a clear behavioral response to jet direction may be attributed to limited behavioral plasticity in hatchery-reared smolts. Hatchery environments typically lack hydrodynamic variability and natural cues, resulting in reduced responsiveness to environmental stimuli (Brown & Laland, 2001; Jonsson & Jonsson, 2011). Moreover, smolts have lower burst-swimming capacity compared to adult kelts (Peake et al., 1997), and may thus perceive turbulent jet flows more as obstacles than guidance cues (Liao et al., 2003). This aligns with evidence that fish avoid turbulent zones unless directional cues are interpretable (Kemp et al., 2005). Despite these limitations, hatchery-reared smolts remain highly relevant for downstream guidance research. Across Europe, supportive restocking and reintroduction programs are essential to compensate for the decline of wild salmonid populations caused by habitat fragmentation, climate stress, and hydropower barriers (Belletti et al., 2020). Developing guidance systems that are effective for hatchery-reared fish is therefore critical to improving post-release survival and ensuring the long-term success of conservation and rewilding efforts.

The co-occurrence of elevated oxidative stress markers in the Right Jet group and the consistent avoidance of the right flume side, despite the absence of statistically significant behavioral differences, suggests that physiological responses may reveal sublethal stress even in the absence of overt behavioral changes.

Conclusion

Our study supports the use of non-lethal blood sampling, leveraging metabolically competent erythrocytes, as a sensitive tool for detecting sublethal oxidative stress in fish exposed to hydrodynamic challenges. This approach complements traditional endocrine assays and allows the integration of oxidative and behavioral indicators from the same individuals, thereby providing a refined, minimally invasive framework for assessing fish welfare in applied contexts (Ruas et al., 2008; Seibel et al., 2021; Pollard et al., 2022).

By integrating physiological markers with behavioral observations, we show that oxidative stress indicators can uncover subtle, direction-dependent metabolic costs associated with jet-induced flow effects that traditional stress markers such as cortisol may fail to capture. Given that baseline cortisol levels in our study were relatively low, this supports the interpretation that the observed oxidative responses reflect genuine, flow-induced metabolic strain rather than handling artifacts.

These findings highlight the importance of combining physiological and behavioral data when evaluating fish guidance systems. Effective passage designs should align hydraulic cues with the energetic and sensory capacities of the fish. For hatchery-reared smolts, this means reducing turbulent barriers and creating low-cost, interpretable flow patterns that minimize physiological strain while improving guidance efficiency. This is especially important in large-scale rewilding and restocking programs, which depend on high post-release survival to achieve conservation outcomes.

Supporting Information

Supplementary material is available online. *Online Resource 1* provides cortisol, hemoglobin, lactate, and AOPP levels across the three treatments. *Online Resource 2* shows channel usage patterns of Atlantic salmon smolts during entry and re-entry into the test arena.

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Author contributions DA, PA, KN, and LG conceptualized the study; DB, GS, AE, LG helped in methodology; SS, VS, and GS validated the study; SS and VS helped in formal analysis; SS, VS, EP, DA, SM, and EN contributed to investigation; DA, PA, KN, AE, and AM helped in resources; SS, VS, and LG curated the data; SS helped in visualization and writing—original draft preparation; VS, EP, DA, SM, EN, PA, KN, AE, LG, AM, and GS contributed to writing—review and editing; GS supervised the study; DA, PA, KN, AM, and LG

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Data availability The data are available on request.

Declarations

Competing interests The authors declare no conflict of interests.

Ethical approval The experiments were carried out according to the guidelines and ethical permissions issued by the Animal Ethical Board of Sweden (5.8.18-13184/2017 and 5.8.18-03390/2019).

