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The effect of total dissolved gas supersaturation on gas bubble trauma in juvenile rainbow trout (*Oncorhynchus mykiss*), juvenile kokanee (*Oncorhynchus nerka*) and two age classes of white sturgeon (*Acipenser transmontanus*).

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Draft

Abstract

Hydroelectric dams are an important source of electricity globally, but they can also cause total dissolved gas (TDG) supersaturation in rivers. Total dissolved gas supersaturation can harm fish through a condition called gas bubble trauma (GBT). Gas bubble trauma has been studied primarily in salmonids, such as rainbow trout and steelhead salmon (*Oncorhynchus mykiss*), but seldomly in non-salmonids like white sturgeon (*Acipenser transmontanus*). We assessed the vulnerability of juvenile rainbow trout (<1 year old), juvenile kokanee (*Oncorhynchus nerka*) (<1 year old), and two ages of white sturgeon (<1 year old and 3+ year old) to GBT. Bubble formation and the time to 50% loss of equilibrium (LOE) was quantified during exposure to nominal levels of 100, 115, 120 and 130% TDG. We predicted that all three species would show similar times to 50% LOE at a given TDG level. However, time to LOE was longer, the proportion of fish with external symptoms of GBT was lower and the proportion of fish with bubbles in the gills was higher or lower (dependant on age) in white sturgeon relative to rainbow trout and kokanee at a given TDG. The physiological basis for the difference is not known. However, it is important to consider species specific differences in TDG sensitivity in the conservation of vulnerable species.

Keywords: gas bubble disease, gill damage, hydroelectric dams, total gas pressure

Introduction

Dam construction has proliferated in recent years with 58,713 large dams registered globally (International Commission On Large Dams 2020). Whereas hydroelectricity is generally considered sustainable, environmental impacts of dams include changes in river morphology and flow, reduced downstream sedimentation (Kondolf et al. 2014), habitat fragmentation, reduced productivity, and changes in biodiversity (Jansson et al. 2000; Kupferberg et al. 2012).

Hydroelectric dams can also generate total dissolved gas (TDG) supersaturation downstream, which can cause tissue damage in aquatic animals as a result of bubble growth, known as gas bubble trauma (GBT) (see review in Weitkamp and Katz 1980).

Total dissolved gas supersaturation can occur at dams when spill water is released, and air is entrained in water that plunges to depth. At depth, hydrostatic pressure increases and the gases dissolve into the surrounding water according to Henry's Law (Brown et al. 2012). This water may then be forced to the surface where hydrostatic pressure is lower and consequently the water is supersaturated with TDG. When aquatic organisms respire TDG supersaturated water, bubbles can nucleate in their tissues and this can cause GBT, which has a range of negative effects.

There are many signs of GBT in fish. Symptoms of GBT include swim bladder overinflation (Shrimpton et al. 1990); non-lethal lesions (Stroud et al. 1975); and bubbles forming under the skin (Fidler 1988), within the lateral line (Mesa et al. 2000), behind the eyes (Bouck 1980), within the buccal cavity and within the cardiovascular system including the gill vasculature (Stroud et al. 1975). Severe cases of GBT can lead to mortality.

The effects of GBT have been studied in many fish species. Salmonids, such as chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), Atlantic salmon (*Salmo salar*), rainbow trout and steelhead salmon (*Oncorhynchus mykiss*), and other freshwater fish

such as rock carp (*Procypris rabaudi*) have been studied extensively (e.g. Abernethy et al. 2001; Ebel et al. 1971; Espmark et al. 2010; Fidler 1985; Liu et al. 2015; Mesa et al. 2000; Nebeker et al. 1979; Pleizier et al. 2020b). These fish often reside in rivers where dams are built and are at risk of GBT. Rainbow trout and steelhead are the most studied salmonid species in the TDG literature (Edsall and Smith 1990; Machado 1988; Pleizier et al. 2020b; Nebeker et al. 1979). Incentives to study TDG effects in these salmonids may include their availability and their cultural and economic significance, but it is unknown whether rainbow trout is a representative model for GBT in all salmonids specifically, and for fish in general. To investigate this, we examined TDG effects in rainbow trout, kokanee (*Oncorhynchus nerka*) and white sturgeon (*Acipenser transmontanus*) as described below.

Kokanee are a species of salmonid that have not been studied in detail in the GBT literature. The effects of high TDG on kokanee have only been measured in one study (Weitkamp et al. 2003). We expect vulnerability to GBT in kokanee to be similar to rainbow trout due to their anatomical similarity.

