PERSPECTIVES ON SUSTAINABLE HYDRO-POWER

Acute effects of gas supersaturation on Atlantic salmon smolt in two Norwegian rivers

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Abstract Total dissolved gas (TDG) supersaturation downstream of hydropower plants may cause gas bubble disease (GBD) and harmful effects in fish. Little is known about tolerance levels of TDG supersaturation on Atlantic salmon (*Salmo salar* Linnaeus, 1758) in natural rivers. The present study investigated the effects of TDG supersaturation on the survival of Atlantic salmon smolts at two field sites in Norway. Here, we kept smolts in cages at increasing distances from hydropower plants known to cause TDG supersaturation and at control sites. We recorded fish mortality and examined for GBD using a stereo microscope. Mortality and symptoms of GBD commenced in fish exposed to an average of 108.3% TDG (maximum 111.0%, water depth 0.55 m) for 2 days. Significant differences in time before mortality at the control sites and test sites commenced at 110.2% TDG (maximum 111.8%) for 3 days. The study indicates that Atlantic salmon may be more vulnerable to TDG supersaturation than Pacific salmonids, which are considered at risk when the TDG is above 110%. In addition, the study provides important data to link effects caused by TDG in the laboratory and in the field.

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Introduction

Both water pressure and temperature are often affected by river regulation and both these factors affect total dissolved gas (TDG). Gas supersaturation of water occurs when TDG pressure exceeds total atmospheric pressure at the water surface (i.e., total dissolved gas saturation $> 100\%$; Weitkamp, 2000). With increasing temperature, gas solubility decreases and TDG increases by approximately 2% per 1°C in water (Marking, 1987). TDG is also affected by water pressure due to the increasing amount of gas that can be dissolved in water with increasing pressure and thus increasing depth (about 10% per m of water depth), according to Henry's law (Henry, 1803). Gas supersaturation can occur naturally in rivers, for example

during rapid solar heating, algal blooms and downstream from waterfalls plunging into deep pools (Marking, 1987). It can also arise from human activity in at least two situations: (1) When air is entrained into the penstock system of hydropower plants (Heggberget, 1984; Pulg et al., 2016, Pulg et al., 2020) and (2) when air is entrained in plunge pools below dams (Ebel, 1969; Backman & Evans, 2002; Weitkamp, 2008; Cao et al., 2019).

Supersaturation of TDG can be harmful to aquatic organisms (Nebeker, 1976; Bouck, 1980; Nebeker et al., 1981; Heggberget, 1984). In fish, the blood will become gas supersaturated when it respires in supersaturated water. When the blood is supersaturated, excess gas can form bubbles in the tissue (emphysema) and in the blood (emboli), which induces a variety of lethal and sub-lethal effects, often referred to as gas bubble disease (GBD; Bouck, 1980). The gas bubbles commonly occur in the gill blood vessels, eyes, mouth, organs, on the head and fins, along the lateral line, and some fish experience exophthalmia ("pop eyes"; Weitkamp & Katz, 1980). The prevalence and severity of gas bubbles on external surfaces of fish may not be a good indicator to classify the extent of GBD (Mesa et al., 2000; Weitkamp, 2008). However, gas bubble formation on fins and gills indicate that the fish have harmful GBD (Weitkamp & Katz, 1980; Mesa et al., 2000).

Research on tolerances to TDG has so far focused on fish species from China and North America. Several field- and laboratory studies on the impact of and tolerance to TDG on endemic fish species have been conducted during the recent years in China (Wang et al., 2015; Cao et al., 2019; Ji et al., 2019; Xue et al., 2019; Deng et al., 2020). These studies were conducted due to sharp declines in several endemic fish populations after construction of hydropower constructions, and indicate that the tolerance to TDG vary among fish species. Species-specific tolerance toward TDG has also been found for Pacific salmonids. Juvenile Chinook salmon (*Oncorhynchus tshawytscha* Walbaum, 1792) exposed to 130% TDG suffered 20% mortality after 3 to 6 h, while juvenile steelhead trout (*Oncorhynchus mykiss* Walbaum, 1792) suffered 20% mortality after 5 to 7 h (Mesa et al., 2000). At 110% TDG, juvenile steelhead trout may develop changes in blood chemistry (Newcomb, 1974), inflation of the gas bladder (Shrimpton et al., 1990) and bubbles on the caudal fin. Juvenile Chinook

salmon can acquire acute GBD already at 106% TDG with weakening of the swimming performance (Schiewe, 1974; Dawley & Ebel, 1975). Other studies did not find acute effects at levels below 110% in Pacific salmonids (Krise & Meade, 1988; Schisler et al., 1999; Geist et al., 2013; Brosnan et al., 2016).

