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RESEARCH ARTICLE

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Does swimming activity influence gas bubble trauma in fish?

Naomi K. Pleizier[1] | Steven J. Cooke[2] | Colin J. Brauner[1]

[1]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

[2]Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, Ontario, Canada

Correspondence
Naomi K. Pleizier, Department of Zoology, University of British Columbia, #4200 - 6270, University Blvd., Vancouver, BC V6T 1Z4, Canada.
Email: pleizier@zoology.ubc.ca

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Abstract

Total dissolved gas (TDG) supersaturation from sources such as hydroelectric dams can cause harmful bubble growth in the tissues of aquatic animals, known as gas bubble trauma (GBT). Locomotion is known to exacerbate bubble growth in tissues during decompression under certain conditions (such as in diving animals), possibly because of increased bubble nucleation. As with decompression sickness, GBT is caused by the supersaturation of tissues with gas, and thus we hypothesize that locomotion promotes bubble nucleation in the tissues of fish exposed to TDG supersaturation. Many previous laboratory studies have tested the effects of TDG on fish exposed to low-velocity, non-directional flow, whereas fish in field conditions are exposed to higher-velocity flows and are likely more active. Therefore, it is important to understand the effects of locomotion on GBT to apply laboratory results to active fish in field conditions. We exposed rainbow trout (*Oncorhynchus mykiss*) to either control (100% TDG) or TDG supersaturation (123% TDG) in either static or flowing water conditions (1.8 Bl/s) and recorded time to 50% loss of equilibrium (LOE). We observed no statistically significant difference in time to 50% LOE between flow conditions. Given the lack of statistically significant difference between static and flowing water, our findings indicate that results from GBT experiments on rainbow trout in non-directional flow are applicable to more active individuals.

KEYWORDS
gas bubble disease, hydroelectric dams, locomotion, total gas pressure

1 | INTRODUCTION

Total dissolved gas (TDG) supersaturation from sources such as hydroelectric dams, pumps in aquatic facilities, and heated industrial effluent can cause bubbles to grow in the tissues of aquatic animals, which results in harmful gas bubble trauma (GBT) and potential mortality (see review in Weitkamp & Katz, 1980). As a result, many studies describe the effects of TDG supersaturation on aquatic animals (see reviews in Pleizier, Algera, Cooke, & Brauner, 2020; Weitkamp & Katz, 1980; Weitkamp, 2008). An important challenge remains in applying results measured in a controlled laboratory setting to animals living in complex field conditions. Water velocity is very rarely reported in the GBT literature, but it is possible that many laboratory studies have been conducted in conditions with no directional water flow, whereas certain species, such as salmonids, are generally active swimmers in field conditions (e.g., Hinch and Rand, 1998; Hinch et al., 2002; Watson et al., 2019). Testing the effect of locomotion on the progression of GBT is therefore important for applying laboratory results to field conditions, especially when the movements of fish are known through the use of data loggers and other devices.

1.1 | Exercise and decompression sickness

We know from decompression experiments in humans and other animals that exercise can promote bubble formation in gas-supersaturated tissues. Decompression sickness in divers is analogous to GBT in some ways because both are caused by bubble formation in tissues as a result of gas supersaturation. In humans, exercise during diving has been demonstrated to exacerbate (Van der Aue, Brinton, &

Kellar, 1945), relieve (Dujić et al., 2005; Jankowski, Nishi, Eaton, & Griffin, 1997; Jankowski, Tikuisis, & Nishi, 2004), or have no effect (Jankowski et al., 2004; Radermacher et al., 1990) on symptoms of decompression sickness, depending on the type of exercise and its timing during the dive. There is also evidence from studies on animals such as rats and bullfrogs (*Rana catesbeiana*, Whitaker, Blinks, Berg, Twitty, & Harris, 1945), crustaceans (*Pachygrapsus crassipes*, *Pagurus hirsutiusculus*, and *Pagurus samuelis*, McDonough & Hemmingsen, 1984a, 1984b), channel catfish (*Ictalurus punctatus*), and woolly sculpin (*Clinocottus analis*, McDonough & Hemmingsen, 1985) that locomotion promotes bubble formation in tissues during decompression and that bubbles form preferentially in limbs that move during decompression (McDonough & Hemmingsen, 1984a, 1984b).

