Received: 1 September 2023 | Revised: 23 February 2024 | Accepted: 2 May 2024
DOI: 10.1002/eco.2665

RESEARCH ARTICLE

WILEY

# Bubbling trouble: Effects of supersaturated water on benthic macroinvertebrates

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**Funding information**
Research Council of Norway, Grant/Award Number: 294742; Norwegian Environment Directorate, Grant/Award Number: M-1126/2018

## Abstract

The saturation of total dissolved gases (TDG) in water remains around 100%. Certain circumstances can lead to TDG values exceeding 100%, resulting in TDG supersaturation (TDGS). TDGS above about 110% can be toxic to animals that rely on water for gas exchange. However, saturation beyond 200% can occur in freshwater downstream of dams and hydroelectric power plants. Despite its impact, TDGS is often overlooked as a hazard to aquatic life, particularly for benthic macroinvertebrates. This study aimed to examine the effects of TDGS on nine species of benthic macroinvertebrates. We used replicated tank studies to manipulate TDGS levels from 100% to 120% and investigated the overall survival and species-specific effects on survival and buoyancy. We also present a summary on the effects of TDGS on invertebrate species previously tested. The results indicate that seven of nine species exhibited increased buoyancy when exposed to TDGS, causing them to float on the water surface. Additionally, a Cox Proportional Hazards model revealed a significant effect of TDGS on the survival of the macroinvertebrates. The sensitivity towards TDGS varied greatly among species of benthic macroinvertebrates, and significant species-specific effects were only observed for *Isoperla grammatica*, *Baetis rhodani* and *Asellus aquaticus*. Among these, the two latter species showed clear dose-related effects caused by TDGS, enabling the assessment of LT50 (time required to kill half of the tested population). *B. rhodani* was most sensitive with a LT50 of 3.7 days at 119% TDGS. Both species had visible air bubbles under the exoskeleton. Our findings highlight that direct and indirect effects on benthic macroinvertebrates can occur even at low to moderate levels of gas supersaturation, likely causing reduced density, decreased species diversity and altered species composition. The emerging evidence strongly supports the implementation of regulations on TDGS in freshwaters.

KEYWORDS
exposure test, gas bubble disease, hazard model, hydropower, management, total dissolved gases

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## 1 | INTRODUCTION

Gases dissolve in water and reach an equilibrium with ambient temperature and pressure, which governs the solubility of gases in water. In most surface freshwater bodies worldwide, the saturation of total dissolved gases (TDG) typically remains around 100%. However, certain circumstances can lead to TDG values exceeding 100%, resulting in a phenomenon known as TDG supersaturation (TDGS). TDGS can occur in specific situations, such as downstream from deep plunge-pools below waterfalls, during periods of high flow in rivers, at the outlets of springs and temporarily during episodes of rapid heating from sunlight, heavy algal blooms and extensive photosynthesis (Harvey, 1975; Weitkamp, 2008). However, a prevailing cause of TDGS is the discharge from hydropower facilities in dammed rivers (Li et al., 2022; Weitkamp & Katz, 1980) or within the penstock systems of hydropower plants (Berg, 1992; Heggberget, 1984; Pulg et al., 2016).

Supersaturation occurs below dams when air entrained in spilled water dissolves under hydrostatic pressure in dam tailraces. In run-of-the river power plants, air becomes saturates in the water under pressure within the tunnel system, and TDGS occurs as the pressure decreases at the outlet of the plant. In rare instances, the level of TDGS may exceed 200% (Heggberget, 1984; Lennox et al., 2022). Typically, saturation returns to 100% within hours or days as excess air forms bubbles during degassing. However, supersaturation may persist at least 30 km downstream from the hydropower plant outlet in rivers (Pulg et al., 2018). The water downstream of these plants serves as crucial habitat and a source of oxygen for aquatic organisms, but TDGS can render the water toxic to animals that rely on it for gas exchange (Marsh & Gorham, 1905).

Water containing supersaturated gas that passes over the gills or integument of animals can lead to the incorporation of excess gas into their bodies, resulting in gas bubble disease. This condition can have both lethal and sublethal effects on animals and is characterized by visible symptoms of embolism in the eyes, skin and other body parts (Goldberg, 1978; Nebeker et al., 1981; Pleizier et al., 2020). Gas bubble disease in animals is analogous to decompression sickness (the bends) in humans. Extensive research has been conducted on gas bubble disease in fish, discerning at least five categories of effects: (1) bubble formation on external surfaces, causing subcutaneous emphysema (Gorham, 1901; Stenberg et al., 2022); (2) gas bubbles blocking tissue and blood vessels, resulting in reduced blood flow and tissue damage (Geist et al., 2013; Marsh & Gorham, 1905); (3) in-body expansion of gas bubbles, causing physical trauma and inflation of the gas bladder (Colotelo et al., 2012; Shrimpton et al., 1990); (4) changes to blood chemistry (Dawley & Ebel, 1975); and (5) behavioural and sublethal effects, such as reduced swimming speed, difficulty maintaining proper buoyancy, reduced growth and immune response (Dawley & Ebel, 1975; Schiewe, 1974; Wang et al., 2018). The impact of TDGS on aquatic macroinvertebrates is not well understood, expect from studies conducted in Montana and Oregon, United States, during the 1970s and 1980s, which examined 16 taxa including water fleas, crayfish, stoneflies, mayflies, caddisflies, mosquitos and non-biting midges (Montgomery & Fickeisen, 1979; Nebeker, 1976; Nebeker et al., 1981; Nebeker, Stevens, & Brett, 1976). Testing of LC50 (lethal concentration required to kill half of the tested population within a given time) for North American species indicates significant variation in tolerance levels among taxa.

Considering the limited understanding of the impacts of gas supersaturation on macroinvertebrates, our objective was to address these knowledge gaps through a comprehensive experimental study. Our research focused on nine macroinvertebrate species from the Palearctic region. To investigate the effects of TDGS, we conducted replicated tank studies, manipulating TDG levels and examining species-specific and treatment-related effects on survival and buoyancy.

## 2 | MATERIAL AND METHODS

### 2.1 | Selection and sampling of species

Nine species of benthic macroinvertebrates were selected for controlled exposure tests at the Industrial and Aquatic Laboratory in Bergen (Table 1). The species were chosen due to their widespread occurrence in the Palearctic region, and we anticipated finding sufficient specimens for testing within a few hours of sampling from nearby sites. The animals were collected by kick sampling in the Apeltun river about 8 km from the laboratory, in the Flesland river about 15 km from the laboratory, in the Aurland river about 150 km from the laboratory and in Lake Langesjøen about 200 km from the laboratory. As there were no artificial sources of supersaturated water upstream of the sampling sites, it is unlikely that the species are adapted to unnatural levels of TDGS. Kick samples were transferred to a tray, and the specimens were sorted in the field using a pipette, identified to species and transferred to containers with cool river water. Within 8 h of initiation of field sampling, the animals were acclimation in tanks containing 100% dissolved gases for 12 h.