The effects of TDG supersaturation on white sturgeon have been studied, but in much less detail than salmonids. In the only study we are aware of (Counihan et al. 1998), white sturgeon embryos were resilient to GBT but the vulnerability of sturgeon larvae varied with the stage of development (Counihan et al. 1998). This is consistent with salmonids, whose embryos are resilient to GBT (Alderdice and Jensen 1985), larvae experience some level of GBT, and juvenile and adults are the most sensitive to GBT (Krise and Smith 1991). White sturgeon feed and live near the deep areas of rivers (Hildebrand et al. 1999) where depth has been shown to provide protective effects to GBT (Antcliffe et al. 2002; Pleizier et al. 2020a). Thus, in the wild, sturgeon may be less likely to be affected by a given TDG level relative to other species

occupying shallower depths. At the same depth, however, we predict that the vulnerability of sturgeon to GBT will be similar to salmonids when exposed to the same TDG supersaturation levels.

All three species in our study (rainbow trout, kokanee and white sturgeon) are found in British Columbian rivers (McPhail 2007) that are affected by hydroelectric dams and are of importance to various industries. Salmonids, including rainbow trout, are economically important commercial fish (Johnson and Walsh 1998) and are ecological keystone species (Helfield and Naiman 2006). Kokanee are a target for recreational fishing and have been the subject of population decline in the past (Askey and Johnston 2013). Some populations of white sturgeon, such as the upper Fraser River population (Schreier et al. 2012), are considered endangered and white sturgeon are the target of recreational catch-and-release fisheries in regions such as the lower Fraser River. Thus, the effects of TDG supersaturated water on these species is of special concern.

The objective of these experiments was to determine differences in GBT vulnerability between two salmonids, rainbow trout and kokanee, and a non-salmonid, white sturgeon, at two different ages. Another objective was to assess whether rainbow trout are a good model for the effects of GBT in salmonids in general, and fish more generally. To investigate these objectives, these three species of fish were exposed to nominal TDG levels of 100, 115, 120 and 130% at similar, shallow depths to determine their time to 50% loss of equilibrium (LOE) and the appearance of bubbles on the exterior and in the gills. We predict that all species at a given TDG level would experience similar times to 50% LOE and a similar progression of GBT symptoms.

Materials and Methods

Experimental subjects

Rainbow trout (n=97) were obtained from the Fraser Valley Hatchery, Abbotsford, British Columbia. Kokanee (n=144) were obtained from the Clearwater Hatchery, Clearwater, British Columbia. White sturgeon were obtained from the Vanderhoof Hatchery, Vanderhoof, British Columbia. Rainbow trout and kokanee were both <1 year old. White sturgeon were of two ages; approximately 103 days post-hatch (n=96) and age 3+ years (n=73). Mean weights were 15.6 (± 0.4 SE) g for rainbow trout, 4.2 (± 0.1 SE) g for kokanee, 17.7 (± 0.4 SE) g for <1 year old sturgeon and 313.8 (± 16.9 SE) g for 3+ year old sturgeon. There were no significant differences in fish weight between TDG treatment groups within the species/age groups. All fish were held at the University of British Columbia at temperatures between 11.0 – 11.5°C at 100% TDG for at least two weeks before experiments. Prior to experiments, rainbow trout, kokanee, and age 3+ year white sturgeon were fed 1.5% of their body weight 3 times a week with EWOS Pacific commercial fish feed. The age <1 year sturgeon were fed hatchery prepared food ad libitum 5 times a week.

Supersaturated water generation

Eight 700 L cylindrical tanks within a 15,000 L recirculation system were used during this experiment. TDG supersaturated water was generated by a 3-meter-tall, 12-inch diameter pressurized packed column (Pentair Aquatic Eco-Systems) as described in Pleizier et al. (2020a). The packed column generated the supersaturated water by exposing water to pressurized air. Air was injected into the top of the column using a compressor. Water from a header tank was pumped into the column using a variable frequency drive pump. A pressure of 30 psi (1 psi = 6.89 kPa) was maintained inside the column using a pressure transducer, which provided feedback to the water pump. The water flowed over high surface area bio-balls in the column to

allow the water gas tensions to equilibrate with the air. Air injection stopped once the water level dropped below a level sensor attached to a sight glass. There was a three second delay before the air valve could reopen. Total dissolved gas supersaturated water from the column was distributed to the test tanks. Differential flow of supersaturated and air equilibrated water was adjusted at each tank by opening and closing valves to create the desired % TDG. The nominal TDG treatment levels were 100% (control treatment), 115%, 120% and 130% (See Table 1 for measured values).

Experimental setup and procedure

Fish were acclimated to air-equilibrated water in the recirculating system and fasted for 48 h prior to the experiments. Rainbow trout and kokanee experiments were conducted simultaneously between 22 – 29 July 2019. Experiments were conducted in two 700 L replicate tanks for each nominal TDG treatment (100%, 115%, 120% and 130%: eight tanks in total). In each of the eight tanks, four plastic cages (34 x 16 x 15 cm) were secured at the surface. Each cage had large holes on the lid and sides covered by plastic mesh to ensure water flow through the cages and equilibration with the respective TDG treatments. Once the target TDG levels were achieved in each of the tanks, fish were netted from their holding tanks, introduced to the cages, and cages were placed directly into the treatment tanks at the target TDG treatment level. Within each tank, two cages contained 9 kokanee each and the other two contained 6 rainbow trout each. One tank contained 13 rainbow trout and two tanks contained 17 and 19 kokanee respectively due to error in counting while netting fish from their holding tanks (Table 2).

