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Title: Understanding gas bubble trauma in an era of hydropower expansion: How do fish compensate at depth?

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Abstract: Hydrostatic pressure is known to protect fish from damage by total dissolved gas (TDG) supersaturation, but empirical relationships are lacking; in this study we demonstrate the relationship between depth, TDG, and gas bubble trauma (GBT). Hydroelectric dams generate TDG supersaturation which causes bubble growth in the tissues of aquatic animals, resulting in sublethal and lethal effects. We exposed fish to 100, 115, 120, and 130% TDG at 16 and 63 cm of depth and recorded time to 50% loss of equilibrium and sublethal symptoms. Our linear model of the log-transformed time to 50% LOE ($R^2 = 0.94$) was improved by including depth. Based on our model, a depth of 47 cm compensated for the effects of 4.1% (±1.3 SE) TDG supersaturation. Our experiment reveals that once the surface threshold for GBT from TDG supersaturation is known, depth protects rainbow trout (*Oncorhynchus mykiss*) from GBT by 9.7% TDG supersaturation per meter depth. Our results can be used to estimate the impacts of TDG on fish downstream of dams, and to develop improved guidelines for TDG.

Introduction

The recent increase in dam construction is altering freshwater habitats worldwide. The International Commission On Large Dams has registered over 59 000 dams with a height that exceed 15 m (ICOLD 2018), and that number is growing as approximately 3700 hydroelectric dams with a capacity over 1MW were planned or under construction as of 2015 (Zarfl et al. 2015). These new dams will reduce the number of large free-flowing rivers by 21% (Zarfl et al. 2015). A recent mapping exercise revealed that only 37% of rivers longer than 1,000 kilometres remain free-flowing over their entire length (Grill et al. 2019). Dams benefit humans by regulating water supply, preventing floods, and generating electricity, but dams also threaten biodiversity (Vörösmarty et al. 2010). Changes such as reduced connectivity, habitat alteration, and changes in flow regimes and sediment transportation can impact fish communities, which is of concern because much of the proposed hydroelectric dam construction will occur in areas of high aquatic biodiversity (Winemiller et al. 2016). Regions with increasing hydroelectric development also tend to be developing countries or emerging economies (Zarfl et al. 2015) where freshwater fisheries are an important source of protein (Mcintyre et al. 2016). One of the ways that dams have the potential to harm aquatic animals is by generating total dissolved gas (TDG) supersaturation, which can affect their health and survival, although this has not received the same attention as other issues like reductions in connectivity from damming.

TDG supersaturation downstream of hydroelectric dams causes gas bubble trauma (GBT) in water-breathing animals (see review in Weitkamp and Katz 1980). Air that mixes with water as it passes through spillways or that is injected into turbines is forced to depth and dissolves in relation to hydrostatic pressure. As that water returns to the surface, where hydrostatic pressure is lower, it is supersaturated with TDG. Water-breathing animals equilibrate with this supersaturated TDG and gases form bubbles at nucleation sites in their tissues; a process somewhat analogous to decompression sickness in SCUBA divers (see review in Blatteau, Souraud, Gempp, and Boussuges (2006)). GBT resulting from TDG supersaturation commonly manifests in fish as gas bubbles in the lateral line, behind the eyes, between the fin rays, under the skin (including in the buccal cavity area), and in the blood (see review in Weitkamp and Katz 1980). TDG supersaturation can also cause swim bladder over-inflation (Shirahata 1966; Fidler 1988; Shrimpton et al. 1990*a*, 1990*b*). These symptoms can lead to indirect effects such as tissue necrosis (Stroud et al. 1975), impaired development (Cornacchia and Colt 1984; Counihan et al. 1998; Geist et al. 2013), increased vulnerability to disease (Stroud et al. 1975; Schisler et al. 2000), increased risk of predation (Mesa and Warren 1997), and positive buoyancy (Shrimpton et al. 1990*a*, 1990*b*). Possibly as a result of positive buoyancy, there is evidence for depth compensation behaviour by fish in TDG supersaturated water (Dawley, Monk, Schiewe, Ossiander, and Ebel 1976; Lund and Heggberget 1985; Shrimpton et al. 1990*b*), which may alleviate GBT.

As depth increases, greater hydrostatic pressure causes bubbles to shrink; when the exterior pressure exceeds the interior pressure of the bubbles, those bubbles will collapse. The depth at which the sum of hydrostatic and atmospheric pressure exceeds the gas pressure of TDG supersaturated water is often used as an approximation of the depth at which bubbles collapse, which is known as the compensation depth. Equation 1 has traditionally been used to define the compensation depth for bubble growth and collapse in animal tissues in TDG supersaturated water,

