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Potential Interactions of Sediment Characteristics and Oxygen Availability: Effects on Embryonic Development of Rainbow Trout *(Oncorhynchus mykiss)*

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Master of Science in Biology

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Abstract

Increasing sediment influx from construction activities threatens salmonid spawning habitats, impacting the development and hatching success of embryos due to finer sediments. This study investigates the interplay between sediment coverage, grain size, and low dissolved oxygen levels on the mortality and hatching success of Rainbow trout (*Oncorhynchus mykiss*) embryos, from fertilization to hatching, using a dual-experiment approach in a laboratory setting. The first experiment evaluated fine sediment characteristics, while the second examined reduced oxygen levels, both focusing on egg mortality, development, growth, and hatching success.

Results indicated that finer sediments (<2 mm) influenced all biological endpoints to varying degrees. Thick layers caused early underdevelopment and death before hatching, while thin layers led to underdeveloped embryos and alevins with reduced growth. Mortality rates and hatching success were dependent on sediment fraction and layer thickness. Developmental delay and growth were primarily influenced by sediment fraction, with thick layers exaggerating these effects. Silt (0.063-0.002 mm) was the most harmful fraction, causing high mortality rates. Sand (0.63-0.2 mm) also significantly impacted parameters, with coarse sand (2.0-1.0 mm) causing high mortality post-hatching due to pre-hatching developmental issues, challenging previous studies. Mixed sediments (<2 mm), containing clay, resulted in the highest early mortality rates.

While a 70% oxygen level posed no significant effects, 40-50% oxygen levels led to high mortality rates, predominantly post-hatching. A notable correlation between finer sediments (<0.63 mm) and reduced dissolved oxygen (DO) levels was observed, suggesting oxygen availability may contribute to developmental delays and mortality in these sediments. However, even with sufficient oxygen, finer sediments still caused developmental delays and mortality, indicating that sediment characteristics and physical effects affect outcomes.

This study supports the theory that fine sediment characteristics and low dissolved oxygen levels detrimentally affect Rainbow trout embryo development. Nevertheless, further research is needed to explore embryo development and survival under sufficient oxygen levels beneath sediments. The findings highlight the need for effective mitigation strategies addressing both sediment size and quantity to reduce fine sediment runoff during construction activities.

1. Introduction

Anthropogenic activities, such as mining, deforestation, agriculture, and construction, have significantly increased sediment flux in freshwater ecosystems (Allan, 2004; Brown et al., 2005; Le Gall et al., 2016; Quinton et al., 2010; Sellier et al., 2020). These ecosystems, including rivers, lakes, and streams, though covering only a small fraction of Earth's surface, are vital for ecological stability and the provision of essential ecosystem services (Baron et al., 2002; Finlayson et al., 2018). They support a rich biodiversity, with numerous endemic species critical for maintaining diverse ecological functions (Abell et al., 2008; Covich et al., 1999; Lapointe et al., 2013). Sediment dynamics, including processes like erosion, transport, deposition, and resuspension, are fundamental to the structure and function of these ecosystems (Apitz, 2012; Larson et al., 2020). While sediment is a natural part of ecosystem processes, human-induced alterations have led to elevated sediment loads and changed sediment distribution patterns, particularly in catchments near these activities (Dearing & Jones, 2003; Donohue & Garcia Molinos, 2009; Maaß et al., 2021). The increase in sediment flux can be detrimental to aquatic life, especially by affecting the habitats of spawning fish and the development of their embryos (Kemp et al., 2011). The declining salmonid populations are partly a consequence of altered habitats and spawning areas (Gibson, 2017; Yeakley, 2014). Hence, it is essential to preserve freshwater ecosystems and mitigate the impact of sediment on embryo development. This can be accomplished through the creation of knowledge-based strategies aimed at reducing these anthropogenic impacts. A comprehensive understanding of the types of sediments, the quantities that are harmful, and the mechanisms behind their effects is crucial for developing effective mitigation strategies.

1.1 Sediment pollution from construction and mitigation strategies

Construction and infrastructure development, such as roadwork, alter surface and soil physical conditions (Chen et al., 2009; Trombulak & Frissell, 2000), leading to increased sediment export and turbidity in nearby water bodies (E. Line et al., 2011). These activities disrupt natural vegetation cover and modify catchment surfaces, resulting in heightened erosion (Burns, 1970; Walling, 2008). Additionally, direct interventions within aquatic systems, including the construction of bridge foundations, pipeline installations, and the discharge of

suspended solids from tunnelling and piling activities, contribute to sediment pollution (Roseth R, 2021). The sediment release from such construction can persist for years, maintaining elevated sediment levels in streams (Burns, 1970). Excess soil particles such as clay, silt, and sand, when washed into water bodies, can act as pollution, altering physical, chemical, and biological processes. This pollution reduces water clarity, modify water flow and temperature (Ryan, 1991) and negatively impact aquatic life (Fossati et al., 2001; Hancock, 2002). Additionally, sediment can carry other pollutants such as metals and nutrients, leading to further chemical contamination and altering aquatic ecosystems (Wetzel et al., 2013; G. Zhang et al., 2017). These anthropogenic shifts in sediment dynamics significantly affect the health and stability of aquatic environments (Dearing & Jones, 2003; Donohue & Garcia Molinos, 2009).

Environmental risk assessments and regulatory guidelines are crucial in managing sediment runoff from construction and infrastructure development. In Norway, the County Governor sets threshold values for specific projects via emission permits, while the Water Regulation outlines threshold values for the ecological and chemical status of water bodies. However, neither regulation specifies limits for suspended materials. Emission permits are often based on regulations and guidelines from organizations such as the European Inland Fisheries Advisory Commission (EIFAC) and the County Governor and the Norwegian Environment Agency (NEA). For instance, EIFAC guidelines state that concentrations below 25 mg/L of suspended particles pose minimal risk to fish, 25-80 mg/L can cause moderate harm, and above 80 mg/L can lead to significant damage. In Norway, risk assessments and permits often relay on studies by Alabaster (1972); Alabaster and Lloyd (1980), report NFF (2009), and (SFT, 1997) (Roseth R, 2021). These assessments typically include monitoring fish, benthic macroinvertebrates, periphyton, and water quality before and after construction activities.

Mitigating the impact of sediment requires sustainable practices that identify and assess harmful effects of sediments and develop strategies to reduce sediment loads, thereby preserving the stability and health of freshwater ecosystems. Integrated strategies focus on both erosion and sediment control, using methods such as slope coverings, sustainable drainage systems (SUDS), and best management practices (BMPs) (Mooselu et al., 2022). Vegetative buffers and cover crops help anchor soil and reduce runoff, while dams and sediment traps slow water flow and capture sediment. Secondary measures, such as silt fences, sediment basins, and ponds, capture eroded materials before they enter water bodies.

(Lundy et al., 2016). These approaches emphasize minimizing land disturbance, preserving natural vegetation, and diverting clean water to prevent contamination. Despite these efforts, challenges in managing sediment load and pollution persist, highlighting the need for continuous advancements.

1.2 Salmonid spawning and environmental requirements

The increased sediment flux and deposition play a critical role in altering of riverbed configurations and the spawning habitats of freshwater fish (Bertin & Friedrich, 2019; Owens et al., 2005). As the sediment composition changes, the riverbed substrate undergoes alterations that can make it inhospitable for the reproductive activities of various fish species.

Salmonids, such as rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and Atlantic salmon (*Salmo salar*), primarily spawn in streams that provide the specific environmental conditions necessary for successful reproduction. These species exhibit selective habitat preferences, typically choosing microhabitats influenced by parameters such as water depth, flow velocity, and substrate composition (Armstrong et al., 2003; Beard & Carline, 1991; Gibson, 1993). Female salmonids create ‘redds’ in spawning beds using vigorous tail movements to displace gravel and small stones, forming depressions in the streambed substrate (Edo et al., 2000; Jones & Ball, 1954). This digging process culminates in a pit where the eggs are deposited, usually buried 15-25 cm deep (Bardonnet & Baglinière, 2000), providing a protective layer that shields the eggs from potential predators and adverse environmental conditions (Jones & Ball, 1954; Ottaway et al., 1981).

Optimal spawning habitats are characterized by cool, oxygen-rich waters and gravel or rocky substrates, which are essential for egg deposition, embryonic development, and egg survival (Armstrong et al., 2003; Douglas Workman et al., 2004). Specifically, small to medium-sized pebble gravel (5 mm – 65 mm) with interstitial spaces is crucial for facilitating effective gas exchange and the removal of metabolic wastes through elevated stream velocities (Barwick et al., 2004; Beard & Carline, 1991; Louhi et al., 2008). Accumulation of metabolic wastes can create toxic conditions within the redd, so efficient removal is critical for maintaining a healthy developmental environment (D. W. Chapman, 1988)

Gas exchange and sufficient oxygen level for efficient energy production, embryo development is important for growth and survival, it is necessary to support the metabolic

demands of developing embryos (Silver et al., 1963). Oxygen plays a pivotal role in metabolism by influencing the activity of key enzymes involved in both aerobic and anaerobic pathways (Matschak et al., 1998). Furthermore, it is crucial for the synthesis of biomolecules and the regulation of gene expression in embryos (Kajimura et al., 2006; Zhang et al., 2017).

1.3 Influence of sediment characteristic on salmonid spawning success

The habitat in which salmonid eggs incubate is highly intricate, with multiple factors simultaneously influencing their development and survival. Therefore, it is crucial to understand how sediment movement affects the gravel environment and, consequently, the development and survival of salmonid embryos.

Fine sediment has been demonstrated to be more detrimental to early fish stages than coarser sediment fractions (D. W. Chapman, 1988; Tappel & Bjornn, 1983). Empirical evidence from both field and laboratory experiments supports these mechanisms, demonstrating that fine sediment decreases the survival rates of salmonid eggs (Argent & Flebbe, 1999; Greig et al., 2005; Hausle & Coble, 1976; Heywood & Walling, 2007; Kemp et al., 2011; Lapointe et al., 2004). The primary hypotheses for this reduction focus on the obstruction of interstitial pathways, which facilitate water percolation to the eggs, leading to reduced intragravel flow velocities and dissolved oxygen levels (DO) (Kemp et al., 2011; Lapointe et al., 2004). These barriers hinder metabolic waste removal and fry emergence, reducing the successful incubation of salmon embryos (Hausle & Coble, 1976; Julien & Bergeron, 2006; Sear et al., 2008; Sternecker & Geist, 2010).

Previous research have found that accumulation of fine sediment in redds impact the availability of oxygen for incubating salmonid embryos, influencing their survival and development (Pattison et al., 2013; Sear et al., 2008; Sear et al., 2017). Greig et al. (2005) found that fine sediment accumulation affects incubation success through several mechanisms: (i) reduced gravel permeability, leading to diminished oxygenated water flow through the incubation environment; (ii) decreased intragravel oxygen concentration due to the infiltration of oxygen-consuming materials; and (iii) hindered oxygen exchange across the egg membrane.

Several factors influence the dissolved oxygen regime within spawning gravels, including the infiltration of fine sediments, groundwater and surface water penetration, thermal conditions, and oxygen consumption by the sediment and associated organic matter (Coble, 1961; Greig et al., 2007; Sear et al., 2014). Fine sediment accumulation within redd gravels decrease their permeability, hindering the exchange between surface and intragravel water, and subsequently reducing the supply of DO essential for salmonid embryonic development (Heywood & Walling, 2007; Meyer et al., 2008). Moreover, larger quantities of sediment increase sediment oxygen demand (SOD) and sediment oxygen consumption (SOC) further reducing DO levels (Sear et al., 2017).

The reduced permeability and obstruction of gravel pores can create hypoxic conditions that are detrimental to embryo survival, particularly given already low DO concentrations within the gravel (Heywood & Walling, 2007; Yamada & Nakamura, 2009). Additionally, the distribution of DO within the redd is influenced by hyporheic flow - the movement and mixing of surface water and groundwater through the sediment - which plays a crucial role in oxygenating the eggs (Cardenas et al., 2016; Coble, 1961). Low DO levels or reducing the influence of either of these, exacerbates the hypoxic environment in the redds, further lowering embryo survival rates (Sear et al., 2014).

Aquatic hypoxia acts as a teratogen (Shang, 2004), causing significant developmental issues in embryos exposed to low oxygen conditions. These embryos can exhibit a range of negative outcomes, including developmental delays, slower growth, increased deformities, reduced larval length, delayed emergence, decreased swimming activity, heightened predation rates during early juvenile stages, and overall lower survival rates and fitness (Alderdice et al., 1958; Bloomer et al., 2016; Geist et al., 2006; Hassell et al., 2008; Roussel, 2007; Shang, 2004; Youngson et al., 2004). Hypoxic condition at early life stages can have long lasting effects trough epigenetic modification, which cause disruption of apoptotic pattern (Shang & Wu, 2004), altered muscle tissues (Matschak et al., 1997), changes in enzyme activities and metabolism (Matschak et al., 1998).

Although some research exists on different oxygen levels and their effects on embryonic development, there is not yet a comprehensive understanding of how low these levels can drop before negative effects are observed, nor of the precise threshold DO levels and oxygen requirements. For instance, brown trout embryos exhibit slower growth and reduced survival rates at 3.0 mg/L of dissolved oxygen at 8.2°C (Roussel, 2007). Rainbow trout also grew

slower at 5.0 mg/L (Ciuhandu et al., 2005). In comparison, steelhead and lake trout embryos show high mortality rates when DO concentrations drop below 2.5-3.5 mg/L at 5-7 °C (Garside, 1959; Silver et al., 1963), with all steelhead dying at 1.6 mg/L (9,5 °C) (Silver et al., 1963). (Sowden & Power, 1985) demonstrated that in redds, survival rates near 0% occur below 4.5 mg/L of DO, with significant improvements as DO levels exceed 5.2 mg/L. These variable results indicate a continuing need to better understand the mechanisms behind sedimentation in gravels and its effects on oxygen levels on embryo viability.

1.4 Challenges and research gaps

Adequate oxygen levels in aquatic environments are essential for the health and survival of freshwater species, especially during their early life stages. Hypoxia poses significant risks to these organisms, highlighting the need to address these environmental issues. The presence of finer sediment in spawning habitats can influence oxygen availability by restricting water flow. Despite efforts to mitigate such impacts, current environmental risk assessment practices still face substantial challenges.

Typically, protocols involve detailed environmental surveys before construction activities and the issuance of emission permits based on a variety of articles and reports. However, these measures often lack consistency and do not establish clear thresholds for emissions. Moreover, the permits may not adequately account for the specific characteristics of individual water bodies, such as their unique ecological, hydrological, and geological features. This inconsistency highlights the need for a more comprehensive understanding of environmental impacts.

Several critical questions remain, such as the impact of specific sediment sizes on embryo development, the periods during which aquatic organisms are most vulnerable, and the sediment thickness that can affect spawning habitats. Addressing these gaps is crucial for improving the precision and effectiveness of environmental risk assessments, ultimately supporting the sustainability of aquatic ecosystems. The insights gained from this experiment can contribute knowledge-based emission standards for suspended solids and turbidity from construction work.

1.5 Aims

The primary objective of this master's thesis is to investigate the intricate relationship between sediment characteristics, specifically size and thickness, and oxygen availability on the development of rainbow trout embryos. The interaction between fine sediment and oxygen availability in spawning environments is complex and multifaceted, particularly in relation to early embryonic development. Previous research has explored the impacts of sediment on hatching success, growth delays, and survival, often correlating these effects with low dissolved oxygen levels. However, a comprehensive analysis that integrates these factors is lacking.

This study addresses this gap by examining the effects of fine sediment layers and hypoxia on embryonic development. It assesses whether these effects are analogous to hypoxic conditions and explores if reduced oxygen concentration could be the primary cause of increased mortality and decreased hatching success. Understanding these dynamics is crucial for the effective management and preservation of salmonid populations.

Research question:

How do different levels of sediment coverage and varying sediment grain sizes affect the survival and hatching success of rainbow trout (Oncorhynchus mykiss) eggs, and to what extent does hypoxia contribute to the mortality of embryos exposed to fine sediments?

Hypotheses:

H0: Sediment coverage of rainbow trout eggs does significantly affect the survival and hatching success of the eggs.
H1: Different levels and size of sediment coverage reduce the survival and hatching success of rainbow trout eggs.
H2: Fine sediments reduce oxygen availability, causing hypoxia and resulting in an increased mortality rate of embryos.

2. Method

This master's thesis explores the impacts of sediment deposition and oxygen deficiency on fish egg development from fertilization to hatching, through a dual-experiment approach. The first part of the experiment assesses the influence of sediment characteristics, including particle size and layer thickness, on egg viability and growth. Simultaneously, the second part of the experiment examines the effects of hypoxia, mirroring conditions beneath sediment layers, on embryonic development during the same experimental period. The methodology encompasses:

1. Exposing eggs to varied sediment thicknesses across four sediment categories (Table 2).
2. Adjusting oxygen concentrations to examine effects in hypoxic environment (Table 2).