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References

- Amérand, A., H. Mortelette, M. Belhomme & C. Moisan, 2017. Silvering and swimming effects on aerobic metabolism and reactive oxygen species in the European eel. *Respiratory Physiology & Neurobiology* 235: 40–44. <https://doi.org/10.1016/j.resp.2016.10.001>.
- Baker, A., C.C. Lin, C. Lett, B. Karpinska, M. H. Wright, & C. H. Foyer, 2023. Catalase: A critical node in the regulation of cell fate. *Free Radical Biology and Medicine* 203: 1–12. <https://doi.org/10.1016/j.freeradbiomed.2023.02.009>
- Balasz, J. C. & L. Tort, 2019. Netting the stress responses in fish. *Frontiers in Endocrinology* 10: 62. <https://doi.org/10.3389/fendo.2019.00062>.
- Barbarossa, V., R. J. P. Schmitt, M. A. J. Huijbregts, C. Zarfl, H. King & A. M. Schipper, 2020. Impacts of current and future large dams on the geographic range connectivity of freshwater fish worldwide. *Proceedings of the National Academy of Sciences* 117: 3648–3655. <https://doi.org/10.1073/pnas.1912776117>.
- Beauchamp, C. & I. Fridovich, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44: 276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8).
- Belletti, B., C. Garcia De Leaniz, J. Jones, S. Bizzi, L. Börger, G. Segura, A. Castelletti, W. Van De Bund, K. Aarestrup, J. Barry, K. Belka, A. Berkhuisen, K. Birnie-Gauvin, M. Bussetтини, M. Carolli, S. Consuegra, E. Dopico, T. Feiferfeil, S. Fernández, P. Fernandez Garrido, E. Garcia-Vazquez, S. Garrido, G. Giannico, P. Gough, N. Jepsen, P. E. Jones, P. Kemp, J. Kerr, J. King, M. Łapińska, G. Lázaro, M. C. Lucas, L. Marcello, P. Martin, P. McGinnity, J. O'Hanley, R. Olivo Del Amo, P. Parasiewicz, M. Pusch, G. Rincon, C. Rodriguez, J. Royte, C. T. Schneider, J. S. Tummers, S. Vallesi, A. Vowles, E. Verspoor, H. Wanningen, K. M. Wantzen, L. Wildman & M. Zalewski, 2020. More than one million barriers fragment Europe's rivers. *Nature* 588: 436–441. <https://doi.org/10.1038/s41586-020-3005-2>.
- Ben Ammar, I., S. Baeklandt, V. Cornet, S. Antipine, D. Sonny, S. N. M. Mandiki & P. Kestemont, 2020. Passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts. *Comparative Biochemistry and Physiology Part a: Molecular & Integrative Physiology* 247: 110745. <https://doi.org/10.1016/j.cbpa.2020.110745>.
- Bertotto, D., C. Poltronieri, E. Negrato, D. Majolini, G. Radaelli & C. Simontacchi, 2010. Alternative matrices for cortisol measurement in fish. *Aquaculture Research* 41: 1261–1267. <https://doi.org/10.1111/j.1365-2109.2009.02417.x>.
- Best, C., E. Faught, M. M. Vijayan & K. M. Gilmour, 2023. Negative feedback regulation in the hypothalamic-pituitary-interrenal axis of rainbow trout subjected to chronic social stress. *General and Comparative Endocrinology* 341: 114332. <https://doi.org/10.1016/j.ygcen.2023.114332>.
- Birnie-Gauvin, K., D. Costantini, S. J. Cooke & W. G. Willmore, 2017. A comparative and evolutionary approach to oxidative stress in fish: a review. *Fish and Fisheries* 18: 928–942. <https://doi.org/10.1111/faf.12215>.
- Borba, J. V., E. Biasuz, G. R. Sabadin, A. C. Savicki, J. Canzian, A. C. Luchiari, I. A. Adedara & D. B. Rosemberg, 2022. Influence of acute and unpredictable chronic stress on spatio-temporal dynamics of exploratory activity in zebrafish with emphasis on homebase-related behaviors. *Behavioural Brain Research* 435: 114034. <https://doi.org/10.1016/j.bbr.2022.114034>.
- Bortoletti, M., L. Maccatrozzo, S. Peruzzi, J. E. T. Strand, M. Jobling, G. Radaelli & D. Bertotto, 2022. Dietary effects on biomarkers of growth, stress, and welfare of diploid and triploid Atlantic salmon (*Salmo salar*) during parr-smolt transformation. *Aquaculture Reports* 24: 101123. <https://doi.org/10.1016/j.aqrep.2022.101123>.
- Brown, C. & K. Laland, 2001. Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology* 59: 471–493. <https://doi.org/10.1111/j.1095-8649.2001.tb02354.x>.

