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Gas bubble disease in Atlantic salmon (*Salmo salar* L.) induced by simulated well boat operations

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ABSTRACT

Decompression-induced gas bubble disease (GBD) has been studied across various species and contexts, but its occurrence in well boat operations in Atlantic salmon aquaculture has not been investigated. In the present study, Atlantic salmon were exposed to pressure conditions designed to mimic the outer operational boundaries of well boat loading (0.4 atmospheres absolute (ata)) and unloading (2.6 ata) in the Norwegian aquaculture industry. The simulated loading, starting from atmospheric pressure (1 ata), with rapid decompression to 0.4 ata resulted in altered positioning, behavioural abnormalities, gas bubbles in blood detected by ultrasound, macroscopically visible gas bubbles in gills and fins, and histopathological lesions. A time-dependent increase in mortality was observed, reaching 100 % after 113 min, with the first mortality recorded at 65 min. Although dissolved oxygen of 100 % and 150 % of air saturation level prior to decompression resulted in different partial pressure ratios (N_2+Ar):O_2 upon exposure to 0.4 ata, the clinical findings were similar. The sub-lethal decompression-induced signs of GBD appeared to be reversible. Compression and exposure to 2.6 ata resulted in upward-tilted swimming, likely representing a behavioural response to maintain buoyancy; however, no other notable behavioural or GBD related signs were observed.

This study clearly demonstrates that a pressure profile representative of certain well boats operations in Atlantic salmon aquaculture can induce acute GBD.

1. Introduction

The rapid expansion of Atlantic salmon (*Salmo salar* L.) farming in Norway has led to significant changes in production infrastructure, including development of substantially larger net pens, fewer but larger harvesting plants, and deployment of larger, more technologically sophisticated well boats (Afewerki et al., 2023). Well boats have become essential in various operational processes, including their original function of transporting fish and their more recent use in delousing treatments employing both medicinal and non-medicinal methods (Sviland Walde et al., 2021).

The increasing size of modern well boats, combined with the occasional need to separate fish from the water during transfer, has resulted in a demand for greater vertical lifting capacity. Loading the well boat from net pens is usually achieved by creating a sub-atmospheric pressure within the loading system. This overcomes the vertical height difference and friction, enabling suction and flow of water and fish through the pipelines and into the wells. In operations where water and fish are separated during loading, the reduced pressure will persist in both the dewatering unit and inside the well until the loading operation is completed (Fig. 1A), due to the continuous connection between these two compartments. Unloading of fish, which may take place several hours after loading, is usually conducted by generating an elevated pressure within the well (>1 atmosheres absolute (ata)) to overcome

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the vertical height difference and flow resistance within the piping system (Fig. 1B).

These pressure fluctuations may lead to gas supersaturation and formation of bubbles. Supersaturation is directly linked to the total gas pressure (TGP) in water. When the TGP exceeds the atmospheric pressure (P_{atm}), the water is supersaturated, a condition where gas bubbles may form (Colt, 1983, 2012). Total gas pressure (TGP) is commonly expressed as a percentage of P_{atm} (modified from Colt (1983)):

$$\mathrm{TGP}\ (\%) = \left[\frac{P_{atm} + \Delta P}{P_{atm}}\right] 100 \quad (1)$$

where the differential pressure (ΔP) can be expressed as:

$$\Delta P = \mathrm{TGP} - P_{atm} \quad (2)$$

Therefore, to accurately determine the TGP (%) under well boat operational conditions, the pressure should be defined as the internal pressure within the gas pocket of the well (P_{well}), rather than the ambient P_{atm} (Figures 1; S 1).

A decompression event (i.e. drop in pressure) can potentially lead to immediate gas supersaturation (Colt, 1986), both in the water and within the body fluids of the fish, as predicted by Henry's law. Gas bubbles formed in the blood (gas embolism) or other tissues (emphysema) of a fish may lead to a clinical condition known as gas bubble disease (GBD) (Weitkamp and Katz, 1980). The probability of gas bubble formation depends mainly on the degree of supersaturation and the duration of exposure (Pleizier et al., 2020a). Gas-filled cavities such as the swim bladder are immediately affected by pressure changes according to Boyle's law (e.g. Brown et al. (2009, 2012)).

In addition to decompression, various mechanisms can lead to gas supersaturation and GBD in aquatic organisms (Colt, 1986, 2012). The physical principles governing the formation of gas bubbles remain consistent. However, the clinical progression of GBD may differ in fish exposed to combined internal/external supersaturation caused by a rapid drop in P_{atm}, compared with fish exposed solely to externally supersaturated water resulting from increased TGP under stable P_{atm} (Beyer et al., 1978). GBD has been extensively studied in salmonids, particularly related to rivers and hydro-power installations, but studies focusing specifically on Atlantic salmon are limited (Pulg et al., 2018). Moreover, our understanding of bubble formation and GBD under decompression scenarios relevant to well boat operations is limited.

Clinical signs of GBD in fish may include behavioural disturbances and loss of equilibrium (Beyer et al., 1976; Velázquez-Wallraf et al., 2022, 2023; Chen et al., 2025), altered vertical distribution in the water column (Dawley et al., 1976), and formation of bubbles in the skin, superficial tissues and internal organs (Colt, 2000; Beyer et al., 1976; Colt, 1986; Weitkamp and Katz, 1980; Brown et al., 2012; Velázquez-Wallraf et al., 2022, 2023). Acute mortality is often attributed to gas emboli occluding major blood vessels in the heart and gills (Beyer et al., 1976). In surviving fish, long-term effects may include necrosis of tissue and secondary bacterial infections (Colt, 1986). Several diagnostic methods for detecting acute gas bubble disease in Atlantic salmon have recently been described by Stensby-Skjærvik et al. (2025).

The primary objective of the study was to investigate whether pressure profiles encountered during well boat loading and unloading operations can induce GBD in Atlantic salmon. Further, the study aimed at assessing reversibility of observed clinical signs and quantifying the clinical impacts of GBD under these conditions. The experimental studies were based on input from industry stakeholders and were designed to simulate realistic, but outer operational boundaries observed during well boat operations in commercial salmon aquaculture; loading at 0.4 ata and unloading at 2.6 ata. As oxygen often is added to the water during crowding of fish and within wells, the 0.4 ata trials were performed using pre-exposure water conditions with Dissolved Oxygen (DO) at both 100 % and 150 % saturation.

2. Materials and methods

2.1. Ethics statement

The study was conducted in accordance with guidelines of the Norwegian Regulation on Animal Experimentation and was approved by the Norwegian Food Safety Authority (ID 29969).

2.2. Experimental design

The study included a total of 10 pressure exposures, referred to as Runs, A–J (Table 1 and Table S 1) performed over a two-week period. The day of pressure exposure of each individual run was designated as day 0 (d0).

Six runs (n = 6) simulated well boat loading under hypobaric pressure of 0.4 ata. These were conducted using two different pre-exposure DO saturation levels: 100 % (n = 3) and 150 % (n = 3). Two runs (n = 2) simulated well boat unloading at a hyperbaric pressure of 2.6 ata with 100 % DO pre-exposure levels. Additionally, two runs at normobaric pressure (1 ata) with 100 % DO of air saturation level served as negative controls (n = 2). The DO levels reported for each run refer exclusively to the conditions prior to the pressure change. This approach reflects realistic operational scenarios in which fish are pumped from water that is sometimes oxygenated beforehand to maintain adequate DO levels. Target DO levels at 100 and 150 % were therefore established before pressure exposure and represent only the pre-exposure saturation—that is, the oxygen level prior to any pressure alteration. In actual well boat operations, DO is not regulated to match changes in pressure within the gas pocket; accordingly, no oxygen was added during pressure exposure in our experiments. This ensured that the test conditions during runs realistically reflected operational practice.

In line with commonly used terminology in aquaculture, 100 % DO refers to water saturated with oxygen in equilibrium with air at 1 ata, where oxygen constitutes 20.95 % of the dry gas mixture. Additional oxygen in the 150 % trials was supplied by infusing pressurized oxygen (Nippon gases, Bergen, Norway) through a microbubble diffusor (Wedge-Lock, Aquamerik, Quebec, Canada). All runs were conducted with seawater (salinity from 33.8 to 34.3 g L^{-1} and starting temperature from 10.2 to 11.35 °C, see Table 4). The actual oxygen partial pressure and concentration in water varied slightly between runs due to differences in atmospheric pressure, temperature and salinity (Table 4).

Although the SI unit for pressure, Pascal (Pa), is standard in most contexts, gas-supersaturation studies typically reports values in mmHg (e.g. Colt (2012)), and recent literature has adopted this convention for ΔP (Pleizier et al., 2021a). In line with this practice, and because the handheld sensor used to measure TGP reports in mmHg, most pressures in the present studies are reported in mmHg. However, the pressure in the pressure chamber's air pocket is given in ata, to make deviations from standard atmospheric pressure (1 ata) easier to interpret. The conditions used in the experimental runs were 0.4 ata (40.5 kPa), 2.6 ata (263.4 kPa) and 1 ata (101.3 kPa), corresponding to approximately 304, 1976, and 760 mmHg, respectively.

The duration of pressure exposure at 0.4 ata was set to a maximum of 150 min to mimic the duration of a loading operation with dewater (Fig. 1A). For the 2.6 ata exposures, the maximum duration was set to 90 min, reflecting the typically shorter duration of unloading operations (Fig. 1B). Of the two control exposures at 1 ata, one lasted 90 min, the other 150 min.

Initially, a humane endpoint (abortion criteria) was established for individual fish that exhibited signs of distress, including lethargy combined with darkened colour, bleeding from the skin, eye or gills, severe exophtalmia, signs of central nervous dysfunction, or significant oedema/wounds. A group level mortality of 25 % was set as a criterion to terminate the exposure. Based on the outcomes of the two first runs, Run I and A, the maximum exposure duration was revised and addition to abortion criteria were introduced, see Table 1 and Section 3.2 Mortality and humane end points.

Table 1
Summary of experimental runs (Run IDs) with planned exposure conditions, including pressure (ata), pre-exposure dissolved oxygen (DO) levels (% of air saturation), and planned duration (minutes). Following Runs I and A, the maximum exposure duration was revised. Actual exposure time was limited by the predefined humane end point criteria (see text for further description). NA: not applicable.

Well boat simulation	Loading						Unloading		Control	
Pressure (ata)	0.4			0.4			2.6		1.0	
Pre-exposure DO (% of air saturation)	100%			150%			100%		100%	
Run ID	A	B	C	D	E	F	G	H	I	J
Planned duration (minutes)	150	150	150	150	150	150	90	90	150	90
Revised max. duration (minutes)	NA	113	113	60	113	113	90	90	NA	90
Actual duration (minutes)	113	79	79	60	65	72	90	90	150	90