1.2 | Mechanisms of bubble nucleation

Mechanisms that may cause bubble nucleation in tissues include tribonucleation and rotational flow. Tribonucleation occurs when two solid surfaces separate, which creates a low-pressure zone where bubbles can nucleate (Campbell, 1968). This process is thought to contribute to bubble formation in joints during decompression (McDonough & Hemmingsen, 1984a, 1984b). As blood velocity increases during locomotion, this would likely result in an increase in rotational flow, which can also create areas of low pressure (Dean, 1944). Rotational flow is known to cause cavitation on features such as artificial heart valves (Avrahami, Rosenfeld, Einav, Eichler, & Reul, 2000; Johansen, 2004). Both mechanisms of bubble nucleation would be expected to increase during exercise and promote GBT.

To our knowledge, there have been only two studies of the effect of locomotion on GBT in fish. Gray, Page, Saroglia, and Festa (1983) found that groups of black bullhead (*Ameiurus melas*) exposed to various levels of TDG supersaturation reached 50% mortality in less time in flowing water compared to static water, whereas there was an interaction between the effects of water flow and TDG on time to 50% mortality for common carp (*Cyprinus carpio*). Carp died more rapidly in flowing water at levels of supersaturation below 133% TDG but flowing water had a protective effect above 133% TDG. Bouck, Nebeker, and Stevens (1976) found that largemouth bass (*Micropterus salmoides*) reached 50% mortality in 25% less time in swimming treatments compared to resting treatments, but swimming had no effect on the time to mortality of sockeye salmon (*Oncorhynchus nerka*). No such studies on the relationship between locomotion and GBT have been conducted on *Oncorhynchus mykiss*.

1.3 | Study objective

The goal of this study is to determine the relationship between locomotion and the progression of GBT in rainbow trout exposed to TDG supersaturated water. We hypothesize that increased locomotion will promote bubble nucleation in tissues. For this reason, we predict that locomotion will accelerate the progression of GBT effects. To test this, we exposed groups of juvenile rainbow trout to air-equilibrated (100% TDG) and TDG supersaturated (123% TDG) treatments in flowing and static conditions and monitored the time to 50% loss of equilibrium (LOE). We used 123% TDG for the supersaturation treatments because this tension generally resulted in LOE in 5 h or more during preliminary experiments, whereas higher TDG levels caused rapid LOE, potentially making it difficult to detect treatment-level effects, and lower TDG levels result in prohibitively long experiments. Rainbow trout were used as study subjects because they are the species most frequently used in GBT laboratory experiments (Pleizier, Algera, et al., 2020), they are active swimmers in the wild (Watson et al., 2019), and *O. mykiss* are particularly sensitive to GBT effects (Kovac, Pleizier, & Brauner, 2021; Pleizier, Algera, et al., 2020) and are thus a good GBT model species for conservation purposes. Our results will help elucidate whether findings from studies conducted in static water can be used to predict the progression of GBT in more active animals.

2 | METHODS

2.1 | Subjects

The rainbow trout in this study were spawned in October 2019 at the Freshwater Fisheries Society of British Columbia's Vancouver Island Trout Hatchery and were transferred to their Fraser Valley Trout Hatchery for final rearing. We acquired the fish on June 25, 2020, and held these fish in a 15,000 L recirculation system at the University of British Columbia. The mean water temperature was 9.8°C during holding and we kept the fish on a 12-h light/dark cycle. We fed fish 1.5% of their body weight in commercial pellet food (EWOS Pacific feed) three times a week prior to the study. We conducted experiments between November 24 and December 18, 2020, and fasted all fish 39–48 h prior to the experiments. The average weight of fish at the time of the study was 24.34 g (±0.56 SE, $n = 192$) and the average fork length was 129 mm (±1 SE, $n = 192$).