Each species was placed in separate cages (size 18 × 15 × 14 cm) using a pipette. The allocation of specimens into cages was performed randomly to prevent systematic variation in size or activity among the groups. The cages were constructed with a lid and two side panels made of 0.5 mm mesh, allowing for the unrestricted flow of water and ensuring equal levels of dissolved gases inside both the tank and the cages. The bottom of the cages was covered with sterilized aquarium gravel (fine gravel), which enabled the animals to attach themselves to a surface or engage in digging behaviour. The gravel was white to ease inspection of the animals, and the weight of the gravel ensured that the cages stabilized at the bottom of the tank where they were positioned.

### 2.2 | Experimental setup

The experimental setup included treatment tanks (size 1 × 1 m and 30 cm water depth) with varying concentrations of TDGS, in addition

**TABLE 1** Benthic macroinvertebrates selected for exposure testing

| Species | Higher classification | Site | Test | Total n | Treat n | Rep |
|---|---|---|---|---|---|---|
| *Asellus aquaticus* | Isopoda | Flesland R | II | 202 | 40 | 2 |
| *Gammarus lacustris* | Amphipoda | Langesjøen L | II | 170 | 34 | 2 |
| *Hydropsyche siltalai* | Trichoptera | Apeltun R | I | 245 | 49 | 3 |
| *Rhyacophila nubila* | Trichoptera | Apeltun R | I | 185 | 37 | 2 |
| *Baetis rhodani* | Ephemeroptera | Apeltun R | I | 191 | 38 | 3 |
| *Ephemerella aurivillii* | Ephemeroptera | Aurland R | III | 110 | 22 | 2 |
| *Amphinemura sulcicollis* | Plecoptera | Aurland R | III | 150 | 30 | 2 |
| *Diura nanseni* | Plecoptera | Aurland R | III | 170 | 34 | 2 |
| *Isoperla grammatica* | Plecoptera | Flesland R | II | 210 | 42 | 3 |

*Note*: Sampling sites (Site) included rivers (R) and one lake (L) in Western Norway. The testing was conducted over three separate test periods (Test). The Total n indicates the total number of specimens per species per test, Treat n represents the number of specimens per species per treatment, and Rep indicates the number of replicate tanks per treatment. See Table 2 for more details.

to control tanks. Supersaturated water at 140% was generated by mixing atmospheric air with cooled water under pressure inside a 1.5 m cone-shaped column. The supersaturated water was then diluted with water containing 100% TDG to generate the treatments including 105%, 110%, 115% and 120% TDGS, where each treatment included three tanks in 2017 and two tanks in 2020. The control treatment included two or three tanks with 100% TDG. We selected low to moderate levels of TDGS that are relevant to river settings, that may or may not cause mortality in Atlantic salmon (*Salmo salar*) (Stenberg et al., 2022) and that are within levels of existing regulations of maximum 110% TDGS in some states in the United States (Weitkamp, 2000). The water flowed constantly through the tanks (flow rate 2,200 L $h^{-1}$ in 2017 and 400 L $h^{-1}$ in 2020) from a pipe and to a submerged drain, allowing oxygenation and some current in the tank. The test was conducted with similar water temperature (6–8°C) as in the rivers and with 12:12 h of light:dark.

We aimed at performing triplicate replications with 15 specimens in each cage, which would require 225 specimens of each species (3 replicates × 5 TDG saturation levels × 15 specimens). However, we had assigned a maximum of 5 h to the field sampling to avoid unduly stressing of the animals after sampling, handling and transport and did not find the aimed number of specimens for all species within the allocated time. Hence, we performed duplicate replications for species with less than 190 specimens (Table 1; Figure 1).

We inspected the tanks twice during the first day, then every 24 h for the subsequent 12 to 14 days. Upon inspection, the cages were raised from the tanks, and we noted the number of specimens with increased buoyancy and the number of dead specimens. A specimen with increasing buoyancy was defined as a living animal that floated on the water surface and that was not able to descent, even when disturbed by water flow from a pipette. Floating specimens were not removed from the cage. Specimens that lay still were transferred to a Petri dish with water and declared dead if they did not move after 10 min, even when disturbed by water flow from a pipette. All dead animals were visually inspected using a stereo microscope for signs of bubbles under the exoskeleton. Also, the species identifications from the field were verified in the laboratory after the test by use of a stereo microscope. Specimens that were misidentified in the field were excluded from the count sum and the data.

## 2.3 | Measurements of supersaturation

TDG saturation and dissolved oxygen were measured every 30 min at the bottom of each tank at 0.3 m depth by a total gas analyser 3.0 (Fisch- und. Wassertechnik), which is based on the Weiss-saturometer principle (Bouck, 1982; Weiss, 1970). See Pulg et al. (2016) and Stenberg et al. (2022) for details on the total gas analyser. The sensors were precalibrated by the manufacturer and were recalibrated at atmospheric pressure prior to the onset of the test. To avoid a bias in monitoring due to bubble formation, each probe was equipped with an aquarium pump generating a constant water flow at the probe to remove potential bubbles. We also measured the TDG saturation manually (portable TGP metre; Point Four™ Tracker) in all tanks each day as control. We measured the TDG saturation in each cage upon completion of the test to check for potential differences between values in the cage and in the tank.

## 2.4 | Data analysis

We tested four TDGS treatments for the nine species, in addition to the control. There were no mortalities in *Rhyacophila nubila*, and this species was excluded from the modelling. Each day, the number of dead individuals was counted, providing a number with which to model the rate of loss for each species as a function of TDGS exposure. Each individual was randomly assigned an ID (because we did not track individuals), and mortalities were sequentially assigned to IDs along with survival times for the purposes of a survival analysis. Survivors were censored at the end of the study. A Cox proportional hazards model was fitted to the survival data with the *coxph* function in the survival package (Therneau, 2020). This hazards model is a

|  | Replicate 1 | Replicate 2 | Replicate 3 |
|---|---|---|---|
| T120 | *B. rhodani* x 18<br>*H. siltalai* x 16<br>*R. nubila* x 18 | *B. rhodani* x 14<br>*H. siltalai* x 16<br>*R. nubila* x 18 | *B. rhodani* x 14<br>*H. siltalai* x 16 |
| T115 | *B. rhodani* x 12<br>*H. siltalai* x 16<br>*R. nubila* x 18 | *B. rhodani* x 12<br>*H. siltalai* x 16<br>*R. nubila* x 18 | *B. rhodani* x 12<br>*H. siltalai* x 16 |
| T110 | *B. rhodani* x 13<br>*H. siltalai* x 17<br>*R. nubila* x 18 | *B. rhodani* x 12<br>*H. siltalai* x 17<br>*R. nubila* x 18 | *B. rhodani* x 10<br>*H. siltalai* x 17 |
| T105 | *B. rhodani* x 14<br>*H. siltalai* x 16<br>*R. nubila* x 19 | *B. rhodani* x 11<br>*H. siltalai* x 16<br>*R. nubila* x 19 | *B. rhodani* x 9<br>*H. siltalai* x 16 |
| T100 | *B. rhodani* x 13<br>*H. siltalai* x 16<br>*R. nubila* x 19 | *B. rhodani* x14<br>*H. siltalai* x 16<br>*R. nubila* x 19 | *B. rhodani* x 13<br>*H. siltalai* x 16 |