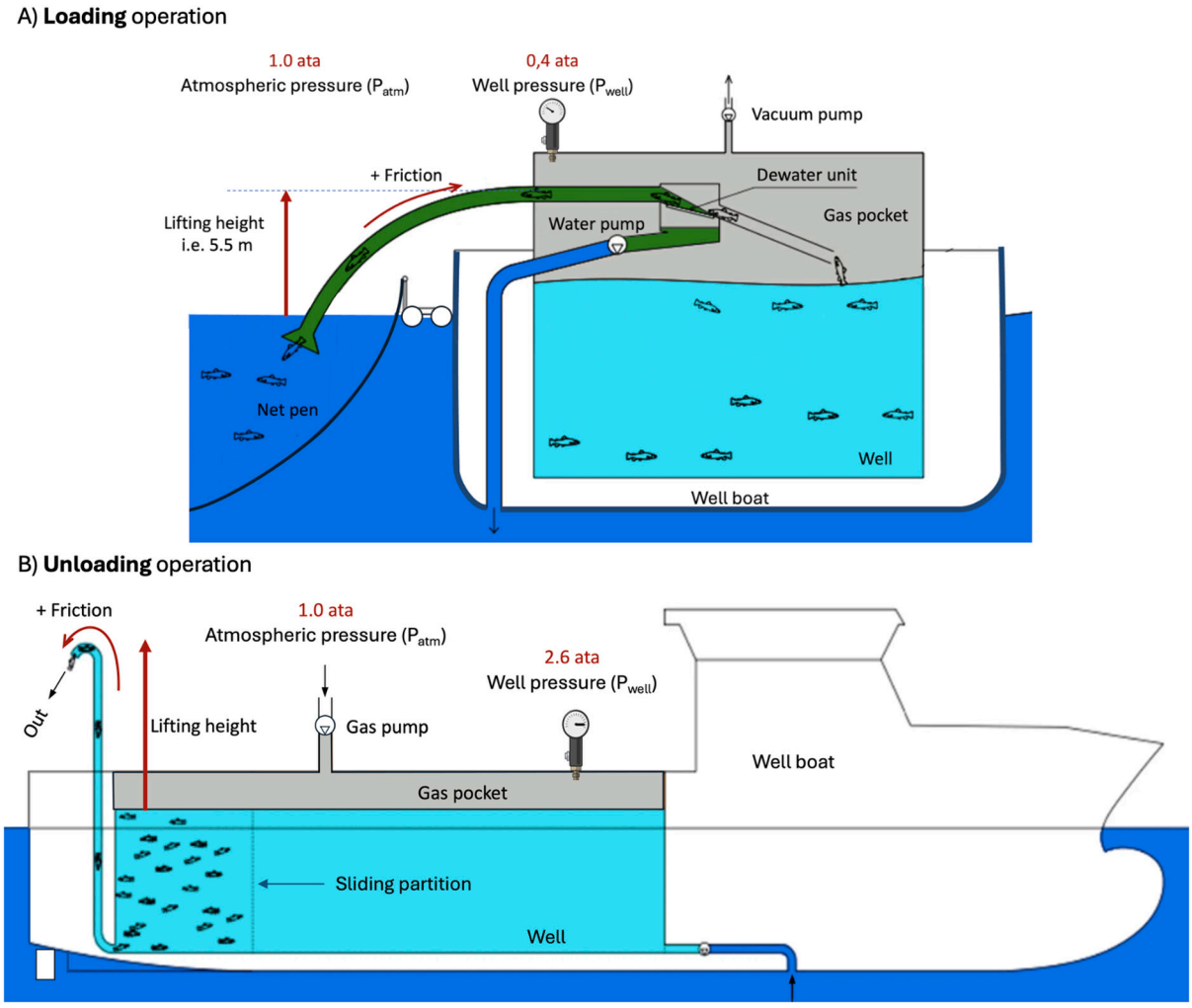

Fig. 1. Schematic representation of well boat operations simulated in the trial. (A) Loading operation: Transfer of fish from a net pen into the well using a dewatering unit that separates the arriving seawater from the well water. A vacuum pump is employed to establish low pressure (in trials 0.4 ata), i.e. hypobaric conditions within the well, creating a pressure difference (and a decompression) that facilitates fish and water transfer. The sub-atmospheric pressure is maintained throughout the loading process until the last fish has entered the well. (B) Unloading operation: Fish are discharged from the well under hyperbaric conditions. An air pump is used to pressurize the well, generating a high pressure (in trials 2.6 ata), which is required to push the fish out of the well and into the receiving system. The elevated pressure enables passive movement of water and fish through the discharge pipeline without mechanical pumping.
Source: Both figures modified from https://bronnbatveilederen.no.

2.3. Pressure chamber and water quality

All 10 runs were carried out with test facilities at NUI (Laksevåg, Bergen, Norway) in a cylindrical pressure chamber made of steel with a volume of 8.9 m^3 (Hyperbaric Test System "Obelix", Optime Subsea AS, Notodden, Norway) (Fig. 2). A cylindrical net cage (designed by Sea-farming Systems, Stavanger, Norway, and manufactured by Egersund Net, Akva Group, Rørvik, Norway) was used to confine the fish to the upper part of the water column (distance from the water surface to the top of the net was approximately 25 cm, and to the bottom approximately 105 cm), well above the compensation depth for the

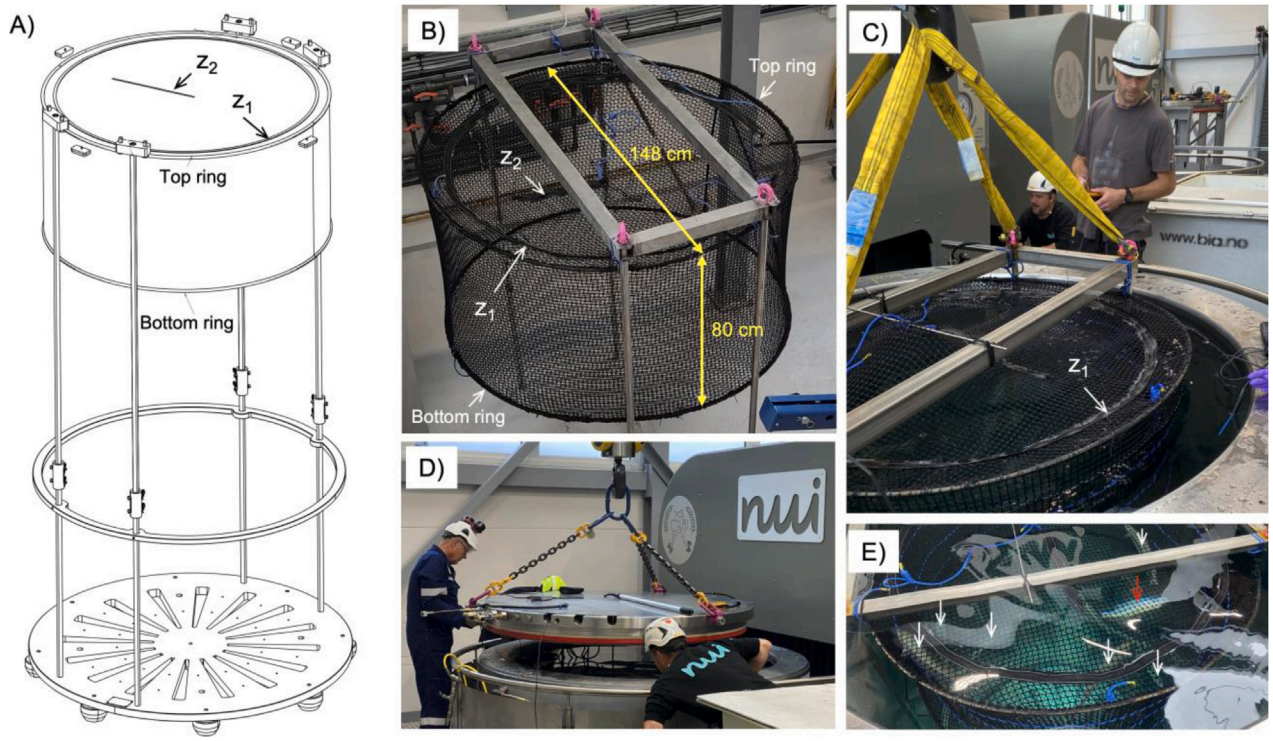

Fig. 2. Experimental set up in the pressure chamber «Obelix» (A) Schematic of the metal rig with the cylindrical test basket (net cage) mounted between the top and bottom rings. (B) The cylindrical net cage had a diameter of 148 cm, height: 80 cm, and mesh size: 30 mm. (C) Lowering the rig with the net cage into the pressure chamber Obelix (internal diameter: 180 cm, height: 350 cm). Fish and equipment were introduced into the cage at this stage via zippers z1 and z2, respectively. (D) The chamber was closed with a metal lid before the start of each pressure exposure. (E) After exposure, the lid was removed. Fish are visible inside the fully submerged net cage (indicated by white arrows); a fish showing loss of equilibrium is marked with a red arrow.

hypobaric pressure runs (Fig. 2, Table 4). The top of the net could be lifted above the surface to allow safe transfer of the fish through a zippered opening in the mesh net (see video recording in Supplementary material). During the runs, the net remained fully submerged so the fish could not access the water surface.

Hyperbaric pressure (2.6 ata) was generated by pumping air into the pressure chamber. To achieve hypobaric pressure (0.4 ata), the same system was used in combination with valves that induced a Venturi effect to evacuate air from the tiny air pocket above the water surface in the chamber. In-chamber pressure measurement was conducted continuously using a High-Precision Digital Pressure Switch - ISE20 (MC Automation AS, Lysaker, Norway) in combination with a Squirrel SQ2040-2F16 Data Logger (Grant 2040 Series, Tempcon Instrumentation, West Sussex, UK). Light was provided continuously during all runs using Hyperbaric 1000 bar led light (COB led 3000K, Cree CXB, NC, USA) and the fish were monitored by a ROV camera (6000msw rated analog ROV camera - 125° field of view) placed in the upper part of the net, and a camera at the side of the middle part of the net (Underwater Camera Fish Finder Alu. 10" Monitor 360° 50M, Blue Marlin, Drøbak, Norway). Camera recordings were stored for later re-evaluation.

Water oxygen, salinity and temperature in the chamber were measured with a WTH Multi 3620 IDS (WTW, Weilheim, Germany). TGP was monitored with an InWater TGP Tracker (InWater Technologies, Campbell River BC, Canada). The TGP measurements and the pressure measurements in the air pocket above the surface in the test chamber were used to calculate ΔP during modified pressure conditions. At normobaric pressure, the InWater TGP tracker was also used to measure the P_{atm}. Calculations of partial pressure ratios (N_2+Ar):O_2, were based on Colt (2012) for water vapour pressure, Weiss (1970) and Colt (1983) for oxygen pressure, Weiss (1974) and Colt (1983) for CO_2 pressure and Millero and Poisson (1981) for density of water. Compensation depth was calculated according to Colt (2012) with the water density component calculated according to Millero and Poisson (1981). Water samples for chemical analysis (pH, alkalinity, CO_2, total organic carbon, Al, Cu, and Zn) were collected from the test chamber just before and after each exposure and analysed by Eurofins Environment Testing Norway (Bergen).

2.4. Experimental fish

Atlantic salmon (*Salmo salar* L.) of the Stofnfiskur strain (n = 122; 670 ± 71 g; 37 ± 1.5 cm fork length) were provided by Stiftelsen Industrilaboratoriet (ILAB; Bergen Norway). On d0 the participating fish were transported the approximately 6 km distance between ILAB and NUI in 650 L tanks, with continuous monitoring of oxygen and temperature (MultiLine 3620 IDS, WTW, Weilheim, Germany). Oxygen was added to maintain approximately 100 % DO saturation in the water at all times. During the transport and holding before and after pressure exposure, the fish were lightly sedated (Aqui-S vet 2 mg L^{-1}; MSD Animal Health Norge AS, Bergen, Norway) to reduce stress. In each run, either 12 or 13 fish were exposed, with a mean (± SD) stocking density of 5.8 ± 0.16 kg/m^3 within the net cage.

2.5. Overview of monitoring and sampling

The behaviour of all fish were monitored during exposure. In addition there were extensive non-lethal and lethal assessment on the animals. If the fish were moribund or had died from exposure, sampling procedures that required euthanasia were also carried out on d0. The remaining fish were examined and sampled either on d1 or d7 after each exposure (see Table 2 and Table S 1). Monitoring and sampling for each method was performed by the same personnel throughout the study.

Table 2
Non-lethal and lethal assessment and sampling on day of exposure (d0), 1 (d1) and 7 (d7) days after exposure.

		d0	d1	d7
Non-lethal	Behaviour	n = 122		
	Ultrasound imaging	n = 120	n = 102	n = 54
	Operational welfare indicators	n = 120	n = 102	n = 54
	Macroscopic evaluation of fins	n = 96	n = 102	n = 54
Lethal	Macroscopic evaluation of gills	n = 17[a]	n = 49	n = 54
	Histopathology	n = 18[a]	n = 36	n = 42

[a] Sampling only from dead or moribund fish.

Table 3
The maximum gas bubble load (mGBL) scoring model used to assess and quantify gas bubble load in the heart and proximal part of the ventral aorta. *Source:* Table reproduced from Stensby-Skjærvik et al. (2025).

Grade	Observations
0	No bubbles
1	1 bubble in 8 consecutive cycles
2	2 bubbles in 8 consecutive cycles
3	3–7 bubbles in 8 consecutive cycles
4	≥8 bubbles in 8 consecutive cycles
5	Near whiteout, individual bubbles still discerned
6	Whiteout: individual bubbles cannot be discerned

2.6. Fish behaviour

The vertical distribution of the fish was assessed at the beginning and at the end of the experiment, as well as every 10 min during the experiment. Fish distribution was categorized into three volume regions: upper (1/4), middle (2/4) and lower (1/4) of the total volume, using built-in boundary lines on the mesh wall that visually divided the mesh net accordingly.