- Calles, O., J. Elhagen, D. Nyqvist, A. Harbicht & P. A. Nilsson, 2021. Efficient and timely downstream passage solutions for European silver eels at hydropower dams. *Ecological Engineering* 170: 106350. <https://doi.org/10.1016/j.ecoleng.2021.106350>.
- Carragher, J. F., J. P. Sumpter, T. G. Pottinger & A. D. Pickering, 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. *General and Comparative Endocrinology* 76: 310–321. [https://doi.org/10.1016/0016-6480\(89\)90163-9](https://doi.org/10.1016/0016-6480(89)90163-9).
- Case, A. J., 2017. On the origin of superoxide dismutase: an evolutionary perspective of superoxide-mediated redox signalling. Multidisciplinary Digital Publishing Institute. *Antioxidants* 6: 82. <https://doi.org/10.3390/antiox6040082>.
- Chatzidimitriou, E., P. Bisaccia, F. Corrà, M. Bonato, P. Irato, L. Manuto, S. Toppo, R. Bakiu & G. Santovito, 2020. Copper/Zinc superoxide dismutase from the crocodile icefish *Chionodraco hamatus*: antioxidant defense at constant sub-zero temperature. *Antioxidants* 9: 325. <https://doi.org/10.3390/antiox9040325>.
- Coleman, R. A., B. Gauffre, A. Pavlova, L. B. Beheregaray, J. Kearns, J. Lyon, M. Sasaki, R. Leblois, C. Sgro & P. Sunnucks, 2018. Artificial barriers prevent genetic recovery of small isolated populations of a low-mobility freshwater fish. *Nature Publishing Group. Heredity* 120: 515–532. <https://doi.org/10.1038/s41437-017-0008-3>.
- Conrad, M., V. E. Kagan, H. Bayir, G. C. Pagnussat, B. Head, M. G. Traber & B. R. Stockwell, 2018. Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes & Development* 32: 602–619. <https://doi.org/10.1101/gad.314674.118>.
- Cornell, S. C., D. M. Portesi, P. A. Veillette, K. Sundell & J. L. Specker, 1994. Cortisol stimulates intestinal fluid uptake in Atlantic salmon (*Salmo salar*) in the post-smolt stage. *Fish Physiology and Biochemistry* 13: 183–190. <https://doi.org/10.1007/BF00004356>.
- Coutant, C. C. & R. R. Whitney, 2000. Fish behavior in relation to passage through hydropower turbines: a review. *Transactions of the American Fisheries Society* 129: 351–380. [https://doi.org/10.1577/1548-8659\(2000\)129%3c0351:FBIRTP%3e2.0.CO;2](https://doi.org/10.1577/1548-8659(2000)129%3c0351:FBIRTP%3e2.0.CO;2).
- Culbert, B. M., A. M. Regish, D. J. Hall, S. D. McCormick & N. J. Bernier, 2022. Neuroendocrine regulation of plasma cortisol levels during smoltification and seawater acclimation of Atlantic Salmon. *Frontiers in Endocrinology* 13: 859817. <https://doi.org/10.3389/fendo.2022.859817>.
- Directive - 2010/63 - EN - EUR-Lex, n.d. <https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng>
- Farag, M. R. & M. Alagawany, 2018. Erythrocytes as a biological model for screening of xenobiotics toxicity. *Chemico-Biological Interactions* 279: 73–83. <https://doi.org/10.1016/j.cbi.2017.11.007>.
- Farrell, A. P., A. K. Gamperl & I. K. Birtwell, 1998. Prolonged swimming, recovery and repeat swimming performance of mature sockeye salmon *Oncorhynchus Nerka* exposed to moderate hypoxia and pentachlorophenol. *Journal of Experimental Biology* 201: 2183–2193. <https://doi.org/10.1242/jeb.201.14.2183>.