Sturgeon experiments were conducted separately for each age-class. Experiments using age <1 year sturgeon were conducted from 3 – 10 October 2019; experiments using age 3+ year sturgeon were conducted from 6 – 12 August 2019. Similarly, there were two 700 L replicate

tanks for each nominal TDG treatment (eight tanks in total). Water height in the tanks was reduced to a depth of 10 cm (approximately 31L of water) and sturgeon were permitted to swim freely in the tanks during the experiments. Air-equilibrated and TDG supersaturated water flowed directly into a bucket where they mixed and overflowed into the respective tanks. Preliminary tests were conducted to ensure % TDG was uniform throughout the water in each tank. Once the target TDG tension had been achieved, 12 <1 year sturgeon or 9 3+ year sturgeon were netted from their holding tanks and introduced into each tank. One tank contained 10 3+ year sturgeon due to error in counting while netting fish from their holding tanks (Table 2). Preliminary trials confirmed that all fish species were quiescent in cages and shallow tanks at the densities used in this study.

All fish remained in the experimental treatment tanks at their respective TDG levels until they reached 50% LOE (see below for the definition of LOE) in each experimental replicate or for a maximum of 7 days at which time the exposure was terminated. The relative flows of TDG supersaturated and air equilibrated water were not adjusted during the experiment once target TDG values were achieved resulting in slight deviations in measured relative to nominal % TDG levels (Table 1). Fish were monitored every hour for the first 12 h, every second hour for the next 12 h, and were subsequently monitored daily.

Loss of equilibrium and fish sampling

At each monitoring time, fish that had lost equilibrium were removed. Fish that experienced both LOE and mortality between monitoring times were recorded as dead and were also sampled. Loss of equilibrium for salmonids in this study was defined as fish that could not remain upright. Sturgeon do not always turn ventral side up at the time of mortality. Sturgeon were determined to have reached LOE when they were swimming erratically or when they were motionless and

could not right themselves after having been prodded gently. Sturgeon were determined to have died if they had stopped ventilating. All fish that had lost equilibrium but were still ventilating at the time of sampling were described as “moribund”. Dead fish were sampled immediately after being removed and fish that had lost equilibrium were sampled after having been euthanized. Fish were euthanized with a lethal concentration of Aquatic MS-222 (200mg/L; Syndel Canada) buffered with sodium bicarbonate (400mg/L; Fisher Scientific) in water of the respective water TDG level. Once a replicate reached 50% LOE (50% LOE includes both moribund and dead animals), the remaining fish in a replicate were removed, euthanized and sampled within 5 min, with the exception of age 3+ year sturgeon which were sampled within 15 min. Fish in the control tanks, which experienced no LOE or mortality, were euthanized and sampled in the same way at the end of the experiment. We recorded time of sampling and whether the fish was dead, moribund or neither at the time of sampling.

Every fish was sampled in the same way during the experiments. Fish were placed on a piece of paper towel with their left, lateral side up. This side of the exterior of the fish was carefully examined without a microscope for symptoms of GBT such as bubbles under the skin, bubbles between the fin rays, and exophthalmia. The 2nd gill arch was excised from the left side of the fish with surgical scissors and placed in a petri dish filled with TDG supersaturated water from the same treatment level. The gill was examined under a dissecting microscope for the presence of bubbles in the gill filaments which could be clearly seen because of their shiny, white appearance. Fish weight was measured and included both the body and the excised gill.

Water Quality

Total dissolved gas was measured using a Point Four Tracker Total Gas Pressure Meter (Pentair Aquatic Eco-Systems) which is accurate to $\pm 2\%$ TDG. The calibration procedure was adapted

from the USGS (Tanner and Johnston 2001) according to Pleizier et al. (2020a). Prior to calibration, the probe was dried for 24 h. The barometric pressure sensor was corrected daily to atmospheric pressure at sea level reported by Environment and Climate Change Canada at the Vancouver International Airport (YVR). The dissolved gas pressure sensor was calibrated with a two-point calibration. The first point was measured at atmospheric pressure. The second point was measured at 200 mmHg (1 mmHg=0.13 kPa) above atmospheric pressure. The probe was enclosed in a capsule and pressurized using a bike pump to 200 mmHg. Once 200 mmHg was recorded by an external pressure gauge attached to the capsule, the probe gauge pressure was recorded. Pressure measurements were corrected using the data from the two-point calibration. While measuring TDG, the TDG probe was gently tapped on the tank wall to ensure bubbles did not adhere to the silastic tubing of the probe, which reduces the accuracy of the reading. The TDG measurement was recorded when the % TDG reading was stable for 2 minutes. Percent TDG, dissolved oxygen and temperature were measured in each of the eight tanks when fish were first stocked into each treatment, at every 24 h thereafter and at the time that a tank reached 50% LOE if this occurred within the first 24 h. Dissolved oxygen levels were measured using a Pro2030 Dissolved Oxygen Sensor (YSI). This probe was calibrated before the start of every experiment according to the user manual. Nitrite, ammonia and pH were measured in two tanks chosen at random at the start and end of each experiment. All tanks received water from the same recirculation system; measuring water quality in two tanks was adequate to characterize conditions in all tanks.