(1)

$$h_c = \frac{(TDG_w - P_{atm})}{\rho \cdot g}$$

in which h_c is the compensation depth, TDG_w is the TDG pressure in the water, P_{atm} is the atmospheric pressure, ρ is the density of water, and g is gravitational acceleration (9.81 m/s^2). The density of water varies with temperature and salinity, but the effect of temperature is small over the range that is typically encountered downstream of dams in riverine systems. Freshwater has a density of approximately 1000 kg/m^3. Based on Eq. 1, the pressure exerted by each meter of freshwater should compensate for an additional 9.7% TDG above saturation and cause bubbles to collapse at that gas pressure. For this reason, for a given TDG_w the degree of supersaturation decreases as depth increases, even if the absolute TDG_w remains constant throughout the water column. In this document, all % TDG values represent the percent supersaturation of the water relative to the surface. For example, at a barometric pressure of one atmosphere, 760 mmHg, the TDG associated with a pressure of 836 mmHg would be referred to as 110% TDG (836/760 x 100) regardless of the depth at which the 836 mmHg TDG was measured.

Equation 1 describes the compensation depth for bubble growth based on an increase in hydrostatic pressure with depth, but the equation does not define the threshold for bubble growth in fish tissues because it does not take bubble physics and the conditions inside the fish into account. Bubbles collapse when the pressure outside the bubble is greater than the pressure inside the bubble. The surface tension at the curved surface of a bubble creates a pressure difference between the interior and the exterior of a bubble, which can be described by the simplified Laplace pressure equation as

(2) $$\Delta P = (2\cdot\sigma)/r$$

in which ΔP is the pressure difference between the inside and the outside the bubble, σ is the surface tension, and r is the radius of the bubble. Additional factors that can affect the threshold for bubble growth inside the fish include blood pressure, and oxygen consumption (and thus reduction in blood Po_2) at the tissues. For example, blood pressures (P_s) in resting rainbow trout (*Oncorhynchus mykiss*) range from 1.4 mmHg above ambient hydrostatic pressure in the cardinal vein to 38 mmHg above ambient in the ventral aorta (Kiceniuk and Jones 1977). The Po_2 in the cardiovascular system of rainbow trout in air equilibrated water ranges from 137 mmHg at rest to 126 mmHg during exercise in the dorsal aorta and from 33 mmHg at rest to 16 mmHg during exercise in the ventral aorta (Kiceniuk and Jones 1977). Thus, at all points in the circulatory system of rainbow trout (except for localized regions of low pressure, such as rotational flow), the additional pressure generated by the pumping of the blood by the heart causes pressures to exceed that of the surrounding ambient water, whereas oxygen consumption by the tissues results in TDG pressures that are below ambient. Fidler (1985) proposed a theoretical equation to represent the TDG threshold for bubble growth in fish tissues which incorporates both the Laplace equation and the conditions in the fish cardiovascular system, which can be rearranged as an equation for the prediction of the compensation depth,

(3)
$$h_c = \frac{TDG_{cv} - (P_{atm} + P_s + (2\cdot\sigma_b)/r + Po_2\cdot(1 - F))}{\rho\cdot g}$$

in which h_c is the compensation depth for bubbles in the blood, TDG_{cv} is the TDG at the site of bubble nucleation in the cardiovascular system, P_s is the system pressure at the point of bubble nucleation, σ_b is the surface tension of fish blood, r is the radius of the critical bubble nucleation sites, Po_2 is the partial pressure of dissolved oxygen in the surrounding water, and F is the oxygen uptake ratio across the gills (other parameters as in Eq. 1). In combination, the factors in Eq. 3 may explain why fish generally do not experience GBT in % TDG saturation levels between 100% and 110% TDG (see meta analysis in Fidler 1988), tensions that would be expected to induce bubble formation in water. Whereas the physical parameters in the equation can easily be measured, the physiological parameters at the location of bubble nucleation are difficult to estimate and will vary regionally within the fish. The anatomical location of the nucleation sites for the bubbles that cause mortality during GBT remain unknown, so the system pressure and TDG_{cv} at these critical locations cannot be defined accurately. Furthermore, the sizes of microbubbles in animals are difficult to quantify *in vivo*. It would be convenient if, once the threshold for bubble growth in fish tissues at the surface was known, Eq. 1 could be used to estimate changes in bubble growth with depth. Experimental data is necessary to determine whether Eq. 1 accurately describes the relationship between depth and GBT effects on fish in TDG supersaturated water.

Experimental studies have confirmed that depth reduces the impacts of TDG supersaturation on GBT in fishes; however, the compensation depth has not been defined experimentally. As predicted, a number of reports indicate that exposure to TDG supersaturation at fixed depths or in deep volition cages provides protection from GBT symptoms and mortality relative to surface exposures for both salmonids (Antcliffe, Fidler, and Birtwell 2002; Dawley et al. 1976; Fickeisen and Montgomery 1978; Knittel, Chapman, and Garton 1980; Lund and Heggberget 1985; Shrank, Dawley, and Ryan 1997) and non-salmonids (Fickeisen and Montgomery 1978; Shrank et al. 1997; Ryan and Dawley 1998). Intermittent exposure to greater depths also reduced GBT symptoms and mortality in salmonids (Weitkamp 1976; Knittel et al. 1980; Antcliffe et al. 2002).