Behaviour was continuously evaluated by at least two researchers and the time and characteristics of any deviant behaviour were evaluated according to Table 1 of OECD Guideline no. 203, *Fish, Acute Toxicity testing* (OECD, 2019), which defines key clinical signs as loss of equilibrium, abnormal swimming behaviour, abnormal ventilatory function, abnormal skin pigmentation, and other visible abnormalities.

2.7. Ultrasound imaging

Immediately after pressure exposure, the fish were transferred from the pressure chamber to a holding tank and sequentially anaesthetized with 100 mg L^{-1} Finquel vet. (MSD Animal Health Norge AS, Norway) in seawater. The time between exposure and examination was kept as short as practically possible. During the ultrasound examination (Vivid iq™, 12L-RS Wideband Linear Array Probe (3.4–12.6 MHz), GE Healthcare, Chicago, IL), the fish was placed in dorsal recumbency in a specially tailored retainer, and the gills were gently flushed with seawater containing 50 mg L^{-1} Finquel vet. Each fish was examined for a minimum of three consecutive periods of no less than 10 s each, with all examinations recorded and stored for later re-evaluation. Assessment was carried out using the maximum gas bubble load (mGBL) scoring model established by Stensby-Skjærvik et al. (2025), (Table 3).

2.8. Operational welfare indicators (OWI)

After the ultrasound examination, fish was transferred to a holding tank containing 100 mg L^{-1} Finquel vet. to achieve deeper sedation. Weight and fork length were then recorded, followed by the evaluation of selected operational welfare indicators (OWI) according to the Fishwell scoring system (Noble et al., 2018). The following parameters were assessed, with scoring ranges indicated in parentheses: opercular damage (0–3), dorsal fin damage (0–3, active (a)/healed (h)), pectoral fin damage (0–3, a/h), caudal fin damage (0–3, a/h), snout damage (0–3), scale loss (0–3), skin haemorrhages (0–3), skin lesions/wounds (0–3), eye haemorrhages (0–3), exophthalmia (0–3) and vertebral deformity (0–3).

2.9. Macroscopic observation of gas bubbles

For all runs except Runs B and I (see Table 1 and S 1), the left pectoral fin and the anal fin were photographed. The presence or absence of gas bubbles was evaluated by visual inspection and their quantity was scored for each fin using the macroscopic fin gas bubble score described by Stensby-Skjærvik et al. (2025): no bubbles observed (score 0), 1–3 bubbles (score 1), 4–10 bubbles (score 2), and more than 10 bubbles (score 3).

In dead and moribund fish (d0), as well as planned euthanized fish (d1, d7), the second left gill arch was excised and photographed (Nikon D3100 digital camera and Micro-Nikkor 60 mmf/2.8 D lens). The quantity of gas bubbles was scored based on the proportion of the affected filaments using a macroscopic gill gas bubble scoring system: no bubbles observed (score 0), <25 % (score 1), 25–50 % (score 2), 50–75 % (score 3) and >75 % (score 4).

2.10. Histology

Following euthanasia with an overdose of 150 mg L^{-1} Finquel vet., the fish was necropsied and samples of the third left gill arch, skin and skeletal muscle (encompassing the sideline at the level of the dorsal fin), heart, pseudobranch, eye and brain (fixated encased by the cranium) were transferred to 10 % neutral buffered formalin (see Table S 1). The same procedure was performed if the fish was dead or moribund after exposure. The occurrence of caverns in the gills resembling possible gas bubble formations was evaluated by the semi-quantitative histological gill gas bubble score as described in Stensby-Skjærvik et al. (2025). For fish exhibiting obvious clinical manifestation of disease that led to euthanasia immediately after pressure exposure (d0), additional organs were sampled in addition to the aforementioned tissues, including kidney, liver, pyloric caecae with pancreatic tissue, mid- and hind gut. If gas bubbles were detected in the fin at the time of sampling, the affected fin(s) were included in the sampling. The samples were stored at room temperature for 24 h, then at 4 °C until further processing according to standard procedures at Fürst medical laboratory, Oslo, Norway. They were then sectioned at 2–3 μm thickness and stained with haematoxylin–eosin saffron (HES) (Suvarna et al., 2018). The resulting slides were scanned on a Pannoramic 100 Flash DX scanner (3DHISTECH, Budapest, Hungary), and histological evaluations were performed digitally on SlideViewer (3DHISTECH).

2.11. Statistical analysis

All statistical analyses were performed with R (version 4.4.3, R Core Team, 2025). Data from the ultrasound study, OWI, and macroscopic observations of gas bubbles in skin, were assessed for normality using Shapiro–Wilk's test and Q–Q plots. As all datasets deviated from normality, non-parametric tests were applied throughout.

Group-level differences were first evaluated using the Kruskal–Wallis test. When significant, pairwise comparisons were performed using Dunn's post hoc test with Bonferroni correction. For specific comparisons between two directly comparable subgroups (matched for exposure time and end points), the Mann–Whitney U test was applied. The significance levels were set to $p < 0.05$.

3. Results

3.1. Pressure and water quality

Measured and calculated key pressure and water quality results are summarized in Table 4. The starting temperature across runs varied within a range of 1.1 °C, and gradually increased by 0.1 to 0.8 °C during exposures. Salinity remained stable at approximately 34 g L^{-1} throughout all runs. Local atmospheric pressure fluctuated slightly over the two-week trial period. Consequently, some of the parameters such as DO concentrations and ΔP values varied slightly across runs as a result of these minor environmental differences. The oxygenation prior to the 0.4 ata 150 % O_2 runs had no or only a negligible impact on total gas pressure (TGP) level.

The partial pressure ratio (N_2+Ar):O_2 was substantially lower (2.27, 2.26 and 2.25) in the 0.4 ata 150 % O_2 runs than the other runs (range from 3.82 to 4.11).

The calculated TGP (%) immediately after reaching the target exposure pressure was similar across all 0.4 ata runs, ranging from 248 % to 258 %. In the 2.6 ata runs TGP values were 38 % and 39 %. Correspondingly, the calculated ΔP at the same time-points ranging from 451 to 481 mmHg for the 0.4 ata runs, and −1215 and −1217 mmHg for the 2.6 ata runs. Compensation depth (Z_{uncomp}) for the 0.4 ata runs was between 5.98 and 6.37 m, immediately after achieved exposure pressure.

TGP/ΔP and oxygen measurements in the pressure chamber decreased during the 0.4 ata runs, but not in a steady nor consistent manner. Therefore, the data acquired during the pressure exposures were deemed unreliable for evaluation, and only measurements conducted prior to and after the exposure were considered in the evaluation.

TGP (%) measurements immediately after exposures were done once the pressure chamber lid had been removed, and the probe was shaken to dislodge any attached bubbles. Some variation in TGP (%) (83 to 95) and ΔP (−37 to −131 mmHg) was recorded following the 0.4 ata runs, which was not attributable to the pre-exposure oxygen levels. After the 2.6 ata runs, the TGP (%) was 101 and 100, and ΔP 9 and −2 mmHg. Data collected after the control runs showed a slight supersaturation of the water (TGP (%) 102 and 101, ΔP 13 and 6 mmHg).

The time frame over which pressure changes were conducted during the runs is consistent with those used in well boat procedures. The decompression to 0.4 ata (4 to 10 s) was faster than compression to 2.6 ata (86 and 95 s). Pressure normalization to 1 ata was completed in 2 to 4 s.

The remaining water quality parameters (pH, alkalinity, CO_2, total organic carbon, Al, Cu and Zn) were within acceptable limits for good fish welfare (Table S 2).

3.2. Mortality and humane end points

In the experiment, mortality was observed on the day of exposure (d0) for all 0.4 ata runs lasting more than 60 min (Table 5), whereas no mortality was observed in any of the other runs, either on d0 or at later time points.

As shown in Table 5, mortality in the 0.4 ata runs increased with longer exposure durations. Following complete mortality in Run A, a conservative limit of exposure to 60 min was set for Run D. Based on the outcomes from these two runs, the maximum exposure duration for the subsequent runs was adjusted to 113 min (Table 1). Furthermore, the humane end point criteria were refined to encompass loss of equilibrium, upward swimming bursts followed by loss of equilibrium, and/or sign of twitching in more than 25 % of the fish group. As a result of this adjustment, all runs with the exception of Run A and Run I had similar exposure durations and humane end points.

3.3. Behaviour

Due to the camera setup and the fact that the downward facing camera became partially occluded with bubbles during the 0.4 ata exposures (see example in Supplementary material: Video 3), concurrent observation of all fish was not always feasible. Some individuals also occasionally moved out of the camera's field of view. The duration of a specific aberrant behaviour of an individual was for the same reasons not always possible to determine. Therefore, the first observation of each category of behaviour was noted consecutively, and the total number of individuals observed at each scheduled observation time was recorded. Abnormal ventilation and abnormal skin pigmentation were difficult to assess due to video quality and resolution, and was therefore not included in the analysis.

The position of the fish in the water column varied substantially between different types of exposures. On average, the proportion of fish observed either swimming in the lower quarter of the water column or resting on the net in normal horizontal orientation, was highest during the 0.4 exposures, lowest during the 2.6 ata exposures and intermediate in the control runs (Table 6, Fig. 3). The observations at the start and the end of each run were not included in the positioning results as the fish may have been disturbed by the logistics of handling the closing and opening of the lid, nor were observations after reaching humane end point criteria.

Within the first minute of all 0.4 ata exposures, all fish adopted a negative pitch characterized by downward-tilted swimming position. This behavioural adaptation was followed by the release of gas from the swim bladder and subsequent normalization of swimming pitch (Fig. 3, see example in Supplementary material, Video 2).

Except for Run D, fish in all other 0.4 ata runs exhibited various behavioural deviations (Fig. 3). In these runs, loss of equilibrium, manifested as abnormal horizontal or vertical orientation, or impaired buoyancy control, was first observed after 56 to 75 min into the exposure. Behaviour compatible with convulsions was first recorded alone or in combination with loss of buoyancy control after 57 to 77 min (see example in Supplementary material Video 5). Hyperactivity, either alone or in combination with hypoactivity and loss of buoyancy control, was first observed after 60 to 76 min. In the two 0.4 ata runs of longest duration (Runs A and C), a combination of loss of buoyancy control, hypoactivity, and abnormal horizontal or vertical orientation was observed after 82 and 73 min, respectively. There was no clear relationship observed between the onset of behavioural deviations and the pre-exposure dissolved oxygen saturation levels (100 % vs. 150 %).

The humane end point encompassing distinct behavioural deviations was met in five of the 0.4 ata runs (i.e except Run D). The recorded mortality was lower than 25 % in four of those runs, and no major changes in fish behaviour were observed during the following 7 days.

In both 2.6 ata exposures, upward-tilted swimming was consistently observed during the entire duration of the exposure (see example in Supplementary material Video 4). Deviant behaviour was only observed in one of the exposures. One individual presented with abnormal horizontal orientation was observed after 21 min and one individual with loss of buoyancy control after 28 min (Fig. 3), both as single cases of short duration. Whether these observations were on the same individual or two different fish were not possible to distinguish.

No behavioural changes or deviations were observed during the two control runs (I and J) (Fig. 3).

3.4. Ultrasound evaluation of gas bubble load

Results from the d0 ultrasound evaluation are presented in Figs. 4 and 5. Gas embolism was observed only in fish exposed to 0.4 ata (both 100 % and 150 % DO) immediately following exposure (d0), except for one fish exposed to 2.6 ata that tested positive on d1 (mGBL score 1). Among fish determined positive for gas embolism, the lowest

Table 4
Key pressure and water quality parameters before, during, and after exposure in all runs. NA: not applicable.