2.2 | Generating TDG supersaturation

We generated TDG supersaturated water using a pressurized stainless-steel column (12″ pressurized packed column for supersaturated oxygen, model number X024656-01, Pentair Aquatic Ecosystems Inc., USA) as previously described in Pleizier, Nelson, Cooke, & Brauner, 2020.

2.3 | Measuring TDG

We measured TDG with a Point Four Tracker Total Gas Pressure Meter (model number 1SSM100, Pentair Aquatic Eco-Systems Inc., USA). We knocked the submerged probe against the bottom of the tank or the flume while taking TDG measurements to dislodge any

bubbles adhering to the Silastic tubing (Pleizier, Cooke, & Brauner, 2021). Once the percent TDG reading of the probe was stable for 2 min we assumed that the gas pressure in the Silastic tube had equilibrated with the water.

2.4 | Calibrating the TDG meter

We calibrated our TDG meter according to a method adapted from the US Geological Survey (Pleizier et al., 2021; Tanner & Johnston, 2001). We corrected the reading of the atmospheric pressure sensor to the atmospheric pressure reported by Environment and Climate Change Canada at the Vancouver International Airport (YVR). Atmospheric pressure reported by Environment and Climate Change Canada is corrected to sea level but as our facility is close to sea level the reading is accurate. We calibrated the TDG pressure sensor in the Silastic tubing of the probe using two points, one at atmospheric pressure and the other in a pressurized chamber at 300 mmHg gauge pressure. This calibration range was equivalent to 100%–139% TDG at atmospheric pressure of 760 mmHg.

2.5 | Swimming treatments

We conducted swimming treatments in a 90 L Loligo Systems swim tunnel (model number SW10200, Loligo Systems, Denmark), which has a swimming chamber with the dimensions 66 (length) × 20 (width) × 20 (height) cm. We calibrated the motor speed settings against water flow using a flowmeter prior to the experiment. We covered the top of the upstream third of the swimming chamber with black plastic to encourage fish to swim in the anterior region of the chamber.

The evening prior to a swimming trial, we introduced 12 fish into the flume, for a mean density of 9.84 g/L, to habituate overnight. The flume contained air-equilibrated water flowing at a velocity of 5.8 cm/s (approximately 0.5 BL/s) with a water replacement rate of 3.7 L/min. We started the experimental treatment the next morning. We conducted each treatment with four replicates each containing 12 fish. One swimming group treated with TDG supersaturation was replaced because TDG during the trial reached 126%, which we considered too high for comparison with the other groups. The two treatments in the flume included swimming at 2 BL/s in 100% TDG and swimming at 2 BL/s in 123% TDG. To initiate the swim trial, we turned on a bright overhead light to promote swimming and increased the water velocity in the flume to 23.0 cm/s (approximately 2 BL/s). This water velocity is approximately 37% of their U_{crit}, which we measured using separate fish during a preliminary study. If it was a control treatment, we waited 40 min before beginning the trial during which time air-equilibrated water was replaced at 3.7 L/min. If it was a TDG supersaturated treatment, we increased TDG in the flume over the course of 40 min. To do this we turned off the air-equilibrated water and opened the valve of a header tank where air-equilibrated water and TDG supersaturated water mixed to achieve the target TDG level of 123% TDG. Based on preliminary experiments we know that the 123% TDG treatment water replaces the 100% TDG water to obtain the target TDG level in the swimming chamber of the flume within 40 min. The replacement rate of TDG supersaturated water in the flume was approximately 10.6 L/min. For all swimming treatments, we monitored the fish for LOE remotely every 10 min using a camera (Geeni HD Hawk 21080p Outdoor Security Camera, model number GN-CW004-PARENT, Merkury Innovations LLC, USA) over the course of 10.5 h or until 50% of the fish in the replicate had lost equilibrium. During each trial, there were between 1 and 7 fish that would not swim but instead rested with their head oriented into the flow and their caudal fin wedged into the stainless-steel mesh at the downstream end of the flume. This behaviour differed from LOE, which we describe below. To promote swimming, we disturbed any resting fish every 10 min as necessary by tapping on the side of the swimming chamber or touching the water overhead. If this did not stimulate swimming, we gently prodded the fish with a metal rod.