**FIGURE 1** Schematic representation of the experimental setup of Test I. Each box represents a tank with submerged cages, and each cage contained a given number of specimens of a single species. The treatment groups (T) were exposed to total dissolved gas (%) according to the number in their name. Tests II and III were implemented in a similar manner. The lower image shows the T115 tank from Replicate 2 with submerged cages.

measure of the relative risk of death occurring in the treatment groups compared to the control group. We treated TDGS as a continuous effect and not a factor. The selection of baseline level species in the model, i.e., the reference point for relative comparisons across species, was done at random as the first species in the alphabet. Assessments of LT50 (time required to kill half of the tested population) were based on mortality plots for species with a significant relationship to TDGS that exhibited low mortality rates in the control tanks.

## 3 | RESULTS

### 3.1 | Levels of supersaturation

The measured levels of TDG in the water varied slightly during the experiments, especially in the two highest supersaturation treatment groups (Figure 2; Table 2). In these, the measured values deviated by a maximum of +1.2 to −4.6 percentage points from the intended values. We report average measured TDG values during the test period when describing effects, which represent the total exposure (Table 2). The temperature varied slightly around 6°C in Test I (5.8–6.3°C) and 8°C in Tests II and III (7.8–8.4°C) (Table 2).

### 3.2 | Effects of supersaturation

A total of 1,633 benthic macroinvertebrate specimens were included in the test. There was a high mortality during the test, except for *R. nubila*, *Gammarus lacustris* and *Ephemerella aurivillii* (Figure 3). The Cox Proportional Hazards model revealed a significant effect of TDG on the survival of the macroinvertebrates ($z = 9.08$, $P < 0.01$) with a hazard ratio of 1.06 (Figure 4), indicating that each percentage point increase in TDG increases the risk of mortality. There were varying effects of TDGS on the species, with significant species-level effects for *Isoperla grammatica*, *Baetis rhodani* and *Asellus aquaticus* (Figure 5) For *B. rhodani*, the LT50 was 3.7 days at an average of 119% TDGS and 9 days at 113.5%. For *A. aquaticus*, the LT50 was 6.5 days at an average of 120% TDGS and 9 days at 115%. *I. grammatica* showed about 40% mortality in the control tanks, which made assessing the LT50 due to TDGS unreliable.

All species except *R. nubila* and *Amphinemura sulcicollis* experienced increased buoyancy and floated at high levels of TDGS (Figures 6 and 7). About half of the dead specimens of *B. rhodani* and *A. aquaticus* had visible air bubbles under the exoskeleton, while no bubbles were found under the exoskeleton in the other species. Most specimens had air bubbles attached to the body surface and legs (Figure 7).

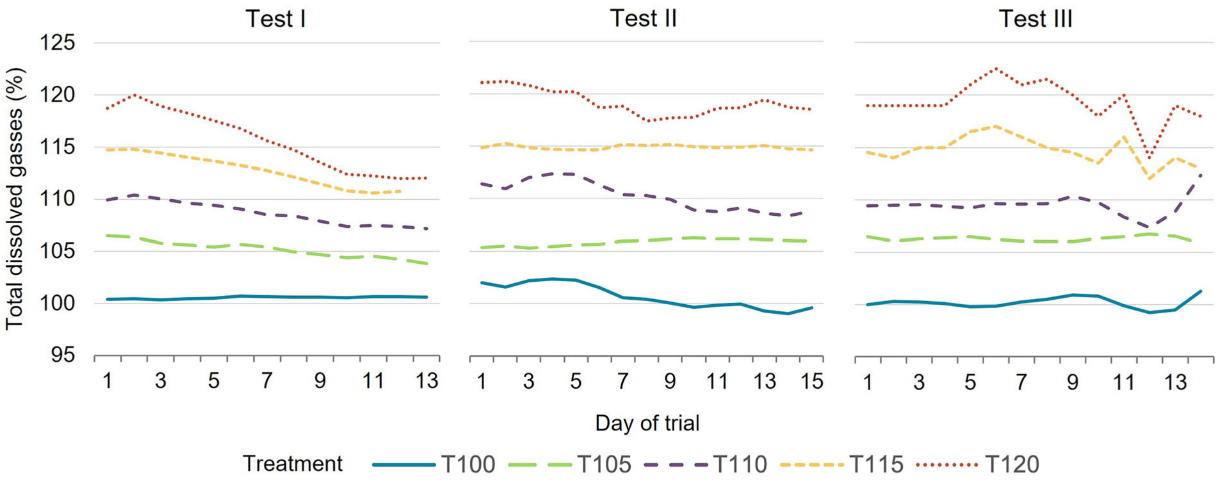

**FIGURE 2** Daily mean total dissolved gases for the treatment groups T105, T110, T115, T120 and T100 (control). The treatment groups were exposed to total dissolved gas (%) according to the number in their name.

**TABLE 2** Details on the three tests that were conducted, including the period when each test was performed (Date), the temperature in the tanks during the test and average (avg) total dissolved gases (TDG, %) with standard deviation (st.dev) for the treatments T105, T110, T115, T120 and T100 (control)

| | Test I | Test II | Test III |
|---|---|---|---|
| Date | 22.11–04.12.2017 | 17.04–02.05.2020 | 12.05–25.05.2020 |
| Temperature (°C) | 5.8–6.3 | 7.8–8.4 | 7.8–8.4 |
| T100 avg ± st.dev TDG | 100.5 ± 0.2 | 100.8 ± 1.1 | 100.2 ± 0.6 |
| T105 avg ± st.dev TDG | 105.2 ± 0.8 | 105.8 ± 0.4 | 106.2 ± 0.4 |
| T110 avg ± st.dev TDG | 108.7 ± 1.1 | 110.3 ± 1.4 | 109.4 ± 1.3 |
| T115 avg ± st.dev TDG | 112.7 ± 1.5 | 114.9 ± 0.5 | 114.7 ± 2.0 |
| T120 avg ± st.dev TDG | 115.4 ± 2.8 | 119.4 ± 1.5 | 116.0 ± 3.13 |