Atlantic salmon (*Salmo salar* Linnaeus, 1758) naturally lives in about 2000 rivers that drain into the North Atlantic Ocean (MacCrimmon & Gots, 1979). During the past two decades, the total number of wild Atlantic salmon has declined dramatically, and several populations are now extinct (Anon, 2018; Mills et al., 2013). Relatively few studies have investigated effects of TDG supersaturation in Atlantic salmon, despite its distribution in rivers that are often exploited for hydropower purpose. In a laboratory study with 15 cm deep tanks, sub-yearling Atlantic salmon were less tolerant to TDG than lake trout (*Salvelinus namaycush* Walbaum, 1792) (Krise & Herman, 1991). Here, Atlantic salmon mortality commenced from 114% TDG, and 50% mortality occurred after 96 h at 117% TDG for parr (mean length 14.8 cm) and 118 to 122% for smaller parr and fry (6.6–2.3 cm). Other studies on gas supersaturation in Atlantic salmon have focused on oxygen (not total gas) saturation (Espmark et al., 2010; Kristensen et al., 2010). Espmark et al. (2010) observed gas bubbles in Atlantic salmon smolts exposed to water supersaturated with 160% oxygen for 14 days. However, salmonids (e.g., Atlantic salmon) exposed to water supersaturated with oxygen develop gas bubbles at higher supersaturation than in water supersaturated with atmospheric gas (Nebeker et al., 1976; Espmark et al., 2010). In Atlantic salmon hatcheries, production problems may commence from 104 to 105% TDG (Marking, 1987). These results suggest that Atlantic salmon is particularly sensitive to TDG supersaturation among salmonids. Potential harmful levels of TDG can occur in rivers with Atlantic salmon several km downstream from hydroelectric installations and dams (Heggberget, 1984; Pulg et al., 2016; Pulg et al., 2020). However, detailed knowledge on effects and tolerances of TDG in Atlantic salmon and most other species of fish are lacking. The difference in tolerance toward TDG among salmonids raises questions whether sensitive species are affected at a lower level than previously thought. Species-specific tolerance to TDG should therefore be known when setting criteria in natural settings for maximum TDG levels.

The aim of this study was to identify the effects of TDG supersaturation exposure on Atlantic salmon in controlled field settings. We placed fish in cages with varying distance from the source of TDG supersaturation (i.e., hydropower plants) in two Norwegian rivers, and thereby exposed fish to varying levels of TDG. Such information is necessary to link the results of laboratory experiments to conditions in the natural habitat, to set thresholds for acceptable levels of TDG and to conduct mitigation measures. In both rivers, the public and environmental protection agencies were concerned for potential harmful effects on fish due to TDG supersaturation.

Materials and methods

Study sites

River Otra is situated in southern Norway and has a catchment area of about 4000 km^2 and an average discharge of 150 m^3 s^{-1} at the river mouth (Fig. 1). The upper part of River Otra, where this study was conducted, has a relict population of the landlocked Atlantic salmon commonly referred to as "Bleke" (*Salmo salar* ssp.) (Wright et al., 2016). Hydropower has been extensively developed in the River Otra (Fig. 1). Brokke power plant is a reservoir hydropower plant with 13 secondary creek intakes, has a head (vertical height between intake and discharge point) of 300 m and produces a maximum of 330 MW at four Francis wheels. The inlet of Hekni power plant is located 11 km downstream of Brokke power plant. Hekni is a run-of-the-river hydropower plant with a head of 38.5 m and a maximum power of 56 MW at two Kaplan wheels, bypassing water over a stretch of 8 km in the main river (Fig. 1).

Lake Evangervatnet is a part of the Vosso watercourse in Western Norway (Fig. 2). The Vosso River has a catchment area of about 1500 km^2 with an average discharge of 108 m^3 s^{-1} at the river mouth. The Atlantic salmon population in the Vosso collapsed in the late 1980s (Barlaup et al., 2018). Since then, intensive abatement measures, such as restocking, have been carried out to restore the population. However, significant stressors for the fish population are still present, such as salmon lice, competition and introgression with escaped farmed Atlantic salmon and hydropower. The Evanger power plant,

approximately 7 km upstream from the river mouth, is a reservoir hydropower plant with a head of 770 m and a maximum power yield of 330 MW at three Pelton wheels.

Cages

The study was conducted by exposing juvenile Atlantic salmon to supersaturated water in cages. Two cages were placed at each of the five study sites in River Otra at 0.1 to 0.55 m water depth (cage type: length 0.45 m, width 0.35 m, height 0.45 m). The depths reflect the typical habitat of juvenile Bleke in this river stretch. Stones were placed in the cages and acted as bottom weight and shelter for the fish. The site Brokke + 0 km was placed at the outlet of Brokke power plant, and the remaining sites were placed at increasing distance downstream: Brokke + 17.5 km (downstream the outlet of Hekni power plant), Brokke + 21 km and Brokke + 30 km (Fig. 1). The last cage was placed 11 km downstream of Brokke (Brokke +11 km) in a residual flow reach between the Tjurrmo dam and Hekni power station. The residual flow reach has a lower discharge (minimum 5 m^3 s^{-1}) than upstream of the dam, and the water is well aerated as it passes the dam. This site has low levels of supersaturation and acted as control.