These studies, not surprisingly, indicate that depth compensates for the impacts of TDG supersaturation on fish but do not provide a quantitative relationship between depth and GBT in fish tissues.

In this investigation we tested the hypothesis that from the threshold for GBT at the surface (approximately 110% TDG, see meta analysis in Fidler 1988), depth compensation in fish can be predicted by the model for depth compensation for large bubbles suspended in water (Eq. 1). Based on this hypothesis we would predict that a depth of 47 cm would compensate for the effects of 4.6% TDG supersaturation. To test this, we exposed rainbow trout to four nominal TDG supersaturation levels (100, 115, 120 and 130 % saturation) at two different depths (16 cm and 63 cm) to determine the relationship between depth and time to 50% loss of equilibrium. If the model for depth compensation is correct, then we predict that the inclusion of depth as a fixed effect should significantly improve the model of the relationship between % TDG saturation and time to 50% loss of equilibrium. We predict that for each meter of depth in freshwater the time to 50% loss of equilibrium should be the same as a surface exposure at a TDG that is 9.7% greater, or conversely that each meter of depth provides the protection equivalent to a reduction of 9.7% TDG supersaturation. Our findings on the relationship between depth and GBT will help managers estimate the impact of TDG generated by dams on fish populations given the levels of TDG and the depth inhabited by fish. This information can be used both to inform offsetting and mitigation strategies, as well as to develop more appropriate TDG guidelines.

Methods

Three-month-old, juvenile, female, Troutlodge jumper strain rainbow trout were obtained from the Little Cedar Falls Hatchery in Nanaimo, British Columbia on October 3, 2018, and held at the University of British Columbia for 15 days prior to experimentation. Tanks were maintained at a mean temperature of 11.3°C. Once the fish arrived at the UBC facility they were fed commercial feed three times a week at a maintenance ration of 1.5% body weight at each feeding. Mean fish weight was 15.3 g at the end of the experiment and two-way ANOVA revealed that the weight did not differ significantly between depth (*P*=0.26), treatment tank (*P*=0.12) or their interaction (*P*=0.12).

The experiments were conducted in 8- 700 L cylindrical tanks held within a 15 000 L recirculation system. Each tank had a diameter of 100 cm and a water depth of 63 cm. During testing fish were held in plastic cages with mesh side panels and a lid (35.5 cm (length) x 23.0 cm (width) x 16.0 cm (height)). The mean stocking densities in the cages were 50.5 kg/m^3, which should reduce adverse effects of dominance hierarchies at low densities and crowding effects at high densities (North et al. 2006). Preliminary video observations of rainbow trout stocked in cages were made to determine the density at which fish did not express stress-type behaviours as a result of crowding. Cages in the deep treatment were weighted to keeps them level with the bottom of the tanks during the experiment. Cages in the shallow treatment floated level with the surface throughout the experiment. The lights were on continuously during the experiment. TDG supersaturated water was generated using one 2.8 m tall, 0.3 m diameter pressurized stainless-steel column packed with bio balls (12” pressurized packed column for supersaturated oxygen, model number X024656-01, Pentair Aquatic Eco-systems). Water was pumped from a header tank into the column, which was pressurized with air at 30 PSI. A pressure transducer mounted on the pressurized packed column provided feedback to a variable frequency drive on the water pump. The system maintained the water depth in the column using a level sensor attached to a sight glass. If the water level rose above the sensor, the air turned on at a flow rate of 11 L/min; if the water level fell below the sensor the air turned off, with a lag period of 5 seconds before the air input valve could be opened again. Supersaturated water from the pressurized column was delivered by PVC pipes to each 700 L tank separately and flow was regulated using needle valves. Air-equilibrated water (100% TDG) was provided to each tank separately using an independent distribution system. The flow rates of the two water types were adjusted and allowed to mix in a 4.5 L bucket before overflowing into the experimental tanks to achieve nominal target TDG tensions of 100, 115, 120 and 130 % saturation. Water flow rate was approximately 6.8 L/min, with a tank water turn-over rate of about 1.2 h. Water drained from the tanks back into the sump of the recirculation system, where it was filtered, de-gassed, denitrified, and temperature controlled before returning it to the experimental system. Tests completed after the experiment indicated that surface cages had TDG levels that were 1% TDG less than the surrounding water and cages at 47-63 cm had TDG levels that were 0.5% TDG less than the surrounding water. As the differences in the TDG levels inside and outside the cages were smaller than the accuracy of the TDG meter (±2% TDG), TDG values were not corrected for cage effects.