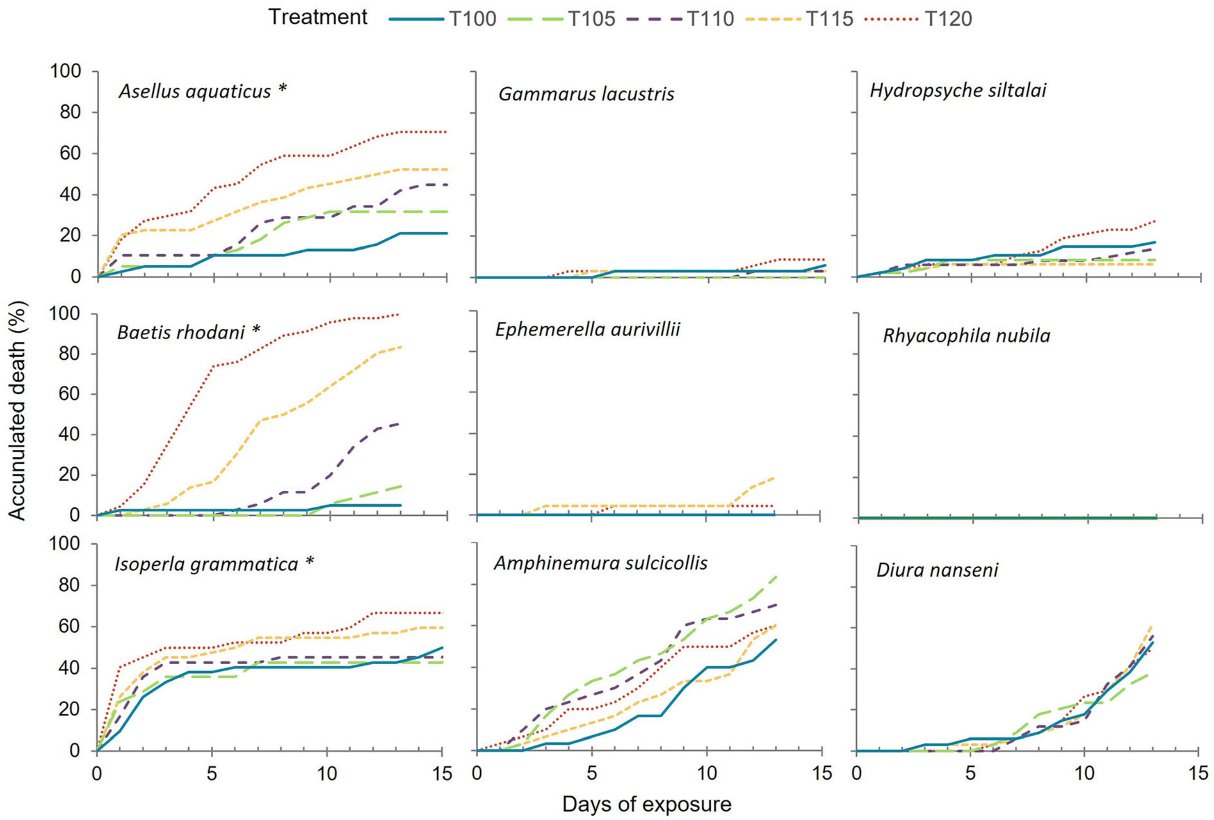

**FIGURE 3** Mortality of benthic macroinvertebrates over time and for all replicates when exposed to water with total dissolved gas supersaturation (%) according to the number in their name. T100 represents the control. Note that no mortality was recorded for *Rhyacophila nubila*. *Treatment had a significant effect on mortality.

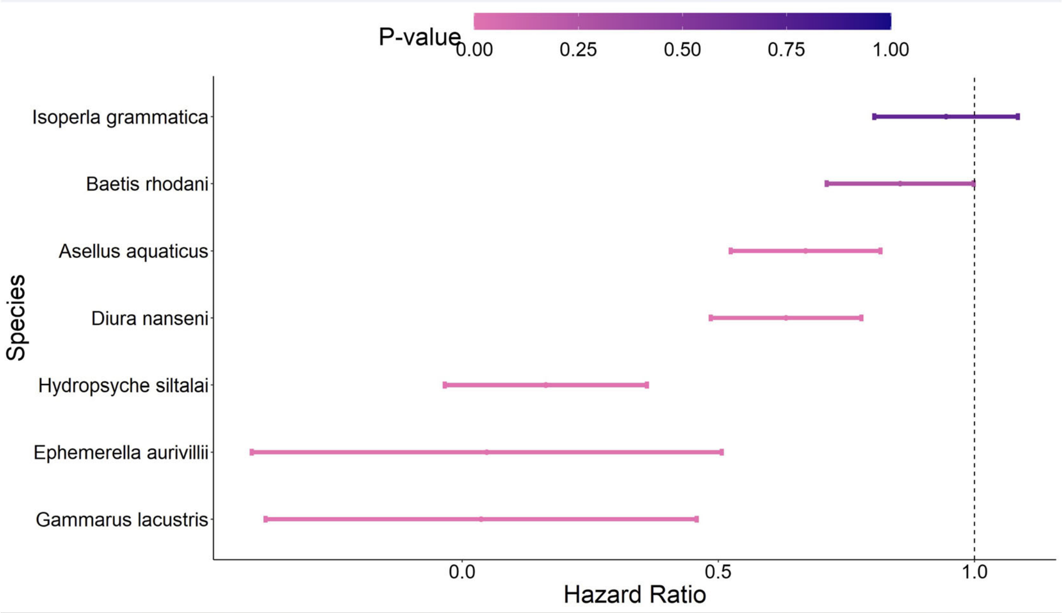

**FIGURE 4** Effect sizes on the mortality of benthic macroinvertebrates in gas supersaturation experiments, as assessed by a Cox proportional hazards model. *Rhyacophila nubila* was part of the experiments but not modelled because no specimens died. *P* values are relative to the baseline *Amphinemura sulcicollis*. The overall hazard ratio in the model was 1.06.

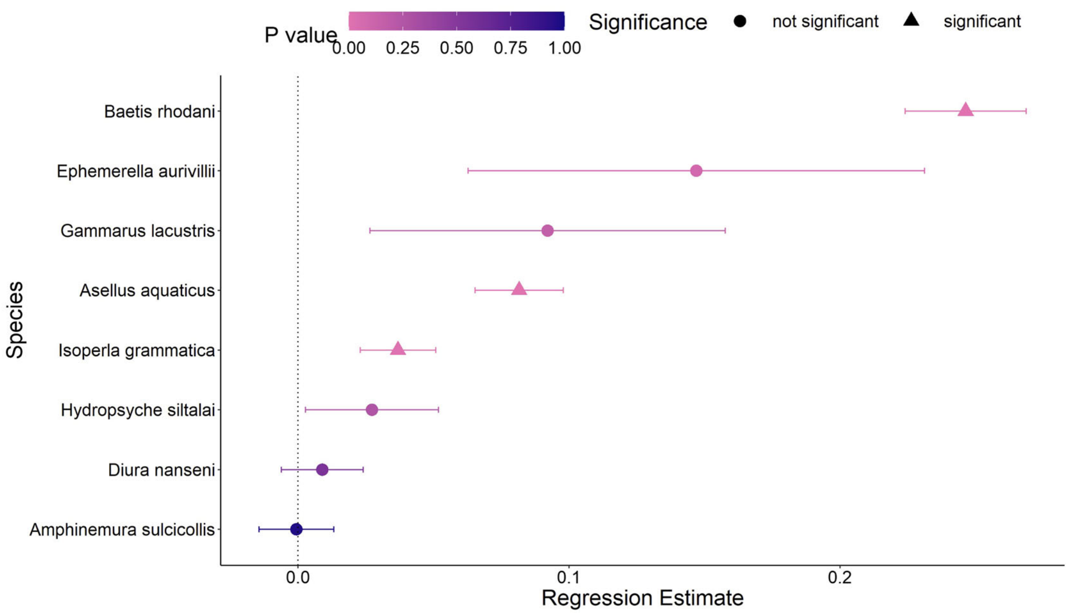

**FIGURE 5** Species-specific effects of gas supersaturation on the mortality of benthic macroinvertebrates, as assessed by a Cox proportional hazards model. *Rhyacophila nubila* was part of the experiment but not modelled because no specimens died.