In contrast to fish that stopped swimming but were still capable of doing so, fish that lost equilibrium lay flat against the mesh at the downstream end of the flume. If the fish maintained this position for at least 5 s we gently prodded the fish with a rod to encourage swimming. If the fish did not resume swimming, we reduced the water velocity to 5.8 cm/s for 20 s and prodded the fish again. If at this point the fish did not swim or maintain equilibrium, we considered it to have lost equilibrium and removed the individual from the flume. After removing a fish, we continued the swimming trial. All fish that we found lying flat against the mesh at the back of the flume were unable to maintain equilibrium at lower water velocities.

2.6 | Static treatments

We conducted static treatments in 100 cm diameter cylindrical tanks filled to a depth of 63 cm and containing 490 L of water. We achieved the TDG supersaturated treatments of 123% by mixing air-equilibrated water and TDG supersaturated water in a bucket that overflowed into the tanks (Pleizier, Nelson, et al., 2020). Control treatment water also overflowed into the tanks from a bucket. The tanks had a mean water replacement rate of 4.5 L/min. We placed six fish in each cage and added two cages to each treatment tank, for a total of 12 fish per replicate. Cages had the dimensions 35.5 (length) × 23.0 (width) × 16.0 (height) cm and fish in the cages were at a mean density of 12.38 g/L. We tested four replicates containing 12 fish each in the tanks with the static flow at both 123% TDG and 100% TDG. Fish were able to make voluntary movements in the cages but were generally quiescent during observations. We monitored the fish every hour and removed any that lost equilibrium. We ended the trial in a TDG supersaturation treatment replicate when 50% of the fish in that replicate had lost equilibrium. We terminated static treatments at 100% TDG when all the static TDG supersaturation replicates tested on that day had reached 50% LOE.

2.7 | Sampling

We sampled all the fish from each treatment group in the same manner. We transferred fish to a bucket and euthanized them using MS-222 buffered with sodium bicarbonate (200 mg/L MS-222 and 400 mg/L sodium bicarbonate) in water at the TDG treatment level. We then removed the fish from the water and examined them on their left side for the presence of GBT symptoms, including bubbles under the skin, bubbles between the fin rays, and exophthalmia (Pleizier, Nelson, et al., 2020). We removed the second gill arch on the left side using scissors, immersed the gill in water at the TDG treatment level, and examined it under a microscope for the presence of bubbles in the gill filaments. We measured the fork length and weight of each fish.

2.8 | Water quality

We measured TDG, barometric pressure, dissolved oxygen, and temperature after each trial in the swimming chamber of the flume and before and after each trial in the static treatment tanks. We did not measure TDG during trials as this would disturb the fish. TDG measurements during preliminary experiments indicated that TDG was stable over time in the flume and in the static tanks and that the addition of fish did not reduce TDG levels by more than 2% TDG, which is within the resolution (±2% TDG) of the TDG meter. We used API kits to measure pH, ammonia, and nitrite in the swimming chamber of the flume at the end of each swimming treatment and in one control and one TDG supersaturated treatment tank at the end of each static treatment. As the static treatment tanks are all supplied by the same recirculating system, we deemed that testing two of the tanks was adequate to characterize pH, ammonia, and nitrite in all tanks. We used dechlorinated Vancouver city water for all treatments.

2.9 | Analysis

We examined the relationship between time to LOE and the swimming treatments using Cox proportional hazards models. These are survival models that fit an exponential relationship between the time to an event (time to 50% LOE in this case) and predictor variables. The equation below describes the Cox proportional hazards mixed effects model,

$$h(t) = h_0(t)\,exp\left(b_1x_1 + b_2x_2 + \ldots b_px_p + a_j\right)$$

in which $h(t)$ is the hazard function, which is the probability of having an event occur at time t given that the subject has survived until that time; h_0 is the baseline hazard, which is the hazard if all the coefficients (b_i) are equal to zero; x_i is the fixed effects, which are the predictive variables that affect the time to an event; b_i is the coefficients of the fixed effects, which indicate the effect size of these predictors; and a_j is the random intercept for the j-th cluster. We then used a hazard function to estimate the hazard ratio for each predictor. The hazard ratio of the categorical fixed effect indicates the hazard rate of each treatment group in comparison to a reference group.