2.1 Location

The experiment was conducted from October 2023 to February 2024 at the Fish Laboratory of the Norwegian University of Life Sciences (NMBU) in a climate room, with certain analyses performed at NMBU.

2.2 Setup

To maintain a controlled environment, the experiment climate room was established at a uniform 6 °C. Additionally, the regulation of ambient lighting was meticulously managed throughout the experiment, activated solely for specific procedures including the collection of samples and monitoring of eggs.

The system was designed to promote effective recirculation, ensuring similar water quality across different groups by incorporating multiple reservoirs, each with a 100-liter capacity. These reservoirs were systematically connected through a consistent pumping mechanism to a header box, measured at 22 cm x 23 cm x 18 cm, with overflow to maintain a constant water level, ensuring a uniform flow and water quality. The design (Figure 1) facilitated the distribution of water across eight distinct boxes via silicon tubes of 4 mm diameter. Within these, a 2 mm diameter inner tube, positioned strategically in the header box, helped regulate the water flow. This setup aimed to emulate river flow, primarily to reduce sediment disturbance within each box while maintaining a uniform flow rate of 200 ml/min across all boxes.

To ensure oxygen saturation, aeration was employed within the tank. To counteract CO_2 accumulation, a tube from the header box to the tank was integrated, enabling water flow to effectively disperse CO_2 concentrations. This combined strategy effectively managed oxygen and CO_2 levels, promoting a stable environment within the tank.

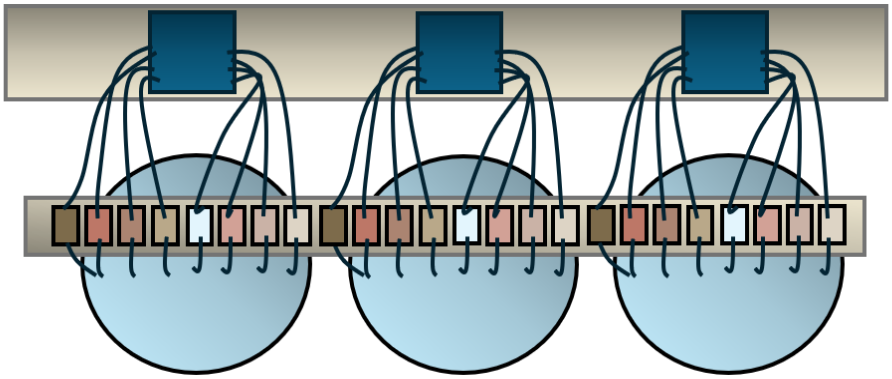

Figure 1 Illustration of the recirculating system. The system consists of a series of reservoirs linked to a central header box, which distributes water uniformly across eight containment units. This design simulates a riverine ecosystem with a continuous flow of water, ensuring consistent environmental conditions.

In the experimental setup, experiment-specific boxes were employed, fabricated from plastic materials with standard dimensional specifications as illustrated in Figure 2. Each experimental box was methodically designed to feature two apertures of specified diameters, precisely drilled to 3 mm and 6 mm, respectively, to facilitate the integration of silicone tubing for experimental manipulations. Additionally, a grid was installed within the boxes to prevent the escape of larvae through the 6 mm opening. The design and adjustment of these drilled holes were crucial for maintaining experimental integrity and ensuring accurate procedural replication.

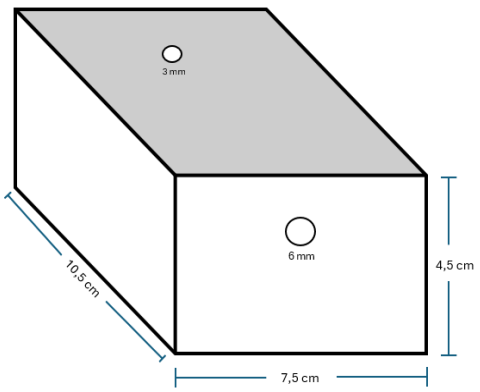

Figure 2 Illustration of the experimental boxes. Each box with standard dimensions, and two drilled holes.

Figure 3 Shows photos of sediment exposure boxes with control unit (left) and sediment units (centre), alongside the oxygen exposure unit (right). This setup illustrates part of the experiment arrangement used to study sediment and oxygen effect.

The experiment included 15 reservoirs: 3 for oxygen and 12 for sediment experiments. The oxygen experiment utilized 12 boxes, with 4 boxes assigned to each oxygen concentration (Figure 3). For the sediment experiment, 96 boxes were used as shown in Figure 3, with 12 boxes designated for each sediment treatment. In addressing the visible challenge of sediment cover, a total of 108 experimental boxes were employed to ensure reliable data and improve observational accuracy. This setup allowed for the targeted removal of specific boxes during designated sampling periods, thereby improving the accuracy of the detailed analysis across both experimental parameters.

2.2.1 Egg preparation

The experiment utilized dry-stripped Rainbow trout eggs and sperm from AquaGen hatchery in Norway (Lerøy Sjøtroll avd. Jakta), transported and stored in ice-chilled polystyrene. Three biological replicates were employed, all dry-fertilized with sperm from a single male. Following dry fertilization, eggs were allocated to treatment boxes, each containing 48 ± 5 eggs. The eggs were placed in water and covered with sediment. Following an additional day to allow the sediments to settle, circulation was started for sediment and oxygen exposure studies.

2.2.2 Control water

The experimental design utilized synthetic very soft EPA water as control water, formulated according to the Environmental Protection Agency (EPA) standards. This control water was used to closely simulate the relatively ion-poor freshwater in Norway. The water, characterized by its minimal mineral content, had a carefully balanced ion composition and neutral pH levels. This created a consistent baseline, with minimum extraneous water variables that could impact the survival rates of the eggs.

2.3 Collection of sediments

Sediment samples were systematically collected from a designated site (60.4634776° N, 10.6397059° E) along the Lygna River, Norway, on September 15, 2024. Using a hybrid approach, the collection methodology combined manual techniques with subsequent sieve analysis (Table 1). Sampling was conducted under calm water conditions to target sedimentation in these pools, ensuring the inclusion of finer sediments in the distribution and minimizing sample displacement caused by high water flow.

The collection process entailed the meticulous extraction of riverbed sediments using a shovel, directly transferring them into designated buckets. These samples underwent an initial on-site sieve analysis, illustrated in Figure 4, employing river water to facilitate the separation process. Post-collection, the sediment samples, encompassing both unsieved and preliminarily sieved materials, were transported to the Norwegian University of Life Sciences (NMBU) for further sieve analysis. The samples were stored in sealed containers under ambient room temperature conditions.

Figure 4 Images of sediment treatment procedures and sieving analysis. The two pictures on the left display the sediment treatment process during sample collection in the rivers and the sieving analysis. The two pictures on the right show the sieving analysis, featuring both the sieve and the pumping mechanism used to differentiate between clay and silt.

2.4 Sediment treatment

The sediments underwent particle size and distribution analysis to isolate different size classes of sediments. Initially, the samples were sieved through a 2 mm mesh to remove the largest material. This was followed by additional sieving through 1 mm, 63 µm, 200 µm, and 63 µm meshes and categorized as showed in Table 1, incorporating reverse osmosis (RO) water to eliminate soluble impurities. In this sieving process, particles with diameters less than 63 µm were systematically differentiated into clay and silt fractions with pumping mechanism illustrated in Figure 4, utilizing Stokes' law to achieve accurate categorization based on their settling velocities.

Table 1 The table presents the sediment categories and their corresponding particle size ranges (mm).

Sediment category	**Particle size range (mm)**
Very coarse sand	2,0 – 1,0
Coarse sand	1 – 0,63
Medium sand	0,63 - 0,2
Fine sand	0,2 – 0,063
Silt	0,063 – 0,002
Clay	< 0,002

Sediments intended for inclusion in the study were subjected (Table 2) to a sterilization process to eliminate biological interferences, achieved through autoclaving over a period ranging from 48 to 72 hours prior to their utilization in the experiments. This ensured the removal of potential biological contaminants that could affect the development of the eggs under study.

2.4.1 Sediment exposure

Sediment used in the experiment was categorized into eight distinct groups based on sediment type and layer thickness, presented in Table 2. Treatment groups were established based on the predominant sediment sizes identified in the analysis. An additional mix treatment incorporated a proportionate combination of all identified sediment types.

Table 2 Overview of the sediment and oxygen exposure treatments for the experiments, including the corresponding particle size ranges and the thickness and mass of layers 1 and 2. For sediment exposure, the treatments included control, coarse sand, mix, sand, and silt, with specified particle size ranges and layer measurements. Layer 1 contained 30 g of sediment, and layer 2 contained 120 g of sediment. For oxygen exposure, the treatments included control, 70-80%, and 40-60% with their respective oxygen concentrations in mg/L.

Experiment	Treatment	Exposure	Layer 1	Layer 2
Sediment exposure	Sediment control	No sediment		
	Coarse sand	2 - 1 mm		8-10 mm *(120 g)*
	Mix	2 - < 0.002 mm	1-2 mm *(30 g)*	8-10 mm *(120 g)*
	Sand	0.63 – 0.2 mm	1-2 mm *(30 g)*	8-10 mm *(120 g)*
	Silt	0.063 - 0.002 mm	1-2 mm *(30 g)*	8-10 mm *(120 g)*
Oxygen exposure	Oxygen control	12.6 mg/L		
	70-80 %	9.9 mg/L		
	40-60 %	4.75 mg/L		

The meticulous application of sediment over the eggs was ensured by precisely following predefined sediment weights – 30 grams for sediment layers of 1-2 mm thickness and 120 grams for 8-10 mm thickness. To facilitate this process and counteract the natural buoyancy of the eggs, a deliberate amount of water was added to each box with fish eggs before sediment was applied. This step was crucial for both easing the sediment layering process and minimizing the upward movement of the eggs, promoting more consistent sediment coverage.

2.5 Oxygen exposure

Oxygen levels were manipulated to create three distinct environments (Table 2). Oxygen concentration was modulated by infusing nitrogen gas into the header tank, The system's design illustrated in Figure 5, which closely followed the layout shown in Figure 1, included significant modifications. The standard header box was replaced with two distinct containment units, each with increased vertical dimensions compared to standard models. This modification extended the contact time of gas bubbles with the water, improving gas exchange, decreasing atmospheric nitrogen release, and increasing nitrogen retention within the system, ultimately lowering oxygen levels.

The first unit, a rectangular container (28 x 20 x 30 cm), operated similarly to the standard header box but maintained oxygen concentrations between 70% and 80%. The second unit, a cylindrical container (65 x 12 cm), aimed to keep oxygen levels between 40% and 50%. Introducing a tall cylinder was crucial for extending gas-water contact time, allowing more precise oxygen control. The setup included two peristaltic pumps: one slowly transferred water to the cylinder, and the other regulated flow to the experimental boxes. This extended water retention in the cylinder, enhancing nitrogen exchange and oxygen level control.

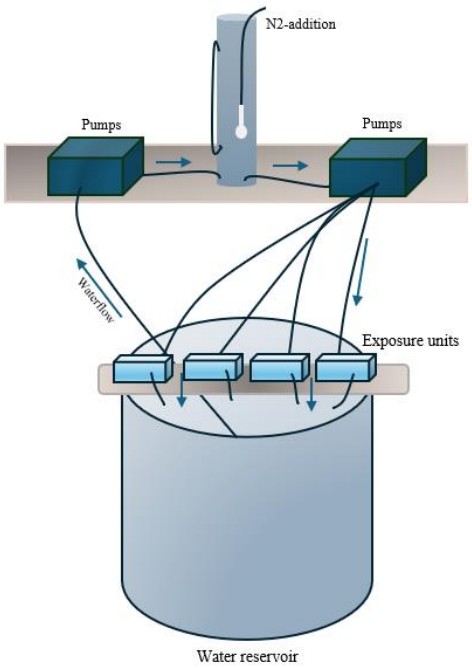

Figure 5 Illustrating the setup features a 100 L reservoir supporting four experimental boxes. A 65 x 12 cm cylinder with a nitrogen pump maintained low oxygen levels. Two peristaltic pumps coordinated water flow: one directed water to the cylinder, and the other regulated flow to the experimental boxes. A silicon tube outside the cylinder displayed the water level, ensuring accurate monitoring.

2.6 Measurements and sampling

2.6.1 Sediment

To determine the sediment size distribution of riverine samples, a granulometric analysis was conducted. The quantity of each size fraction was measured in Liters (L). Subsequently, the percentage of each fraction relative to the total sample was calculated. Further analysis was performed using phi-sorting and phi-curve techniques to obtain sediment distribution profile.

2.6.2 Water

2.6.2.1 Logging

Water quality parameters such as temperature, oxygen levels, and conductivity were monitored every week throughout the experiment using WTW multi 3420 with conductivity and oxygen probe. A temperature logger, Hobo Pendant temperature logger (UA-001-64), was placed within the header box of each system to enable continuous temperature monitoring and calculation of day degrees. Implemented water changes were conducted when the ion strength increased by 10% (from 40 to 44 µS/cm) to maintain optimal levels within the desired range.

2.6.2.2 Manual measurement

Water samples were collected from each reservoir three times during the experimental period, each time before water replacement. Using a syringe, the water was drawn and then filtered through a 0.45 µm syringe filter. For each sampling, two 15 ml tubes of the filtered water were collected from each reservoir, with one sample being acidified and the other left non-acidified. These filtered water samples were then analysed for various components. Dissolved organic carbon (DOC) content was measured using a TOC Organic Carbon Analyzer (Model TOC Vcph/CPN) from Shimadzu Corporation. Major anions, such as sulphate, chloride (Cl^-), and nitrate (NO_3^-), were quantified using ion chromatography (ICS-6000) with a Dionex ADRS 600 2mm Suppressor and a Dionex ICS-6000 CD Conductivity Detector. Additionally, after acidification with 5% ultrapure HNO_3, the concentrations of major cations and trace metals were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 9800). This systematic approach ensured comprehensive analysis of the water quality in each reservoir and comparison with the synthetic EPA water.

2.6.3 Oxygen

2.6.3.1 Manuel measurements

Detailed assessments of oxygen profile in sediments were meticulously conducted in three separate boxes for each experimental group, including sediment boxes, control boxes, and boxes for the oxygen experiment. To facilitate these measurements, O2 Microsensor (Unisense A/S, based in Aarhus, Denmark) was utilized, reinforced with thin steel needles (3-5 µm) for enhanced resistance to sediment and prevent disruption of sediment upwelling. The sensors were connected to UniAmp Multi Channel meters (Unisense A/S), ensuring precise data collection for both oxygen levels and temperature. A specialized setup incorporating an adjustable lift table with a grab mechanism was employed for all boxes (Figure 6). This apparatus was designed to lower the oxygen sensor into the boxes and maintain stability for accurate measurements.

For the measurement of oxygen concentrations in the water of boxes without sediment, a probe was carefully lowered into the water, almost reaching the bottom without touching the box. For the sediment boxes, a sequence of three to four depth-specific measurements was carried out, gradually delving deeper with each subsequent measurement. Each probing session lasted for 30 minutes, ensuring a thorough and systematic data collection process that enabled the evaluation of oxygen concentrations at various sediment depths.

Figure 6 Illustrates the setup featured an O_2 microsensor (Unisense A/S) with reinforced steel needle (3-5 µm). It incorporated a specialized adjustable lift table with a grab mechanism, allowing for precise positioning and stability.

2.6.4 Eggs and larvae

2.6.4.1 Sampling method

Table 3 demonstrates the sampling schedule and experimental focus. For the sediment experiment, one of the four parallel boxes was removed at each of the three sampling event, resulting in the complete collection of all boxes from three different reservoirs (Figure 1). In contrast, for the oxygen experiment where all eggs were visible, sampling began at sampling 2, where one box from each exposure was taken out. At sampling 3, three boxes from each exposure were removed.

Table 3 Details the sampling schedule, degree days (DD), dates, sediment and oxygen boxes used, and the focus of each sampling. Sampling 1 (215–230 DD) used 24 sediment boxes to analyse the eye stage and development. Sampling 2 (260–294 DD) included 24 sediment boxes and 3 oxygen boxes, focusing on pre-hatching and metabolite analysis. Sampling 3 (430–444 DD) involved 24 sediment boxes and 9 oxygen boxes, examining post-hatching development and larval growth.