	Exposure pressure and pre-exposure DO level	0.4 ata 100% O_2			0.4 ata 150% O_2			2.6 ata 100% O_2		1 ata 100% O_2	
	Run	Run A	Run B	Run C	Run D	Run E	Run F	Run G	Run H	Run I	Run J
Prior to exposure	Temperature (°C)	11.1	10.3	11.3	10.5	10.4	10.8	10.2	10.2	11.3	10.5
	Salinity (g L^{-1})	34.1	34.0	33.8	34.1	34.0	33.8	34.1	34.0	34.3	33.8
	P_{atm} (ata)	0.997	1.005	1.000	1.003	1.011	1.000	0.997	1.012	0.999	1.004
	TGP (%)	100	99	99	100	100	103	100	99	100	101
	ΔP (mmHg)	−2	−5	−5	−1	−1	25	3	−10	2	4
	O_2 (%)	94	96	95	147	146	151	98	94	98[a]	93
	Calculated partial pressure ratio (N_2+Ar):O_2	4.01	3.96	3.96	2.27	2.26	2.25	3.82	4.01	3.84	4.11
During exposure	Time from 1 ata to achieved exposure pressure (seconds)	7	8	7	4	9	10	86	95	NA	NA
	Calculated TGP (%) after achieved exposure pressure	249	250	248	250	252	258	39	38	NA	NA
	Calculated ΔP after achieved exposure pressure (mmHg)	452	455	451	457	463	481	−1215	−1217	NA	NA
	Calculated Z_{uncomp} after achieved exposure pressure (metres)	5.99	6.03	5.98	6.06	6.14	6.37	NA	NA	NA	NA
	Mean pressure (ata)	0.400	0.392	0.400	0.393	0.397	0.420	2.577	2.584	0.997	0.990
	s.d. of pressure	0.024	0.015	0.012	0.011	0.015	0.012	0.100	0.108	0.001	0.001
After exposure	Time from end of exposure to 1 ata (seconds)	3	3	2	2	3	2	4	3	NA	NA
	TGP (%)	95	90	86	92	88	83	101	100	102	101
	ΔP (mmHg)	−37	−78	−109	−64	−90	−131	9	−2	13	6
	O_2 (%)	86[b]	93	93	131	133	146	98	91	96[b]	91

[a] Oxygen was not measured prior to sealing the pressure chamber and the run started, therefore the value recorded 3 min after the start of the run was used.
[b] Oxygen was not measured after end of exposure at a time comparable to the other runs, therefore the value recorded approximately when exposure ended was used.

Table 5
Mortality and exposure time of the runs at hypobaric pressure at d0:

0.4 ata, pre-exposure DO 100%			0.4 ata, pre-exposure DO 150%		
Run	Exposure time (minutes)	Dead or moribund fish/exposed fish (mortality %)	Run	Exposure time (minutes)	Dead or moribund fish/exposed fish (mortality %)
Run A	113	12/12 (100%)	Run D	60	0/12 (0%)
Run B	79	1/12 (8.3%)	Run E	65	1/13 (7.7%)
Run C	79	3/12 (25%)	Run F	72	1/12 (8.3%)

Table 6
Percent of observed fish that were either swimming in the lower quarter of the water column or resting on the net with a normal horizontal orientation (s.d.: standard deviation); runs grouped according to exposure.

Exposure pressure and pre-exposure DO level	Observed population in the lower quarter and in normal orientation (%)	s.d.	Number of observations	Fish per observation	Total fish observations
Run A, B and C (0.4 ata 100% O_2)	79.9%	0.143	21	7 to 12	194
Run D, E and F (0.4 ata 150% O_2)	67.3%	0.135	18	8 to 12	172
Run G and H (2.6 ata 100% O_2)	19.6%	0.165	16	8 to 12	160
Run I and J (1 ata 100% O_2)	52.1%	0.275	23	8 to 12	244

mGBL-score among dead or moribund individuals was 2, while the highest score for the surviving fish was 3. All fish with an mGBL score of 4 or higher (n = 16) either died during the exposure or were classified as moribund and euthanized immediately afterward. Among the 0.4 ata exposed fish on d0, 48.6 % were found to have gas emboli in one or more heart chambers. When comparing different levels of oxygen saturation in decompression runs with similar exposure times and humane end points, 41.7 % of fish from Run B and C (100 % O_2, n = 24) and 45.8 % from Run E and F (150 % O_2, n = 24) were evaluated as positive for gas embolism on ultrasound. The mean mGBL-score between these two groups was comparable with no statistical difference (Mann–Whitney U test, p-value = 0.936), with mean mGBL-score of 1.13 (SD 1.92) for Run B and C, and 0.88 (SD 1.23) for Run E and F. An incidental finding was hyperechogenic structures possibly representing gas bubbles in the liver (Fig. 5).

3.5. Operational welfare indicator (OWI) scores

Overall, the most prevalent types of lesions observed were active and healed fin damage, exophthalmia, scale loss, and skin hemorrhages. Among these, only active and healed fin damage, and exophthalmia were significantly different between the groups across time points (Kruskal–Wallis, $p < 0.05$). Active fin damages was more prevalent in the control group at all sampling points. A low level of exophthalmia was observed in most fish at all sampling points, but the only significant difference between the groups was observed at d1 with a higher score for the 0.4 ata 100 % O_2 and 2.6 ata exposures (Dunn's post hoc, $p < 0.001$). Healed fin damage was evaluated as unrelated to the experiment. No systematic pattern of altered OWI score was observed with type of treatment, and the deviation in terms of positive OWI scores is assumed to be mainly caused by handling during transportation.

3.6. Macroscopic evaluation of gas bubbles in fins and gills

Macroscopically visible gas bubbles in fins (Fig. 8A) were only observed for fish exposed to 0.4 ata 100 % and 0.4 ata 150 % O_2 at d0 (Fig. 6). On d1 and d7 there were no clearly discernible bubbles in the fins. Due to this, only data from d0 were included in the analysis.

Gas bubbles were observed in both dead and moribund fish, as well as among remaining fish. Bubbles in either or both the pectoral and anal fins was a consistent finding in all dead and moribund fish. Notably, the presence of bubbles in fins in Run A, Run G and Run I at d0 (see Table S 1) was not evaluated for technical reasons. Of the 60 evaluated fish exposed to 0.4 ata (Run B to F), bubbles in the fins were observed in 57 individuals (95 %).

When evaluating groups with comparable exposure time and humane end point (see Table S 1), the prevalence of macroscopically visible gas bubbles in the fins was 87.5 % for the 0.4 ata 100 % O_2 (Run

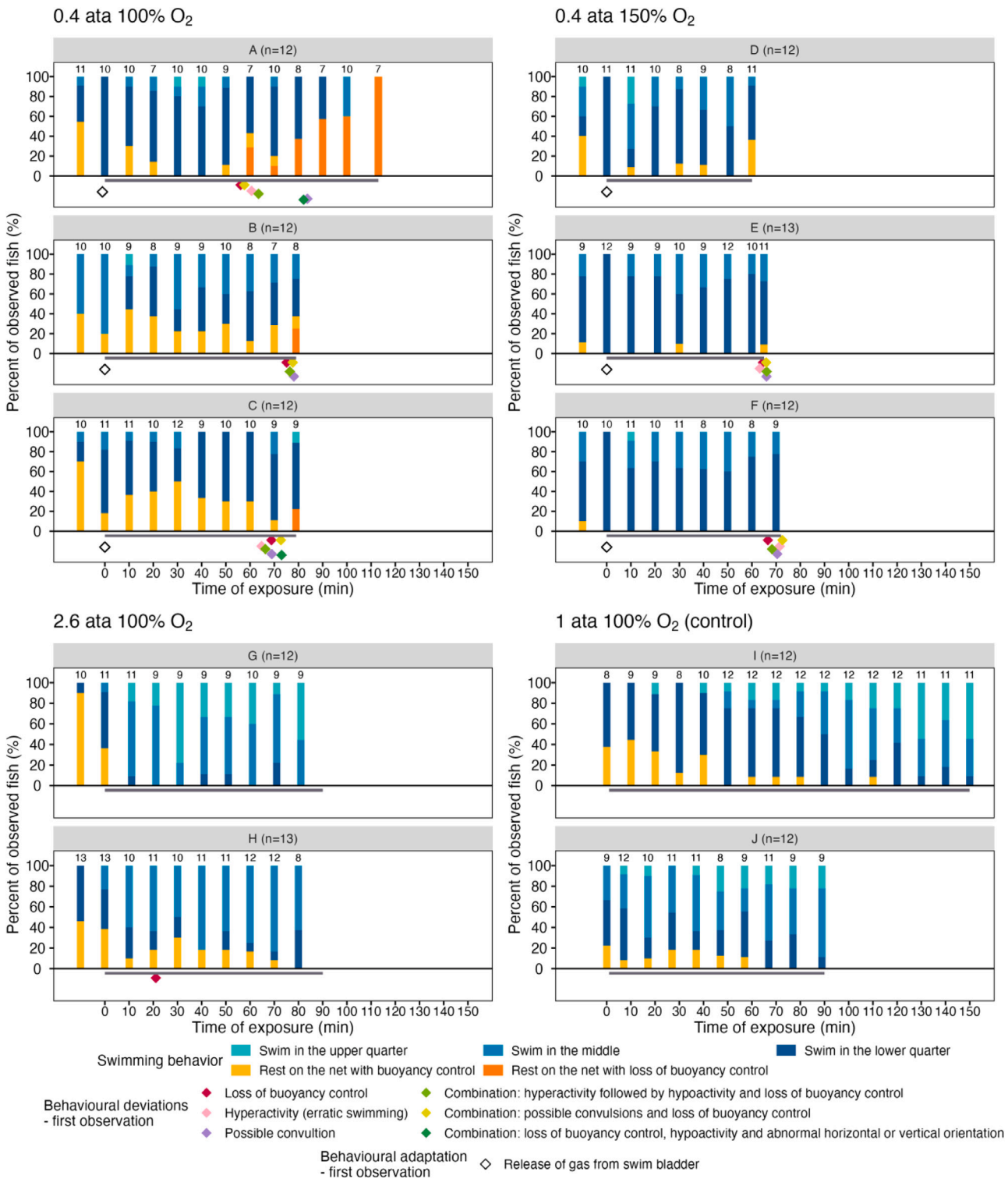

Fig. 3. Behavioural observations immediately before, and during the 0.4 ata 100% O_2, 0.4 ata 150% O_2, 2.6 ata 100% O_2, 1 ata 100 % O_2 (control) runs (pressure in ata, O_2 indicating pre-exposure DO % levels, see Table 1). Run duration indicated by horizontal bars, time 0 indicates start of run. Percent of observed fish positioned in the upper quarter, middle half or lower quarter of the available volume, or resting on the net in equilibrium or with loss of equilibrium, are indicated on vertical bars. Observed number of fish observed at each timepoint is displayed above each vertical bar. The first observation of a behavioural response (release of gas from swim bladder) is indicated by an unfilled diamond, and the first observation of a behavioural deviation is indicated by coloured diamonds.

B and Run C, n = 24), and 100 % for the 0.4 ata 150 % O_2 (Run E and Run F, n = 24). When comparing the fin gas bubble score between the two 0.4 ata exposure groups, a significantly higher score was observed for the 0.4 ata 150 % O_2-group both for the examined pectoral fin (Mann–Whitney U test, p = 0.018) and the anal fin (Mann–Whitney U test, p = 0.005).

Macroscopically visible gas bubbles in the gills (Fig. 8D) were only observed in dead and moribund fish exposed to 0.4 ata 100 % (n =

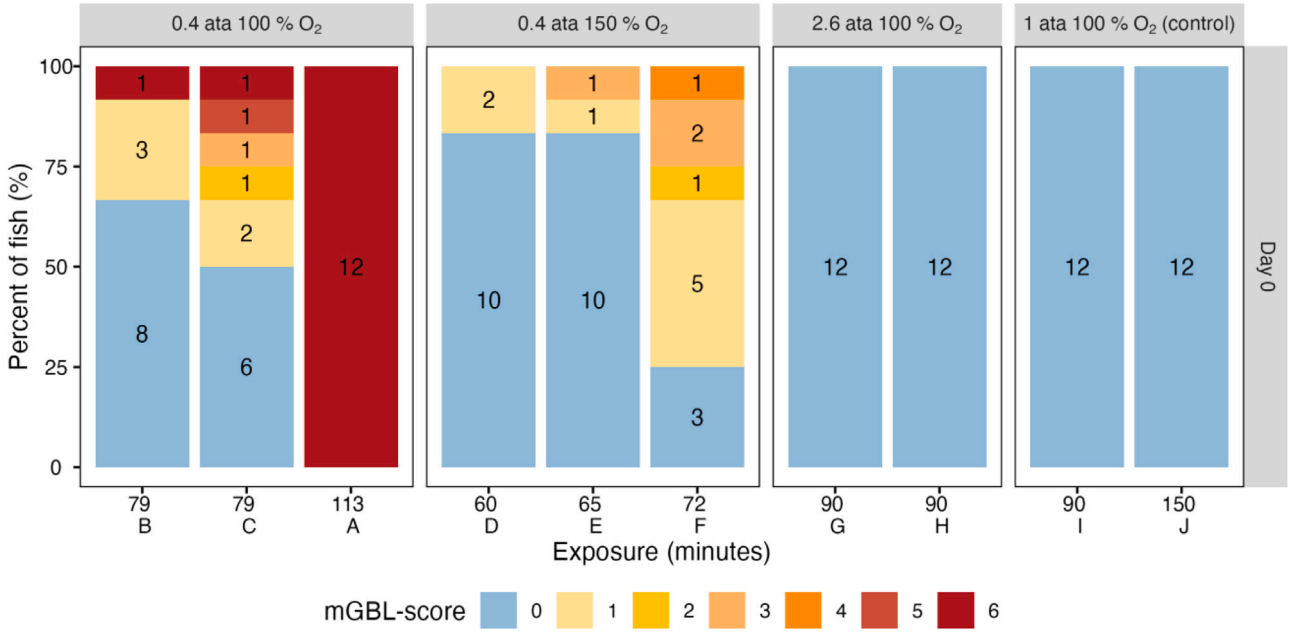

Fig. 4. Percent of fish with different gas bubble loads (mGBL-score) determined by ultrasound on d0 for the different experimental scenarios. Panels show exposure pressure and pre-exposure DO level. Duration of the runs is stated on the *x*-axis, and order of runs arranged by duration. The numbers in each column indicate the number of fish with a given score.