We modelled time to LOE using the 'coxme' package from the R environment (version 2.2–14; Therneau, 2019a). The full model included swimming treatment as a fixed effect and replicate as a random effect. We used AIC values to compare the full model to two reduced models, one without a fixed effect for swimming treatment and one without replication as a random effect. We selected the model with the lowest AIC value and ran it again using the coxph function (package 'survival', version 3.1–8; Therneau, 2019b), with random intercepts specified as offsets, to estimate coefficient standard errors and confidence intervals, and to test proportional hazards and linearity.

3 | RESULTS

Fish weight ($F[1] = 26.63$, $p < .001$) and fork lengths ($F[1] = 23.77$, $p < .001$) differed significantly between swimming and static treatments with an average weight of 21.64 *g* (±0.61 g SE, $n = 96$) and an average fork length of 125 mm (±1 mm SE, $n = 96$) for the fish in the swimming treatments and 27.03 *g* (±0.85 *g* SE, $n = 96$) and 133 mm (±1 mm SE, $n = 96$) for the fish in the static treatments (Table 1). There were no significant differences in lengths ($F[1] = 0.25$, $p = .62$) and weights ($F[1] = 0.20$, $p = .65$) between TDG treatments. Fish in the swimming treatment were thus exposed to water flowing at 1.8 BL/s (23 cm/s) during the trial.

The mean TDG level at the end of the trials was 102% (±0 SE, mean pressure above ambient air pressure [ΔP] = 16 mmHg) for the air-equilibrated TDG treatments and 123% (±0 SE, ΔP = 173 mmHg) for the TDG supersaturated groups (Table 2). The mean temperature for all treatments was 10.1°C (±0.1 SE); the mean temperature for the TDG supersaturated treatment groups was somewhat higher (10.4°C ± 0.0 SE) than the air-equilibrated TDG treatments (9.8°C ± 0.0 SE) in both static and swimming treatments (Table 2). The pH was 6.6 for all treatment groups. Ammonia was 0.25 ppm in the last replicate of the static treatment in both TDG supersaturated and air-equilibrated conditions but was 0.00 ppm for all other samples. Nitrite was 0 ppm for all treatment groups.

TABLE 1 Mean fork length (FL) and weight of rainbow trout (*Oncorhynchus mykiss*) in each treatment group

Treatments	Average of FL (mm)	Average of weight (g)
Static	133	27.03
100% TDG	133	26.91
123% TDG	133	27.15
Swimming	125	21.64
100% TDG	126	22.23
123% TDG	124	21.05
Grand Total	129	24.34

TABLE 2 Water quality was measured before and after experimental trials

Treatments	ΔP (mmHg, ±SE)	BP (mmHg, ±SE)	%TDG, ±SE	Dissolved oxygen (mg/L, ±SE)	Temperature (°C, ±SE)
Static					
100% TDG beginning of trial	20 (±3)	765 (±1)	103 (±0)	13.48 (±0.09)	9.7 (±0.0)
100% TDG end of trial	16 (±4)	760 (±3)	102 (±0)	13.06 (±0.04)	9.8 (±0.0)
123% TDG beginning of trial	179 (±2)	766 (±1)	123 (±0)	15.04 (±0.08)	10.2 (±0.1)
123% TDG end of trial	172 (±3)	759 (±4)	123 (±1)	14.73 (±0.08)	10.3 (±0.1)
Swimming[a]					
100% TDG end of trial	16 (±1)	768 (±1)	102 (±0)	12.83 (±0.12)	9.8 (±0.0)
123% TDG end of trial	174 (±6)	767 (±4)	123 (±0)	14.91 (±0.07)	10.4 (±0.0)

Note: ΔP is the difference in pressure between barometric pressure and the total dissolved gas pressure, BP is barometric pressure and %TDG is the per cent total dissolved gas.

[a]We did not measure water quality parameters at the beginning of swimming trials because we ramped up TDG treatments in the flume at the beginning of trials and taking measurements in the flume during trials at the end of the ramping period would have been disruptive to the study subjects.