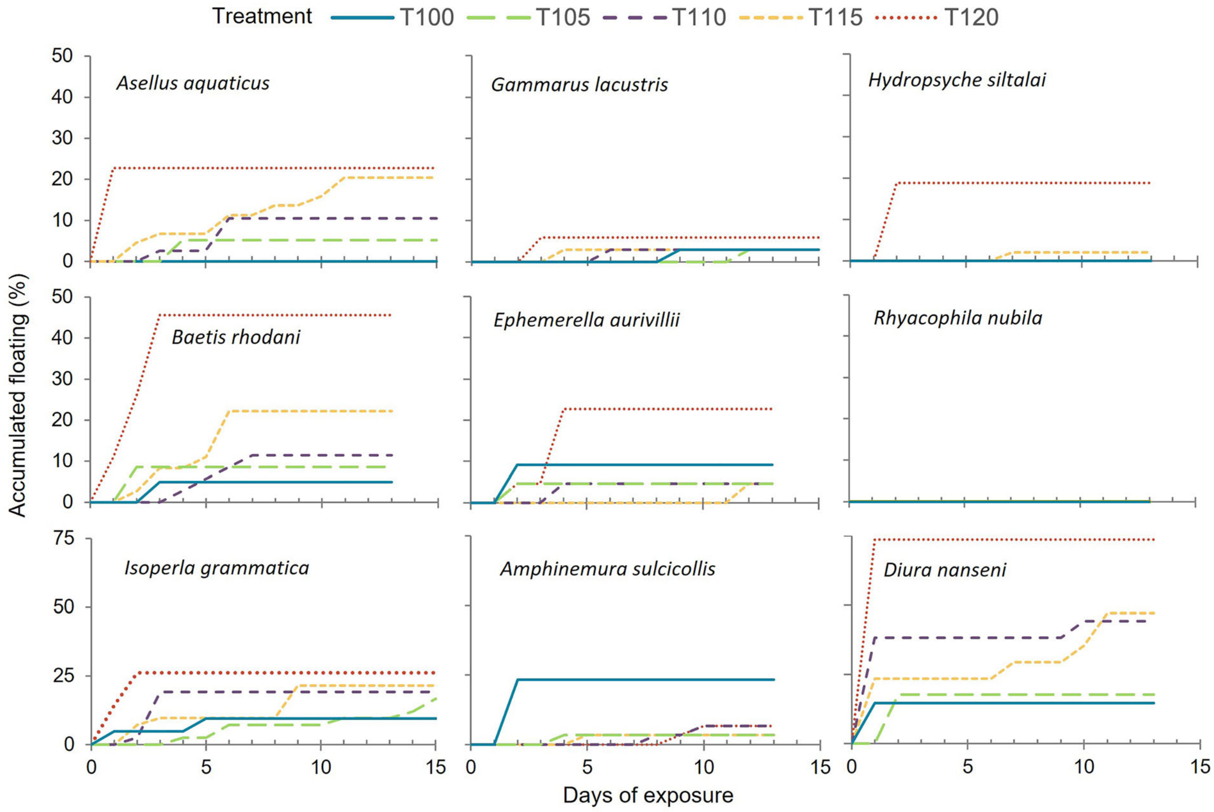

**FIGURE 6** Accumulated proportion of the specimens that experienced increased buoyancy (accumulated floating) over time for all treatments (T) when exposed to water with total dissolved gas supersaturation (%) according to the number in their name. T100 represents the control. Some specimens died without floating, implying that the proportion floating is a minimum estimate. Note the divergent y-axis scale in the lowermost row