- Feio, M. J., R. M. Hughes, S. R. Q. Serra, S. J. Nichols, B. J. Kefford, M. Lintermans, W. Robinson, O. N. Odume, M. Callisto, D. R. Macedo, J. S. Harding, A. G. Yates, W. Monk, K. Nakamura, T. Mori, M. Sueyoshi, N. Mercado-Silva, K. Chen, M. J. Baek, Y. J. Bae, R. D. Tachamo-Shah, D. N. Shah, I. Campbell, N. Moya, F. O. Arimoro, U. N. Keke, R. T. Martins, C. B. M. Alves, P. S. Pompeu & S. Sharma, 2023. Fish and macroinvertebrate assemblages reveal extensive degradation of the world's rivers. *Global Change Biology* 29: 355–374. <https://doi.org/10.1111/gcb.16439>.
- Funk, N., J. Knott, J. Pander & J. Geist, 2024. Fish behavior at the horizontal screen of a novel shaft hydropower plant. *River Research and Applications* 40: 1219–1230. <https://doi.org/10.1002/rra.4302>.
- Friard, O., Gamba, M. 2016. BORIS a free versatile open-source event-logging software for video/audio coding and live observations. *Summary Methods in Ecology and Evolution* 7(11): 1325-1330. <https://doi.org/10.1111/mee3.2016.7>
- Jonsson, B. & N. Jonsson, 2011. Population enhancement and population restoration. In Jonsson, B. & N. Jonsson (eds), *Ecology of Atlantic Salmon and Brown Trout: habitat as a template for life histories* Springer, Netherlands: 567–632.
- Kemp, P. S., M. H. Gessel & J. G. Williams, 2005. Fine-scale behavioral responses of Pacific Salmonid Smolts as they encounter divergence and acceleration of flow. *Transactions of the American Fisheries Society* 134: 390–398. <https://doi.org/10.1577/T04-039.1>.
- Khare, A., N. Chhawani & K. Kumari, 2019. Glutathione reductase and catalase as potential biomarkers for synergistic intoxication of pesticides in fish. *Biomarkers* 24: 666–676. <https://doi.org/10.1080/1354750X.2019.1651902>.
- Liao, J. C., D. N. Beal, G. V. Lauder & M. S. Triantafyllou, 2003. Fish exploiting vortices decrease muscle activity. *American Association for the Advancement of Science. Science* 302: 1566–1569. <https://doi.org/10.1126/science.1088295>.
- Livingstone, D. R., F. Lips, P. G. Martinez & R. K. Pipe, 1992. Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis*. *Marine Biology* 112: 265–276. <https://doi.org/10.1007/BF00702471>.
- Nyqvist, D., L. A. Greenberg, E. Goerig, O. Calles, E. Bergman, W. R. Ardren & T. Castro-Santos, 2017. Migratory delay leads to reduced passage success of Atlantic salmon smolts at a hydroelectric dam. *Ecology of Freshwater Fish* 26: 707–718. <https://doi.org/10.1111/eff.12318>.
- Pacchini, S., E. Piva, S. Schumann, P. Irato, D. Pellegrino & G. Santovito, 2023. An experimental study on antioxidant enzyme gene expression in *Trematomus newnesi* (Boulenger, 1902) experimentally exposed to perfluoro-octanoic acid. *Multidisciplinary Digital Publishing Institute. Antioxidants* 12: 352. <https://doi.org/10.3390/antiox12020352>.
- Pavlova, A., L. B. Beheregaray, R. Coleman, D. Gilligan, K. A. Harrison, B. A. Ingram, J. Kearns, A. M. Lamb, M. Lintermans, J. Lyon, T. T. T. Nguyen, M. Sasaki, Z. Tonkin, J. D. L. Yen & P. Sunnucks, 2017. Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: a call for assisted gene