Sampling	Degree day/Date	Sediment boxes	Oxygen boxes	Focus
Sampling 1	215 – 230 DD 14. December 2023	24 boxes		Eye stage and developmental analysis
Sampling 2	360 – 294 DD 9. January 2024	24 boxes	3 boxes	Pre-hatching, metabolites, and developmental analysis
Sampling 3	430 – 444 DD 18. January 2024	24 boxes	9 boxes	Post-hatching, development analysis, assessing hatching success and larval growth

During the experiment, sediment treatment boxes were removed at three different stages. The first sampling, conducted at 215-230 degree days (DD), covered the period from fertilization to the eyed stage, when the embryos had developed visible eyes. The second sampling, at 360-380 DD, represented the period from fertilization to pre-hatching, just before the embryos began to hatch. The final sampling, at 430-440 DD, encompassed the period from fertilization to hatching, including when the embryos were either in the process of hatching or had already hatched.

For sediment boxes, eggs and sediment within the box underwent meticulous separation using sieves and control water at the time of sampling. The eggs were then carefully collected for subsequent analysis. Additionally, boxes without sediment were subjected to direct biological endpoint analysis.

The sediment application resulted in unintended stratification of eggs within the boxes with 8-10 mm layers, causing segregation into upper and lower sediment layers. To assess developmental variations accurately across these depths, a method was employed for the vertical segregation of eggs and larvae. Eggs located in the upper sediment layers were separated first, followed by those in the lower strata. This meticulous approach allowed for precise identification of developmental impacts associated with each sediment stratum, ensuring our analysis robustly reflected the complex environmental conditions.

2.6.4.2 Biological endpoints

2.6.4.2.1 Development

At each sampling point, eggs and larvae were examined and compared with the control group. Those exhibiting developmental delays were categorized as underdeveloped, with the aim to identify any delays in development. During each sampling phase, at least three eggs were randomly selected for detailed analysis under a microscope. This analysis focused on observing morphological differences among the groups and examining developmental stages in greater detail. Additionally, morphological abnormalities were examined under the microscope, allowing for assessment of developmental anomalies and group disparities.

2.6.4.2.2 Egg survival

Egg mortality was carefully monitored and recorded approximately every other day by identifying and removing non-viable eggs to prevent fungal growth, characterized by their opaque and whitened appearance, indicative of non-viable eggs. However, the main analysis of egg mortality was conducted during the three primary sampling intervals. Survival rates were calculated by subtracting the total count of non-viable eggs from the total number of fertilized eggs initially placed in each experimental setup.

2.6.4.2.3 Affected

To analyse the combined effects of developmental delay and mortality, the term "affected eggs" was introduced, encompassing both mortality and underdevelopment. By defining affected eggs as the sum of these factors, a comprehensive measure was provided, reflecting the interplay and improving understanding of how developmental delays contributed to increased mortality.

2.6.4.2.4 Hatching Success

Hatching was systematically observed at bi-daily intervals where larvae was visible; however, the primary evaluation of hatching success was conducted during the specified third sampling period. Larvae were classified as either emerging or fully emerged, collectively categorized as 'hatched'. Additionally, larval mortality, characterized by a whitening appearance and the lack of circulatory activity, was meticulously recorded. These comprehensive observations strengthened the calculation of hatching rates and overall success, providing a detailed understanding of the developmental outcomes.

2.6.4.2.5 Growth

During the post-hatching phase, all larvae were measured to quantify growth metrics, including weight and length. Larval weight was determined using a scale, while length measurements were obtained by photographing the larvae in a petri dish placed over graph paper and reading the length from the images for accuracy (Figure 7). These metrics are vital for evaluating larval health and developmental progress. This data enables systematic comparisons across treatment groups, highlighting discrepancies in growth rates due to experimental conditions and providing insights into early-stage larval development.

Figure 7 Demonstrates the procedure for measuring fish larvae length, employing graph paper marked in millimetres for precision. Larvae are positioned within a petri dish to ensure accurate and consistent measurements.

2.6.4.2.6 Metabolites analysis

The content of organic acids and carbohydrates in fish eggs was analysed utilizing a modified high-performance liquid chromatography (HPLC) method, adapted from Grønnevik et al. (2011). This modification was tailored to precisely measure lactate and metabolites associated with oxidative stress and information about hypoxia. The samples underwent snap-freezing in liquid nitrogen to preserve biochemical integrity, followed by storage at -80°C. Prior to analysis, the samples were carefully thawed and standardized to contain five to seven eggs, achieving a total mass of approximately 500 mg. A precise incision in the chorion facilitated the homogenization process. Adhering to this modified protocol, which accommodates a sample mass half that used by Grønnevik et al. (2011), ensured rigorous preparation and accurate measurement.

2.7 Statistic analysis

All preparation and statistical analyses of data were done using R version 2023.06.2 (R Core Team, 2023). All statistical tests were conducted using a significance threshold (p-value) of 0.05.

Analysis of Covariance, ANCOVA, was used to determine the influence of sediment fraction, thickness, and sampling time on fish egg underdevelopment, mortality, and overall health. This method was chosen to identify the factors significantly affecting egg development. Following ANCOVA, Tukey's Honest Significant Difference (HSD) test was performed to evaluate the impacts of sediment fractions, which merged the 1-2 mm and 8-10 mm thickness levels, and to determine which of the sediment fractions were significant compared to the control. The test also quantified the differences between the two thickness levels and determined which of the three sampling times showed significant differences from each other

To examine the effects of different treatments on percent underdeveloped, mortality and affected at each sampling point, linear regression was used as the primary analytical method. This approach was chosen for its ability to provide clear, interpretable results and directly compare each treatment to the control group. Data were organized by sampling points, with percent underdeveloped, mortality and affected eggs as the dependent variables and treatments as the independent variables. Separate linear regression models were fitted for each sampling point, and for each biological endpoints, to estimate the effect size of each

treatment. This approach ensured that the findings were specific to each time point, providing a detailed understanding of how treatment effects varied over time.

To determine if there were statistically significant differences among the treatment groups regarding their effects on biological endpoints, specifically growth and hatching, a log transformation was applied selectively to data sets that exhibited skewness to better meet the assumption of normality. When the transformed or original data satisfied the normality assumption, Analysis of Variance (ANOVA) was employed. Specifically, a one-way ANOVA was used to compare the means of more than two groups based on one independent variable. Post-hoc analysis with Tukey's Honest Significant Difference (HSD) test was conducted to identify which specific treatment groups differed significantly from each other.

However, when the assumption of normality was not satisfied, even after transformation, the Kruskal-Wallis rank sum test was applied to determine if there were statistically significant differences among the treatment groups. For post-hoc analysis, pairwise comparisons were performed using the Wilcoxon rank sum test with continuity correction. To control for the false discovery rate, the p-values were adjusted using the Benjamini-Hochberg (BH) method.

To compare the hatching dynamics among different treatments, a nonlinear regression analysis was conducted using sigmoid curves to model the relationship between degree days (DD) and the percentage of eggs hatched. The sigmoid function used was defined as follows:

$$\frac{L}{1 + e^{-k(DD-x0)}}$$

L represents the maximum hatching percentage, k denotes the rate at which hatching occurs, x0 indicates the inflection point where hatching begins to rise rapidly. The parameters were estimated for each treatment group. Initial parameter values were determined based on the data distribution and prior fitting of the control group. A two-way ANOVA was performed to compare a reduced model (without treatment interactions) to a full model (including treatment interactions) to test the significance of the treatment effects. The full model included interaction terms for each treatment's effect on the parameters L, k, x0.

To investigate the relationship between sediment treatments and oxygen levels in the thickest sediment layers, a series of statistical tests were conducted. A linear regression model was

applied to evaluate the nature and strength of the relationship between sediment treatment and oxygen level, and ANOVA was used to determine the p-value of the interaction.

3. Results

3.1 Environmental factors

3.1.1 Sediments

The granulometric analysis of the riverine sediment samples revealed a bimodal distribution of silty sand presented in Table 4, predominantly comprising sand, silt and some clay. Sand was the dominant grain size, accounting for 61.5% of the total sediment mass. Notably, medium sand constituted the largest portion of this fraction, making up 29%. In contrast, coarser and finer sand fractions were less prevalent. The silt fraction accounted for 31.3% of the sediment composition. Finer materials, such as fine sand and clay, were present in smaller proportions, comprising 8.4% and 7.2% of the sediment samples, respectively.

Table 4 Presents the distribution of sediment categories based on particle size range (mm), quantity (L), and percentage (%). The sediment categories include very coarse sand, coarse sand, medium sand, fine sand, silt, and clay.

Sediment category	Particle size range (mm)	Quantity (L)	Percent (%)
Very coarse sand	2,0 – 1,0	0,9	10.8
Coarse sand	1 – 63	1,1	13.3
Medium sand	0,63 - 0,2	2,4	29
Fine sand	0,2 – 0,063	0,7	8.4
Silt	0,063 – 0,002	2,6	31.3
Clay	< 0,002	0,6	7.2

3.1.2 Water

The water throughout the experiment had a pH at 6.9 ± 0.2, electrical conductivity of the water at 44 ± 2.6 µS/cm, dissolved oxygen concentration level at 15.1 ± 0.21 mg/L and DOC concentration was 0.3 ± 0.04 mg/L. The temperature was maintained at 6.2 ± 0.5 C° throughout the experiment.

The water samples were analysed for major cations and anions. Measured values for chloride, sulphate and nitrate were within or close to certified values and were acceptable for information purposes.[1] Water that interacted with sediment (S) exhibited similar concentrations of major anions and cations to water without sediment (WS) (Table 5), but with a slight increase in ionic strength. Notably, there was an increase in the concentrations of sulphate, calcium, and dissolved organic carbon (DOC) in comparison to the experimental water.

Metal concentrations were quantified using ICP-MS for the key metals listed in Table 5. Throughout the duration of the experiment, interaction with sediment led to a raised concentration of Mn, and a slight increase in concentrations of Al, Cr, and Fe. Nevertheless, metal concentrations were low, ensuring no critical exposure for the eggs.

Table 5 Summarizing the concentrations of major cations, anions, and trace metals in water samples in contact with sediment and fish eggs, and water in contact only with fish eggs. The values are presented as means for water in contact with sediment (S-Mean) and water without contact with sediment (WS-Mean), each with three replicates (N=3) throughout the experiment.

Major cations and anions								
	Cl- (mg/L)	**SO4 (mg/L)**	**NO3 (mg/L)**	**Na (mg/L)**	**Mg (mg/L)**	**K (mg/L)**	**Ca (mg/L)**	**DOC**
S-Mean (N =3)	0.7 ± 0.07	8.3 ± 0.61	0.2 ± 0.05	3.9 ± 0.03	1.2 ± 0.1	0.4 ± 0.1	2.0 ± 0.3	0.73 ± 0.4
WS-Mean (N=3)	0.8 ± 0.16	8.6 ± 0.37	0.2 ± 0.04	3.9 ± 0.17	1.3 ± 0.05	0.3 ± 0.1	2.4 ± 0.85	0.7 ± 0.25
Metals								
	Al (µg/L)	**Cr (µg/L)**	**Mn (µg/L)**	**Fe (µg/L)**	**Cu (µg/L)**	**Zn (µg/L)**	**Pb (µg/L)**	
S-Mean (N =3)	3.72 ± 1.4	0.44 ± 0.01	115.1 ± 97	7.7 ± 4.5	0.8 ± 0.3	4.3 ± 2.2	0.05 ± 0.03	
WS-Mean (N=3)	2.29 ± 0.5	< 0.07	0.2 ± 0.2	0.3 ± 0.14	0.8 ± 0.8	7.8 ± 4.3	0.02 ± 0.01	

[1] The certified values can be found in Appendix A1.

3.1.3 Dissolved oxygen levels

The measurements of oxygen levels under different sediment treatments and oxygen exposure conditions are displayed in Table 6. The sediment treatments revealing variations based on sediment type and particle size. Sediment control, which had no sediment but used similar water as the sediment boxes, had an oxygen concentration at 12.5 mg/L. The sediment treatments impacted the dissolved oxygen (DO) levels at various depths within the sediment columns. The top represents the first 2-4 mm, the middle 4-6 mm and the base at 6-8/10 mm. The silt 8-10 mm treatment showed the greatest reduction in DO levels, with a 69.5% decrease from the top to the base, 44.9% at the top to the middle, and 44.6% at the middle to the base. In contrast, the coarse sand 8 mm treatment had the smallest reduction, with only a 4.97% total decrease in DO levels. Intermediate reductions were observed for the mix 8-10 mm and sand 8-10 mm treatments, with total decreases of 44.2% and 54.5%, respectively.

In the oxygen control treatment, the middle layer had a mean oxygen level of 12.6 mg/L, serving as a baseline. Under 70% and 50% oxygen treatments, oxygen levels dropped to 9.9 mg/L and 4.7 mg/L, respectively.

Table 6 Presents a summary of dissolved oxygen (DO) concentrations (mg/L) in both the sediment and oxygen experiments. In the sediment experiment, the table lists the control water and sediment samples with varying layer thicknesses, from thinnest to thickest. For the thickest sediment layer, oxygen levels are measured at three different positions within the box: top (2-4 mm), middle (4-6 mm), and bottom (6-8/10 mm), along with the percentage decrease in oxygen levels. In the oxygen experiment, the table shows the DO levels for the control group and groups exposed to 70% and 50% oxygen levels.

Treatment	**DO level *(mg/L)***		**Oxygen top *(mg/L)***	**Oxygen Middle *(mg/L)***	**Oxygen Bottom *(mg/L)***	**Decrease (%)**
Sediment experiment						
Sediment Control	12.5	Coarse sand 8 mm	11.7	11.3 (-3.1%)	11.1 *(-1.9%)*	-4.9
Silt 1-2 mm	10.8	Mix 8-10 mm	10.6	7.7 (-27.3%)	5.9 *(-23.2%)*	-44.2
Sand 1-2 mm	10.7	Sand 8-10 mm	9.9	6.9 (-30.5%)	4.5 *(-34.5%)*	-54.5
Mix 1-2 mm	8.8	Silt 8-10 mm	6.1	3.3 (-44.9%)	1.8 *(-44.6%)*	-69.5
Oxygen experiment						
Oxygen Control	12.6					
70 %	9.9					
50 %	4.7					

3.2 Biological endpoints

3.2.1 Sediment experiment

3.2.1.1 Underdevelopment

Throughout the experimental period, developmental anomalies were consistently monitored, with considerable delays particularly affecting the initial and intermediate stages. The overall effects of ANCOVA analysis on fraction, thickness, and sampling showed that fraction significantly impacted underdevelopment ($F(4,62) = 4.949$, $p = 0.00298$), while the thickness of the sediment layer did not have a significant effect. When focusing specifically on fraction sizes, it was found that both sand (Tukey's HSD, $p = 0.005$) and silt (Tukey's HSD, $p = 0.02$) had significant impacts on underdevelopment. Moreover, the developmental stages themselves had a highly significant impact on underdevelopment (ANCOVA, $F(2,62) = 24.48$, $p = 1.45e\text{-}8$).

During the first sampling (215-230 DD), which covered the period from fertilization to the eyed stage, the treatment with a thick layer of sand had over 50% delayed eggs and showed a marked increase in underdevelopment compared to the control group (one-way linear model, $\tau = 46.7$, $p < 0.00001$).

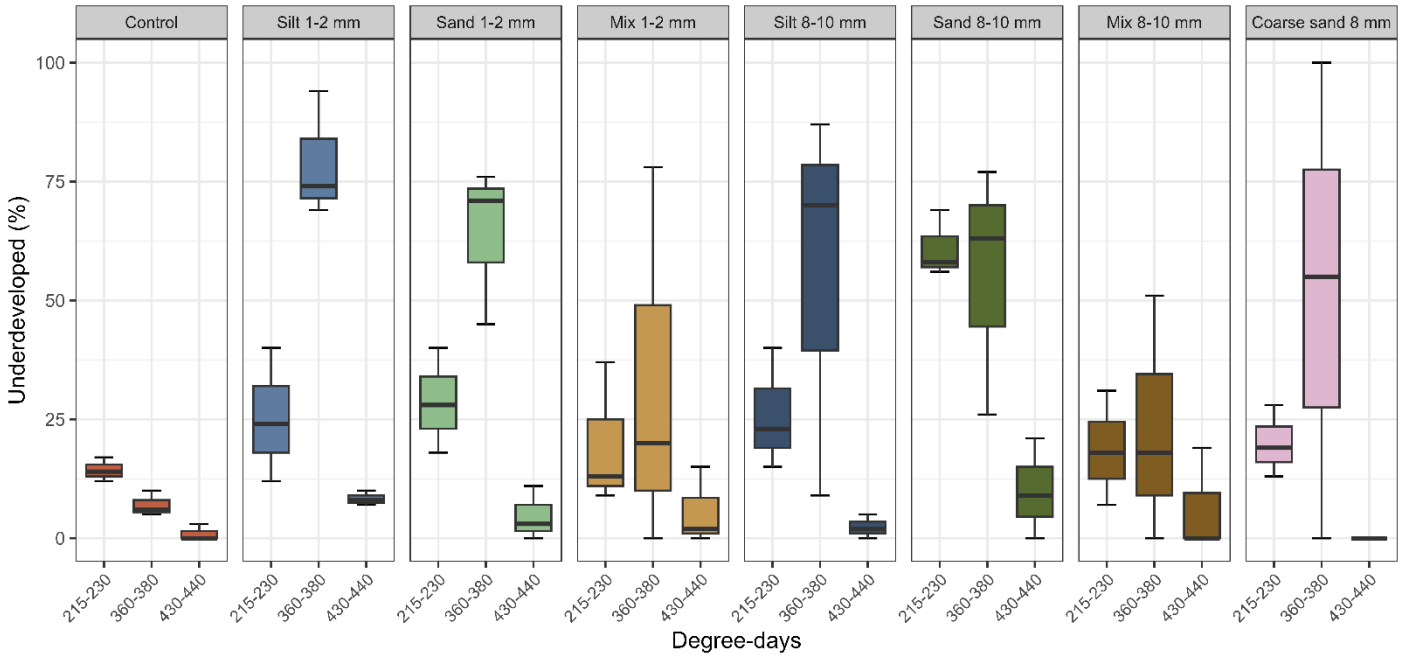

Figure 8 Illustrating the comparison of underdeveloped eggs under different sediment exposure conditions at various sampling points, from fertilization to hatched larvae. The sample size (N) is 3 for each treatment group. Sampling at 215-230 DD represents the fertilization to eyed stage, 360-380 DD represents fertilization to pre-hatching, and the final sampling at 430-440 DD shows the outcome of the entire experiment from fertilization to hatching. The initial stages exhibited the most delayed embryos.