Analysis

Data were analysed using R (R Core Team 2019) and figures were created in RStudio using the R package “ggplot2” (Wickham 2016). The data for age <1 year sturgeon were analyzed

215 separately from the age 3+ year sturgeon. Control groups were not included in the statistical
216 analyses as no mortality was observed. Data for each pair of cages within a tank were combined
217 and treated as one replicate during analysis due to cages being pseudo-replicates within each
218 tank. Each treatment tank was treated as an independent observation in the dataset. The TDG
219 values used in the analysis were the mean TDG values measured in each treatment replicate tank
220 for each species/age group. Mean weight for each fish species/age group was calculated.
221 ANOVA tests and Tukey-Kramer post-hoc tests (“TukeyHSD()” function) were conducted using
222 R to check for significant differences in mean fish weight or mean temperature between
223 treatment tanks.

224 Linear regressions between the natural logarithm of the time to 50% LOE and the factors %
225 TDG, species/age group and their interaction were calculated in R using the “lm()” function. A
226 type III ANOVA was generated in R (Anova() function in the R package “car”, Fox and
227 Weisberg 2011) for the relationships between time to 50% LOE and %TDG, species/age group
228 and their interaction. If the P-value for the interaction term in the model was non-significant ($\alpha =$
229 0.05), the interaction term was removed, and a type II ANOVA was created for a model which
230 included the main effects only. Assumptions of the ANOVA were checked using the Levene’s
231 test. Normality was assessed using quantile plots in R (“qqnorm()” and “qqline()” functions).
232 Tukey-Kramer post-hoc tests were conducted using adjusted values to identify significant
233 differences in time to 50% LOE between species/age groups.

234 The proportions of fish in each treatment with bubbles present on their exterior or within their
235 gills at the time of sampling was calculated for each species/age group in each treatment tank.
236 Linear regressions and ANOVAs of the proportion of fish with bubbles on their exterior and the
237 proportion of fish with bubbles within their gill filaments were built using the same methods as

the model of the time to 50% LOE. These models also included % TDG as the covariate, and species/age group as a categorical variable.

Ethics Approval

All experiments and sampling were conducted in accordance with the guidelines of the Canadian Council on Animal Care as administered by the University of British Columbia (UBC) (A15-0266).

Results

Treatment conditions

TDG levels during each experiment are reported in Table 1. Dissolved oxygen levels ranged from 11 mg·L⁻¹ to 15 mg·L⁻¹ between treatments. Temperatures ranged between 9.8 – 11.9 °C. For the rainbow trout and kokanee, mean temperatures were significantly cooler in control treatments (10.6 °C and 10.8 °C) compared to both 120% TDG treatments (11.1 °C and 10.9 °C); one control treatment (10.6 °C) was significantly cooler than one 130% TDG treatments (11.0 °C) ($p < 0.001$). For the <1 year old sturgeon, mean temperatures were significantly cooler in both control treatments (11.0 °C and 10.6 °C) than one 130% TDG treatment (11.1 °C) ($p < 0.001$). The water pH varied between 6.6 – 6.8 in all experiments. Ammonia and nitrate levels were undetectable in all experiments.

Time to 50% Loss of Equilibrium

Times to 50% LOE are listed in Table 2 for each treatment group as well whether fish were dead or moribund at the time of sampling. There was a significant effect of species/age group on time to 50% LOE ($F(3,20)=4.44$, $p=0.02$) (Table 4, Figure. 1). An interaction between TDG and

species/age did not improve the model of time to 50% LOE ($F(3,19)=0.61$, $p=0.61$) (Table 3). Adjusted means for time to 50% LOE were significantly different between rainbow trout and <1 year old sturgeon ($p=0.02$). The difference between rainbow trout and 3+ year old white sturgeon was not significant but was the next lowest p-value ($p=0.09$). Both age classes of white sturgeon exhibited a longer time to 50% LOE than rainbow trout and kokanee salmon, but at high TDG levels (130%), this difference became smaller, and all fish reached 50% LOE within 2 – 6 h.