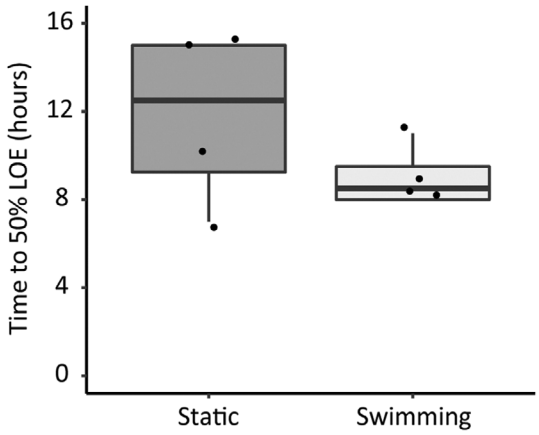

FIGURE 1 Time to 50% loss of equilibrium (LOE) for rainbow trout (*Oncorhynchus mykiss*) exposed to 123% total dissolved gas in either static flow or 23.0 cm/s (1.8 BL/s) flow. Each data point represents one replicate; each replicate contained 12 fish.

3.1 | Time to 50% LOE

No fish lost equilibrium in the air-equilibrated (102% TDG) treatments. The mean time to 50% LOE in the static treatment in TDG supersaturated water was 12 h (±2, $n = 4$ replicates with 12 fish per replicate) compared to 9 h (±1, $n = 4$ replicates with 12 fish per replicate) for the swimming treatment in TDG supersaturated water (Figures 1 and 2). The model of time to 50% LOE was not significantly improved by including swimming treatment as a fixed effect (ΔAIC decreased by 2 with the removal of a fixed effect for swimming treatment; Table 3), which indicates that there was no significant difference in the time to 50% LOE between static and swimming treatments. The time to 50% LOE in the static treatment replicates ranged between 7 and 15 h to 50% LOE between replicates, whereas the time to 50% LOE for the swimming treatment ranged between 8 and 11 h (Figure 1). We observed bubbles in the gills and under the skin of most fish exposed to TDG supersaturation at the time of sampling (Table 4). None of the fish in the air-equilibrated treatments had symptoms of GBT.

4 | DISCUSSION

Based on our hypothesis we predicted that locomotion would accelerate the progression of GBT. The swimming treatment did not significantly improve the model of the time to 50% LOE, which indicates that locomotion did not have a significant effect. We compare our results to those of other studies and discuss potential sources of differences. We explore the implications of our findings for interpreting TDG effects in wild free-swimming fish and propose directions for future study.

4.1 | Comparison to previous studies

Based on our observations, mortality caused by TDG supersaturation generally occurs within 1 h of LOE. Thus, time to LOE can be compared with the time to mortality data, the latter of which has been used extensively as an endpoint in the literature. In contrast to our results, we note that GBT outcomes in the study by Gray et al. (1983) were reached earlier in the present relative to the absence of swimming at TDG levels below 133% TDG. Based on the line of best fit, black bullhead exposed to 123% TDG would reach a median time to mortality (LT50) in 21 h in static flow and in 7 h swimming at 1 BL/s (Gray et al., 1983). The latter is similar to our mean values of 9 h until time to 50% LOE during swimming, but the former is considerably longer than our mean value of 12 h until time to 50% LOE in the static exposure. In comparison, common carp had a considerably longer time to LT50 (Gray et al., 1983) than rainbow trout and black bullhead in both swimming and static treatments, possibly indicating species differences. The error bars in the plots for both black bullhead and common carp suggest that variation around the mean LT50 values did not differ greatly between the two swimming treatments. Although we