As development progressed to the pre-hatching stage, the second sampling revealed further distinctions among treatments. Overall, there was a 5-percentage point difference in underdevelopment from the first to the second sampling (ANCOVA, Tukey's HSD, $p = 0.004$). The one-way linear model showed that the thinnest layers of silt and sand significantly delayed development, with percentage point increases of 74 ($p = 0.01$) and 57 ($p = 0.03$) compared to the control, respectively. Although the thickest layers of silt and sand also showed increases in underdevelopment, 48.3 percentage points each, these results did not reach statistical significance despite having the lowest p-values among the non-significant treatments ($p = 0.073$).

By the final sampling period, encompassing hatching, developmental outcomes had largely stabilized. Notably, there was a significant decrease in underdevelopment by 36 percentage points difference from the first to the third sampling (ANCOVA, Tukey' HSD, $p = 0.001$), and a -41-percentage point difference from the second to the third sampling (ANCOVA, Tukey's HSD, $p < 0.00001$). None of the treatments showed significant levels of underdevelopment. Most eggs had either hatched or exhibited mortality, leaving only a small fraction categorized as delayed.

3.2.2.2 Mortality

Throughout the experiment, mortality rates among embryos varied depending on the thickness of the sediment layer and the sediment fraction. The sediment control group, unexposed to sediments, maintained a high and stable survival rate across developmental stages (Figure 9). Mortality rates slightly increased from eyed stage (215-230 DD) to post-hatching (430-440 DD). The overall survival rate was 89 ± 4.8%.

The ANCOVA analysis revealed that both fraction ($F(4,64) = 2.79$, $p = 0.033$) and thickness ($F(1,64) = 39.64$, $p = 6.66e\text{-}7$) had significant impacts on mortality rate. Specifically, the thickest layer (8-10 mm) exhibited a highly significant difference compared to the thinnest layer (Tukey's HSD, $p = 7.6e\text{-}6$). When focusing solely on fraction, no specific fraction was significant, only the mix treatment had a significant impact on mortality (Tukey's HSD, $p = 0.013$). Additionally, different developmental stages also had a significant effect on mortality ($F(2,63) = 4.123$, $p = 0.02$).

At the eyed stage (215-230 DD), only the mixed treatment with an 8-10 mm thickness significantly affected mortality rate, resulting in an increase of 50.33 percentage points (one-

way linear model, p = 0.016). As development progressed to the pre-hatching stage (360-380 DD), the mixed treatment showed an increase in mortality rate to 51.66 percentage points (one-way linear model, p = 0.024).

By the post-hatching stage (430-440 DD), the overall effects on mortality were pronounced for the thickest layer of sediments. Mortality rates significantly increased with a difference of 16.45 percentage points from the first eyed stage to the post-hatching stage (Tukey's HSD, p = 0.035) and from the pre-hatching stage to the post-hatching stage a difference of 15.62 percentage points was observed (Tukey's HSD, p = 0.047). The mixed treatment at 8-10 mm continued to show a significant impact ($\tau = 59.6$, p = 0.0045), while sand at 8-10 mm exhibited a 53 percentage point increase (one-way linear model, p = 0.0097). Coarse sand at 8 mm resulted in a 42.33 percentage point increase in mortality (one-way linear model, p = 0.0032), while the last thick treatment, silt, showed a 34 percentage point non-significant increase (one-way linear model, p = 0.07).

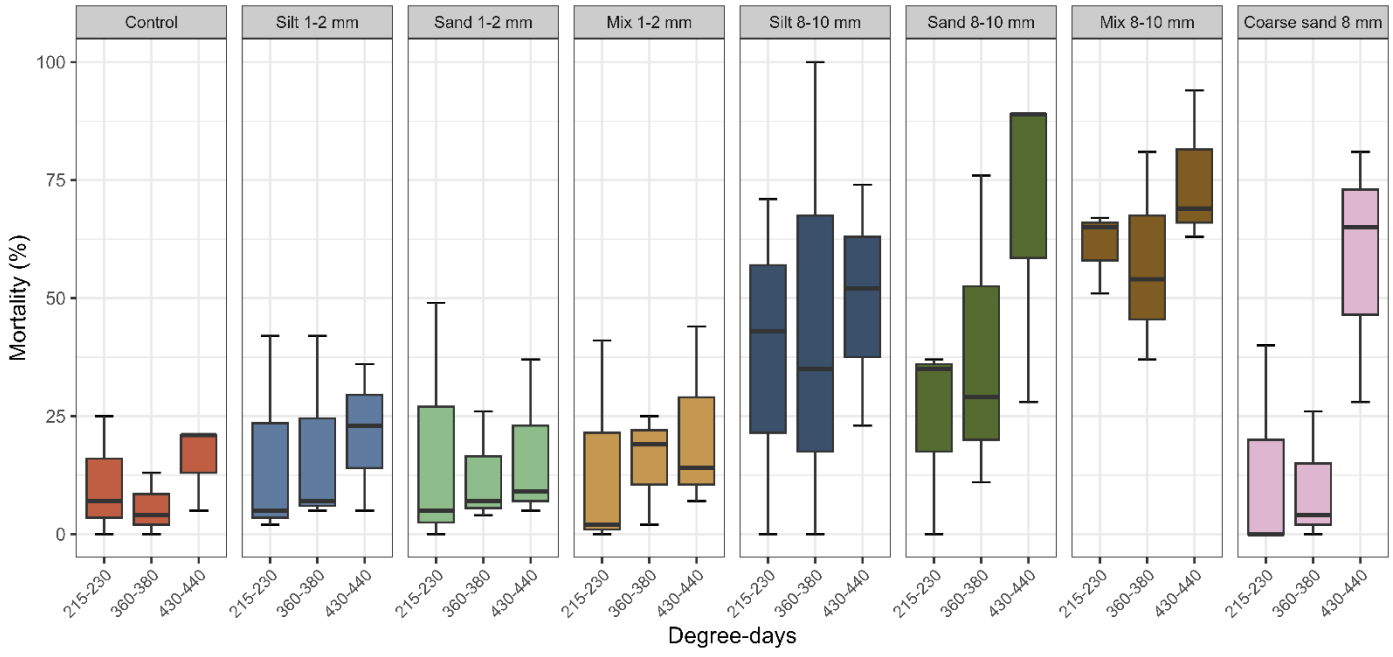

Figure 9 Presents the mortality rates (%) of eggs and larvae subjected to different sediment exposure conditions, from fertilization to hatched larvae. The sample size (N) is 3 for each treatment group. Sampling at 215-230 DD represents the fertilization to eyed stage, 360-380 DD represents fertilization to pre-hatching, and the final sampling at 430-440 DD shows the outcome of the entire experiment from fertilization to hatching. Notably, eggs exposed to the thickest sediment layer (8-10 mm) exhibited higher mortality rates compared to other treatment groups, and at the end of the experiment the mortality rate significantly increased (p = 0.035). The mixture of sediment (mix treatment) exhibited the most pronounced increase, and early mortality rate (p = 0.0045)

3.2.2.2.1 Mortality rate at the base of treatment boxes

To analyse the mortality rate of eggs positioned at the base of the thickest sediment layer (8-10 mm) in the treatment box, it was essential to consider the uneven distribution and stratification due to the buoyancy of the eggs. The silt and mixed sediment treatments had the highest number of surface-placed eggs in the thick layer. The weighted mortality rate in Figure 10, is the average mortality rate at the last sampling adjusted for the number of eggs positioned at the base in each treatment box, provided a more accurate comparison by accounting for varying numbers of eggs. The results shows that eggs in mixed sediment had the highest mortality rate at 95%, followed by silt at 81%. Sand had a mortality rate of 66%, and coarse sand had the lowest at 56%.

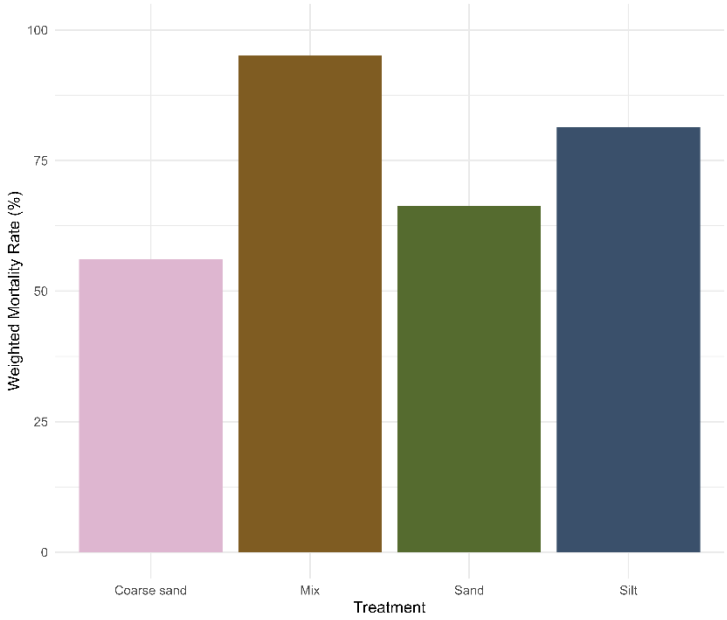

Figure 10 Illustrates bar chart representing the weighted mortality rates (%) for eggs positioned at the base of the exposure boxes of treatment 8-10 mm layers. The treatments include coarse sand, a mix of different sediments, sand, and silt. The weighted mortality rate is adjusted for the number of eggs at the base, providing a more accurate comparison. The y-axis shows the weighted mortality rate ranging from 0% to 100%, and the x-axis lists the different 8-10 mm treatments.

Figure 11 illustrates the impact of sediment coverage on the developmental lag and mortality rates of eggs. Photos (a) and (b) show eggs in the mixed sediment treatment, with (a) depicting eggs at the base of the sediment layer and (b) showing eggs on the surface. Eggs in photo (a) experienced more delayed development compared to embryos in photo (b). High mortality rates were detected for embryos under the thick silt layer in photo (c), while those on the surface in photo (d) were delayed compared to the control group (e) but did not exhibit mortality.

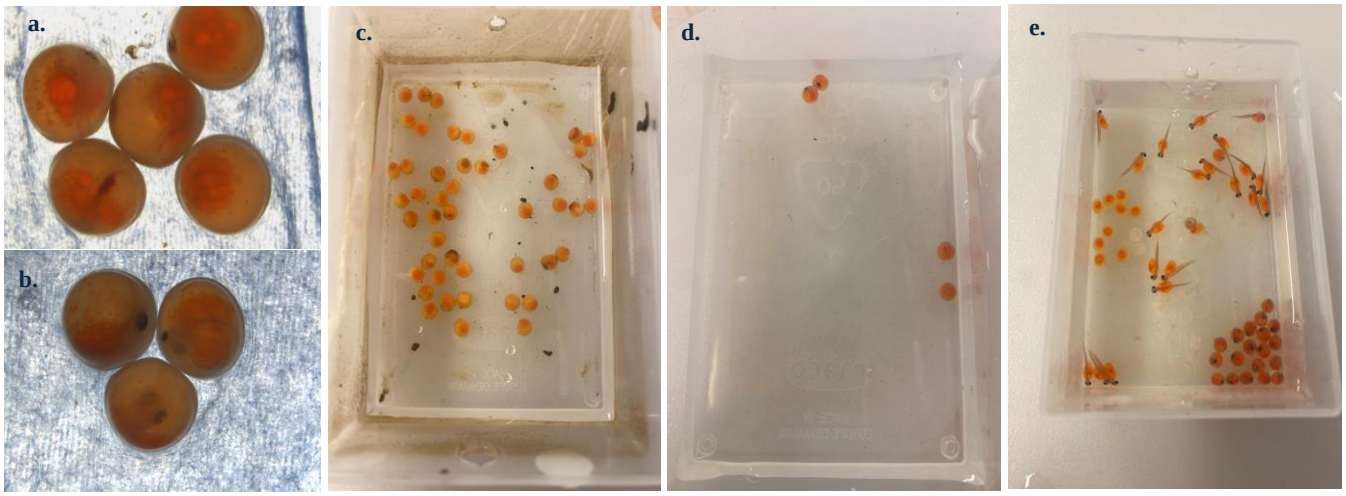

Figure 11 Illustrates the differences in egg positioning and development within the 8-10 mm treatment experimental boxes. Panel (a) shows eggs positioned at the base of the mix treatment box, while panel (b) shows eggs positioned at the surface of the mix sediment layer. The comparison indicates that eggs in (a) are more lagged in development compared to those in (b). Panel (c) illustrates the eggs' mortality and development at the base of the silt treatment box, and panel (d) depicts the mortality and development at the surface of the silt sediment layer, demonstrating differing outcomes based on positioning. Panel (e) shows the control group, where eggs have already begun hatching.

3.2.2.3 Affected

To analyse the combined effects of developmental delay and mortality a comprehensive measure of affected eggs provided a detailed view of how developmental delay effects mortality, and how sediment fraction, thickness, and sampling period impact embryo development and viability. The affected eggs through-out the experiment, with focus on the different developmental stages are illustrated in Figure 12.

The ANCOVA, for the whole experiment, revealed that sediment fractions significantly affected the percentage of affected eggs compared to the control ($F(62, 4) = 6.286$, $p = 0.00026$). Specifically, silt showed a significant difference of 43.6 percentage points (Tukey's HSD, $p = 0.00054$), sand exhibited a difference of 47.83 percentage points (Tukey's HSD, $p = 0.00012$), and the mixture of sediments showed a difference of 38.05 percentage points (Tukey's HSD, $p = 0.0033$) compared to the control. However, the coarse sand fraction, with a difference of 32 percentage points, was not statistically significant (Tukey's HSD, $p = 0.0519$). Thickness also had a highly significant effect on the affected eggs ($F(1, 62) = 22.9$, $p = 1.1e-05$). There was a notable difference of 28.25 percentage points between the thinnest and thickest layers (Tukey's HSD, $p = 0.000097$).

The varying sediment sampling periods or experimental phases significantly influenced the observed effects ($F(2, 62) = 1.758$, $p = 0.003$). These analyses revealed notable differences in the outcomes across the different time points. The transition from the eyed stage to pre-hatching revealed a difference of 18.58 percentage points (Tukey's HSD, $p = 0.03$), while from pre-hatching to post-hatching, there was a decrease of 24.5 percentage points (Tukey's HSD, $p = 0.0032$). However, no significant difference was seen from eyed stage to post-hatching.

The one-way linear regression of the effects of each treatment at each sampling (Figure 12) revealed that some treatment had significant increase of affected eggs from the control at different stages. At the eyed stage, the sand 8-10 mm treatment exhibited 90% affected eggs ($\tau = 59.6$, $p = 0.004$), and the mix 8-10 mm treatment had median of 83% affected eggs ($\tau = 54.3$, $p = 0.0075$). In general, the thinnest layers primarily caused developmental delays, while the thickest layers began to exhibit increased mortality.

By the pre-hatching stage, the increase in affected eggs continued. The thinnest layers of silt and sand showed significant increases from the control. Silt had a median of 98% ($\tau = 80$, $p = 0.0041$) and sand with a median of 83% ($\tau = 62.6$, $p = 0.018$), respectively, primarily due to developmental delays rather than mortality. In contrast, the thickest layers of silt ($\tau = 82$, $p = 0.0034$), sand ($\tau = 80$, $p = 0.0041$), and mixed ($\tau = 67$, $p = 0.012$) sediments exhibited both developmental delays and increased mortality rates.