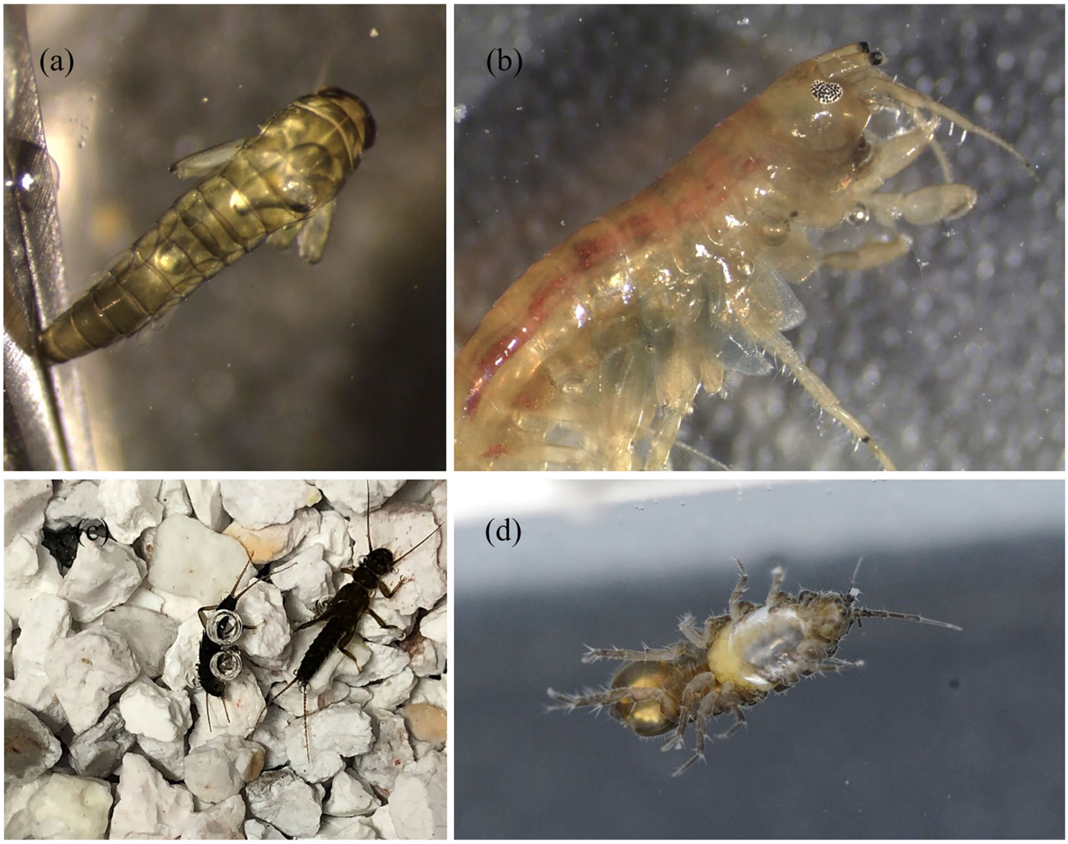

**FIGURE 7** Images from the laboratory testing. (a) *Baetis rhodani* with bubbles under the exoskeleton in the thorax and carapace. This specimen died after exposure to an average of 110% total dissolved gas supersaturation for 12 days. (b) *Gammarus lacustris* with air bubbles between the ventral surface and the coxal plate. (c) *Diura nanseni* with air bubbles attached to the legs. The specimen was clinging to the gravel to prevent floating. (d) A floating specimen of *Asellus aquaticus* with an air bubble under the exoskeleton causing abdominal distention

## 4 | DISCUSSION

### 4.1 | Varying levels of supersaturation

The measured TDGS in the tanks was not stable throughout the test (Figure 2; Table 2). This variability is not unusual in such experiments since the TDGS water is generated in a pressurized tank and then diluted to the intended values. Factors contributing to the observed variation in TDGS include minor temperature variations, fluctuations in TDG levels in the water source and changes in atmospheric pressure during dilution and degassing. To address this variability, we report the average measured TDG that a species was exposed to over a specific duration. This average reflects the cumulative exposure (dose and duration) and is a commonly used metric for linking TDGS to gas bubble disease (Pleizier et al., 2019; Weitkamp & Katz, 1980). It should be noted that there is a possibility of periodic bubble formation on the probes in experiments conducted at shallow water depths (0.3 m). However, it is likely that the data reflect real TDG-values since the permanent probes were controlled visually and by manual measurements and since a water current was applied and directed at the probes to remove bubbles. Compared to snap sampling of TDG during experiments, which is common in research on TDGS, our permanent logging represents more reliable measurements and underlines the importance of monitoring to avoid misinterpretation of TDGS.

### 4.2 | Effects on mortality and buoyancy

The overall survival in the current test was correlated to TDGS with a Hazard ratio of 1.06, indicating that each percentage point increase in TDG increases the risk of mortality. Significant species-level mortality correlated with TDGS was observed in *I. grammatica*, *B. rhodani* and *A. aquaticus*. Also, *A. aquaticus*, *G. lacustris*, *Hydropsyche siltalai*, *B. rhodani*, *E. aurivillii*, *Diura nanseni* and *I. grammatica* showed increased buoyancy with TDGS. The overall mortality in the current test was high. We cannot pinpoint the cause of mortality in the control tanks as it was clearly not linked to TDGS. In *B. rhodani* and *A. aquaticus*, air bubbles typically formed under the carapace after exposure (Figure 7). When bubbles had formed in or on the animals, they lost control of their buoyancy and floated. Death occurred 1.5 to 2 days after the onset of buoyancy problems for these two species. Studies conducted in North America also reported that TDGS can cause increased buoyancy in macroinvertebrates (Montgomery &

Fickeisen, 1979; Nebeker, 1976). Results from the buoyancy tests suggest that studies examining the impact of TDGS in the laboratory should incorporate evaluations of effects related to buoyancy.

Our results highlight two distinct effects caused by TDGS. First, TDGS can have indirect effects when air bubbles form in the internal organs and on the body surface of specimens, causing floatation. The bubbles that form in the body primarily consist of nitrogen (Nebeker, Bouck, & Stevens, 1976), and their formation is a function of supersaturation and duration of exposure. Specimens with increased buoyancy that float will be displaced downstream in river stretches affected by TDGS, increasing their mortality due to starvation and predation. Additionally, abiotic stressors may induce a behavioural drift response, where specimens intentionally leave the substrate and enter the water column to escape unfavourable conditions (Jackson et al., 2007; Larsen & Ormerod, 2010). This response would require the ability to sense TDGS. Second, effects of TDGS can be direct through increased mortality, most likely resulting from bubbles (emboli) that obstruct the passage of body fluids in internal organs or from bubbles in the gut causing blockage of food and subsequent starvation (Nebeker, 1976).

Although field studies on the effects of TDGS on benthic macroinvertebrates are limited, our findings suggest that even low to moderate levels of TDGS, starting at approximately 110%, can have extensive direct and indirect effects, leading to reduced density, decreased species diversity and altered species composition. For instance, *B. rhodani* is a widespread and abundant species of benthic macroinvertebrate and found in fast-flowing rivers in the western Palearctic region. Our study demonstrates that *B. rhodani* exhibits increased mortality already at 110% gas supersaturation. Furthermore, while the mortality of *D. nanseni* was not significantly correlated with TDGS, 75% of the specimens of *D. nanseni* experienced increased buoyancy after 1 day of exposure to an average TDGS of 119%. Temporal trends in the biodiversity of benthic macroinvertebrates across Europe indicate that these organisms are especially vulnerable downstream of dams (Haase et al., 2023). Our finding offers a possible explanation for this phenomenon, which might be linked to gas supersaturation.

## 4.3 | Synopsis of laboratory tests

Mortality caused by TDGS has now been studied in nine European and 16 North American freshwater macroinvertebrate species. A summary of the effects on all tested species presented in Table 3. Additionally, gas bubble disease in marine macroinvertebrates has been explored, although these studies have not specified TDGS levels. It should be noted that studies terminated due to experimental setup issues, such as Montgomery and Fickeisen (1979), are excluded from our report and that methodological differences among studies exist. Previous studies often had limitations, including low sample size and intermittent TDGS measurements. Furthermore, laboratory tests assessing acute TDGS toxicity have inherent limitations: they cannot be generalized to other species, often overlook sub-lethal or chronic effects and do not account for multiple stressors, which can significantly affect individual behaviour and population structure (Lemm et al., 2021). Results from laboratory and controlled cage studies may not directly translate to natural river environments. For instance, the zoobenthos of rivers typically crawl and cling to the substrate to withstand the pressure from running water. In this natural setting, increased buoyancy can lead to flotation and subsequent starvation or predation. It is not straightforward to measure these effects in laboratory settings. Nevertheless, laboratory studies still offer valuable insights into the expected sensitivity of macroinvertebrate taxa to TDGS.