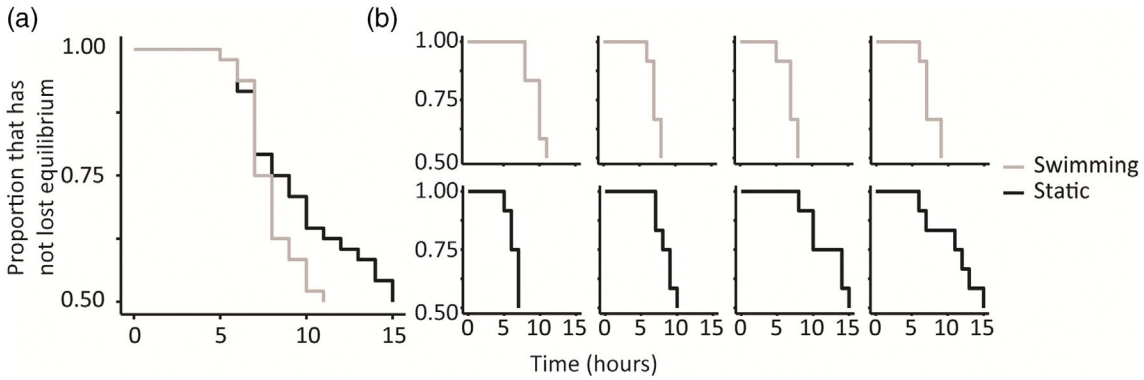

FIGURE 2 Survival plots of the time to loss of equilibrium of rainbow trout (*Oncorhynchus mykiss*) exposed to 123% total dissolved gas in either static flow or 23.0 cm/s (1.8 BL/s) flow. (a) Survival plot of all replicates combined; each line on the plot represents the time to 50% loss of equilibrium of a total of 48 fish. (b) Survival plots of the time to 50% loss of equilibrium of individual replicates; each replicate contained 12 fish. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Cox proportional hazards models of the time to 50% loss of equilibrium of rainbow trout (*Oncorhynchus mykiss*) exposed to 123% total dissolved gas

Model	Effects	Coefficient	Hazard ratio	95% confidence interval	Coefficient *p*-value	AIC
Model 1	Swimming	0.81	2.24	−0.89, 2.50	.35	246
	Replicate	-	-	-	-	
Model 2	Replicate	-	-	-	-	244
Model 3	Swimming	0.71	2.04	0.08, 1.35	.03	278

TABLE 4 Proportions of fish with symptoms of gas bubble trauma at the time of sampling (time at 50% loss of equilibrium for TDG supersaturation treatments or at the end of the trial for 100% TDG treatments)

Treatments	% of all fish with bubbles in the gills at the time of sampling ($n = 48$)	% of fish that lost equilibrium with bubbles in the gills ($n = 24$)	% of fish with bubbles on the exterior at the time of sampling ($n = 48$)	% of fish that lost equilibrium with bubbles on the exterior ($n = 24$)
Static, 100% TDG	0	0	0	0
Static, 123% TDG	67	100	75	100
Swimming, 100% TDG	0	0	0	0
Swimming, 123% TDG	65	96	88	96

compare our results with the values in Gray et al. (1983) at 123% TDG based on the lines of best fit, we caution that their dataset has only one treatment mean below 123% TDG in the static treatment (with considerable variation within that group), and no values below 125% TDG for the swimming treatment. Thus, estimates of LT50 values at 123% TDG based on the line of best fit should be interpreted with caution.

Bouck et al. (1976) also observed species differences in LT50 between fish exposed to swimming and non-swimming treatments. The authors report that largemouth bass forced to swim in a current of unknown velocity at 140% TDG reached LT50 in 19 h in the swimming treatment compared to 26 h in non-swimming treatments. The text is unclear as to whether LT50 for swimming fish was tested at other TDG levels. In the same study, the tolerance of sockeye salmon to TDG supersaturation did not differ between swimming treatments. Swimming speeds and TDG levels were not reported for the experiments on sockeye salmon. Based on both these results and our own, we speculate that salmonids are less vulnerable to the effects of locomotion on GBT than other species. However, because so few details are given about the methods and the results in the Bouck et al. (1976) study, it is difficult to compare their results to the current study, and clearly further studies are required.