TDG levels were measured using a Point Four Tracker Total Gas Pressure Meter (Pentair Aquatic Eco-Systems). The meter measures TDG by comparing the atmospheric pressure to the pressure of the gas that diffuses into the silastic tubing of the probe. The TDG meter was calibrated at the beginning of the experiment according to a protocol adapted from the USGS (Tanner and Johnston 2001). A two-point calibration of the TDG pressure sensor was performed. For the first point the gauge pressure of the TDG pressure sensor was measured using the dry probe at atmospheric pressure. The second point was measured by putting the probe in a pressure chamber and comparing the change in pressure measured by the TDG pressure sensor to the measurement of a separate pressure gauge when the chamber was at 200 mmHg above atmospheric pressure. TDG measurements from the experiment were corrected based on the measurements from the TDG pressure sensor calibration. The range of the two-point calibration is equivalent to 100-126% TDG, spanning most of the range used in this study. The TDG pressure sensor probe was also submerged in carbonated water to test for damage; very rapid increases in TDG indicate rips in the silastic tubing of the probe. The atmospheric pressure sensor was calibrated with the current atmospheric pressure reported by Environment and Climate Change Canada at the Vancouver International Airport. During the experiment, TDG was measured while knocking the probe continuously on the bottom or side of the tank to dislodge any bubbles that may have formed on the silastic tubing of the probe. It was assumed that the gas pressure in the silastic tubing of the probe was equilibrated with gas pressure in the water when the percent TDG remained stable for two minutes.

The treatment consisted of exposing fish at nominal TDG levels of 100, 115, 120 and 130% saturation (see Table 1 for measured TDG levels) in cages held at the surface (0-16 cm, the range representing the top and bottom of the cage) or at depth (47-63 cm). Fish were fasted for 48 h prior to the experiment and 6 fish were placed in each cage. Cages were allocated to treatments using a random number generator. Each tank had 2 cages at each depth, for a total of 12 fish at each depth/TDG treatment level (Table 2). TDG exposure start dates were staggered as indicated in Table 1. Fish were monitored for loss of equilibrium every hour for the first 12 h of exposure, every two hours from 12 to 24 h, and every 24 hours from day 2 to day 7. Cages were periodically disturbed to release bubble build-up. TDG was measured in each tank daily and again if the treatment reached 50% loss of equilibrium before 24 h of exposure. Oxygen and temperature were measured daily (Table 1) and pH, ammonia, and nitrite were measured in two tanks both at the beginning and at the end of the experiment.

At the first observation that a fish had lost equilibrium the cage was brought to the surface, the fish was quickly removed for sampling, and the cage was returned to its depth. This procedure was completed within 1 minute. If fish from multiple cages lost equilibrium simultaneously, the fish remained in their respective treatments until they could be sampled. Moribund fish were euthanized in water taken from the treatment tanks using a lethal dose of MS-222 (200 mg/L MS-222 and 200 mg/L sodium bicarbonate). Fish were placed on their left side and examined for exophthalmia, and for gas bubbles under the skin externally and in the buccal cavity, and between the fin rays. Fish were not monitored for bubbles in the lateral line because Dawley et al. (1976) observed that the removal of mucous can cause bubbles to form in the lateral line of rainbow trout that were not exposed to TDG supersaturation. The second gill arch was removed and immersed in the respective TDG water and examined under a dissecting microscope for the presence/absence of bubbles in the gill vasculature. Each fish was weighed, including the excised gill arch. When 50% of the fish in a depth/TDG treatment had lost equilibrium, the remaining fish were sampled in the same manner as the fish that had lost equilibrium. Control treatment fish were all sampled at the end of the experiment. All samples were collected in accordance with the guidelines of the Canadian Council on Animal Care as administered by the University of British Columbia (A15-0266).

The lm function from the R environment (R Core Team 2018) was used to build the linear model of the log-transformed time to 50% loss of equilibrium. The models were selected based on Akaike's information criterion (AIC). The full model included percent TDG as a continuous variable and depth as a categorical variable (levels were shallow depth, where the fish had access in the cage to 1-16 cm of depth, and deep depth, where the fish had access in the cage to 47-63 cm of depth). The full model was compared to two reduced models, one which only included % TDG saturation as a fixed effect, and the other which only included depth as a fixed effect. The best model of these three was compared to an additional model with an added interaction between the % TDG saturation and depth. This model was compared to a model with an added quadratic term for percent TDG. The final model was examined for influential cases and outliers by looking at the values of the standardized residuals, Cook's distance, DFBetas, DFFit, hat values, and covariance ratios. The assumption of independence was tested using the Durbin-Watson test, and the assumption of no multicollinearity was tested using the variance inflation factor (VIF), average VIF, and tolerance (1/VIF). The *z*-scores of the skew and kurtosis of the standardized residuals were examined to determine whether they were significant at $\alpha = .05$ (Field et al. 2012).

Results

TDG levels and water quality were stable in most treatments throughout the experiment (Table 1). Tank 8 was removed from the experiment because TDG dropped from 118% to 113% in the first 24 h of the experiment. The molar ratio of oxygen to nitrogen ranged from 0.51 to 0.53 between all treatments. Mean temperatures over the duration of the experiment ranged from 10.2 to 11.0 °C between TDG treatments (Table 1). Ammonia and nitrite were undetectable, and pH was 6.6 in all treatments at both the beginning and the end of the experiment.