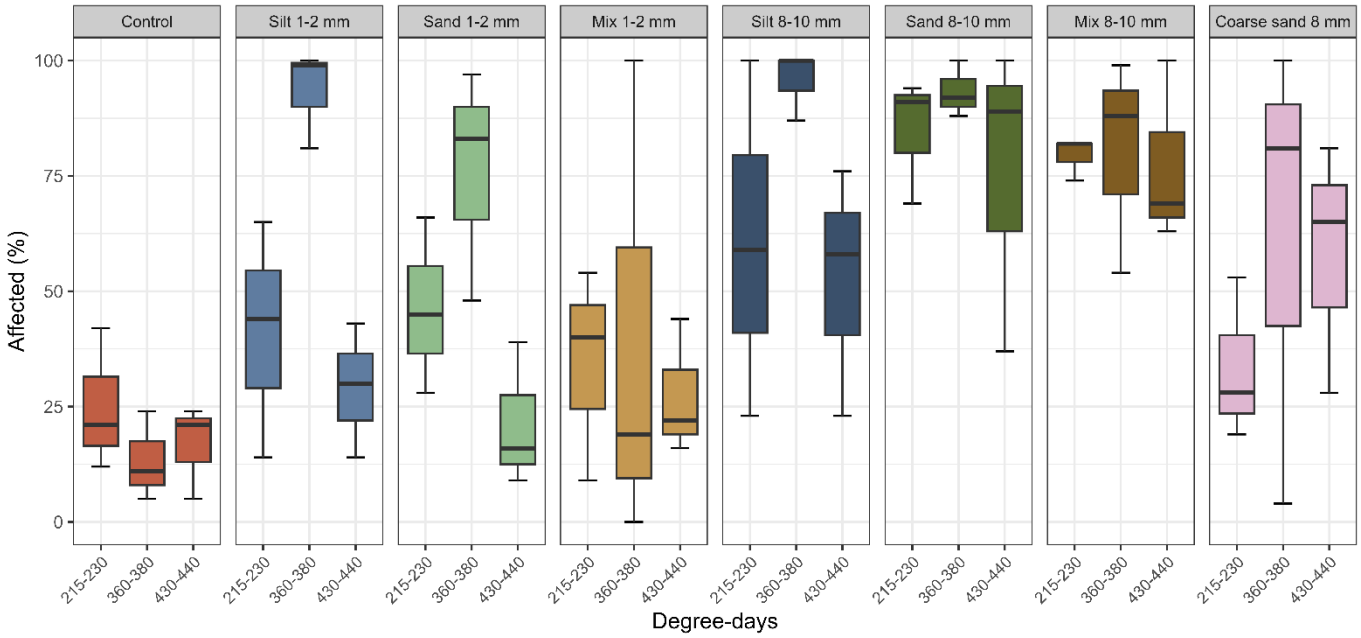

Figure 12 Illustrates the percentage of eggs and larvae affected (underdeveloped + mortality) by different sediment exposure conditions, , from fertilization to hatched larvae. The sample size (N) is 3 for each treatment group. Sampling at 215-230 DD represents the fertilization to eyed stage, 360-380 DD represents fertilization to pre-hatching, and the final sampling at 430-440 DD shows the outcome of the entire experiment from fertilization to hatching. The results show that eggs exposed to the thickest sediment layer (8-10 mm) had a significantly higher overall affected rate compared to other groups. The comprehensive analysis show that thinnest layer experienced underdevelopment at pre-hatching, while the thickest layer experienced early underdevelopment and an increase in mortality which increased thought out the experiment.

At the post-hatching stage, the thinnest sediment layers led to minimal developmental delays and low mortality rates, with most eggs successfully beginning to hatch. While significant developmental delays were observed for silt and sand treatments at the pre-hatching stage, these delays diminished by the post-hatching stage for the thinnest sediment layers. However, for the thickest layers, some developmental delays persisted, but most eggs either experienced mortality or successfully hatched. Significant impacts were seen for the thickest layer of sand ($\tau = 58.6$, $p = 0.004$), mixed sediments ($\tau = 60.6$, $p = 0.0034$), and coarse sand ($\tau = 41.3$, $p = 0.033$). The last thickest layer, silt, showed a non-significant increase of 35.6 percentage points from the control ($p = 0.061$). Overall, the results demonstrated that developmental delays for embryos were prevalent at the pre-hatching stage, particularly for the thinnest layers, but decreased by the post-hatching stage. While the thickest sediment layers showed high and early mortality rates, increasing at post-hatching stage.

3.2.2.4 Hatching success

A comparative analysis of embryo commenced hatching, revealed notable differences between each treatment group and the control group, illustrated in Figure 13. The control group exhibited a hatching success rate of 85%. Hatching began at 335 DD, marking the onset of emergence under the experimental conditions. By 368 DD, 50% of the eggs had successfully hatched (Table 7).

All treatment groups exhibited lower hatching success and a later initiation of hatching compared to the control group (Figure 13, Table 7). Eggs covered with a thin layer of sediment (1-2 mm) began hatching earlier overall than those covered by a thicker layer (8-10 mm). Notably, none of the eggs under the thick sediment layer reached the 50% hatching point, except for those in coarse sand (8 mm). Among all treatments, the sand (8-10 mm) group had the lowest number of total eggs hatched (Table 7).

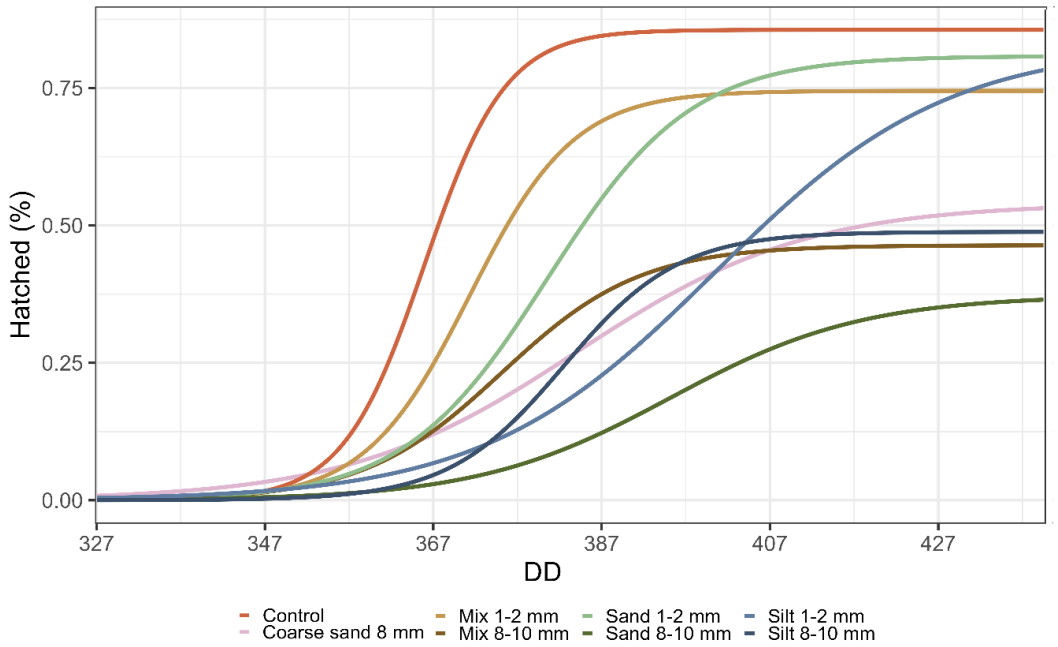

Figure 13 Presents sigmoid curves illustrating the percentage of eggs hatched (%) as a function of degree days (DD) for various substrate types. The control group (red) is compared against different substrates, including mix 1-2 mm (yellow), mix 8-10 mm (brown), sand 1-2 mm (light green), sand 8-10 mm (dark green), silt 1-2 mm (blue), silt 8-10 mm (dark blue), coarse sand 8 mm (pink). The curves shows that all treatment groups exhibited lower hatching success and a later initiation of hatching compared to the control group.

A two-way ANOVA showed significant differences between the control group and several treatment groups. Specifically, the treatments mix 1-2 mm ($p = 0.032$), silt 8-10 mm ($p = 2.7e^{-9}$), sand 8-10 mm ($p = 7.7e^{-7}$), mix 8-10 mm ($p = 1.2e^{-10}$) and coarse sand 8 mm ($p = 0.002$) resulted in significant changes in the maximum hatching percentage, meaning these treatments altered the maximum percentage of eggs that hatched compared to the control.

Additionally, all the groups, silt 1-2 mm ($p < 0.01$), sand 1-2 mm ($p < 0.001$), mix 1-2 mm ($p = 0.02$), silt 8-10 mm ($p < 0.001$), sand 8-10 mm ($p < 0.001$), mix 8-10 mm ($p = 0.048$) coarse sand 8 mm ($p = 0.048$), significantly affected the timing of rapid hatching. Furthermore, the treatment silt 1-2 mm ($p = 0.01$) significantly changed the hatching rate, suggesting a different speed of hatching compared to the control. These findings highlight that specific treatments not only impact the maximum hatching percentages but also alter the timing and rate at which hatching occurs.

Table 7 Presents the hatching dynamics of embryos and larvae exposed to three different sediment treatments. It includes data on the initiation of hatching (DD), the point at which 50% of the embryos had hatched (DD), hatching success (%), and larval mortality (%).

Treatment	**Initiation (DD)**	**50 % Hatched (DD)**	**Hatching success (%)**	**Mortality (%)**
Sediment Control	335	368	85	0
Silt 1-2 mm	340	406	82	0
Sand 1-2 mm	343	385	80	2.3
Mix 1-2 mm	344	376	74	0
Silt 8-10 mm	356	NA	48	0
Sand 8-10 mm	354	NA	37	2.6
Mix 8-10 mm	344	NA	46	0
Coarse sand 8 mm	363	418	52	2.2

3.2.2.5 Growth

The growth metrics of fish larvae, assessed immediately post-hatching at 430-437 degree-days (DD), showed significant differences in length and weight compared to the control (Table 8). The length of the control organisms predominantly ranged from 17 to 20 mm, with a median of 19 mm. In terms of weight, there was a slightly greater variation, yet most larvae weighed between 0.065 to 0.085 g, with a median weight of 0.071 g. Analyses revealed consistently lower mean and median values for both length and weight in all treatment groups (Figure 14). Significant differences in larval length were observed across all treatment groups, while in weight analysis, all groups except the mixed sediment treatment exhibited statistically significant differences from the control (Table 8).

Table 8 Presents the length (mm) and growth (g) of larvae exposed to various sediment treatments at 430-347 degrees post-hatching, along with the associated p-values.

Treatment	*Observations*	*Length (Median)*	*P-value (Kruskal-Wallis)*	*Observations*	*Weight (Mean + SD)*	*P-value (ANOVA)*
Control	N = 100	19.0		N = 84	0.071 ± 0.009	
Silt 1-2 mm	N = 92	13.5	< 2e-16	N = 82	0.059 ± 0.008	$1.3e^{-10}$
Sand 1-2 mm	N = 104	16.75	< 2e-16	N = 93	0.063 ± 0.012	$8.3e^{-7}$
Mix 1-2 mm	N = 92	18.0	1.4e-05	N = 79	0.069 ± 0.007	0.88
Silt 8-10 mm	N = 70	15.5	< 2e-16	N = 62	0.063 ± 0.007	$5.5e^{-5}$
Sand 8-10 mm	N = 42	13.0	< 2e-16	N = 41	0.061 ± 0.008	$1.0e^{-6}$
Mix 8-10 mm	N = 51	17.5	1.4e-06	N = 51	0.067 ± 0.008	0.24
Coarse sand 8 mm	N = 53	15.0	4.9e-14	N = 48	0.060 ± 0.007	$6.4e^{-9}$

Length distribution analysis (Figure 14a, 13b) demonstrated that larvae from sediment treatments exhibited a wide range of lengths. The presence of outliers, particularly lengths below 10 mm, reflects considerable variability within the treatment groups. Mixed sediment groups (1-2 mm and 8-10 mm) achieved the highest median lengths of 18 mm and 17.5 mm, respectively. In contrast, sand (8-10 mm) and silt (1-2 mm) treatments recorded significantly lower median lengths of 13 mm and 13.5 mm, respectively (Table 8)

The analysis of weight distributions revealed that larvae subjected to sediment treatments generally exhibited lower weight metrics compared to the control, with less pronounced variation (Figure 14c, 13d). Notably, sand (8-10 mm) and silt (1-2 mm) treatments had the lowest mean weights, consistent with the length trends. In contrast, mixed sediment groups (1-2 mm and 8-10 mm) had higher median weights relative to other treatments (Table 8).

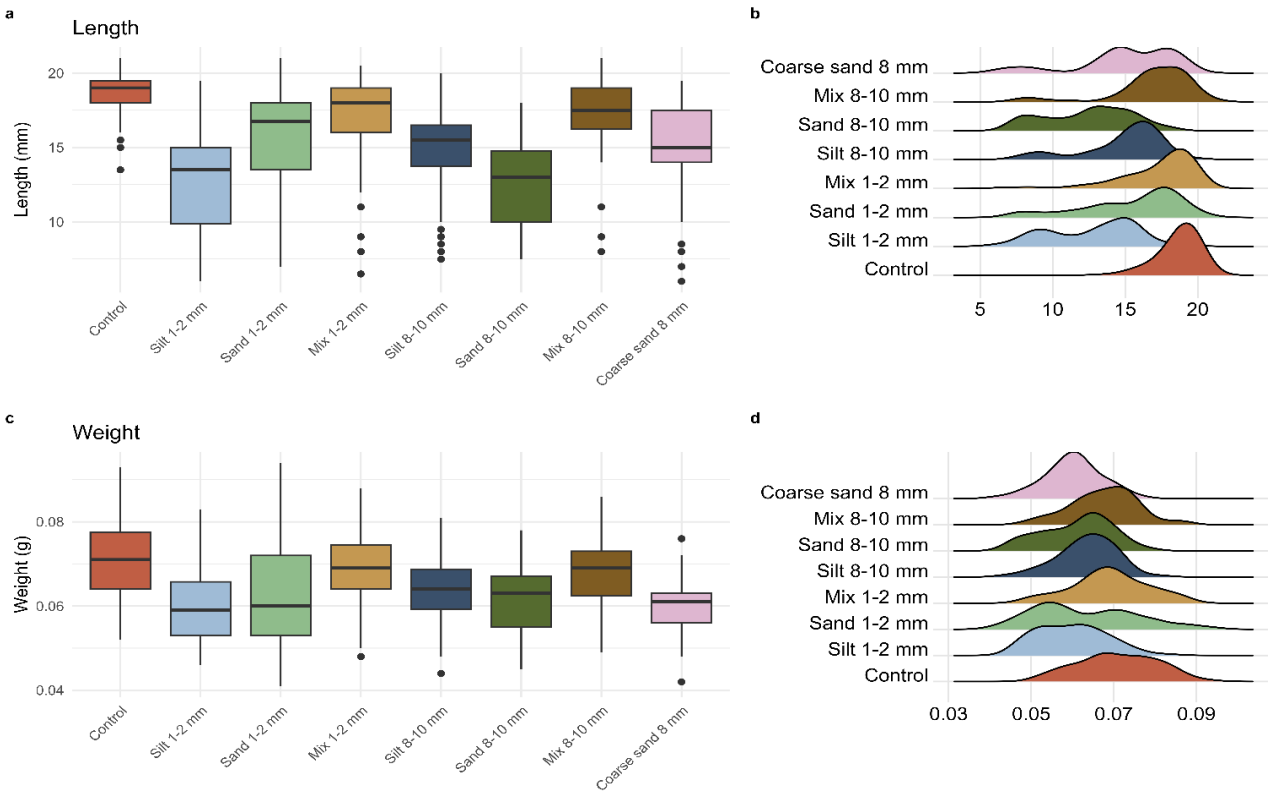

Figure 14 Illustrates the growth dynamics, with length (mm) and weight (g), of larvae under different sediment treatments using boxplots and geom ridges, revealing both central tendencies and the spread of the data, as well as the overall distribution shapes for each treatment group. Plot (a) presents a boxplot of larval length, while plot (b) provides a corresponding geom ridge plot for the same data. Similarly, plot (c) features a boxplot of larval weight, and plot (d) depicts the geom ridge plot for weight data. Each treatment group is represented, allowing for a comprehensive comparison of larval growth across different sediment conditions.