Symptoms of GBT

All fish were examined for external symptoms of GBT and for bubbles in the blood vessels of the 2nd gill arch at the time of LOE or once the treatment had reached 50% LOE. The most common external symptom of GBT in all species was bubbles between the fin rays of the caudal fin; bubbles were found infrequently between the fin rays of the pectoral fins. Bubbles were also frequently present in the blood vessels of the gill filaments. The interaction between TDG and species/age improved the model of the proportion of fish with external GBT symptoms ($F(3,27)=10.72$, $p<0.001$) (Table 5; Figure 2). Adjusted means for proportion of fish with exterior bubbles were significantly higher for kokanee compared to <1 year old white sturgeon ($p<0.001$), higher for rainbow trout compared to <1 year old white sturgeon ($p<0.001$) and higher for rainbow trout compared to 3+ year old white sturgeon ($p=0.03$). There was also a significant effect of TDG and species/age group on the proportion of fish with bubbles in gill filaments ($F(3,28)=9.07$, $p<0.001$) (Table 6; Figure 3). The interaction between TDG and species/age did not improve the model of proportion of fish with bubbles in gill filaments ($F(3,27)=1.42$, $p=0.26$) (Table 5; Figure 3). Adjusted means for proportions of fish with bubbles in gill filaments were significantly higher for 3+ year old white sturgeon compared to <1 year

old white sturgeon ($p < 0.001$), higher for rainbow trout compared to <1 year old white sturgeon ($p = 0.004$) and higher for 3+ year old white sturgeon compared to kokanee ($p = 0.05$).

Discussion

While rainbow trout and kokanee exhibited a similar time to 50% LOE at a given TDG level as predicted, both age-classes of white sturgeon had a longer time to 50% LOE at a given TDG level (Fig. 1), and the time to 50% LOE was significantly longer for <1 year old sturgeon compared to rainbow trout. These data indicate that rainbow trout and kokanee share similar pathogenesis of GBT for both time to 50% LOE and observable symptoms of GBT. White sturgeon had a longer time to 50% LOE than the salmonids and had fewer external symptoms of GBT, but the proportion of fish with bubbles in the gills was significantly higher in the 3+ year old white sturgeon compared to <1 year old white sturgeon and kokanee, and also significantly lower in the <1 year old white sturgeon compared to rainbow trout.

The physiological basis for white sturgeon's longer time to 50% LOE in TDG supersaturated water compared to rainbow trout is not known. A possible explanation for this could be each species' physiological response to hypoxemia (reduced internal oxygen levels). Oxygen uptake by the fish in this study may have been impeded by the formation of bubbles within the gill filaments, which could have led to LOE. White sturgeon significantly reduce their metabolism in hypoxic environments, which may help them tolerate low oxygen uptake for longer periods of time (Crocker and Cech 1997) and thus they may be quite tolerant of hypoxemia. Conversely, rainbow trout are a highly active species and are not very tolerant to environmental hypoxia (Scott et al., 2015) and thus likely sensitive to hypoxemia.

303 The effects of TDG on rainbow trout have been studied numerous times under various
304 conditions. Our results for time to 50% LOE can be compared to time to 50% mortality, a
305 common metric used in earlier studies, as we observed LOE within an hour of mortality for most
306 fish in this and previous experiments. Nebeker et al. (1979) observed mortality of steelhead
307 smolts within 1 – 6 h at 130% TDG. Machado (1988) also observed that rainbow trout fry (9
308 weeks old) reached 50% mortality in <2 h at 130% TDG and above. These values are similar to
309 the time to 50% LOE values in our study. Bouck et al. (1976) found similar minimum values for
310 time to death, 8 – 36 h, at 120 and 125 % TDG for rainbow trout parr. The minimum values
311 found by Bouck et al. (1976) are similar to our time to 50% LOE of 8 – 10 h; however, the
312 maximum values for time to death at 120% and 125% TDG found by Bouck et al. (1976) were
313 higher than ours.

314 The effects of TDG on kokanee have been studied on only one occasion as part of a field
315 experiment (Weitkamp et al. 2003), but kokanee are the same species as anadromous sockeye
316 salmon, which have been studied in two additional occasions. Sockeye salmon experience
317 increased mortality under elevated TDG levels similar to other salmonids, like rainbow trout
318 (Bouck et al. 1976). The time to mortality observed by Bouck et al. (1976) for sockeye at 120%
319 TDG was 36 h and 167 h at 115% TDG. Nebeker et al. (1976) showed adult sockeye salmon
320 mortality to increase significantly between 115 and 120% TDG. However, time to mortality at
321 both these treatment levels were higher than our findings for times to LOE. They found mortality
322 at 120% TDG from 77 – 120 h and mortality at 115% TDG from 523 – 835 h, whereas we found
323 mortality at 120% TDG from 8 – 10 h and mortality at 115% TDG from 8 – 72 h. This difference
324 may be due in part to the greater depth exposure of fish in Nebeker et al. (1976) study, where
325 fish has access to at depth of 60 cm, whereas our fish were restricted to 10 cm. If fish in the

study by Nebeker et al. (1976) were routinely at a depth 50 cm deeper than our study, the protective effects of depth against GBT would partly explain the difference between studies (Pleizier et al. 2020a). This difference may also be due to the age of sockeye salmon used by Nebeker et al. (1976). These studies show kokanee have similar vulnerability to other salmonids but can vary depending on various factors such as depth and age.