All control treatment fish maintained equilibrium throughout the experiment, and the fish in the 117.5% TDG treatment at 47-63 cm depth did not reach 50% loss of equilibrium before the experiment ended after 168 hours of exposure (Fig. 1, Table 2). Generally, the fish in all treatments remained inactive at the bottom of the cage unless disturbed. We observed fish in TDG supersaturated water moving rapidly and erratically shortly before losing equilibrium. The control treatment fish did not exhibit symptoms of GBT, whereas 93% of the fish that lost equilibrium in the TDG supersaturation treatments exhibited gas bubbles in the blood vessels of the gills and 89% of the fish that lost equilibrium in the TDG supersaturation treatments exhibited gas bubbles on their external surfaces (Table 2). For all fish exposed to TDG supersaturation, both those that had reached LOE and those that had not, we observed external GBT on 79% of all fish and gas bubbles in the blood vessels of the gills of 57% of all fish. Gas bubbles on the exterior of the fish occurred most frequently between the fin rays. All the treatments at 47-63 cm depth reached 50% loss of equilibrium after the 0-16 cm depth treatments in the same tank (Fig. 1).

The optimal model for the natural logarithm of the time to 50% loss of equilibrium included both % TDG saturation and depth (R^2= 0.94, AIC 9, Fig. 2, Table 3); however, it was not improved by including the interaction between % TDG saturation and depth (AIC 11). A quadratic term for TDG did not improve the fit of the model (AIC 9, table 3). The time to 50% loss of equilibrium increased in the 47-63 cm depth treatment relative to the 0-16 cm depth treatment, such that the time to 50% loss of equilibrium in 47-63 cm at a given % TDG saturation is equivalent to the time to 50% loss of equilibrium at 0-16 cm at a % TDG saturation that is 4.1% (±1.3 SE) less. Certain data points exerted more influence than is desirable (Field et al. 2012), but given the small sample size and the good fit of the model we do not consider this a cause for concern. Nonetheless, neither skew nor kurtosis of the standardized residuals were significant at $\alpha = .05$.

Discussion

The results support our hypothesis that depth compensation for bubble growth in the tissues of fish exposed to TDG supersaturated water can be estimated based on the model for large bubbles suspended in water (Eq. 1) from the threshold for GBT in surface waters. Given the pressure exerted by freshwater (0.097 atm per meter depth), we predicted that a depth of 47 cm would compensate for the effects of 4.6% TDG supersaturation. Our data indicates that 47 cm of depth compensated for 4.1% (±1.3 SE) TDG supersaturation, such that the time to 50% loss of equilibrium at a depth of 63 cm was equivalent to the time to 50% loss of equilibrium at 16 cm at a level of supersaturation that is 4.1% (±1.3 SE) TDG less (*i.e.* a right shift of 4.1% TDG in Fig 2 due to a depth of 63 cm relative to 16 cm). Our data suggests that estimating compensation depth based on Eq. 1 is valid once the threshold TDG for GBT at the surface is known. For this reason, additional parameters from Eq. 3 that are difficult to estimate, such as bubble radius, system pressure, and the TDG pressure at the nucleation point, may not be necessary in estimating time to effects of GBT in TDG supersaturated water at different depths once those effects have been accounted for at the surface. That is, in fish at the surface, bubbles do not form until TDG exceeds a threshold that may be predicted by Eq. 3 (*i.e.* around 110%; the threshold is species and context specific, see below for further clarification), but beyond that, Eq. 1 may be used to calculate further depth compensation (see below for further elaboration). We can describe the relationship between the TDG threshold for GBT and depth as an equation,

(4)

$$TDG_{threshold} = TDG_{st} - h\left[\frac{TDG_{w} - P_{atm}}{\rho \cdot g}\right]$$

in which $TDG_{threshold}$ is the TDG threshold for the emergence of a GBT symptom of interest, TDG_{st} is the TDG threshold for the emergence of the symptom of interest at the surface, and h is the depth (other parameters as in Eq. 1). In Eq. 4, TDG_{st} can be determined experimentally or estimated using Eq. 3. It is important to note that our model is based on experiments that were conducted in depths no greater than 63 cm. It would be worthwhile to repeat the experiment with exposures at greater depths; however, achieving such depths were beyond the scope of our facility.