3.2.2.6 Correlation between sediments and oxygen

Statistical analysis was conducted to investigate the relationship between sediment treatments and oxygen levels in 8-10 mm sediment layers. The overall ANOVA analysis showed a p-value at $< 2\times10^{-16}$. A linear regression model indicated that sediment treatments explained 98.3% of the variance in oxygen means (R-squared = 0.983, adjusted R-squared = 0.9825). Specifically, treatments with coarse sand, mix, sand, and silt reduced oxygen levels by 1.14, 4.74, 5.46, and 9.10 units, respectively, compared to the control group, all with p-values below 2×10^{-16}, except coarse sand with $p = 4.4\times10^{-8}$.

3.2.2 Oxygen experiment

3.2.2.1 Mortality and affected

The analysis of varying oxygen levels on mortality revealed distinct differences across treatments from fertilization to hatched Figure 16a. The oxygen control group (100% DO, 12.62 mg/L), exhibited a moderate survival rate, averaging 75% ± 13.8% (Table 9). However, mortality rates varied significantly, ranging from 10% to 40%, with a median of 25%. This variability was pronounced during elevated degree days and hatching.

The 70% oxygen treatment (9.89 mg/L) demonstrated substantially lower mortality, with a median of 5.5%, compared to the control group. Conversely, the 50% oxygen treatment (4.75 mg/L) resulted in a significantly higher mortality rate compared to both the control group (One-way ANOVA, Tukey's HSD, p = 0.0034) and the 70% oxygen treatment (One-way ANOVA, Tukey's HSD, p = 4.3e-4), with median of 84.5%.

Table 9 Presents the mortality (%) and affected (%) embryos and larvae exposed to three different dissolved oxygen concentrations. Additionally, it shows the median length (mm) and wight (g) of larvae post-hatching.

Treatment	Median Mortality (%)	Mean + SD Affected (%)	Median Length (mm)	Median Weight (g)
Control *(12.6 mg/L)*	25	24.7 ±13.9	20	0.076
70% *(9.9 mg/L)*	5.5	6.7 ± 2.9	20	0.081
50% *(4.7 mg/L)*	84.5	83.5 ± 11.9		

These mortality rates were consistent with the percentage of affected individuals, which also varied across treatments. Both the control and the 70% oxygen treatments had lower percentages of affected individuals, then 50% oxygen treatments (Table 9). The 50% oxygen treatment showed a significantly higher affected rate than both the control (One-way ANOVA, Tukey's HSD, p = 7.7e-4) and the 70% oxygen treatment (One-way ANOVA, Tukey's HSD, p = 4.1e-5), with a median of 84.5% embryos affected.

These observations are visually supported by Figure 15 which presents the physiological state and developmental progression of embryos and larvae under different dissolved oxygen concentrations. In the figure, photo (a) shows embryos in exposed to 70% DO, appearing more viable compared to those in photo (b), which shows the 50% oxygen environment where the embryos are visibly more compromised. Embryos in the 50% oxygen environment exhibit a noticeable reduction in coloration, appearing whiter and less vibrant compared to those in the 70% oxygen environment. Photos (c), (d), and (e) contrast the developmental stages of larvae from different treatments: photo (c) shows larvae from the 100% oxygen control group exhibiting normal development, such as larvae in photo (d) from the 70% oxygen group, and photo (e) highlights larvae from the 50% oxygen group with noticeable yolk-sac abnormalities.

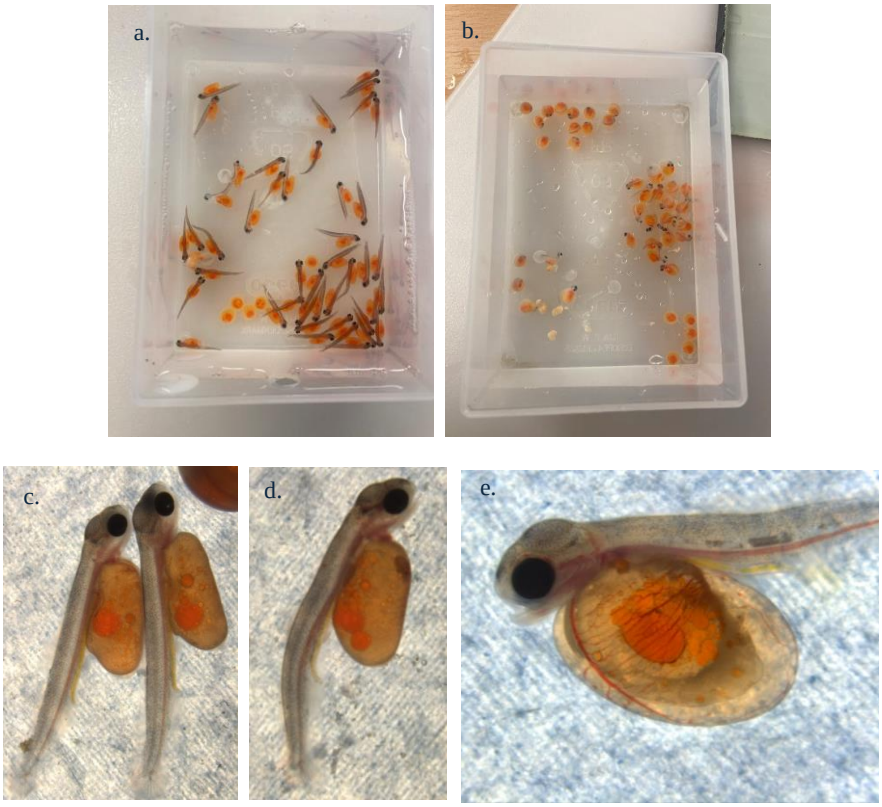

Figure 15 Illustrates the effects of three different dissolved oxygen concentrations on embryos and larvae. Photo (a) shows an experimental box with embryos exposed to 70% DO (9.86 mg/L), while photo (b) shows an experimental box with embryos exposed to 50% DO (4.75 mg/L), where embryos is more affected. Photo (c) depicts larvae from the control group with 100% DO (12.62 mg/L). Photo (d) shows larvae exposed to 70% DO, and photo (e) shows larvae exposed to 50% DO, with noticeable differences in yolk-sac morphology.

3.2.2.4 Growth

The physical growth parameters measured as length and weight showed less differences than mortality and affected percentages (Figure 16b, c). Since all the larvae at the 50% oxygen level exhibited full mortality, this group could not provide data. Although the median length in all treatments was consistently around 20 mm (Table 9) analysis revealed a statistically significant difference (One-sided Wilcoxon rank-sum test, $p = 3.4\times10^{-5}$). Similarly, weight measurements were relatively stable across the control and 70% oxygen groups (0.076 g and 0.081 g, respectively). The analysis showed a significant difference (One-way ANOVA, Tukey's HSD, $p = 2.9\times10^{-6}$).

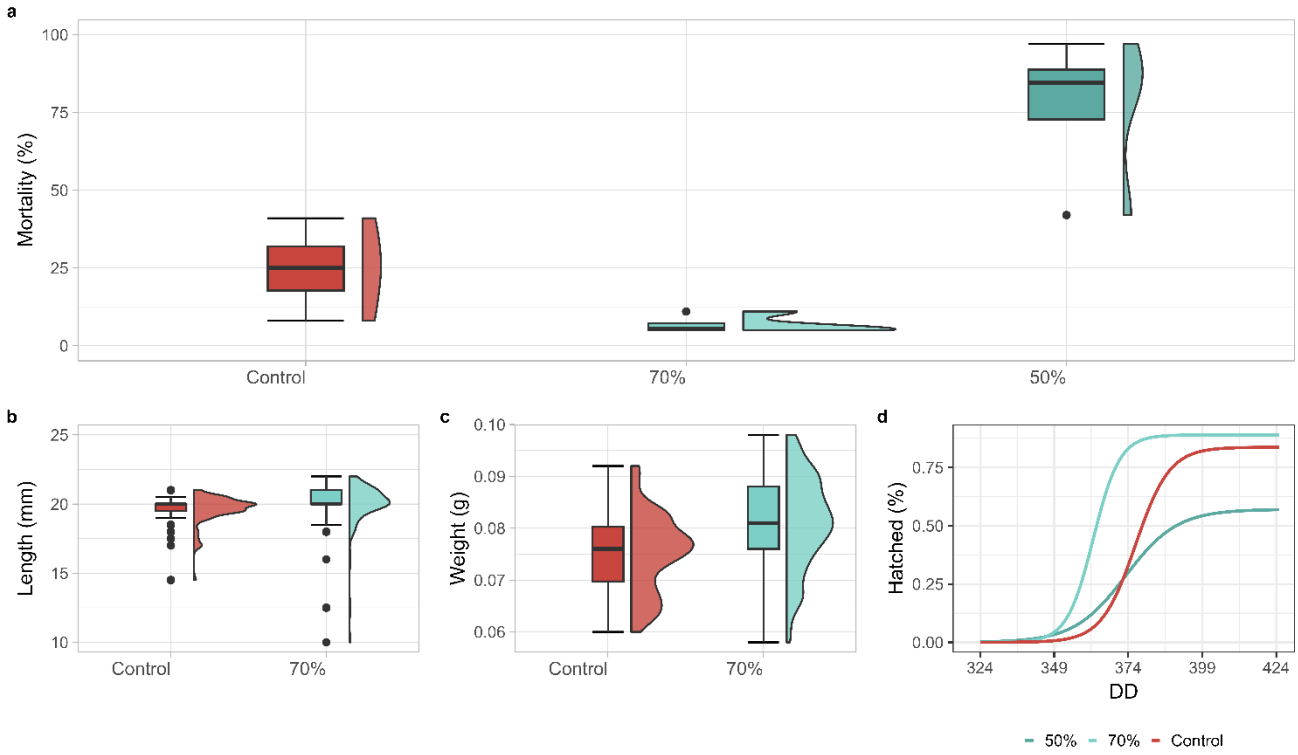

Figure 16 Shows the impact of different oxygen levels (control, 70%, and 50%) on mortality, length, weight, and hatching. (a) Box plots of mortality percentages with higher median mortality in the 50%. (b) Combined box and violin plots for lengths (mm) showing a wider distribution and higher median length in the 70% group. (c) Combined box and violin plots for weights (g) with a wider distribution and higher median weight in the 70% group. (d) Sigmoid hatching curves displaying the percentage of eggs hatched (%) over degree-days (DD).

3.2.2.5 Hatching success

The analysis of hatching rates, depicted in Figure 16d with sigmoidal curves across degree-days, showing that both exposer group did differ from control group. Both of the exposure group had an earlier hatching initiation than control, albeit 50% oxygen exposure did not reach 50 % hatched larvae until 390 DD (Table 10).

The nonlinear regression analysis (Two-way ANOVA) revealed significant differences in the hatching dynamics among the treatment groups. The control group maximum hatching percentage was 83.66%. The 50% treatment significantly reduced the maximum hatching percentage by 33.69%. Furthermore, the timing of rapid hatching for the 50% treatment did not show a significant difference compared to the control. Additionally, the 70% treatment significantly advanced the timing of rapid hatching by 14.24 DD. There were no significant effects on the maximum hatching percentage for the 70% treatment. The hatching rate was also not significantly affected by either the 70% or 50% treatments. Overall, the 50% treatment significantly decreases the maximum hatching percentage, while the 70% treatment significantly shifts the timing of rapid hatching earlier.

Table 10 Presents the hatching dynamics of embryos and larvae exposed to three different dissolved oxygen (DO) concentrations. It includes data on the initiation of hatching (DD), the point at which 50% of the embryos had hatched (DD), hatching success (%), and larval mortality (%).

Treatment	**Initiation (DD)**	**50 % Hatched (DD)**	**Hatching success (%)**	**Larvae Mortality (%)**
Control *(12.62 mg/L)*	361	379	83	7.3
70% *(9.89 mg/L)*	353	363	88	2.2
50% *(4.75 mg/L)*	359	390	57	47.6

3.2.2.6 Metabolism

High-performance liquid chromatography (HPLC) analysis revealed notable differences in compound concentrations between the oxygen-controlled sample and the sample with the lowest oxygen level (Table 11). The oxygen-controlled sample exhibited a lower citric acid concentration, with a 55.6% increase observed in the lowest oxygen sample. Conversely, the lowest oxygen concentration resulted in reduced levels of glucose, lactic acid, uric acid, and DL-pyroglutamic acid, with percentage differences of -2.1%, -37.7%, -6%, and -5% respectively.

Table 11 Presents the concentrations of citric acid, glucose, lactic acid, uric acid, and DL-pyroglutamic acid in two different oxygen conditions: the control group and the 50% oxygen group. The table also shows the percentage differences between these two conditions.

	Citric acid	Glucose	Lactic acid	Uric acid	DL-pyroglutamic acid
Oxygen Control	747.7 ± 55	235 ± 38	430 ± 38	1.52 ± 0.3	24.2 ± 3.2
50 %	1163.6 ± 112	230 ± 38.5	267.5 ± 41	1.43 ± 0.5	23 ± 2.2
% Differences	55.6	-2.1	-37.7	-6	-5

4. Discussion

This study examined the impact of sediment particle size, layer thickness, and oxygen concentration on the development of rainbow trout (Oncorhynchus mykiss) embryos from fertilization to hatching. Two parallel experiments were conducted: one focused on different sediment treatments with specific sediment sizes, and the other analysed the impact of lowered oxygen levels. The hypothesis suggested that sediment coverage reduces oxygen availability, adversely affecting fish egg development, with finer sediments being more harmful due to lower oxygen levels.

4.1 Sediment type on embryonic development

Coarse sand (2.0-1.0 mm) led to underdevelopment and significantly high mortality rate pre-hatching. Eggs incubating in coarse sand showed developmental delays, with only 53% hatching success, resulting in dead eggs rather than larvae. The growth of surviving alevins was significantly impacted, resulting in shorter and lighter individuals, indicating early developmental issues. Hausle and Coble (1976) observed delayed emergence and lighter alevins but reported higher survival rates than our study, when using a gravel mixture with 20% coarse sand. This suggests that coarse sand has distinct impacts, with developmental delays, resulting in mortality, inhibiting growth and affecting the hatching phase.

Sand fractions demonstrated significantly effects on underdevelopment, while thin layers increased development delay at the mid-stage, this was not significant. However, thick sediment layers significantly affected the eyed stage, causing over a 50% delay. Only thick sand sediment showed significant effects on mortality, particularly high during the hatching phase. These findings align with Witzel and MacCrimmon (1983) and Pattison et al. (2015), who noted that egg survival is highly sensitive to suspended sand particles. The high percentage of underdeveloped eggs is evident in the significant impact of both thin and thick sand layers on smaller alevins and altered hatching phase. Julien and Bergeron (2006) also observed that medium and coarse sand adversely affects pre-eyed, eyed, and hatching stages, leading to increased mortality rates and significantly affecting the hatching success. In contrast, Gatch et al. (2020) found no significant reduction in hatching success with larger fine sediment particles. However, Reiser and White (1988) highlighted fine sediments up to 0.84 mm negatively affected hatching success, reinforcing the idea that sand-induced delays can have sublethal or lethal effects.

The most severe effects were observed with silt, which caused multifaceted impacts on all stages of development, especially considering the stratification of eggs under thick layers. Underdevelopment was significantly affected by silt, as even a thin layer could disrupt early developmental stages, corroborating Julien and Bergeron (2006) observation that fine sediment (<0.063 mm) adversely impacts these stages. This delayed development was the most substantial among all the fractions used in this study. Furthermore, the presence of thick silt layers was associated with a pronounced increase in mortality rates, exceeding 90%, thereby highlighting the critical influence of silt accumulation on embryonic viability. In contrast, Gatch et al. (2020) found that only 2 mm cover had high mortality rate (71%) on walleye, which could be more sensitive than rainbow trout. Notably, silt exposure affected embryos throughout all developmental stages, significantly impairing growth rates and hatching success. This inhibition of growth, along with the substantial delays and reductions in hatching success, underscores the detrimental impact of silt on overall reproductive success.

The mixture of fine sediment, characterised as silty sand with clay content (38.5% particles <0.063 mm and 61.5% particles 2.0-0.063 mm), underscores the detrimental effects of a mixture of fine sediment particles (< 2 mm) on embryonic development. Initially, these fine sediments did not cause developmental delays, but significant mortality began from the eyed stage, progressively increasing through all stages and reaching 100% under sediment conditions of 8-10 mm depth. This aligns with previous studies by Greig et al. (2005) and Sear et al. (2008), which show that even a small percentage of fine sediment in a mixture can lead to high mortality rates. Additionally, research by Franssen et al. (2012) and Lapointe et al. (2004) suggests that the finest particles, especially with clay, mixed with coarser sand exacerbate viability of eggs. The study showed a significant reduction in length, indicating that growth was affected by the sediment mixture. However, the insignificant impact on weight suggests that surviving alevins were predominantly from surface-laid eggs, where minimal sediment coverage allowed for better development. None of the eggs at the base survived, and the lack of significant developmental delays in thin layers supports the idea that thin sediment layers facilitate healthier embryonic development.