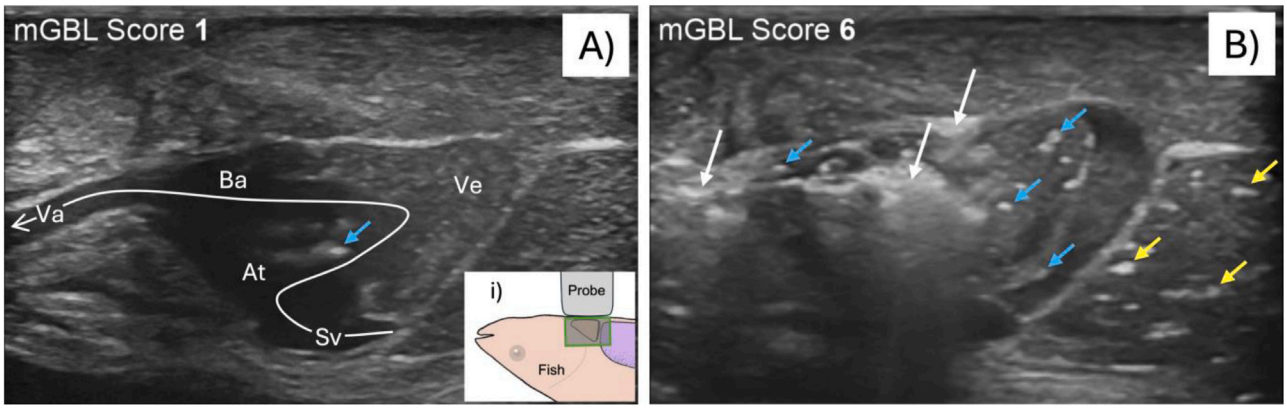

Fig. 5. Representative ultrasound images of the heart region in fish exhibiting different levels of gas bubble disease (GBD), with a mean gas bubble load (mGBL) score of 1 (A) and 6 (B). (A) Fish with mild GBD (mGBL-score 1) and normal cardiac function with 1 gas bubble in 8 consecutive cycles. Key anatomical regions labelled are: Sv: sinus venosus, At: atrium, Ve: ventricle, Ba: bulbus arteriosus, Va: ventral aorta. The white line traces the typical path of a gas bubble through the heart, and the blue arrow shows a single gas bubble. Inset (i) displays the ultrasound probe placement on the fish. The green area corresponds to the visible area in the ultrasound image. (B) Fish with a severe mean gas bubble load (mGBL-score 6). Blue arrows indicate examples of individual gas bubbles. White arrows indicate "whiteout" areas, where severe gas emboli accumulation has occurred and individual bubbles have merged or could not be identified as distinct structures. Possible gas bubbles in the liver are indicated with yellow arrows.
Source: Modified from Stensby-Skjærvik et al. (2025).

11) and 0.4 ata 150 % O_2 (n = 1) at d0 (Fig. 7), and only in fish with gas bubbles in heart detected by ultrasound. For dead and moribund fish, the prevalence of macroscopically visible bubbles in the gills was 66.7 %. When evaluating groups with comparable exposure time and humane end point (see Table S 1), the prevalence of gas bubbles in the gills was 12.5 % for the 0.4 ata 100 % O_2 (Run B and C), and 8.3 % for the 0.4 ata 150 % O_2 (Run E and F). As an incidental finding, a distinctly brilliant and warm red colour of the gill tissue was noted among the dead and moribund fish (Figure S 2), a finding that has also been observed in a decompression study by Beyer et al. (1976).

3.7. Histopathology associated with gas bubble disease

No histological lesions could fully explain the observed clinical findings of dead and moribund fish. Notably, histological evaluation was only performed after lethal sampling of the fish, implying that only dead and moribund fish were sampled on d0, while planned sampling was performed on d1 and d7. In the fins, the lesions on d0 and d1 consisted of either empty caverns interpreted as space-occupying gas bubbles (Fig. 8A and B), or fibrinous subepithelial inflammatory processes. At d7, moderate influx of polymorph-nuclear leucocytes (presumable neutrophilic granulocytes) was seen in fibrinopurulent inflammatory processes as well as spongiosis of the epidermis (Fig. 8C) of fish with macroscopically visible lesions at d0 and d1.

The most conspicuous finding was the occurrence of caverns in the vascular structures of the gills, structures suspected to represent space-occupying lesions from gas bubbles (embolism). The occurrence of caverns in the gills was graded with the semi-quantitative histological gill gas bubble score, but due to the low number of observations, no

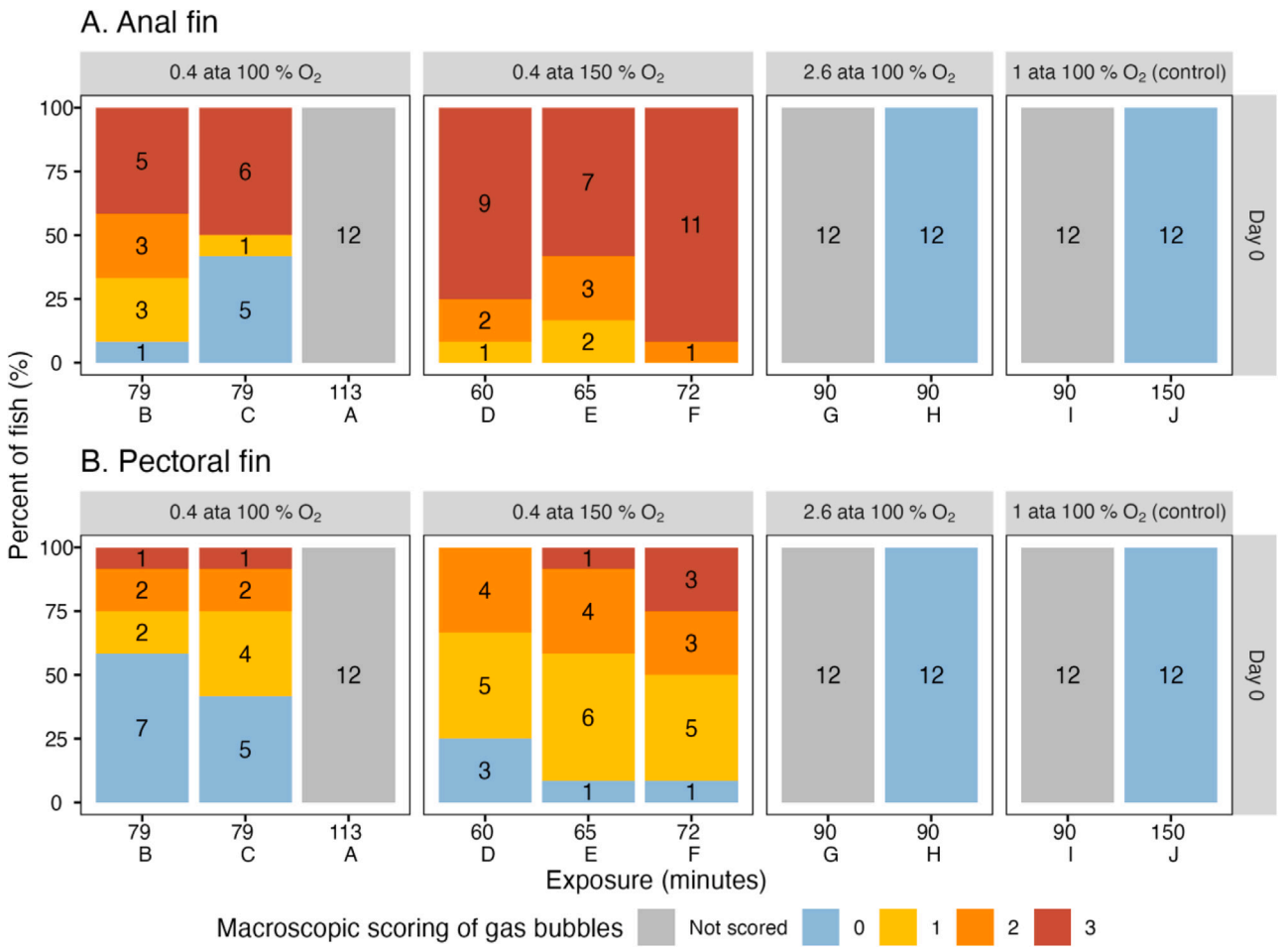

Fig. 6. Percent of fish with visible gas bubbles based on macroscopic evaluation in (A) anal fin and (B) pectoral fin on d0 (n = 12), for the different experimental scenarios. Panels show exposure pressure and pre-exposure DO level. Duration of the runs is stated on the *x*-axis, and order of runs arranged by duration. The numbers given in each bar indicate the number of fish with a given score.

statistical evaluations were performed between the different groups. As can be seen from Fig. 9, caverns were not an exclusive finding in decompressed fish. However, although caverns appeared in the filament arteries of fish from all groups, the decompressed fish had a seemingly higher score than other groups. Furthermore, in dead and moribund fish, bubbles were found in the whole length of the gill filaments, while in the 2.6 ata 100 % O_2 and the control group, bubbles were only seen in the distal-most part of the filaments. This is in line with macroscopic observations of the gills of dead or moribund fish at d0, where gas bubbles were observed along the whole length of the afferent and efferent filament arteries (Fig. 8D). Further, an exclusive finding in histological gill sections from dead and moribund fish was the apparent transitions of caverns from the afferent filament artery into the afferent lamellar arterioles and the lamellar capillaries (Fig. 8E and F).

None of the examined tissues—skin, skeletal muscle, heart, pseudobranchs, brain or eyes revealed lesions that could be ascribed to GBD.

4. Discussion

This study demonstrates that exposure to sub-atmospheric (hypobaric) pressure (0.4 ata), such as may occur during certain well boat loading operations, can induce decompression-related GBD in Atlantic salmon, particularly when the duration exceeds one hour. The duration of gas supersaturation was more critical than the inert gas to oxygen ratio in determining clinical effects. Ultrasound and behavioural observations served as useful indicators of GBD, while histological findings need to be evaluated with care.

In the vast literature on GBD in fish, few studies have focused on decompression-induced conditions. Some studies have examined the effects of returning fish equilibrated to hyperbaric conditions back to atmospheric pressure (D'Aoust and Smith, 1974; Beyer et al., 1976; D'Aoust et al., 1980). Decompression from atmospheric to hypobaric conditions as a cause of GBD was first described for fish by Marsh and Gorham (1904), who summarized findings from Gorham (1899), in which scup (*Stenotomus chrysops*) died after 45 min of exposure to approximately 0.33 ata. To the authors' knowledge, no published studies to date have examined the effects of decompression of Atlantic salmon from normobaric to hypobaric conditions. Such a scenario is particularly relevant to well boat operations in aquaculture. The present study demonstrates that decompression-protocols representative of those used in the well boat industry can induce acute GBD in Atlantic salmon.