Several previous studies have reported LC50- values. For example, Nebeker et al. (1981) found that the mayfly *Timpanoga hecuba* had an LC50 of 129% TDGS for a 96 h exposure period. Some species, including several in our study, have also been tested without conclusive results. Likely, exposure levels were too low. For example, Nebeker et al. (1981) were unable to establish LC50 for two species of dipterans and one species of caddisfly, but these species had better survival than *T. hecuba*. Some studies also report sub-lethal levels of TDGS. For example, *Daphnia magma* had a 10-day LC50- value of 118% TDGS, and sub-lethal effects occurred at 115% TDGS when air in the gut caused blockage of food, leading to starvation (Nebeker, 1976). Table 3 shows that macroinvertebrate tolerance to TDGS varies widely. The water flea *D. magma* and the mayfly *B. rhodani* are among the most sensitive, with 50% mortality occurring within 4 and 9 days at about 114% TDGS, respectively. In contrast, the caddisfly *Dicosmoecus gilvies* has a higher tolerance, reaching a 10-day LC50 at 150% TDGS.

It would be valuable to identify shared characteristics, such as taxonomic entities or functional traits, among sensitive taxa that could serve as field indicators of TDGS. Sufficient data and careful metric selection are needed to reliably measure human impacts in macroinvertebrate communities (Sinclair et al., 2024). However, we were unable to identify such characteristics from the list of tested species, except for speculating whether sensitive taxa are proficient swimmers. If true, these taxa likely spend time above the riverbed, exposing them to higher TDGS levels compared to species that do not ascend from the substrate. Additionally, shear stress at the interphase between the water column and substrate might influence the mixing of supersaturated and saturated water, and hence the exposure. Then, taxa that crawl on the substrate or dig, such as certain dipterans (Table 3), will be less exposed to TDGS, while taxa that swim above the substrate, such as *B. rhodani*, will be more exposed to TDGS. Ecological strategy theory (Leiva et al., 2022; Pianka, 1970; Townsend & Hildrew, 1994) suggests that r-strategists, which are organisms adapted for rapid colonization of new habitats and include burrowers and crawlers with a small streamlined and flexible body, could be more resilient in harsh conditions with high levels of TDGS. Conversely, k-strategists, characterized by longer lifespans, larger bodies and a preference for stable habitats, could be more sensitive to TDGS. Most macroinvertebrates, including multivoltine species, like *B. rhodani*, are considered r-strategists, whereas semivoltine species, like *Dicosmoecus nanseni*, may be less r-selected. To identify shared traits and

**TABLE 3** Published results on the sensitivity of freshwater macroinvertebrates to total dissolved gas supersaturation (TDGS)

| Order | Species | Region | Test | n | Results | Ref. |
|---|---|---|---|---|---|---|
| Isopoda | *Asellus aquaticus* | Norway | LT50 | 202 | 120% 6.5 d, 115% 12 d | 1 |
| | | Norway | Buoyancy | 202 | 121% 1 d 23%[a], 115% 11 d 20%[a] | 1 |
| Amphipoda | *Gammarus lacustris* | Norway | LT50 | 170 | 118% > 15 d[b] | 1 |
| | | Norway | Buoyancy | 170 | 121% 3 d 7%[a] | 1 |
| Anomopoda | *Daphnia magna* | Oregon | LC50[c] | NA | 123% 4 d, 120% 7 d, 118% 7 d | 2 |
| | | Oregon | LC50[d] | NA | 114% 4 d | 2 |
| Decapoda | *Pacifastacus leniusculus* | Oregon | LC50 | NA | 147% 4 d, 145% 7 d, 133% 10 d | 2 |
| Plecoptera | *Amphinemura sulcicollis* | Norway | LT50 | 150 | 116% > 13 d[b] | 1 |
| | | Norway | Buoyancy | 150 | 116% > 13 d[b] | 1 |
| | *Diura nanseni* | Norway | LT50 | 170 | 116% > 13 d[b] | 1 |
| | | Norway | Buoyancy | 170 | 116% 1 d 74%[a] | 1 |
| | *Isoperla grammatica* | Norway | LT50 | 210 | 118% > 15 d[e] | 1 |
| | | Norway | Buoyancy | 210 | 121% 2 d 26%[a] | 1 |
| | *Acroneuria californica* | Oregon | LC50 | NA | >135% 10 d | 2 |
| | *Acroneuria pacifica* | Oregon | LC50 | NA | >125% 10 d[e] | 2 |
| | *Pteronarcys californica* | Oregon | LC50 | NA | >125% 10 d[e] | 2 |
| | | Montana | Mortality | 50 | >140% 10 d[b] | 3 |
| | | Montana | Buoyancy | 9 | 123% 5 d 22%[f] | 3 |
| | *Isogenus* sp. | Montana | Mortality | 17 | >140% 10 d[b] | 3 |
| | *Claassenia sabulosa* | Montana | Buoyancy | 3 | 107% 5 d 100% | 3 |
| Ephemeroptera | *Baetis rhodani* | Norway | LT50 | 191 | 119% 3.7 d, 114% 8 d | 1 |
| | | Norway | Buoyancy | 191 | 119% 3 d 46%[a], 114% 6 d 22%[a] | 1 |
| | *Ephemerella aurivillii* | Norway | LT50 | 110 | 116% > 13 d[b] | 1 |
| | | Norway | Buoyancy | 110 | 116% 4 d 23%[a] | 1 |
| | *Timpanoga hecuba* | Oregon | LC50 | 16 | 129% 4 d | 4 |
| | *Cloeon* sp. | Montana | Mortality | 50 | >140% 10 d[b] | 3 |
| Trichoptera | *Hydropsyche siltalai* | Norway | LT50 | 245 | 115% > 13 d[b] | 1 |
| | | Norway | Buoyancy | 245 | 119% 2 d 19% | 1 |
| | *Rhyacophila nubila* | Norway | LT50 | 185 | 115% > 13 d[b] | 1 |
| | | Norway | Buoyancy | 185 | 115% > 13 d[b] | 1 |
| | *Dicosmoecus gilvies* | Oregon | LC50 | 10 | 150% 10 d | 4 |
| | *Hydropsyche* | Montana | Mortality | 50 | >140% 10 d[b] | 3 |
| | *Lepidostomatidae* | Montana | Mortality | 50 | >140% 10 d[b] | 3 |
| | *Lepidostomatidae* | Montana | Buoyancy | 5 | 123% 5 d 0% | 3 |
| | *Neophylax* sp. | Montana | Buoyancy | 15 | 123% 5 d 80%[f] | 3 |
| Diptera | *Culex peus* | Oregon | LC50 | 10–20 | >144% 4 d[b] | 4 |
| | *Cricotopus* sp. | Oregon | LC50 | 10–20 | >141% 4 d[b] | 4 |
| | Chironomidae | Montana | Mortality | 50 | >140% 10 d[b] | 3 |

*Note*: The sample size (n) represents the total number of specimens in the test. The results for LT50 (time required to kill half the of the tested population) and LC50 (lethal concentration required to kill half of the tested population) are presented as percentage of TDGS and the number of days (d) to reach the result. The results of buoyancy testing include the percentage of TDGS, days (d) to reach the result and percentage of specimens that float. The references (Ref.) include: (1) the current study, (2) Nebeker (1976), (3) Montgomery and Fickeisen (1979) and (4) Nebeker et al. (1981).