Depth compensation for GBT has been demonstrated in several studies, but the assumption that depth compensation for GBT in TDG supersaturated water can be predicted using the model for large bubbles suspended in water (Eq. 1) has not previously been experimentally tested. Knittel et al. (1980) found that correcting for depth based on the assumption of 9.7% TDG compensation per meter of depth improved the R^2 value of their dose-response curve from 0.95 to 0.97 when modeling the time to 50% mortality data for *O. mykiss* held at three different depths. The evidence from Knittel et al. (1980) supports our hypothesis but does not indicate whether the assumption of 9.7% TDG compensation per meter of depth is accurate. Fickeisen and Montgomery (1978) restricted fish to depths in TDG supersaturated water and found that greater depth increased the time to mortality, but the authors did not test their assumption of 10% TDG compensation per meter depth. Other studies have demonstrated the effects of depth on time to mortality and time to GBT symptoms (Antcliffe et al. 2002; Dawley et al. 1976; Lund and Heggberget 1985; Ryan and Dawley 1998; Shrank et al. 1997), but because most treatment groups were not restricted to small ranges of depth, the depth that the fish inhabited is not known, confounding quantitative estimates of the protection of depth on GBT. Jensen et al. (1986) modeled the data for time to 50% mortality from 8 studies and found that including depth improved their models, but in their models the effect of depth on the estimated TDG thresholds for 50% mortality at 50 days and 20 days was less than 9.7% TDG per meter depth. To our knowledge our study is the first to experimentally investigate the model of depth compensation for time to loss of equilibrium in fish (but see Shrimpton et al. (1990*a*, 1990*b*) for a model of swim bladder over-inflation in TDG supersaturation).

Our model describes the relationship between time to 50% loss of equilibrium and depth within the range of TDG supersaturations that we tested; however, our model does not explain the threshold of effect of TDG supersaturation at the surface in fish. In a review of time to mortality data as a function of TDG supersaturation, Fidler (1988) suggested that a threshold for GBT induced mortality for salmonids greater than 50 mm in length exists at approximately 76 mmHg TDG above saturation (110% TDG at an atmospheric pressure of 760 mmHg). The physiological basis for this threshold is not fully known, but may include the factors discussed previously, such as internal cardiovascular pressures and tissue oxygen consumption. Thus, a more complex model such as Eq. 3 may be useful for predicting the threshold of effect of TDG supersaturation on fish, whereas our model (Eq. 4) may be adequate to describe the effect of depth on time to 50% loss of equilibrium at TDG levels above the experimentally determined surface threshold.

Changes in the internal environment of fish may also affect bubble growth in tissues and should be considered when applying the relationship between depth and GBT to fish in different states. For example, our model is based on data from fish that were mostly inactive, whereas exercise can have multiple different effects on bubble growth. Exercise may promote bubble nucleation either through tribonucleation (McDonough and Hemmingsen 1984*a*, 1984*b*; Mcdonough and Hemmingsen 1985) or possibly low-pressure regions formed by rotational flow (see review in Blatteau et al. (2006)). Conversely, the oxygen content of the blood decreases and the blood pressure increases during exercise; both of these factors could potentially reduce the likelihood of bubble growth in the cardiovascular system. To our knowledge, only two studies have investigated the effects of exercise on the progression of GBT, the results of which indicate that exercise can decrease the time to mortality in some species but not others (Bouck et al. 1976), depending on the level of TDG saturation (Gray et al. 1983).

Extrinsic factors should also be considered when assessing the impact of TDG supersaturation on wild fish. Although depth appears to be of particular importance (Jensen et al. 1986), other variables such as temperature (Antcliffe, Birtwell, and Fidler 2003; Bouck et al. 1976; Ebel et al. 1971; Fickeisen, Montgomery and Hanf Jr 1974; Nebeker et al. 1979) and dissolved oxygen to nitrogen ratios (Jensen 1988; Nebeker et al. 1976; Nebeker et al. 1979; Rucker 1975) have also been demonstrated to affect the severity and progression of GBT.

Including depth as a factor can improve estimates of TDG supersaturation effects on fish downstream of dams. If freshwater compensates for the effects of approximately 9.7% TDG per meter, we can use this relationship to estimate the effects of TDG supersaturation on GBT symptoms and mortality of fish given the depth that they inhabit. If the effects of TDG over time on a species of a certain size class are well characterized by experiments done at the surface, we can assume that for each meter of increased depth, the GBT effects will be reduced to the same degree as lowering TDG supersaturation by 9.7% TDG. Knowledge of the depth of a body of water and the depth use of a population would allow us to estimate time to loss of equilibrium, and thus the death of fish in TDG supersaturated water downstream of dams. This information can be used to determine the extent of the impact of TDG on fish populations and the need for hydroelectric utilities to offset or mitigate this impact. Depth effects could also be incorporated into TDG guidelines, such that the allowable % TDG threshold increases by 9.7% per meter depth.

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Table 1. Water quality of each total dissolved gas (TDG) and depth treatment exposure during the experiment.