- flow. *Evolutionary Applications* 10: 531–550. <https://doi.org/10.1111/eva.12484>.
- Peake, S., F. W. Beamish, R. S. McKinley, D. A. Scruton & C. Katopodis, 1997. Relating swimming performance of lake sturgeon, *Acipenser fulvescens*, to fishway design. *NRC Research Press. Canadian Journal of Fisheries and Aquatic Sciences* 54: 1361–1366. <https://doi.org/10.1139/f97-039>.
- Pettinau, L., E. Seppänen, A. Sikanen & K. Anttila, 2022. Aerobic exercise training with optimal intensity increases cardiac thermal tolerance in Juvenile Rainbow Trout. *Frontiers in Marine Science*. <https://doi.org/10.3389/fmars.2022.912720>.
- Pfalzgraff, T., I. Lund & P. V. Skov, 2022. Prolonged cortisol elevation alters whole body and tissue metabolism in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part a: Molecular & Integrative Physiology* 263: 111098. <https://doi.org/10.1016/j.cbpa.2021.111098>.
- Piva, E., S. Schumann, S. Dotteschini, G. Brocca, G. Radaelli, A. Marion, P. Irato, D. Bertotto & G. Santovito, 2022. Antioxidant responses induced by PFAS exposure in freshwater fish in the Veneto Region Multidisciplinary Digital Publishing Institute. *Antioxidants* 11: 1115. <https://doi.org/10.3390/antiox11061115>.
- Pollard, S., J. C. Anderson, F. Bah, M. Mateus, M. Sidhu & D. B. D. Simmons, 2022. Non-lethal blood sampling of fish in the lab and field with methods for dried blood plasma Spot Omic analyses. *Frontiers in Genetics*. <https://doi.org/10.3389/fgene.2022.795348>.
- Riley, W. D., 2007. Seasonal downstream movements of juvenile Atlantic salmon, *Salmo salar* L., with evidence of solitary migration of smolts. *Aquaculture* 273: 194–199. <https://doi.org/10.1016/j.aquaculture.2007.10.022>.
- Rizzotti, D., C. Manfrin, M. Gerdol, S. Greco, G. Santovito & P. G. Giulianini, 2022. Morphological analysis of erythrocytes of an Antarctic teleost under heat stress: bias of the stabling effect. *Journal of Thermal Biology* 103: 103139. <https://doi.org/10.1016/j.jtherbio.2021.103139>.
- Roza e Silva, M. L., R. T. Pereira, A. L. Arvigo, F. S. Zanuzzo & R. E. Barreto, 2020. Effects of water flow on ventilation rate and plasma cortisol in Nile tilapia introduced into novel environment. *Aquaculture Reports* 18: 100531. <https://doi.org/10.1016/j.aqrep.2020.100531>.
- Ruas, C. B. G., C. D. S. Carvalho, H. S. S. de Araújo, E. L. Gaeta Espíndola & M. N. Fernandes, 2008. Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. *Ecotoxicology and Environmental Safety* 71: 86–93. <https://doi.org/10.1016/j.ecoenv.2007.08.018>.
- Rummer, J. L. & C. J. Brauner, 2015. Root effect haemoglobins in fish may greatly enhance general oxygen delivery relative to other vertebrates. *PLoS ONE* 10: e0139477. <https://doi.org/10.1371/journal.pone.0139477>.
- Sadoul, B. & B. Geffroy, 2019. Measuring cortisol, the major stress hormone in fishes. *Journal of Fish Biology* 94: 540–555. <https://doi.org/10.1111/jfb.13904>.
- Schumann, S., E. Negrato, A. Marion, G. Santovito & D. Bertotto, 2024. Stress evaluation using physiological biomarkers on fish tested in the hydraulic facility. In Kalinowska, M. B., M. M. Mrokowska & P. M. Rowiński (eds), *Advances in hydraulic research* Springer, Cham: 353–362.
- Schwevers, U. & B. Adam, 2020. *Fish protection technologies and fish ways for downstream migration*, Springer, Cham:
- Seibel, H., B. Baßmann & A. Rebl, 2021. Blood will tell: what hematological analyses can reveal about fish Welfare. *Frontiers in Veterinary Science* 8: 616955. <https://doi.org/10.3389/fvets.2021.616955>.
- Shahjahan, Md., Md. H. Uddin, V. Bain & Md. M. Haque, 2018. Increased water temperature altered hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish *Pangasianodon hypophthalmus*. *Fish Physiology and Biochemistry* 44: 1309–1318. <https://doi.org/10.1007/s10695-018-0522-0>.
- Stoilova, V., E. Bergman, D. Aldvén, R. E. Bowes, O. Calles, N. Nyquist, D. Nyqvist, P. Rowinski & L. Greenberg, 2025. Downstream guidance performance of a bubble curtain and a net barrier for the European eel, *Anguilla anguilla*, in an experimental flume. *Ecological Engineering* 215: 107599. <https://doi.org/10.1016/j.ecoleng.2025.107599>.
- Sun, J., W. Du, M. C. Lucas, C. Ding, J. Chen, J. Tao & D. He, 2023. River fragmentation and barrier impacts on fishes have been greatly underestimated in the upper Mekong River. *Journal of Environmental Management* 327: 116817. <https://doi.org/10.1016/j.jenvman.2022.116817>.
- Weber, J.-M., K. Choi, A. Gonzalez & T. Omlin, 2016. Metabolic fuel kinetics in fish: swimming, hypoxia and muscle membranes. *Journal of Experimental Biology* 219: 250–258. <https://doi.org/10.1242/jeb.125294>.
- Witko-Sarsat, V., M. Friedlander, T. Nguyen Khoa, C. Capeillère-Blandin, A. T. Nguyen, S. Canteloup, J. M. Dayer, P. Jungers, T. Drüeke & B. Descamps-Latscha, 1998. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *Journal of Immunology (Baltimore, Md.: 1950)* 161: 2524–2532.
- Zöschg, H., W. Dobler, M. Aufleger & B. Zeiringer, 2023. Evaluation of hydraulics and downstream fish migration at run-of-river hydropower plants with horizontal bar rack bypass systems by using CFD. *Multidisciplinary Digital Publishing Institute. Water* 15: 1042. <https://doi.org/10.3390/w15061042>.

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