Decompression from 1 ata to 0.4 ata induced behavioural deviations, clinical signs, and mortality in five out of six runs. No deviations in behaviour were observed during the first 55 min of these runs. While no mortality occurred during the 60-minute run, mortality was observed in the 65-minute run, and 113 min of exposure at 0.4 ata resulted in 100 % mortality. These findings clearly highlight

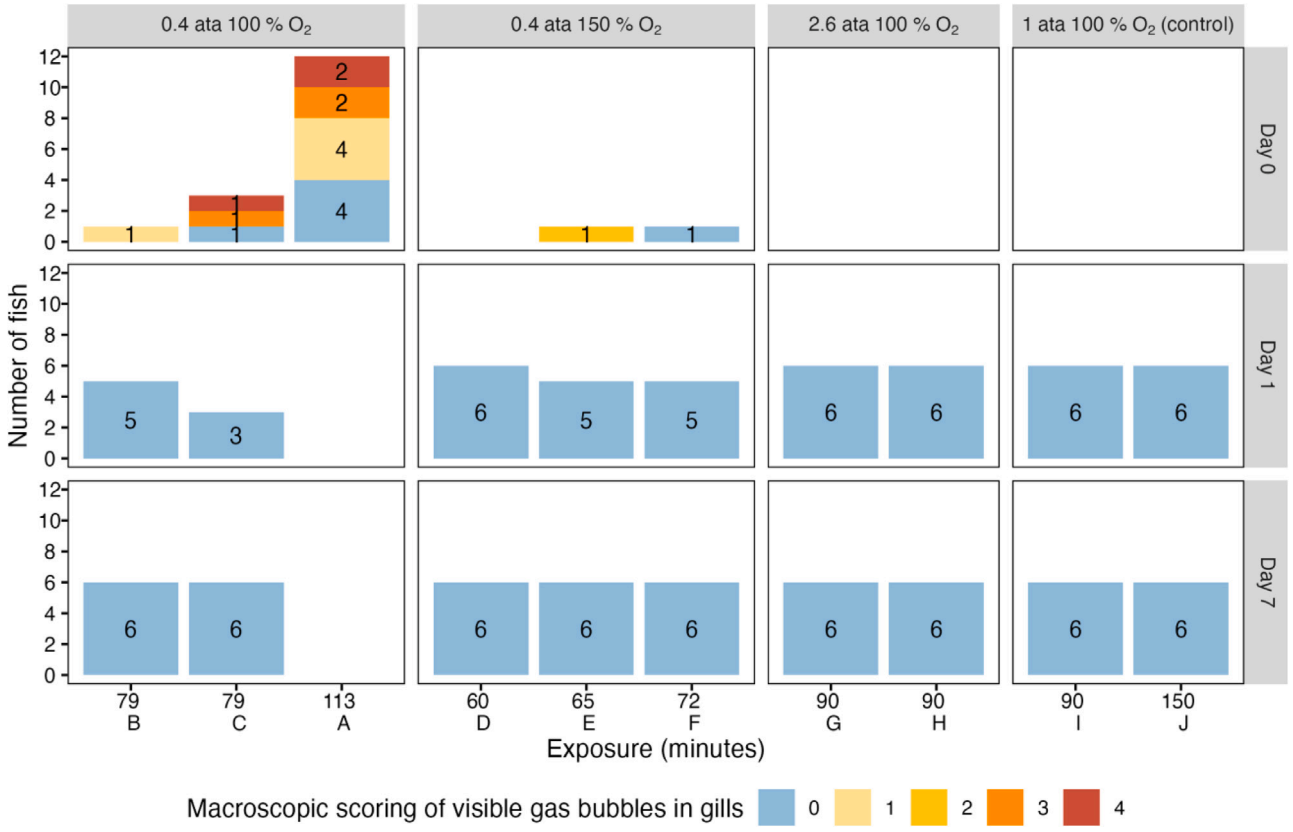

Fig. 7. Number of fish possessing bubbles based on macroscopic evaluation of gills, shown instead of percentages due to the low sample size (n = 1–6). Panels show exposure pressure and pre-exposure DO level for the different experimental scenarios. Duration of the runs is stated on the *x*-axis, and order of runs arranged by duration. The numbers given in each column indicate the number of fish with a given score.

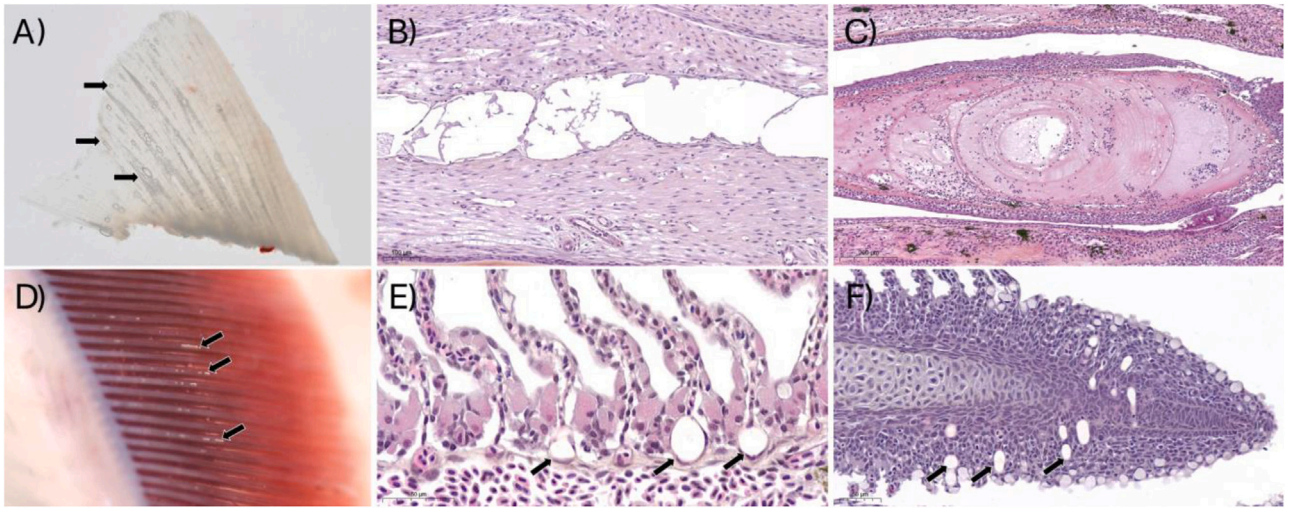

Fig. 8. Macroscopic (A and D) and histopathological (B, C, E and F) findings in fins (A–C) and gills (D–F) associated with gas bubble disease (GBD). Image A, B and D-F are from moribund fish. Arrows indicates examples of circular to elliptical caverns that are suspected to represent space-occupying gas bubbles (gas embolism). (A) Gas bubbles in the anal fin (0.4 ata 100 % O_2 at d0). (B) Extensive cavern formations in distended vascular structures of the anal fin: note the valve-like structures in the vessels (0.4 ata 100 % O_2 at d0). (C) Extensive fibrinopurulent inflammation in the anal fin and initial spongiosis of the epidermis (0.4 ata 150 % O_2 at d7). (D) Gas bubbles in the efferent filament arteries of the gill (0.4 ata 150 % O_2 at d0). (E) Example of caverns in afferent lamellar arterioles (0.4 ata 100 % O_2 at d0); this was a consistent finding in dead and moribund fish. (F) Caverns in the distal part of the filament seemingly protruding into the lamellar capillaries (0.4 ata 150 % O_2 at d0). All DO notations refer to pre-exposure DO levels (see Table 1).

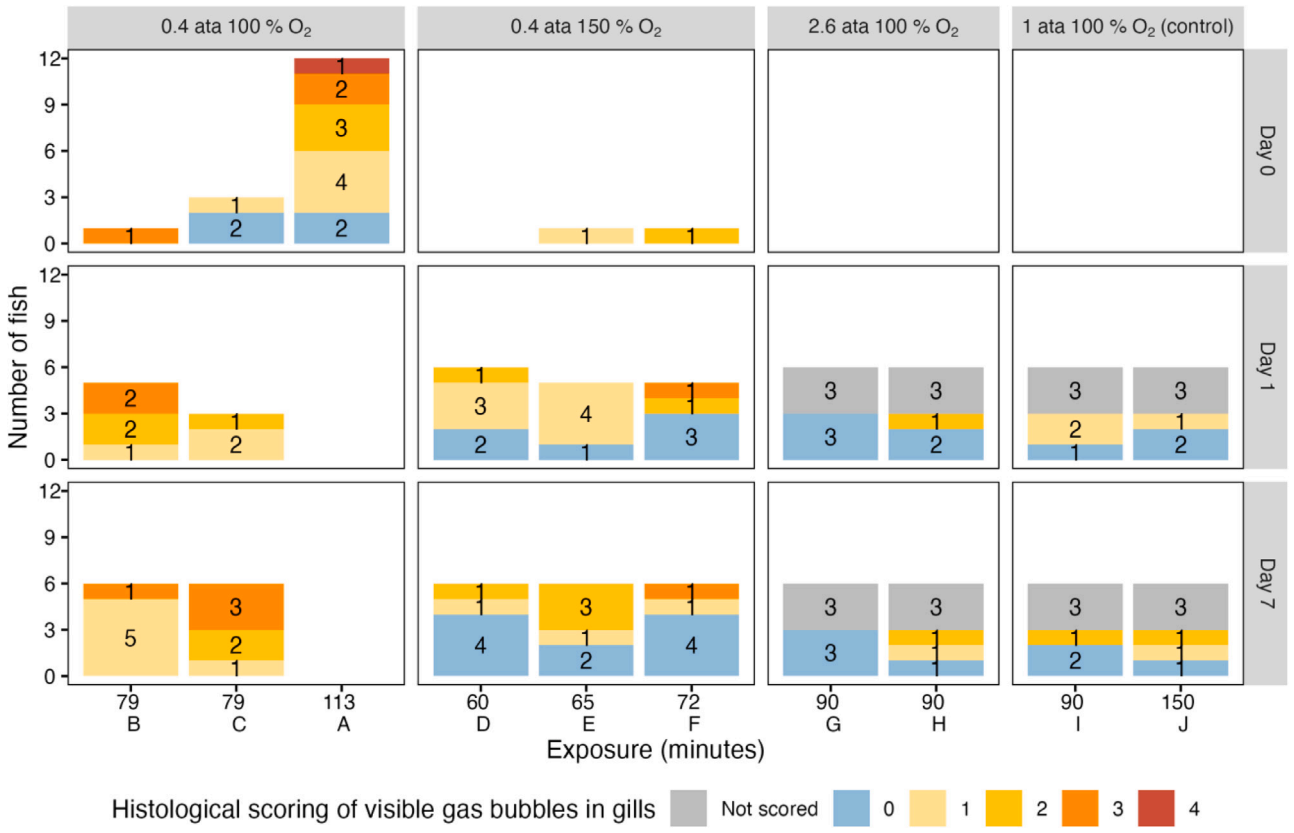

Fig. 9. Numbers of fish with different occurrence of caverns evaluated as the semi-quantitative histological gill gas bubble score at d0, d1 and d7, shown instead of percentages due to the low sample size (n = 1–6). Panels show exposure pressure and pre-exposure DO level for the different experimental scenarios. Duration of the runs is stated on the *x*-axis, and order of runs arranged by duration. The numbers given in each column indicate the number of fish with a given score.

the biological importance of exposure time in the development of decompression-induced GBD.

The kinetics of gas bubble formation across different tissues is sparsely documented in the literature, and the time constants for loading and unloading gas into solution likely vary with tissue, gas species, and pressure conditions. Nevertheless, it is notable that the present results, obtained under combined internal/external supersaturation, show a similar timeframe to the observations of Beyer et al. (1976) under internal supersaturation alone, where equilibration of critical tissues was suggested to occur within 60–90 min. The observed behavioural deviations may have resulted from gas bubble accumulation in specific anatomical sites. Lodging of gas bubbles in the heart, gills, and fins, as observed in this study, might have caused discomfort. Other authors have observed the presence of bubbles in the central nervous system (Beyer et al., 1976), which may play an important role in the behaviour changes observed in affected fish.