Tank number	Treatment start date	Depth treatment (cm)	Mean %TDG (±SE)	O_2 (mg/L)	Mean temperature (°C)	Number of TDG measurements
1	October 18	0-16	117.9 (±0.2)	12.8 (±0.1)	10.8 (±0.1)	4
1	October 18	47-63	117.5 (±0.2)	12.7 (±0.1)	10.6 (±0.1)	8
2	October 22	0-16	122.2 (±0.4)	12.8 (±0.1)	10.4 (±0.2)	2
2	October 22	47-63	122.2 (±0.2)	12.9 (±0.1)	10.4 (±0.1)	3
3	October 18	0-16	125.2 (±0.5)	13.2 (±0.0)	11.0 (±0.1)	2
3	October 18	47-63	126.0 (±0.3)	13.5 (±0.3)	11.0 (±0.1)	2
4	October 22	0-16	134.0 (±0.8)	14.0 (±0.3)	10.7 (±0.0)	2

4	October 22	47-63	134.0 (±0.8)	14.0 (±0.3)	10.7 (±0.0)	2
5	October 18	0-16	117.2 (±0.4)	12.7 (±0.1)	10.7 (±0.1)	3
5	October 18	47-63	118.2 (±0.9)	12.6 (±0.1)	10.6 (±0.1)	5
6	October 18	0-16	102.4 (±0.1)	11.2 (±0.0)	10.2 (±0.1)	8
6	October 18	47-63	102.4 (±0.1)	11.2 (±0.0)	10.2 (±0.1)	8
9	October 18	0-16	102.2 (±0.1)	11.2 (±0.1)	10.2 (±0.1)	8
9	October 18	47-63	102.2 (±0.1)	11.2 (±0.1)	10.2 (±0.1)	8

Table 2. The time to 50% loss of equilibrium (LOE) of rainbow trout (*O. mykiss*) and proportions of fish with gas bubble trauma at the time of sampling (time at 50% loss of equilibrium or at 168 hours of exposure) in different total dissolved gas (TDG) and depth treatments.

Tank no.	Depth treatment (cm)	Mean %TDG (±SE; range)	Number of fish in the treatment	Time to 50% LOE (hours)	% of fish with bubbles in the gills at time of sampling	% of fish with bubbles on the exterior at time of sampling
1	0-16	117.9 (±0.2; 117.4-18.5)	12	72	66.7	91.7
1	47-63	117.5 (±0.2; 116.8-118.5)	12	-	16.7	83.3
2	0-16	122.2 (±0.4; 121.8-122.5)	12	20	75.0	75.0
2	47-63	122.2 (±0.1; 121.8-122.5)	12	48	66.7	75.0
3	0-16	125.2 (±0.5; 124.6-125.7)	12	9	66.7	75.0
3	47-63	126.0 (±0.2; 125.7-126.4)	12	24	58.3	91.7
4	0-16	134.0 (±0.8; 133.2-134.8)	12	5	91.7	100
4	47-63	134.0 (±0.4; 133.2-134.8)	12	6	75.0	91.7

5	0-16	117.2 (±0.4; 116.5-117.8)	12	48	75.0	83.3
5	47-63	118.2 (±0.7; 116.5-121.7)	12	96	25.0	83.3
6	0-16	102.4 (±0.1; 102.0-103.0)	12	-	0	0
6	47-63	102.4 (±0.1; 102.0-103.0)	12	-	0	0
9	0-16	102.2 (±0.1; 101.8-102.5)	12	-	0	0
9	47-63	102.2 (±0.1; 101.8-102.5)	12	-	0	0

Table 3. Comparison of linear models of the natural logarithm of the time to 50% loss of equilibrium data for rainbow trout (*O. mykiss*) held at different total dissolved gas (TDG) saturations and depths. Depth is a categorical variable with two levels, shallow (fish had access to depths between 0 and 16 cm) and deep (fish had access to depths between 47 and 63 cm); the coefficient for depth is the intercept for the deep treatment.

Fixed effects	Coefficient	Coefficient SE	Standardized β	AIC	*P*
Model 1				9	
Intercept	23.11	2.17			<0.001
TDG	-0.16	0.02	-0.96		<0.001
Depth, deep	0.66	0.21	0.32		0.022
Model 2				32	
Intercept	2.99	0.51			<0.001
Depth, deep	0.36	0.77	0.18		0.65
Model 3				16	
Intercept	22.42	3.20			<0.001
TDG	-0.16	0.03	-0.92		<0.001
Model 4				11	
Intercept	21.99	3.03			<0.001
TDG	-0.15	0.02	-0.91		0.002
Depth, deep	3.35	4.70	1.63		0.51
TDG x depth	-0.02	0.04	-0.13		0.59
Model 5				9	

Intercept	81.05	49.85			0.16
TDG	-1.11	0.81			0.23
TDG^2	0.003	0.003			0.29
Depth, deep	0.88	0.21			0.009