To our knowledge, there is only one other published study on the effects of TDG supersaturation on white sturgeon. Counihan et al. (1998) assessed the effects of TDG supersaturation on white sturgeon larvae beginning at hatching. The authors found mortality at high elevated TDG levels (131%) after 13 days, compared to our finding of 50% LOE within 3 h at 130% TDG in our study. Counihan et al. (1998) found no mortality over 10 days at 118% TDG in white sturgeon larvae. The juvenile sturgeon in our study experienced some LOE at 113 and 111% TDG, but these treatments did not reach 50% LOE within 7 days. It may not be appropriate to compare the effects of elevated TDG between larval and juvenile white sturgeon as it appears that larvae are much less vulnerable to the effects of TDG supersaturation than juveniles.

We were interested in determining whether rainbow trout are a good model species for the effects of TDG supersaturation in fish. Our results indicate that rainbow trout are more sensitive to GBT than white sturgeon, as they have shorter times to 50% LOE in TDG supersaturation and a greater proportion show external symptoms of GBT. As such, rainbow trout are not a representative model for GBT in white sturgeon in the sense that their vulnerability to GBT is similar, but they appear to be a suitable model for conservation purposes because they are more sensitive to GBT than sturgeon. Pleizier et al. (2020b) conducted a systematic review and meta analysis of laboratory studies on GBT in freshwater fish and found rainbow trout and steelhead were consistently the most or second most sensitive species in terms of time to GBT outcomes,

including 50% and 10% mortality and external symptoms of GBT. This suggests that rainbow trout are a sensitive model species for the effects of elevated total dissolved gas and GBT, which could serve an important role in conservation efforts. Based upon the comparisons between GBT studies using rainbow trout and other species described above, practitioners should be aware that the effects of TDG differ with laboratory conditions, such as depth, and the age and stage of development of fish (Pleizier et al. 2020b).

All species exhibited short times to 50% LOE at high levels of TDG supersaturation in this study. This is important considering the levels of TDG supersaturation observed in rivers downstream of hydroelectric dams. For example, the Peace Canyon Dam forebay (Peace River, British Columbia) has shown an increase from 104% TDG to consistently above 118% TDG during a spill event from the upstream Bennett Dam (Peace River, British Columbia) (Kamal et al. 2016; McArthur 2014). The Mica Dam (Columbia River, British Columbia) has TDG levels between 120 – 130% during generating periods and Plate et al. (2016) suggest that rainbow trout and kokanee below the dam may be at high risk of GBT. Based on our results, TDG supersaturation generated by hydroelectric dams can result in harmful effects on salmonids, which was previously known, but also in sturgeon in near surface exposures. Elevated TDG created by hydroelectric dams could be a serious concern given that some populations of white sturgeon are endangered or threatened (Committee on the Status of Endangered Wildlife in Canada 2012) and that rainbow trout and kokanee are ecologically important species. The physiological basis for the differences in TDG sensitivity between sturgeon and salmonids is an area for further study, and the susceptibility to GBT of other species endemic to TDG impacted systems should be investigated.

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Tables

Table 1: Mean percent total dissolved gas (%TDG) and mean temperature (T°C) in each tank of rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and white sturgeon (*Acipenser transmontanus*) aged <1 year and 3+ years. Each value is the mean of all recorded values. Values are reported as means ± standard error of the mean. Values in parentheses represent the difference in pressure (in mmHg; Δ P) measured between the water and the atmosphere.