Our findings demonstrate that finer sediments, especially those containing clay and silt, are the most detrimental to embryonic development and survival. Argent and Flebbe (1999) and Lapointe et al. (2004) found that silt significantly reduces survival rates for freshwater fish embryos, aligning with our results. Greig et al. (2005) also reported similar findings regarding the detrimental impact of silt. Medium sand caused significant harm across various developmental stages, though its impact was not as severe as that of silt or the silty sand mixture with clay. Interestingly, coarse sand had notably developmental delay, with mortality rates high at the later stage. This study indicates that finer sediment fractions do affect developmental stages and can cause high mortality rates, and that the fraction size matters . Conversely, our findings contrast with Sear et al. (2016) and Louhi et al. (2023), who reported no significant correlation between specific sediment size fractions and mortality. However, our result is consistent with studies of Greig et al. (2005), Kemp et al. (2011) and Levasseur et al. (2006), which found that finer sediments cause significant effects on developmental stages and mortality. The severity of impact increased as sediment size decreased, with all sediments under 2 mm proving harmful, and the finest sediments having the most exaggerated effects across all developmental stages.[2] This supports the hypothesis that finer sediments cause more severe developmental impacts.

4.2 Different dissolved oxygen concentrations on embryonic development

This study underscores the critical importance of dissolved oxygen (DO) levels in fish egg development. Lower oxygen concentration (50%, 4.7 mg/L) resulted in significantly higher mortality and developmental issues compared to control (100%, 12.6 mg/L) and moderate (70%, 9.9 mg/L) oxygen concentration. These findings align with Bjornn (1991) recommendation that DO levels should not fall below 5 mg/L to support healthy fish development. Increased mortality and developmental delays under hypoxic conditions are consistent with the work of Wood et al. (2020) and Garside (1959), who documented severe impacts on fish eggs at low DO levels (< 4.5 mg/L). The embryos exhibited a white coloration; however, it cannot be definitively concluded that this is caused by a loss of

[2] The comparable analysis of thick sediment treatment, and the state of the alevins that successfully hatched can be found in Appendix B1

pigmentation without further examination. It is worth noting that other experiments have observed similar results, potentially caused by oxidative stress (Hahn et al., 2014).

Interestingly, the study also noted earlier hatching in low oxygen conditions, which contradicts the findings of Ciuhandu et al. (2005), Hamor and Garside (1976), and Wood et al. (2020), who indicated that hypoxia during early salmon development can slow growth and delay hatching. However, this observation corroborates Czerkies et al. (2001) and Latham and Just (1989), suggesting that environmental stressors like hypoxia can induce premature hatching, as seen in Wedekind and Müller (2005). Despite earlier hatching, the high mortality rates post-hatching, as described by Wood et al. (2019), emphasize the necessity of sufficient oxygen levels throughout the entire developmental process.

A moderate DO reduction to 9.89 mg/L (70% of original DO) appeared to have no effect on the development of embryos. Davis (1975) states that DO concentrations of 9.75 mg/L are fully protective of larvae and mature eggs, and Wood et al. (2020) found that moderate hypoxia did not reduce growth, development, or survival compared to normoxia. In this study, 70% DO had higher survival rates and improved growth and development compared to the control group.

However, Shumway et al. (1964) noted that embryos reared at low and intermediate oxygen concentrations hatched later and were smaller compared to those reared at air-saturation levels, which contradicts these results. Hamor and Garside (1976) found that developmental rate and survival increased directly with higher DO concentrations and increased water exchange rates. The unexpectedly high mortality rates in the control group raise concerns about potential tank effects, as the control group in the parallel experiment did not exhibit such high mortality. Although there were no significant differences between the two control groups, this discrepancy suggests that the high mortality in the oxygen control group might be an anomaly due to unique environmental stressors in that tank, especially given that the survival rate was below 80%. Due to the low survival rate in the control group, comparisons with the 70% and 50% groups should be interpreted with caution. The apparent advantage of the 70% group may be misleading, and the 50% group could appear less significant than it actually is. Supporting this, Miller et al. (2008) found that chronic exposure to moderate hypoxia (around 70% DO) led to decreased DO levels within the boundary layer around rainbow trout embryos, associated with lower metabolic rates and adaptive physiological changes. This highlights the complex relationship between dissolved oxygen levels and

embryonic development, demonstrating that optimal DO conditions are crucial for maintaining fish health and promoting proper development.

The measurement of metabolites offers insights into the metabolic adjustments that occur under hypoxic conditions. Notably, the significant increase in citric acid concentration under 50% oxygen levels with a 55.6% rise suggests a substantial increase in metabolic pathways, potentially indicating an adaptive response to maintain energy production when oxygen availability is limited. The relatively stable glucose level (-2.1% change) and the more pronounced reduction in lactic acid levels (-37.7% decrease), can indicate that the embryos are trying to make more efficient energy use mechanisms, aligning with (Panepucci et al., 2001). For instance, the decrease in lactate levels could be due to its conversion back to pyruvate for oxidation in the citric acid cycle or its utilization in gluconeogenesis (Léger et al., 2021)

These findings are consistent with the (Bergstedt et al., 2021) on hypoxia tolerance and metabolic coping strategies in Nile tilapia (*Oreochromis niloticus)*, where a critical oxygen threshold leads to a loss of homeostasis, but significant metabolic changes occur before this point. The observed reduction in glucose and lactic acid levels, indicate a reduction in metabolic rate, without an accumulation indicative of loss of equilibrium (LOE), supports the notion that the metabolic system has not reached a critical failure point. Nevertheless, it indicates a strategic adjustment, to increase citric acid and cope with the reduced oxygen supply. This adaptive response highlights the organism's ability to modulate its metabolic pathways to maintain homeostasis and energy balance under varying oxygen conditions.

4.3 Mechanisms behind sediment-induced negative effects

4.3.1 Metals and toxic effects

Sediments can be substantial contributors to environmental pollution, often containing metals, trace metals, and endocrine disruptors, which pose potential risks to embryo development (Wetzel et al., 2013). Fine sediment particles, particularly those less than 63 µm such as clay and silt, demonstrate a high affinity for soluble metals (Collins et al., 1997). Although the water chemistry exhibited marginally increased metal concentrations upon contact with sediments, the overall levels were insufficient to significantly impact embryo development. Nevertheless, further toxicity testing on embryos is imperative to reach definitive conclusions.

4.3.2 Sediment impact on oxygen level: Biological effects, influence of stages and depth

The findings of this study highlight the nuanced relationship between fine sediments and dissolved oxygen (DO) levels, demonstrating the adverse effects on embryo mortality and development. Our findings indicate that fine silt, with 8-10 mm layer, significantly reduces DO concentrations to critically low levels of 2.34 mg/L in the middle and 1.85 mg/L at the base. This reduction in DO is particularly alarming, because is under the level for hypoxic conditions (Vaquer-Sunyer & Duarte, 2008), and . It also correlates with increased mortality and developmental issues among embryos (Greig et al., 2005; Kemp et al., 2011; Reiser & White, 1988). These findings align with Silver et al. (1963) and Fuda et al. (2007), who reported complete mortality in steelhead and rainbow smelt embryos at DO concentrations between 1.6-1.9 mg/L. The consistency across these studies underscores the sensitivity of aquatic embryos to low oxygen environments, exacerbated by the presence of fine sediments. Additionally, the broader implications of these results are supported by Sowden and Power (1985), who identified that DO levels below 4.5 mg/L significantly impede normal embryonic development. This suggests that not only the presence of fine sediments but also the specific DO thresholds are critical for embryo survival. The detrimental effects observed at DO levels under 5 mg/L across both silt and sand substrates indicate a prevalent issue where fine sediments hinder oxygen availability.

The oxygen requirements of rainbow trout are lowest shortly after fertilization (< 1 mg/L) and increase gradually, peaking just before hatching (7.5 mg/L) (Rombough, 1988). Although salmonids, including rainbow trout, can endure fluctuating oxygen levels to some extent chronic exposure to sediment and low oxygen levels are showed highly lethal (Wood et al., 2020). These results reveal that later stages, particularly just before and during hatching, have high mortality rate and may indicate that the oxygen levels beneath the sediment layers contribute to the effects seen under thick layers. These findings highlight the critical need to support hatching success and reduce mortality rates by ensuring optimal oxygen conditions and minimizing sediment exposure, especially as oxygen demands increase with developmental progression.

Balancing the energy budget is crucial during all life stages (Hunt von Herbing & Pan, 2022). The observed morphological differences, such as paler alevins and reduced growth, could be

attributed to the energy trade-off resulting from environmental stressors like sediment and low oxygen levels. These stressors likely increase the metabolic cost, diverting energy away from both growth and pigmentation towards more critical survival processes (Hahn et al., 2014; Hunt von Herbing & Pan, 2022). The paleness of larvae in both experiments, however more pronounced at 50% oxygen reduction, can indicate an early trade-off. Some coloration was observed in yolk-sac alevins exposed to sediments, which could either indicate a trade-off where pigmentation starts later, or that the sediment-exposed embryos were delayed in development and had not yet reached the stage where colour begins to appear. However, control groups at earlier day-degrees were smaller, albeit had already started to develop pigmentation, whereas alevins exposed to sediment at later day-degrees were larger yet lacked visible coloration. This suggests that the observed paleness and reduced growth could be due to a trade-off in energy allocation, prioritizing survival over growth and pigmentation under stress. Future research should focus on the metabolic mechanisms underlying these changes in embryos. However, due to time constraints, the measurement om metabolites for embryos under sediment was not possible in the current thesis. Understanding these mechanisms could provide deeper insights into how energy allocation impacts developmental outcomes under environmental stress.[3]

Thin sediment layers elicit sub-lethal effects that significantly influence growth parameters in aquatic organisms. Despite their minimal thickness, even small amounts of sediment can disrupt normal development, particularly resulting in smaller and lighter alevins and altering the onset of rapid hatching. Gatch et al. (2020) corroborate these findings, demonstrating that even minimal sediment deposition can retard growth and reduce hatching success rates. The occurrence of smaller and lighter alevins has been identified as a detrimental factor affecting both emergence and swimming abilities, as well as their capacity to avoid predation, which is crucial for fry survival (Roussel, 2007). The specific microenvironment in the boundary layer adjacent to the eggs within the thinnest sediment layers remains challenging to characterize. However, it is plausible that even a thin sediment layer could hinder the embryos' mobility especially when starting the hatching process, potentially exacerbating growth reduction and accounting for the observed shifts in rapid hatching timing. Further research is necessary to

[3] The comparison of coloration between sediment control, 50% exposure and the sediment exposure are presented in Appendix B2.

elucidate the underlying mechanisms by which thin sediment layers affect embryonic development.

In contrast, thick sediment layers pose more severe effects, primarily affecting earlier developmental stages and resulting in higher mortality rates among embryos. The exacerbation of low dissolved oxygen (DO) concentrations in thicker layers suggests that oxygen levels play a crucial role in the adverse effects observed under fine sediment exposure. This leads to higher mortality rates and lower hatching success, as demonstrated by (Sear et al., 2016) and corroborated by the findings of this study.

This underscores that while both thin and thick sediment layers disrupt normal developmental processes, the severity and timing of the resulting developmental delays, morphological differences, hatching success and mortality vary considerably. Thin layers primarily impact growth rates and the timing of rapid hatching without causing substantial mortality, thus permitting embryo survival. This indicates that minimal sediment deposition in spawning gravels can lead to sub-lethal effects, influencing later fry stages without necessarily reducing oxygen concentration (Julien & Bergeron, 2006; Yamada & Nakamura, 2009). Conversely, thick sediment layers induce early developmental issues, resulting in significantly higher mortality rates. The marked reduction in oxygen levels under thick layer and the ensuing lethal effects support the hypothesis that finer sediments reduce oxygen availability, thereby reducing the number of viable embryos and alevins (Greig et al., 2005).[4]

4.2.3 Physical effects

The indication that fine sediments significantly lower DO levels corroborates previous studies (Greig et al., 2005; Kemp et al., 2011; Reiser & White, 1988). However, the results show that the detrimental effects of fine sediments, both in thin and thick layers, on embryos are more severe than those caused by reduced oxygen levels alone. This suggests that factors beyond reduced oxygen levels are at play. For instance, sand, with slightly lower oxygen levels than the 50% oxygen level in the controlled oxygen experiment, exhibited more pronounced adverse effects, indicating that additional factors inherent to the sediments exacerbate the harm. Mixed sediments containing a small amount of clay had slightly higher DO levels than

[4] Comparison between alevins exposed to thin and thick layer of silt are presented in Appendix B3.

the 50% level, albeit resulted in much higher embryo mortality. This points to the possibility that clay in the mixture could block micro-pores in egg capsules, hindering oxygen diffusion (Greig et al., 2005). Even trace amounts of fine sediments (0.2%) have been documented to reduce survival and cause developmental issues (Levasseur et al., 2006). This reinforces the idea that certain sediment characteristics are intrinsically harmful to embryos.

While previous research (Levasseur et al., 2006) suggested that sediments finer than 0.063 mm are particularly harmful and larger sediments up to 2 mm are less impactful, the current findings challenge this perspective. While studies like Greig et al. (2005), Julien and Bergeron (2006), Bertin and Friedrich (2019) and Reiser and White (1988) suggest that primary impact of coarse sand is that this fraction hinders fry emergence due to small pores, our findings indicate that physical barriers alone do not account for the observed effects. However, our observations indicate that embryos under coarse sand experienced delayed development and increased mortality even before hatching. This was evidenced by the presence of dead embryos, suggesting that factors beyond physical blockade are at play. Despite high DO levels under coarse sediments, which were sufficient according to our 70% modified oxygen level experiment, embryos still exhibited significant adverse effects. This indicates that the detrimental impacts of coarse sand are not solely due to oxygen deprivation but may also involve other mechanisms. The physical characteristics of coarse sand particles may induce mechanical stress or other conditions unfavourable to embryonic development, contributing to pre-hatching mortality.

The potential effects caused by exposure to thin layers, coarse sand and the more severe effects observed in oxygen experiments require careful consideration of sediment characteristics. One possible explanation is that, despite washing and separation processes, residual fine sediments such as silt and clay may still be present, contributing to the adverse effects (Greig et al., 2005; Lapointe et al., 2004; Levasseur et al., 2006). In this study, we observed a thin layer of silt forming a coat around the eggs, with silt and clay adhering to the embryos, which was also noted by Julien and Bergeron (2006). Furthermore, Stuart (1953) noted that the chorion of brown trout eggs lost their smooth, glossy exterior as they attracted finer silt particles, becoming completely covered by a dark coat of sediment.[5]

[5] The thin coating of silt and the adhered of particles to the chorion is presented in Appendix B4.

Finer sediments can degrade the local microhabitat around eggs by trapping metabolic waste products and other chemicals (Greig et al., 2005; Greig et al., 2007). Despite adequate oxygen levels, poor water flow can prevent the removal of waste products, particularly before hatching when metabolic processes are increasing. This can lead to increased mortality and developmental issues, which aligns with the increase of developmental delay and mortality rate at later stages. Coarse sand may allow oxygen diffusion but can also create micro-environments with insufficient water flow (Donald Wallace Chapman, 1988). These stagnant areas can trap contaminants, leading to harmful localized buildups even if overall water quality appears acceptable. Around embryos, a boundary layer of minimal water movement can exacerbate the issue by increasing local concentrations of harmful substances (Miller et al., 2008). Further research should focus on mapping waste products not only in surface water but also within sediment layers, creating a gradient to identify microenvironments with poor water removal or transient hypoxia. This approach, combined with chemical analysis of interstitial water to detect trace metals and other chemical species, could help identify additional factors beyond oxygen availability and hindered emergence.

4.3 Limitation and future studies

In a controlled environment, this study isolates specific variables such as sediment type and oxygen concentration to focus on their direct effects on fish egg development. By minimizing external environmental fluctuations, the study ensures that the observed effects are primarily due to sediment exposure. However, the sensitivity of controlled environments to small changes can complicate the interpretation of results, as any inadvertent inconsistencies might lead to unexpected outcomes, highlighting a limitation in replicating natural complexity.