Dead or moribund fish had among the highest ultrasound scores, a high prevalence of caverns in gill lamellar afferent arterioles, and were the only ones that had macroscopic visible gas bubbles in gills, collectively suggest possible mechanisms contributing to mortality. Distribution of gas bubbles in more proximal parts of the afferent filament artery, as well as transition of gas bubbles into the afferent lamellar arterioles, emerged as important histological diagnostic criteria for GBD. The presence of gas bubbles confined to the distal-most afferent filament artery without transition to the lamellar arterioles, also seen in the control groups and 2.6 ata groups, indicates an artefact. This might have resulted secondarily from suction of air through severed blood vessels as a result of residual heart activity, although alternative explanations cannot be excluded. Gas displacement of blood from the afferent artery within the gill filament was also reported by Machado et al. (1987) in moribund rainbow trout (*Salmo gairdneri*) suffering from GBD. The lack of pressure-related exophthalmia in the present study could be related to the fact that this condition typically occurs in chronic, but not acute forms of GBD (Weitkamp and Katz, 1980).

When comparing the effects of decompression at different oxygen levels under comparable humane end points criteria, a higher occurrence of macroscopically visible gas bubbles in the fins was observed in the 0.4 ata, 150 % exposure group. In contrast, ultrasound-based mGBL were comparable between the two groups. However, the number of fish in each group was limited, and it cannot be excluded that the effects represent natural variation. In general, for a given level of gas supersaturation, a lower partial pressure ratio of $(N_2+Ar):O_2$ is considered more favourable compared to a higher ratio (Colt, 1986; Pleizier et al., 2020a), as oxygen is actively consumed in metabolic processes and replaced by CO_2 with a much higher solubility and therefore less impact on TGP. The 0.4 ata exposures at 150 % oxygen saturation had a lower partial pressure ratio $(N_2+Ar):O_2$ compared to the 0.4 ata at 100 % oxygen. On the contrary, the elevated level of oxygen would, at least in theory, result in a higher TGP (%). However, this effect was likely partially off-set by high oxygen levels driving dissolved nitrogen gas from the water into the air, as described by Nebeker et al. (1979). This is supported by the observations that ΔP showed no consistent differences across oxygen levels in the 0.4 ata runs. In fact, the results appeared relatively comparable between the two exposures. A common misconception in the aquaculture industry is that only nitrogen contributes to development of GBD. This myth should be dispelled, as several studies have demonstrated that total gas supersaturation with high oxygen-tension can also cause severe GBD (Nebeker et al., 1979; Espmark et al., 2010). The importance of total gas supersaturation, rather than nitrogen gas supersaturation alone, as a critical factor in GBD development was already emphasized by Weitkamp and Katz (1980). The present findings further support

this, demonstrating a dominant effect of total gas supersaturation and only a minor contribution from the individual gases.

Systematic observation of fish behaviour can provide valuable insight into fish welfare. Although the available water volume in the pressure chamber was limited, the present study indicates that Atlantic salmon tend to swim deeper in decompression-induced supersaturated water. Interestingly, the fish exposed to 2.6 ata were swimming higher in the available net cage volume compared to those exposed to 0.4 ata or the control fish, despite the apparent continuous challenge of maintaining buoyancy and avoiding sinking, as indicated by their upward-tilted swimming behaviour. The rapid changes in pressure from 1 ata to either 0.4 ata or 2.6 ata result in equally rapid changes in the volume of gas-filled cavities within the fish's body, such as the swim bladder, in accordance with Boyle's law. The expansion of the swim bladder volume and associated increase in buoyancy explains the involuntary upward movement of the fish during pressure reduction, and also the observed release of gas from the swim bladder shortly after the pressure reduction (decompression). Correspondingly, the volume reduction of the swim bladder explains the fish's involuntary downward movement in the water column during pressure increase.

A study on juvenile rainbow trout (*Oncorhynchus mykiss*) support that fish lack a sensory mechanism to detect acute gas supersaturation (Pleizier et al., 2021b). This finding is also supported by a study on several salmonid species, which found no evidence of active avoidance of supersaturated water (Weitkamp et al., 2003). In contrast, Stevens et al. (1980) reported that some salmonid species avoided supersaturated water, and Dawley et al. (1976) found that chinook salmon and steelhead tended to position themselves at a greater depth with increasing supersaturation. The findings in the present study align with the latter. One possible explanation for the observed behaviour is the gradual relief of distress/discomfort as the fish approach the compensation depth. However, the net-pen design in the current study restricted the fish to go deeper than 1 m, whereas the calculated compensation depth in the 0.4 ata exposured were approximately 6 m.

Similar deviant behaviour as observed in this experiment, such as erratic or spastic movements, disorientation, and loss of equilibrium, have been reported in other studies involving decompression-induced supersaturation and subsequent GBD both in salmonids (D'Aoust and Smith, 1974; Beyer et al., 1976; Pleizier et al., 2020b) and in non-salmonid fish (Velázquez-Wallraf et al., 2022). In juvenile rainbow trout (*Salmo gairdneri*), exposed to gas supersaturation, loss of equilibrium, abnormal buoyancy, and apparent aimless swimming were observed before the onset of the convulsive stage. Convolutions were characterized by side- and belly-up swimming, violent whirling movements, and intermittent periods of inactivity followed by spasmodic convulsions (Machado et al., 1987). In the present study, the convulsive stage was followed by mortality, frequently accompanied by an open mouth and flared gills and operculum. These signs might indicate asphyxia caused by gas emboli obstructing vital blood vessels. Another interesting finding from Machado et al. (1987) was the presence of emphysematous, space-occupying lesions in the semicircular canals and surrounding the medulla oblongata in a subset of the moribund fish. The acute formation of such space-occupying lesions in the central nervous system or the vestibular system may explain some of the observed abnormal behaviour prior to mortality from GBD in the present experiment. In goldfish (*Carassius auratus*) with GBD, affected blood vessels have been observed in the brain (Velázquez-Wallraf et al., 2022), and interestingly, deviant behaviour was also observed. In this experiment, no deviations were noted in the free-dissected brains although the vestibular system and meninx primitiva surrounding the medulla oblongata were not subjected to detailed investigations. Hence, the presence of emphysematous, space-occupying lesions in these sites cannot be excluded. Understanding the mechanisms of GBD-associated deviant behaviour would be a valuable supplement to clinical fish health assessments.

As noted by Fidler (1988), the signs of GBD can be surprisingly varied. Although bubble formation is governed by the same physical principles, there are probably differences in the clinical presentation of the disease, depending on whether the gas supersaturation is internal, external, or combined internal/external. The 0.4 ata runs in the present study induced a combined internal/external supersaturation, as both the body fluids of the fish and the surrounding water became supersaturated simultaneously and to a similar extent as a result of decompression. Beyer et al. (1976, 1978) have taken a systematic approach to the pathological outcome according to the direction of the net excess gas flux. Their studies conducted on Pacific salmonids provide valuable insight, however, no comparable studies have been conducted on Atlantic salmon. Another factor that can influence the signs of GBD is whether the condition is present in its acute or chronic form (Weitkamp and Katz, 1980). Therefore, diagnostic efforts must consider the variability in clinical signs, and it cannot be ruled out that some signs of GBD might have been overlooked by the present study.

Thorough measurement of relevant water quality parameters is essential for safe and acceptable handling of fish in aquaculture, including well boat operations. When evaluating the level of gas saturation in water, it is recommended to report both ΔP and TGP (%) or the corresponding atmospheric pressure (Colt, 1983, 2012; Pleizier et al., 2021a), along with water temperature, salinity (Colt, 1983, 2012) and dissolved oxygen (Colt, 1983). In the present experiments, the measured TGP levels were neither stable nor consistent during the 0.4 ata runs, as TGP initially increased, followed by a gradual reduction during the exposure. Such a decline can be explained by the formation of gas bubbles in the water that subsequently escaped as gas bubbles released at the water surface during the exposure. Additionally, the TGP measurements were also probably affected by bubble accumulation on the silastic tubing of the TGP-probe, a known factor that can interfere with the readings (Colt, 1983; Pleizier et al., 2021a). The silastic tubing of the TGP sensor may also have been affected by pressure changes. The lack of consistency in TGP readings may have been influenced by fish-induced movements of the sensor, causing bubbles temporarily to dislodge from the tubing, or by other unknown factors that the modified pressure exerted on the probe. Similarly, the oxygen readings also varied with pressure during the 0.4 ata runs. These variations may have resulted from a combination of bubble accumulation on the sensor, pressure-induced technical errors, and actual changes in oxygen concentrations.

As previously discussed, the current experimental design and net cage restricted the fish to a maximum depth of approximately 1 m below water surface. Access to deeper water is known to have a potential preventive and/or curative effect on fish affected by GBD, due to the compensatory effect of increased hydrostatic pressure on bubble formation and stability (Dawley et al., 1976). The uncompensated TGP (TGP_{uncomp}) accounts for hydrostatic pressure by incorporating depth into the calculation, providing a more accurate assessment of gas supersaturation at different water column levels (Colt, 1983). The compensation depth is defined as the depth at which hydrostatic pressure offsets the pressure differential (ΔP) and below this point, the ambient pressure is sufficient to prevent gas bubble formation (Colt, 2000). In our trials, a decompression from 1 ata with TGP (%) = 100 to 0.4 ata, resulted in a calculated TGP of approximately 250 %, a compensation depth of about 6 m, and the TGP_{uncomp} of about 200 % at 1 m below the surface. For operational purposes, it is important to note that the commonly used approximation of a 9.7 % reduction in supersaturation per 1 m depth, is only applicable in freshwater under standard atmospheric pressure (Pleizier et al., 2021a), and does not hold true under modified pressure conditions as those in the 0.4 ata exposures with seawater in this study. Therefore, a correct evaluation of compensation depth, or TGP_{uncomp} at a given depth in a well boat operating under modified pressure must be calculated specifically for each scenario, and not based on standard approximations.

In this experiment, either hypobaric (0.4 ata), normobaric (control, 1 ata) or hyperbaric (2.6 ata) conditions were tested separately for a period of one hour or longer. These pressure levels were selected to reflect conditions during specific stages during well boat operations. However, well boat transportation involves multiple procedures in which fish are repeatedly subjected to a sequence of pressure changes, starting with hypobaric exposure during loading, followed by hyperbaric exposure during unloading, before returning to normobaric conditions upon transfer (Espmark et al., 2016). Repeated exposure to gas supersaturation may have an additive effect on the fish and increase the probability of gas bubble formation. Such cumulative effect has been reported in Rock carp (*Procypris rabaudi*) (Wang et al., 2015). Although most signs observed during or immediately after the 0.4 ata runs in the present study were no longer evident after 1 or 7 days, the possibility of additive effects in Atlantic salmon cannot be ruled out. It is possible that subclinical signs or underlying mechanisms, initiated but below observable thresholds in the present study could have had a negative impact on the ability to tolerate a subsequent exposure to gas supersaturation. Fidler (1988) describes that nucleation sites originating from a previous GBD condition may lower the threshold for bubble formation on consecutive exposure to gas supersaturation. There may also be a counteracting effect, adaptation, as described for humans and mammals (Risberg, 2021). Although few deviating observations were made during hyperbaric runs, it is important to consider that, during well boat operations, oxygen is often infused into the pressurized water to prevent hypoxia. Previous studies have clearly demonstrated potential harmful effects of oxygen or air infusion under pressure, which can lead to gas supersaturation and GBD when the system, with fish, is returned to normobaric conditions (Beyer et al., 1976).

Possible complications or long-term effects of decompression-induced GBD in fish are to a large extent unknown. Currently, the best available estimates are derived from experiments investigating GBD resulting from gas supersaturation at atmospheric pressure. A study on juvenile chinook salmon (*Oncorhynchas tshawytscha*) showed that swimming performance was impaired after exposure to supersaturated water (Schiewe, 1974). Antcliffe et al. (2003) reported that both rainbow trout and coho salmon were rendered stuporous, i.e with reduced responsiveness, after exposure to supersaturated water with a TGP of 125 %. This is also an area of interest for Atlantic salmon as swimming capacity might be crucial for fish during and after a well boat operation. Severe GBD might affect the metabolic capacity in fish (Yuan et al., 2025) possibly by impairing blood circulation in the gills causing a reduced aerobic scope, and for several non-salmonid species impaired swimming performance is observed in gas supersaturated water (Pleizier and Brauner, 2024). In addition, complications such as skin lesions and secondary bacterial skin infections have been reported in association with GBD (reviewed by Speare (2010)). Although such complications were not prevalent within the 7 days post exposure in the present study, a prolonged observation may be necessary to develop visible wounds. Interestingly, several long-term studies on GBD resulting from supersaturated waters at atmospheric pressure have reported no significant differences in growth and condition factor between exposed and control fish (Dawley et al., 1976). Moreover, it is documented that fish can recover from GBD (Dawley et al., 1976), including cases of decompression-induced GBD (Gorham, 1899), as also observed in the present study. Recovery from decompression-induced GBD has been extensively described for humans and mammals (Mitchell, 2024).