	Rainbow Trout		Kokanee		<1 yr Sturgeon		3+ yr Sturgeon	
Nominal % TDG	% TDG (Δ P)	T (°C)	% TDG (Δ P)	T (°C)	% TDG (Δ P)	T (°C)	% TDG (Δ P)	T (°C)
100	101±0.3 (8±2)	10.8 ±0.03	101±0.3 (8±2)	10.8 ±0.03	100±0.05 (1±0)	11.0 ±0.1	99±0.08 (-6±1)	11.2 ±0.1
100	101±0.2 (6±2)	10.6 ±0	101±0.2 (6±2)	10.6 ±0	100±0.1 (1±1)	10.6 ±0.1	98±0.08 (-9±1)	11.1 ±0.1
115	120±2.2 (155±17)	11.0 ±0.05	120±2.2 (155±17)	11.0 ±0.05	113±0.2 (100±2)	11.0 ±0.1	112±0.3 (92±2)	11.4 ±0.12
115	113±0.3 (102±2)	10.9 ±0.05	113±0.3 (102±2)	10.9 ±0.05	111±0.2 (90±2)	10.8 ±0.1	115±1.1 (122±8)	11.3 ±0.17
120	122±0.6 (166±4)	11.1 ±0.1	122±0.6 (166±4)	11.1 ±0.1	117±0.8 (127±6)	10.9 ±0	116±0.2 (124±1)	11.3 ±0.2
120	122±0.3 (166±2)	10.9 ±0.03	122±0.3 (166±2)	10.9 ±0.03	125±0.7 (192±6)	10.9 ±0	117±0.9 (132±7)	11.4 ±0
130	130±0.1 (234±1)	11.0 ±0	130±0.1 (234±1)	11.0 ±0	128±1.2 (212±9)	11.0 ±0.05	130±2.3 (228±18)	11.5 ±0
130	131±0.09 (238±1)	11.0 ±0.1	131±0.09 (238±1)	11.0 ±0.1	129±0.3 (217±3)	11.1 ±0	129±1.8 (221±14)	11.4 ±0.2

519 Table 2. Numbers of rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and
 520 white sturgeon (*Acipenser transmontanus*; aged <1 year and 3+ years) in each total dissolved gas
 521 (TDG) treatment replicate, time to 50% loss of equilibrium (LOE) and the percent of fish that
 522 were moribund or dead at the time of sampling.

Species/age group	% TDG ($\pm$ SE)	Number of fish in the treatment	Time to 50% LOE (hours)	% moribund at time of sampling (n)	% dead at time of sampling (n)
Rainbow trout	101 $\pm$ 0.3	12	96*	0 (0)	0 (0)
Rainbow trout	101 $\pm$ 0.2	12	96*	0 (0)	0 (0)
Rainbow trout	120 $\pm$ 2.2	12	8	0 (0)	58.3 (7)
Rainbow trout	113 $\pm$ 0.3	13	48	15.4 (2)	69.2 (9)
Rainbow trout	122 $\pm$ 0.6	12	7	25 (3)	25 (3)
Rainbow trout	122 $\pm$ 0.3	12	9	8.3 (1)	41.7 (5)
Rainbow trout	130 $\pm$ 0.1	12	3	0 (0)	58.3 (7)
Rainbow trout	131 $\pm$ 0.09	12	2	0 (0)	50 (6)
Kokanee	101 $\pm$ 0.3	18	96*	0 (0)	0 (0)
Kokanee	101 $\pm$ 0.2	18	96*	0 (0)	0 (0)
Kokanee	120 $\pm$ 2.2	17	8	17.6 (3)	47.1 (8)
Kokanee	113 $\pm$ 0.3	18	72	0 (0)	50 (9)
Kokanee	122 $\pm$ 0.6	19	8	10.5 (2)	42.1 (8)
Kokanee	122 $\pm$ 0.3	18	10	11.1 (2)	38.9 (7)
Kokanee	130 $\pm$ 0.1	18	4	0 (0)	55.5 (10)
Kokanee	131 $\pm$ 0.09	18	3	22.2 (4)	33.3 (6)
<1 yr sturgeon	100 $\pm$ 0.05	12	168*	0 (0)	0 (0)
<1 yr sturgeon	100 $\pm$ 0.1	12	168*	0 (0)	0 (0)
<1 yr sturgeon	113 $\pm$ 0.2	12	168*	0 (0)	33.3 (4)
<1 yr sturgeon	111 $\pm$ 0.2	12	168*	0 (0)	16.7 (2)
<1 yr sturgeon	117 $\pm$ 0.8	12	24	0 (0)	50 (6)
<1 yr sturgeon	125 $\pm$ 0.7	12	9	0 (0)	58.3 (7)
<1 yr sturgeon	128 $\pm$ 1.2	12	7	16.7 (2)	41.7 (5)
<1 yr sturgeon	129 $\pm$ 0.3	12	6	25 (3)	25 (3)
3+ yr sturgeon	99 $\pm$ 0.08	9	144*	0 (0)	0 (0)
3+ yr sturgeon	98 $\pm$ 0.08	9	144*	0 (0)	0 (0)
3+ yr sturgeon	112 $\pm$ 0.3	9	120	11.1 (1)	66.7 (6)
3+ yr sturgeon	115 $\pm$ 1.1	9	48	22.2 (2)	33.3 (3)
3+ yr sturgeon	116 $\pm$ 0.2	9	48	33.3 (3)	11.1 (1)
3+ yr sturgeon	117 $\pm$ 0.9	9	18	0 (0)	55.6 (5)
3+ yr sturgeon	130 $\pm$ 2.3	10	5	22.2 (2)	66.7 (6)
3+ yr sturgeon	129 $\pm$ 1.8	9	5	22.2 (2)	44.4 (4)

523 * Treatments that did not reach 50% LOE before the treatment was terminated.