Natural systems involve a complex interplay of factors such as gravel permeability, sedimentary oxygen demands, and variable hydraulic gradients, which influence oxygen flux and egg survival. Temperature also plays a crucial role; as higher temperatures reduce oxygen levels in the water, narrowing the margin to the critical oxygen threshold necessary for survival. This situation is exacerbated by the increased metabolic rates of embryos at higher temperatures, which demand more oxygen (Alderdice et al., 1958). Consequently, sedimentation becomes more critical under these conditions, as it can further reduce oxygen availability and impede normal development. Additionally, when sediment contains organic matter, microbial activity within the sediment can increase oxygen consumption through

decomposition processes. This heightened microbial oxygen demand competes with developing embryos for the limited available oxygen, thereby reducing oxygen availability for embryonic development and increase the effects on development and further (Greig et al., 2007; Sear et al., 2016; Sear et al., 2014). Understanding these natural environmental factors is essential for making accurate implications, especially during construction work. For instance, Sear et al. (2014) found that groundwater can maintain dissolved oxygen (DO) levels and mitigate the impact of surface sedimentation. When groundwater influence is minimal, sediment composition becomes critical due to its effect on water flow and velocities. Spawning habits of salmonids will temporally clean the gravel for fine sediment (Peterson & Quinn, 1996); however, resuspension of fines can heighten the suspended sediments in the redd potentially to its former level. Thereby will the architecture of the gravel bed and the redd, be correlated and strongly controlled by near-bed sediment composition and flux (Sear et al., 2008)

To gain comprehensive insights, these natural elements must be incorporated into field studies. Future research should investigate the specific mixtures and percentages of fine particles and the underlying causes of sediment effects when sufficient oxygen is present. By focusing on the composition and ratios of fine particles and their impact on DO levels, this will improve the understanding of how to protect fish populations during sensitive developmental periods. Such research will provide more accurate and applicable findings for environmental management and conservation efforts during construction activities.

4.4 Broader applications

The findings from this study underscore the critical importance of careful sediment management in road construction to protect fish embryos throughout the entire spawning period, particularly from sediments under 2 mm, and not just the finest materials. Effective strategies are essential to reduce sediment influx while ensuring that gravel beds remain suitable for egg incubation.

Sediment management guidelines face challenges due to the diverse range of environments they must cover, including rivers, lakes, and coastal areas. Each of these environments has unique characteristics that complicate the application of a one-size-fits-all approach. Moreover, global sediment management targets tend to be overly generalized, often overlooking local environmental factors such as specific ecological needs, the composition,

chemical and biological properties of the water bodies, and human activities (Greig et al., 2005; Owens et al., 2005; Roseth R, 2021). This lack of localized consideration results in recommendations that may not be effective or suitable for all locations.

Avoiding construction activities during spawning periods is crucial to mitigate harm to embryos. Understanding the vulnerability of different developmental stages to sediment exposure is essential. This study confirms that both the quantity and duration of sediment exposure adversely impact fish egg health, with thicker layers posing greater damage. Sedimentation poses significant risks to fish embryos during both early and late developmental stages, though the nature and severity of these impacts differ. During early development or from fertilization, prolonged exposure to sediment exacerbates mortality rates (Argent & Flebbe, 1999), and as indicated by this study's findings that early developmental delays result high mortality rate and smaller alevins, potentially affecting fry survival. At later stages, with an increased oxygen demand (Rombough, 1988), sedimentation can block and reduce oxygen levels, jeopardizing hatching success; however, embryos at this stage are generally more developed and resilient. Wedekind and Müller (2005) found that embryos exposed to stressors can hatch earlier to avoid these suboptimal conditions, thus enhancing survival rates. Early exposure to sediment leads to higher mortality and developmental anomalies, while late exposure causes oxygen limitation issues and triggers adaptive responses such as early hatching. Therefore, further investigation into sediment exposure to embryos at different life stages is needed, and construction activities should be scheduled outside critical spawning periods to minimize disturbance. In Norway, avoiding sediment disturbance during spawning is crucial, underscoring the need to protect the entire spawning season to ensure fish population health.

Implementing erosion control measures, such as silt fences and sediment basins, can help prevent fine sediments from entering water bodies during construction. Secondary measures, including silt fences, sediment basins, and ponds, capture eroded materials before they enter water bodies. Fine sediments like silt and clay can remain suspended in the water column longer than coarser sediments like sand, which is more likely to settle into gravel beds and disrupt spawning grounds. Silt and clay, which is highly detrimental, eventually settle in slower-flow areas, such as gravel beds, river bends, lakes, and estuaries (Greig et al., 2005; Owens et al., 2005; Rodrigues et al., 2012). There is also a need to look at sediment source and mass, as well as particles. Sear et al. (2016) found that sewage and road verge were

highly detrimental, and that organic matter was essential to consider for spawning habitat quality. This should be taken in consideration when implementing control measures and mitigating suspended soils in water bodies.

Habitat restoration efforts, such as enhancing riverbank vegetation to reduce erosion and sediment runoff, are vital in managing sediment impact. Restoring natural stream flows helps maintain sediment transport dynamics, favouring the removal of fine particles from spawning areas (Wohl et al., 2015). Regular monitoring of sediment levels and water quality in critical habitats is necessary to ensure ongoing protection and adapt management practices accordingly.

Establishing stringent regulations to decrease sediment discharge from industrial, agricultural, and road construction activities is crucial for protecting aquatic ecosystems. The impact of fine sediments extends beyond just oxygen levels; their physical presence can directly harm developing embryos and preserve adverse effects. Comprehensive assessments of river conditions, including the influence of groundwater on oxygen levels in spawning areas, should inform emission permits and regulatory frameworks to minimize environmental impacts.

Setting low limits on sediment discharge can mitigate the adverse effects of fine sediments on fish embryos and overall water quality. These regulations are essential to safeguard aquatic ecosystems and ensure the health and sustainability of these vital environments.

4.5 Concluding remarks

This study underscores the pivotal roles of sediment characteristics and oxygen availability on the development of fish embryos. Sediment coverage significantly affects the survival and hatching success of rainbow trout eggs. The results showed varying degrees of impact, heavily influenced by the levels, thickness, and size of the sediment which supports the hypotheses.

Key findings from these experiments reveal that both sediment particle size and layer thickness significantly influence fish egg development, mortality, growth, and hatching success. Developmental delays for embryos were prevalent at the pre-hatching stage, particularly in the thinnest layers, resulting in small and light alevins. Conversely, the thickest sediment layers exhibited early developmental delays and mortality rate which increased at post-hatching stage, culminating in small, light fry and low hatching success. Notably, all sediment types tested (<2 mm) adversely affected the development process to varying degrees. Silt emerged as the most damaging isolated sediment fraction tested in this study, causing significant underdevelopment, negatively impacting growth and hatching success, and resulting in high mortality in thick layers. While sand also caused considerable harm, it was less detrimental in thinner layers, though it still slowed growth. Embryos exposed to coarse sand experienced delayed development and increased mortality before hatching, contradicting previous studies that primarily noted physical barriers affecting alevins and fry. The mixture of sand (<2 mm), containing clay, had the highest impact on mortality. This highlights the importance of considering sediment components collectively, as it indicates the detrimental effects that finer sediments, such as clay, can have when mixed with coarser substrates.

This study found that low oxygen level caused high mortality rate. Finer sediments decrease oxygen levels, and higher quantities of fine sediment correlated with lower oxygen, suggesting that finer sediment can exacerbate these harmful effects if oxygen levels are inadequate. This supports the hypotheses that fine sediment lower oxygen levels, which intensify the effects. However, the observed impacts on embryo development even at sufficient oxygen levels suggest that factors beyond oxygen deprivation are involved. The precise mechanisms through which sediment impacts embryo development remain somewhat unclear. Further research should focus on physical effects as blocking pores in chorion, insufficient water flow, microenvironments in the pockets, and the boundary layer to provide more insight into the mechanisms at play when oxygen levels are sufficient. Nonetheless,

these findings indicate that sediment characteristics significantly contribute to embryo mortality and developmental delays, necessitating further investigation.

For construction and infrastructure projects near rivers, it is crucial to recognize the significant threats posed by sediments smaller than 2 mm to fish embryo survival. Avoiding the disruption of spawning periods and minimizing the introduction of suspended solids into aquatic ecosystems are essential steps. Minimizing sediment discharge should be a priority, as even thin layers of sediment can cause substantial harm. Comprehensive assessments of river conditions, including the influence of groundwater on oxygen levels in spawning areas, are essential. Emission permits should be based on these assessments, with a focus on minimizing environmental impacts. A thorough understanding of river ecosystems and the implementation of stringent sediment control measures can help protect fish populations and maintain healthy aquatic environments.

This study underscores the need for nuanced conservation and management practices that address both sediment composition and oxygen availability in redds. Continued research and adaptive management strategies will be essential to mitigate the impacts of fine sediments and promote the sustainability of aquatic ecosystems.

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APPENDIX A

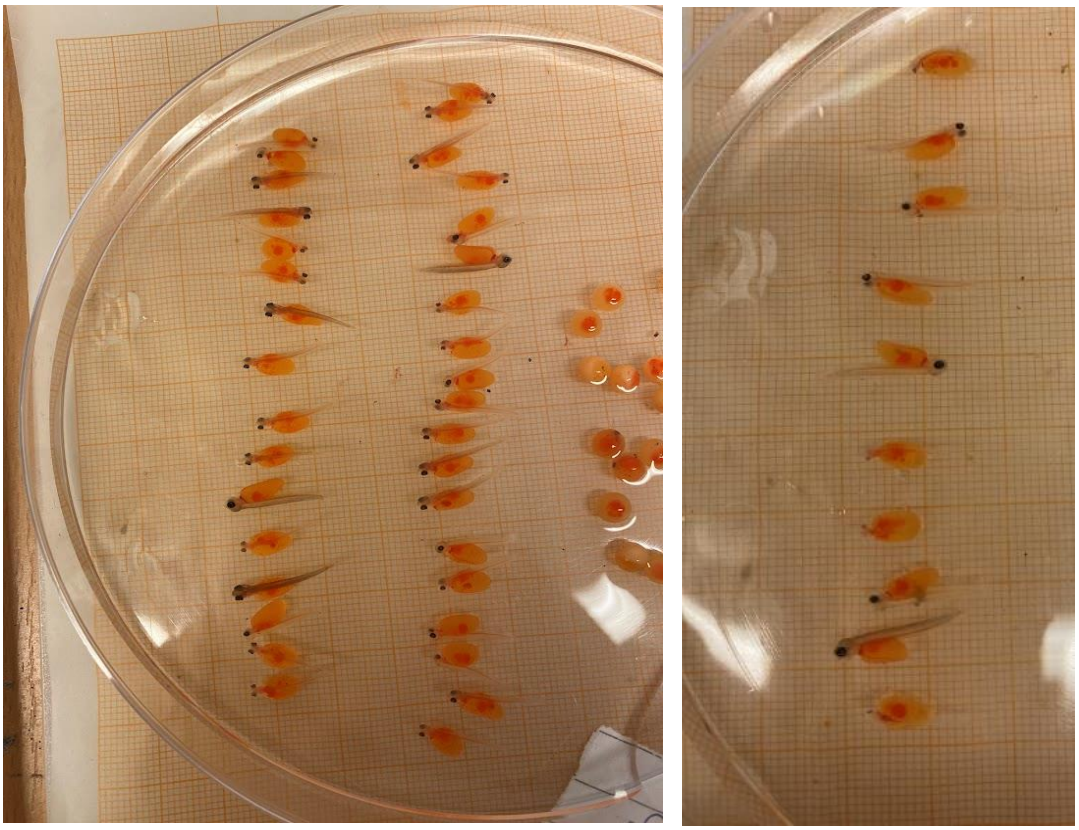
Appendix A1 Compares the measured values of chloride (Cl), sulphate (SO4), and nitrate (NO3) concentrations with certified reference material (CRM) values, including the percentage bias. Measurements were taken for three different reference materials (RM1, RM2, and QS3060), showing the deviation of the measured values from the CRM values.

Parameter	Measured	CRM-value	% Bias
Cl mg/L	RM1 – 76,79	RM1 - 74.0 ± 3.8	3.77 %
	RM2 - 15,66	RM2 - 16.6 ± 1.5	-5.67%
	QS3060 – 164,49	QS3060 - 159 ± 1	3.54%
SO4 mg/L	RM1 – 81,60	RM1 - 76.3 ± 4.2	6.95%
	RM2 – 16,47	RM2 - 18 ± 1.4	-8.50%
	QS3060 – 84,33	QS3060 - 77.0 ± 0.6	9.51%
NO3 mg/L	RM1 – 11,88	RM1 - 2.86 ± 0.30	315.66%
	RM2 – 56,85	RM2 - 11.7 ± 1.0	386.33%
	QS3060 – 50,62	QS3060 - 46.3 ± 0.4	9.32%

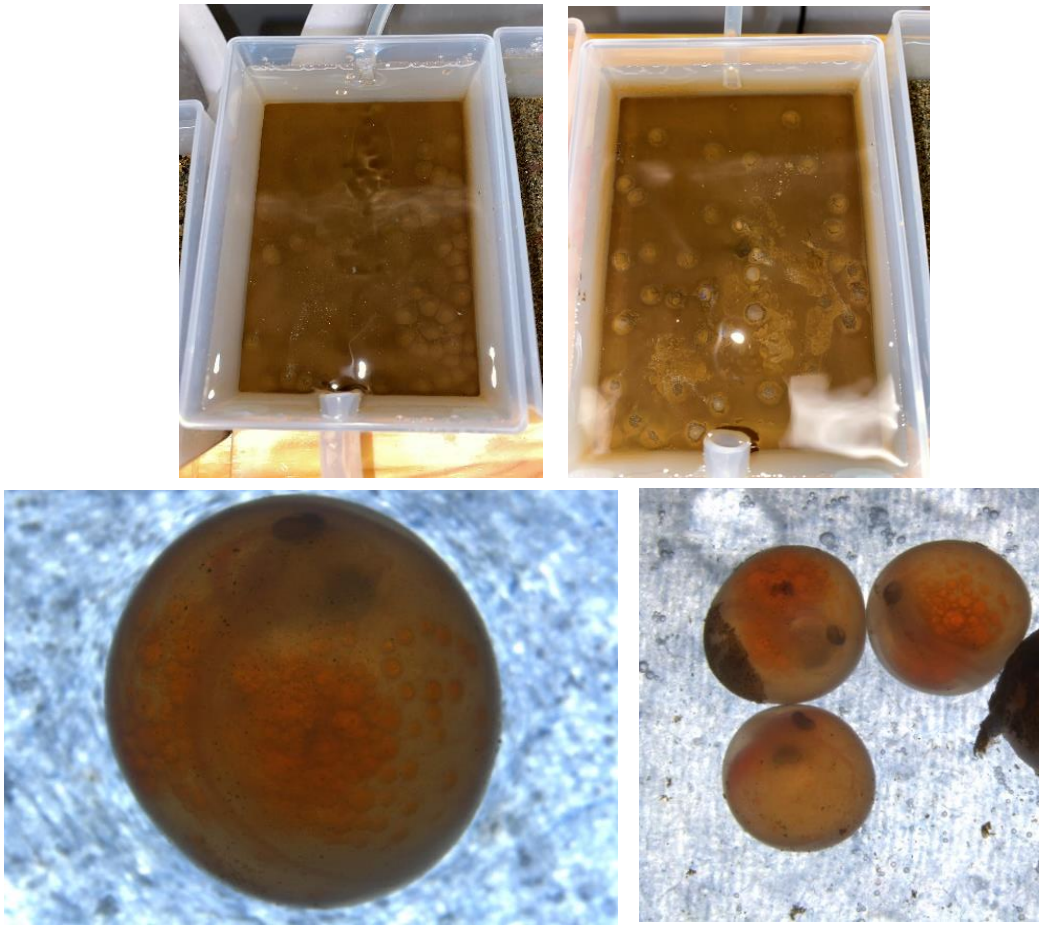
APPENDIX B

Appendix B1 Shows the effect of sediment layer thickness (8-10 mm) on fish embryos, with different sediment types represented from left to right: the control group (no sediment), coarse sand, mixed sediment, sand, and silt. The adverse effects become more pronounced as the sediment size decreases from coarse sand to silt, fewer larvae indicate higher mortality rate, with the control group on the far left showing the baseline condition without sediment, highlighting the contrast in effects caused by the varying sediment sizes with thick layer.

Appendix B2 Showing the impact of hypoxic conditions and sediment exposure on colour of fish eggs and the appearance of hatched alevins. The top left image shows eggs under hypoxic conditions at 360 DD, with significant whitening of the larvae inside the eggs. The top right image depicts eggs exposed to a sand fraction, also at 250 DD, demonstrating similar, albeit less evident, effects. The bottom left image shows eggs exposed to silt at 420 DD, resulting in pale appearances of the hatched alevins. The bottom right image shows the control group at 360 DD, displaying more coloured larvae and embryos, indicating healthier development compared to the hypoxic and sediment-exposed groups.



Appendix B3 Showing the difference from thin layer to thick layer of silt. Highlighting the increase developmental delay with reduced growth in both layers, with increasing mortality and reduced hatching success with thick layers.



Appendix B4 Illustrating the coating effect of silt and mixed sediment on fish eggs. The top images show the eggs in silt with both thick (left) and thin (right) layers, revealing a noticeable coating effect around the eggs. The bottom images show close-ups of the eggs exposed to silt and mixed sediment (including clay), indicating that particles have attached to the chorion of the eggs.

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