Many facets of decompression-induced GBD in fish are still unexplored or unclear, underscoring that further research in this area is needed, particularly given its relevance for the aquaculture industry. The data in this study suggest that certain measures can be implemented to reduce the risk of clinically significant GBD during well boat operations. Most importantly, minimizing the deviation from standard atmospheric pressure required for loading over a dewater system, and to reduce the duration the fish are exposed to sub-atmospheric pressure, is likely to be beneficial. However, additional trials are necessary before more precise operational guidelines can be established.

5. Conclusion

The present study demonstrates that pressure exposure relevant to well boat operations in the aquaculture industry can induce decompression-related GBD in Atlantic salmon. The duration of exposure to a high level of total gas supersaturation emerged as a more critical factor than the partial pressure ratio of $(N_2+Ar):O_2$. The findings suggest that exposure to a pressure of 0.4 ata puts Atlantic salmon at risk of developing clinically relevant GBD if the duration exceeds approximately one hour.

Ultrasound detection of gas bubbles enabled semi-quantitative assessment of the clinical impact of GBD. In addition, behavioural deviations and positioning served as useful supporting indicators. However, histological examinations, one of the main tools in salmonid aquaculture diagnostics, needs to be interpreted with care. Most signs of GBD observed during or immediately after decompression were no longer detectable one day after exposure or one week later, although some histopathological lesions persisted.

To safeguard fish welfare and minimize GBD risks, efforts should be directed towards reducing the duration and intensity of decompression protocols experienced by farmed Atlantic salmon during well boat handling.

CRediT authorship contribution statement

Torolf Storsul: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ole-Kristian Hess-Erga:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alf Seljenes Dalum:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Silje Stensby-Skjærvik:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kåre Segadal:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Linda Andersen:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Camilla Karlsen:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Sara Calabrese:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Lauris Boissonnot:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Alf Reidar Sandstad:** Writing – review & editing, Methodology, Conceptualization. **Ivar Rønnestad:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

No competing financial interests or personal relationships that could have influenced the work reported in the present paper are reported by the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.744195.

Data availability

All data are stored at Norwegian Institute for Water Research (NIVA) according to internal guidelines and are available on request.

References

Afewerki, S., Asche, F., Misund, B., Thorvaldsen, T., Tveteras, R., 2023. Innovation in the Norwegian aquaculture industry. Rev. Aquac. 15 (2), 759–771.

Antcliffe, B.L., Birtwell, I.K., Fidler, L.E., 2003. Lethal and sublethal responses of rainbow trout (Oncorhynchus mykiss) and coho (Oncorhynchus kisutch) fry to elevated dissolved gas supersaturation and temperature. Can. Tech. Rep. Fish. Aquat. Sci. 2500.

Beyer, D.L., D'Aoust, B.G., Smith, L.S., 1976. Decompression induced bubble formation in salmonids: comparison to gas bubble disease. Undersea Biomed. Res. 3.

Beyer, D., D'Aoust, B., Smith, L., Casillas, E., 1978. Decompression and isobaric supersaturation in fluid-breathing vertebrates: Timed response via bio-assay, hematology, and ultrasonic bubble detection. In: Shilling, C., Beckett, M. (Eds.), Proceedings of the Sixth Symposium on Underwater Physiology. Federation of American Societies for Experimental Biology, Bethesda.

Brown, R.S., Carlson, T.J., Welch, A.E., Stephenson, J.R., Abernethy, C.S., Ebberts, B.D., Langeslay, M.J., Ahmann, M.L., Feil, D.H., Skalski, J.R., Townsend, R.L., 2009. Assessment of barotrauma from rapid decompression of Depth-Acclimated Juvenile Chinook Salmon bearing radiotelemetry transmitters. Trans. Am. Fish. Soc. 138 (6), 1285–1301.

Brown, R.S., Pflugrath, B.D., Colotelo, A.H., Brauner, C.J., Carlson, T.J., Deng, Z.D., Seaburg, A.G., 2012. Pathways of barotrauma in juvenile salmonids exposed to simulated hydroturbine passage: Boyle's law vs. Henry's law. Fish. Res. 121–122.

Chen, X., Zhang, Z., Qin, Y., Liu, S., Wang, Y., Feng, J., Li, K., 2025. An in-situ study in the Xijiang River basin revealed adverse effects of total dissolved gas supersaturation on fish. Ecotox. Environ. Safe 289, 117663.

Colt, J.E., 1983. The computation and reporting of dissolved gas levels. Water Res. 17 (8), 841–849.

Colt, J., 1986. Gas supersaturation - Impact on the design and operation of aquatic systems. Aquac. Eng. 5 (1), 49–85.

Colt, J., 2000. Gas bubble disease. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. John Wiley & Sons, Inc., New York, pp. 403–408.

Colt, J., 2012. Dissolved Gas Concentration in Water: computation as Functions of Temperature, Salinity and Pressure. Elsevier.

D'Aoust, B.G., Smith, L.S., 1974. Bends in fish. Comp. Biochem. Physiol. A 49.

D'Aoust, B., Stayton, L., Smith, L., 1980. Separation of basic parameters of decompression using fingerling salmon. Undersea Biomed. Res. 7 (3), 199–209.

Dawley, E., Monk, B., Schiewe, M., Ossiander, F., Ebel, W., 1976. Salmonid Bioassay of Supersaturated Dissolved Air in Water. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, 1976, pp. 1–39.

Espmark, Å.M., Hjelde, K., Baeverfjord, G., 2010. Development of gas bubble disease in juvenile Atlantic salmon exposed to water supersaturated with oxygen. Aquaculture 306 (1), 198–204.

Espmark, Å.M., Midling, K.Ø., Nilsson, J., Humborstad, O.B., 2016. Effects of pumping height and repeated pumping in Atlantic Salmon Salmo salar. Nat. Resour. 07.

Fidler, L.E., 1988. Gas Bubble Trauma in Fish (Ph.D. thesis). University of British Columbia.

Gorham, F.P., 1899. Some physiological effects of reduced pressure on fish. J. Boston Soc. Med. Sci. 3 (9), 250–254.

Machado, J.P., Garling Jr., D.L., Kevern, N.R., Trapp, A.L., Bell, T.G., 1987. Histopathoiogy and the pathogenesis of embolism (gas bubble disease) in rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 44.

Marsh, M.C., Gorham, F.P., 1904. The Gas Disease in Fishes. Rep. U.S. Bur. Fish, pp. 343–376.

Millero, F.J., Poisson, A., 1981. International one-atmosphere equation of state of seawater. Deep-Sea Res. Ocean. A 28 (6), 625–629.

Mitchell, S.J., 2024. Decompression illness: a comprehensive overview. Diving Hyperb. Med. 54 (1Suppl), 1–53.

Nebeker, A.V., Kent Hauck, A., Baker, F.D., 1979. Temperature and oxygen-nitrogen gas ratios affect fish survival in air-supersaturated water. Water Res. 13 (3), 299–303.

Noble, C., Gismervik, K., Iversen, M.H., Kolarevic, J., Nilsson, J., Stien, L.H., Turnbull, J.F., 2018. Welfare Indicators for Farmed Atlantic Salmon: Tools for Assessing Fish Welfare. Nofima, pp. 1–351.

OECD, 2019. OECD Guidelines for the Testing of Chemicals, Section 2: Effect on Biotic Systems. Test Guideline No. 203: Fish Acute Toxicity Testing. Technical Report, Organisation for Economic Cooperation and Development (OECD), pp. 1–23.

Pleizier, N.K., Algera, D., Cooke, S.J., Brauner, C.J., 2020a. A meta-analysis of gas bubble trauma in fish. Fish Fish. 21, 1175–1194.

Pleizier, N.K., Brauner, C.J., 2024. Causes and consequences of gas bubble trauma on fish gill function. J. Comp. Physiol. B 194 (5), 739–747.

Pleizier, N.K., Cooke, S.J., Brauner, C.J., 2021a. A simple chamber design for calibrating weiss saturometers and recommendations for measuring and reporting total dissolved gases. Water Resour. Res. 57 (8).

Pleizier, N.K., Nelson, C., Cooke, S.J., Brauner, C.J., 2020b. Understanding gas bubble trauma in an era of hydropower expansion: How do fish compensate at depth? Can. J. Fish. Aquat. Sci. 77.

Pleizier, N.K., Rost-Komiya, B., Cooke, S.J., Brauner, C.J., 2021b. The lack of avoidance of total dissolved gas supersaturation in juvenile rainbow trout. Hydrobiologia 848.

Pulg, U., Isaksen, T.E., Velle, G., Vollset, K., Stranzl, S., Espedal, E.O., Bye-Ingebrigtsen, E., Barlaup, B.T., 2018. Gassovermetning i Vassdrag–En Kunnskapsoppsummering. LFI-Laboratorium Ferskvannsø Kologi Og Innlandsfiske.

Risberg, J., 2021. Acclimatization to diving: a systematic review. Undersea Hyperb. Med. 48 (2), 127–147.

Schiewe, M.H., 1974. Influence of dissolved atmospheric gas on swimming performance of Juvenile Chinook Salmon. Trans. Am. Fish. Soc. 103 (4), 717–721.

Speare, D., 2010. Disorders associated with exposure to excess dissolved gases. In: Leatherland, J.F., Woo, P.T.K. (Eds.), Fish Diseases and Disorders, Volume 2: Non-Infectious Disorders. CABI, pp. 342–356.

Stensby-Skjærvik, S., Boissonnont, L., Dalum, A., Storsul, T., Hess-Erga, O., Calabrese, S., Segadal, K., Andersen, L., Wahl-Ovesen, H., Rønnestad, I., 2025. Assessment of diagnostic tools to detect acute gas bubble disease in atlantic salmon (Salmo salar L.). J. Fish Dis. e14142.

Stevens, D.G., Nebeker, A.V., Baker, R.J., 1980. Avoidance responses of salmon and trout to air-supersaturated water. Trans. Am. Fish. Soc. 109 (6), 751–754.

Suvarna, S.K., Layton, C., Bancroft, J.D., 2018. Bancroft's Theory and Practice of Histological Techniques, eighth ed. Elsevier.

Sviland Walde, C., Bang Jensen, B., Pettersen, J.M., Stormoen, M., 2021. Estimating cage-level mortality distributions following different delousing treatments of Atlantic salmon (salmo salar) in Norway. J. Fish Dis. 44 (7), 899–912.

Velázquez-Wallraf, A., Caballero, M.J., Fernández, A., Betancor, M.B., Saavedra, P., Hemingway, H.W., Bernaldo de Quirós, Y., 2023. Biomarkers related to gas embolism: Gas score, pathology, and gene expression in a gas bubble disease model. PLoS One 18 (7), e0288659.

Velázquez-Wallraf, A., Fernández, A., Caballero, M.J., Arregui, M., González Díaz, Ó., Betancor, M.B., Bernaldo de Quirós, Y., 2022. Establishment of a fish model to study gas-bubble lesions. Sci. Rep. 12 (1), 6592.

Wang, Y., Liang, R., Tuo, Y., Li, K., Hodges, B., 2015. Tolerance and avoidance behavior towards gas supersaturation in rock carp procypris rabaudi with a history of previous exposure. N. Am. J. Aquac. 77 (4), 478–484.

Weiss, R., 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep-Sea Res. Ocean. Abstr. 17 (4), 721–735.

Weiss, R., 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Mar. Chem. 2 (3), 203–215.

Weitkamp, D.E., Katz, M., 1980. A review of dissolved gas supersaturation literature. Trans. Am. Fish. Soc. 109 (6), 659–702.

Weitkamp, D.E., Sullivan, R.D., Swant, T., DosSantos, J., 2003. Behavior of resident fish relative to total dissolved gas supersaturation in the lower clark fork river. Trans. Am. Fish. Soc. 132 (5), 856–864.

Yuan, Q., Du, J., Li, K., Zhu, B., Liang, R., Wang, Y., 2025. Gas bubble trauma of Schizothorax prenanti at various life stages induced by total dissolved gas supersaturation. Ecotox. Environ. Safe 303, 118862.