It is possible those different swimming speeds have different effects on the progression of GBT and that this may have contributed

to differences between our study, where we observed no significant effect of locomotion and others where locomotion promoted GBT effects. Fish in our swimming treatment swam at 1.8 BL/s (23 cm/s) which was equivalent to approximately 37% of U_{crit} based on our preliminary measurements. In the study by Gray et al. (1983), common carp with a mean length of 8.0 cm swam at a speed of 1 BL/s (8 cm/s). Katopodis and Gervais (2016) did a review of U_{crit} values for common carp and calculated a mean value of 70.4 cm/s, which is about 4.6 BL/s based on the reported body length (15.2 cm). Assuming the U_{crit} values in BL/s reviewed by Katopodis and Gervais (2016) are relevant for the population of carp used in the study by Gray et al. (1983), these were tested at a swimming speed that was 22% of U_{crit}. Thus, although our fish were tested at a higher speed in proportion to U_{crit} compared to carp, the effect on time to 50% LOE was smaller for the rainbow trout in our study, which indicates a possible species difference.

4.2 | Implications and future directions

The 3 h difference in the meantime to 50% LOE between the swimming and the static treatment is small and not statistically significant. However, the difference between time to LOE between swimming and static treatments is potentially greater at lower TDG levels (e.g., Gray et al., 1983), during which fish survive longer exposures. In future studies, it would be of interest to compare swimming treatments to static treatments at lower TDG levels to determine whether the effects of locomotion on GBT are more pronounced in these conditions.

Another consideration is whether the swimming speed tested in our experiment reflects rainbow trout's swimming behaviour in the field. Watson et al. (2019) tracked triploid rainbow trout (mean length 49.7 cm) that were at least several years old using acoustic telemetry tags. They measured a mean swimming speed of 0.61 BL/s during the summer, with a maximum speed of 1.24 BL/s. In a study by Warner and Quinn (1995), adult rainbow trout (mean length 41.4 cm) tracked during the summer and fall using radio tags had a mean swimming speed of 0.3 BL/s and a maximum speed of 0.6 BL/s. James and Kelso (1995) tracked two adult rainbow trout (53.5–46.3 cm body length) for 1.58–6.25 h, respectively, during the summer using radio tags and observed a mean swimming speed of 0.3 BL/s and a maximum speed of 1 BL/s. Thus, the swimming speed used in our experiment (1.8 BL/s) appears to be higher than those routinely used by rainbow trout in the field; although we note that smaller fish are generally able to maintain greater speeds in terms of BL/s than larger fish and that rainbow trout in these field studies were larger than those in this study. Testing the effect of swimming at higher velocities could produce different results in terms of vulnerability to GBT, but these velocities may not reflect the behaviours of rainbow trout in the field. However, experiments at greater swimming velocities may be relevant for more active species and populations, such as anadromous salmonids, which use bouts of rapid swimming to cross velocity barriers (Hinch and Rand, 1998; Hinch et al., 2002).

Whereas locomotion did not have a significant effect on the results, other factors warrant further study. One such factor is depth, which has an important protective effect against GBT (e.g., Pleizier, Nelson, et al., 2020). The effect of exposure to TDG supersaturation at a constant depth can be modelled based on the increase of hydrostatic pressure with depth (Pleizier, Nelson, et al., 2020). However, fish are unlikely to inhabit constant depths in rivers. Antcliffe, Fidler, and Birtwell (2002) studied the effects of intermittent depth use on the progression of GBT in rainbow trout, but it is unknown whether the pattern of depth exposure in this study is similar to that of fish in rivers. It would be of interest to conduct tracking studies to determine the depth use of fish, and to use the results to design studies of the effect of intermittent depth use on GBT.

5 | CONCLUSIONS

Our results indicate that locomotion does not have a significant effect on the progression of GBT in rainbow trout. If locomotion does not have an effect on time to GBT this simplifies the application of lab results conducted in low-flow conditions to active wild fish. Thus, future research can focus on other factors relevant to the application of GBT data from lab experiments to conditions in rivers downstream of dams, such as the effect of intermittent depth use.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in UBC Research Data Collection, Scholars Portal Dataverse at https://doi.org/10.5683/SP2/HHBOLL.

ORCID

Naomi K. Pleizier https://orcid.org/0000-0001-5255-082X

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