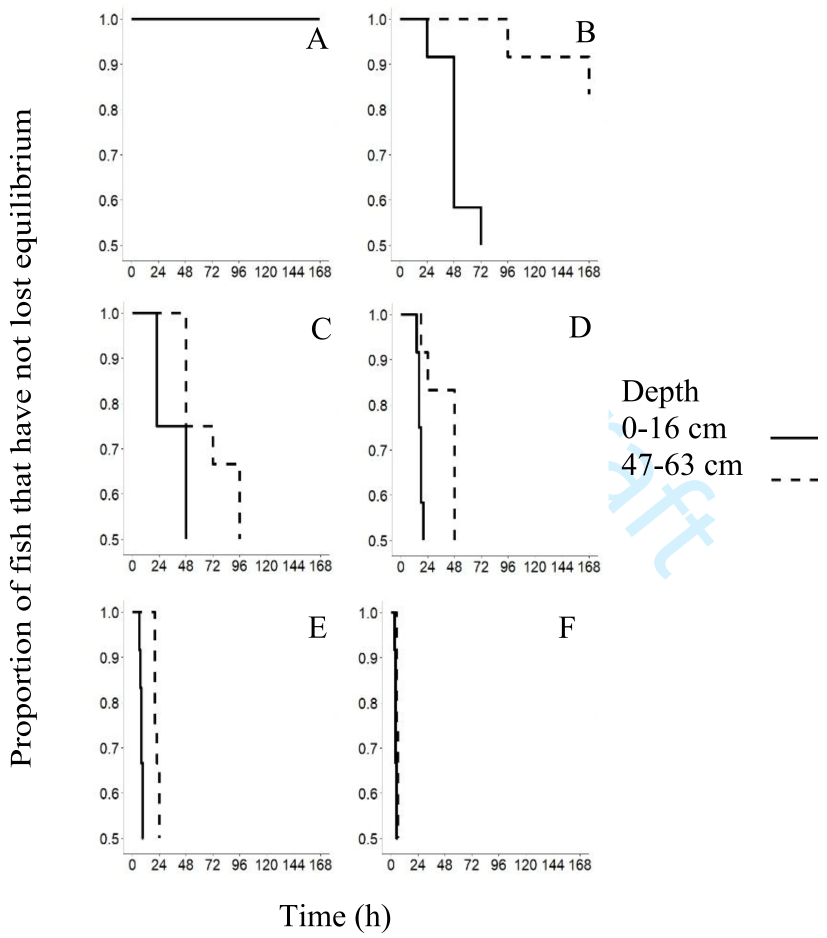

Figure 1. The proportion of rainbow trout (*O. mykiss*) that lost equilibrium at different depths and total dissolved gas treatments. Fish were exposed to nominal TDG tension treatments of (A) of 100% in tanks 6 and 9; (B) 115% in tank 1; (C) 115% in tank 5; (D) 120% in tank 2; and (E) 130% in tank 3, and (F) 130% in tank 4. Measured TDG values are reported in Table 1. Each line

represents two cages containing 6 fish that have been pooled, for a total of 12 fish per treatment. Depth refers to the range of depths available to the fish in each treatment.

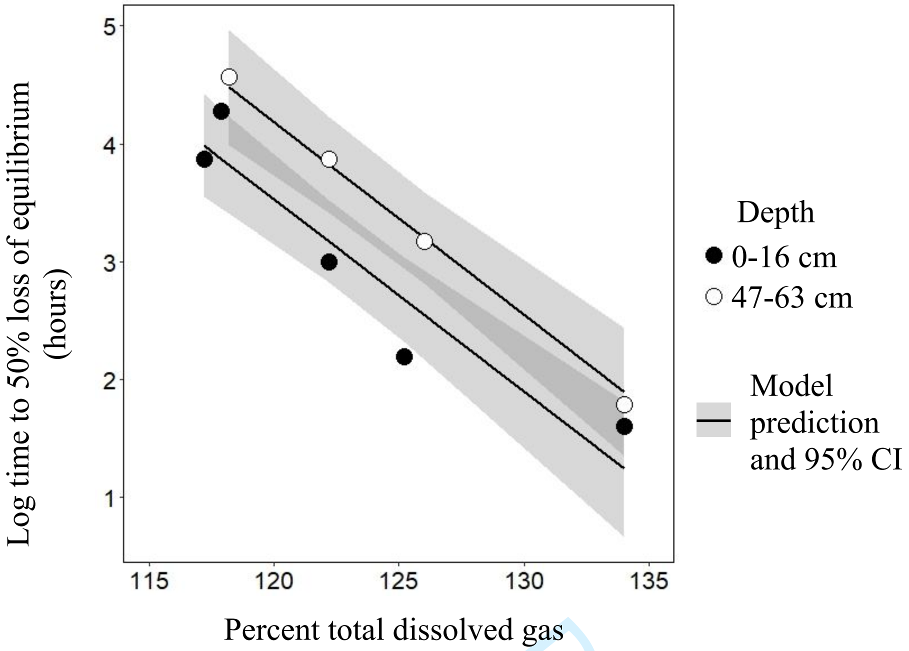

Figure 2. Data and linear model for the natural logarithm of time to 50% loss of equilibrium (±95% CI) for rainbow trout (*O. mykiss*) with percent total dissolved gas and depth as fixed effects. Each data point represents one replicate of 12 fish. Depth refers to the range of depths available to the fish in each treatment.