[a] Some specimens died without floating, implying that the proportion floating is a minimum estimate.

[b] No death was related to TDGS/no buoyancy problems were related to TDGS.

[c] In still water with food.

[d] In flowing water with no food.

[e] TDGS was a significant cause of death, but LC50/LT50 was not achieved or not possible to assess.

[f] Not specified if the larvae that floated were exposed to TDGS 123%, 117% or 107%.

strategies among sensitive taxa, it is necessary to conduct comprehensive field experiments that involve quantitative sampling of benthic macroinvertebrates in both impacted and non-impacted sites. Such studies would provide more definitive insights into the traits and behaviours that correlate with TDGS sensitivity and help develop effective indicators for monitoring TDGS effects in aquatic ecosystems.

### 4.4 | Responses in fish and macroinvertebrates

While studies on fish have been instrumental in uncovering the causes of gas bubble disease in laboratory and field trials, it is crucial to gain an understanding of the entire ecosystem of gill-breathing animals. Research on fish has emphasized interspecific differences in vulnerability to gas supersaturation (Pleizier et al., 2020), with salmonids being more susceptible compared to cyprinids. Similarly, our findings reveal significant variations among macroinvertebrates, which should be considered in the context of the meta-community for rivers experiencing frequent occurrences of gas supersaturation.

Although fish and macroinvertebrates belong to different taxonomic groups and have distinct physiology, they exhibit similar symptoms of gas bubble disease. This includes the formation of gas bubbles in the cardiovascular system of fish and the circulatory system of macroinvertebrates. These bubbles can result in emboli and increased mortality when exposed to high levels of TDGS. Macroinvertebrates still appear to be less sensitive to TDGS compared to most species of fish. Several species of macroinvertebrates exhibit no increased mortality up to 140% TDGS and their specific tolerance to TDGS remains unknown. Atlantic salmon smolt develop gas bubble disease and die already from 108% TDGS (Stenberg et al., 2022), while chinook salmon (*Oncorhynchus tshawytscha*) experience mortality from about 120% TDGS (Mesa et al., 2000). Among the approximately 30 fish species tested so far, the most tolerant species is speckled dace (*Rhinichthys osculus*), which reach LC50 after 4 days at 140% TDGS (Nebeker et al., 1980).

The effects of TDGS may be moderated by water depth in the natural environment compared to laboratory settings. TDGS, being a physical condition, is compensated by hydrostatic pressure, resulting in a decrease of approximately 10% saturation per metre increase in water depth (Pleizier et al., 2019; Weiss, 1970). Mobile animals can therefore escape harmful TDGS by moving deeper (Lennox et al., 2022; Pleizier et al., 2019). They can also seek refuge in side channels or move upstream or downstream from affected areas (Weitkamp et al., 2003). Benthic macroinvertebrates may be more exposed to TDGS than fish, as they have lower mobility and cannot easily escape. For avoidance behaviour to be effective, the organisms must be able to sense hazardous levels of supersaturation indirectly or directly and respond through migration. This behaviour is most likely present in certain fish species, while others may lack such ability or other behavioural traits may dominate (Lennox et al., 2022; Lund & Heggberget, 1985; Weitkamp, 2000). There is no documented evidence of avoidance behaviour in macroinvertebrates. A differing avoidance ability could potentially explain the stronger selection for tolerance in benthic macroinvertebrates compared to fish. However, it should be noted that benthic macroinvertebrates utilize habitats deeper in the water column or substrate than most fish, suggesting weaker selective pressure for developing TDGS tolerance in benthic macroinvertebrates. Additionally, inherent physiological differences between fish and macroinvertebrates, particularly the closed circulatory system in fish, may contribute to differences in sensitivity. Fish exposed to TDGS develop emboli in their veins (Pleizier et al., 2020), which can obstruct blood vessels in vital organs, such as the brain and gills. Arthropods have an open circulatory system where the occurrence of emboli causing blockages is less likely. Furthermore, fish typically develop subcutaneous emphysema, while the exoskeleton of arthropods may provide protection against surface injuries.

When it comes to chronic effects caused by TDGS, Colt (1986) documented delayed mortality occurring one to two months after exposure in fish. There are currently no data available on chronic effects in macroinvertebrates. Additionally, there is a lack of data for additive effects of stressors involving TDGS in macroinvertebrates. The stress induced by TDGS may potentially be amplified or reduced by biotic factors, such as competition or infections, or by abiotic factors, such as acidity or discharge. In Atlantic salmon, no additive effects were observed between sublethal levels of TDGS and environmental acidification (Höglund et al., 2024).

### 4.5 | Implications

Studies on the occurrence and effects of TDGS on fish in rivers have been conducted primarily in the United States, Canada, China and Norway (e.g., Backman & Evans, 2002; Liu et al., 2013; Stenberg et al., 2022; Wang et al., 2015). In contrast, research on its effects on macroinvertebrates has been limited to the United States and Norway (Table 3). Regulations have been implemented to limit TDGS to 110% in many states in the United States (Weitkamp, 2000), primarily due to its adverse effects on fish. However, the prevalence of TDGS downstream from dams and power plants in most parts of the world, and its effects on macroinvertebrates, remains largely unknown. We recommend routine monitoring of TDGS downstream from hydroelectric power plants, particularly if the water is opalescent or where sensitive fish species (Pleizier et al., 2020) and benthic macroinvertebrates (Table 3) are unexpectedly absent or less abundant.

Despite the global increase in dams and run-of-the-river power plants (IEA, 2021; Perera et al., 2021; Zarfl et al., 2015) and the documented negative effects of TDGS on the aquatic biota, most countries lack regulations or criteria for maximum TDGS levels. The accumulating evidence underscores the urgent need for implementing TDGS regulations in freshwater environments worldwide. This is particularly critical given the stagnation in the recovery of European freshwater biodiversity, as highlighted by Haase et al. (2023). Establishing such regulations will be a significant step towards mitigating the impacts of TDGS on aquatic ecosystems and preserving global biodiversity.

## ACKNOWLEDGEMENTS

This research could not have been performed without the help from Torunn Landås, Arne Johannessen, Ingrid Hornnes, Jessica Anne Hough, Mathilde Blanche Setnes, Veronica Aarvik Bøe and Lina Florian during assembly of the test cages, sampling in the field and sampling in the lab. The Industrial and Aquatic Laboratory (ILAB) in Bergen provided technical assistance during the trials. The study was funded by the Research Council of Norway through the project SUPERSAT (294742): hydropower induced supersaturation in freshwaters: effects on ecosystems, mitigation and solutions, and by the Norwegian Environment Directorate through project M-1126/2018 (Pulg et al., 2018).

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be openly available in Zenodo upon acceptance of the manuscript.

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**How to cite this article:** Velle, G., Isaksen, T. E., Lennox, R. J., & Pulg, U. (2024). Bubbling trouble: Effects of supersaturated water on benthic macroinvertebrates. *Ecohydrology*, e2665. https://doi.org/10.1002/eco.2665