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524 Table 3: Type III ANOVA table of the natural log time to 50% loss of equilibrium of rainbow
 525 trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and two age-classes (<1 and 3+
 526 years) of white sturgeon (*Acipenser transmontanus*) exposed to various levels of total dissolved
 527 gas (TDG) supersaturation. Species/age group is a categorical variable for both species and two
 528 age-classes of white sturgeon (<1 and 3+ years).

	Sum of Squares	Degrees of Freedom	F-value	p-value
TDG	5.36	1	30.62	<0.001
Species/Age Group	0.41	3	0.77	0.53
TDG x Species/Age Group	0.32	3	0.61	0.61
Residuals	2.80	16		

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Table 4: Type II ANOVA table of the natural log time to 50% loss of equilibrium of rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and two age-classes (<1 and 3+ years) of white sturgeon (*Acipenser transmontanus*) exposed to various levels of total dissolved gas (TDG) supersaturation. Species/age group is a categorical variable for both species and two age-classes of white sturgeon (<1 and 3+ years). The interaction term between the covariate (TDG) and the factor (Species/age group) is removed.

	Sum of Squares	Degrees of Freedom	F-value	p-value
TDG	29.66	1	180.31	<0.001
Species/Age Group	2.19	3	4.44	0.02
Residuals	3.12	19		

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Table 5: Type III ANOVA tables of the proportion of rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and two age-classes (<1 and 3+ years) of white sturgeon (*Acipenser transmontanus*) with symptoms of gas bubble trauma (GBT) and various levels of total dissolved gas (TDG) supersaturation. Species/age group is a categorical variable for both species and two age-classes of white sturgeon (<1 and 3+ years). The top table represents proportions of fish that exhibited external symptoms of GBT. The bottom table represents proportions of fish that exhibited bubbles forming within their gill filaments.

External GBT				
	Sum of Squares	Degrees of Freedom	F-value	p-value
TDG	0.0002	1	0.010	0.92
Species/Age Group	0.47	3	8.13	<0.001
TDG x Species/Age Group	0.61	3	10.72	<0.001
Residuals	0.46	24		
Bubbles in the gill filaments				
	Sum of Squares	Degrees of Freedom	F-value	p-value
TDG	0.41	1	25.25	<0.001
Species/Age Group	0.04	3	0.84	0.49
TDG x Species/Age Group	0.068	3	1.42	0.26
Residuals	0.39	24		

Table 6: Type II ANOVA tables of the proportion of rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), and two age-classes (<1 and 3+ years) of white sturgeon (*Acipenser transmontanus*) with internal symptoms of gas bubble trauma (GBT) and various levels of total dissolved gas (TDG) supersaturation. Species/age group is a categorical variable for both species and two age-classes of white sturgeon (<1 and 3+ years). The data represents proportion of fish with bubbles within their gill filaments.

Bubbles in the gill filaments				
	Sum of Squares	Degrees of Freedom	F-value	p-value
TDG	2.63	1	156.19	<0.001
Species/Age Group	0.46	3	9.07	<0.001
Residuals	0.45	27		

Figures

Figure 1: The relationship between the time to 50% loss of equilibrium (LOE) in hours and percent total dissolved gas (TDG) for rainbow trout (*Oncorhynchus mykiss*; n = 12 per replicate), kokanee (*Oncorhynchus nerka*; n = 18 per replicate), <1 year white sturgeon (*Acipenser transmontanus*; n = 12 per replicate), and 3+ year white sturgeon (n = 9 per replicate). The relationships are plotted on a log-linear graph, with the y-axis scaled to log base 10. Each data point represents a replicate. Regressions were plotted using the “geom_smooth” function with the “lm” function in R.

Figure 2: The relationship between the proportion of rainbow trout (*Oncorhynchus mykiss*; n = 12 per replicate), kokanee (*Oncorhynchus nerka*; n = 18 per replicate), <1 year white sturgeon (*Acipenser transmontanus*; n = 12 per replicate), and 3+ year white sturgeon (n = 9 per replicate) with external symptoms of gas bubble trauma (GBT) and percent total dissolved gas (TDG) at time of loss of equilibrium (LOE). Each data points represents one replicate's proportion. Regressions were plotted using the “geom_smooth” function with the “lm” method in R.

Figure 3: The relationship between the proportion of rainbow trout (*Oncorhynchus mykiss*; n = 12 per replicate), kokanee (*Oncorhynchus nerka*; n = 18 per replicate), <1 year white sturgeon (*Acipenser transmontanus*; n = 12 per replicate), and 3+ year white sturgeon (n = 9 per replicate) with bubbles in the filaments of the second gill arch and percent total dissolved gas (TDG) at time of loss of equilibrium (LOE). Each data points represents one replicate's proportion. Regressions were plotted using the “geom_smooth” function with the “lm” method in R.