In Lake Evangervatnet, two cages were placed at 0 to 1.15 m depth at four sites with different distances to the Evanger power plant (cage type: radius 0.32 m, height 1.15 m). The depth distribution reflects the typical depth distribution of juvenile salmon in such lakes, including out-migrating smolts. Cages were placed at three sites downstream of the power plant (Evanger + 0 km, Evanger + 0.5 km, Evanger + 5.4 km; Fig. 2) and at one control site upstream of Evanger power plant (Evanger − 1.4 km).

Fish

10 Atlantic salmon smolts (wild Bleke strain hatched and reared at Syrtveit hatchery, average length 12.6 cm, average weight 18.7 g) were placed in each cage in River Otra and 15 Atlantic salmon smolts (wild Vosso strain hatched and reared at Voss hatchery, average length 13.2 cm, average weight 21.2 g) were placed in each cage in Lake Evangervatnet. The fish could feed on naturally drifting invertebrates passing though the cages during the exposure period, and were not fed.
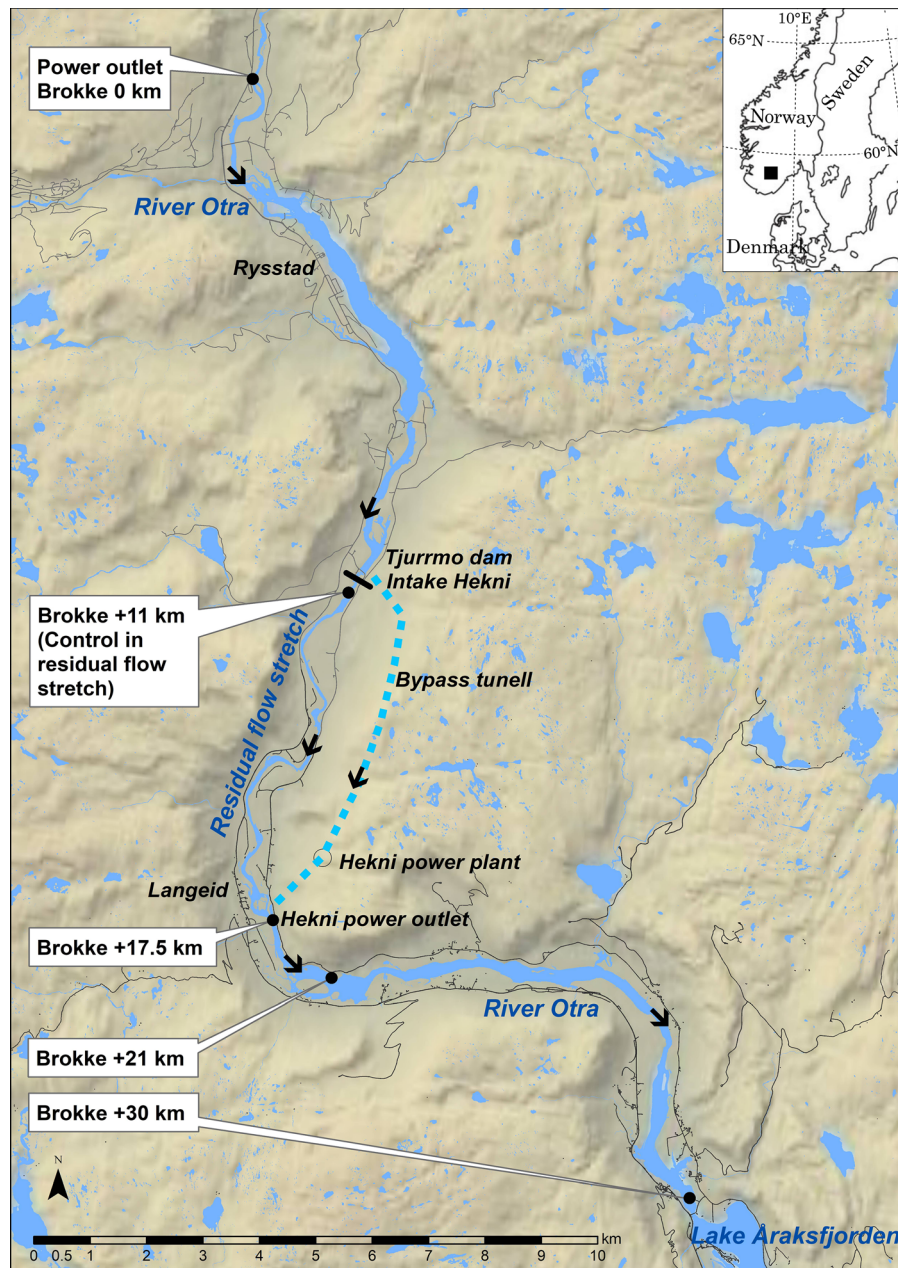
Fig. 1 River Otra, southern Norway, indicating Brokke- and Hekni hydropower plants, and the study sites (black dots) at Brokke + 0 km, Brokke + 11 km (control site), Brokke + 17.5 km, Brokke + 21 km and Brokke + 30 km. Arrows indicate the direction of the water current

The experiments were conducted with permission from the animal experimentation administration of the Norwegian Food Safety Authority (Permission nos. 16/86290 and 16-20221).

Exposure period and fish examination

The experiment took place between June 6 and June 14, 2016 in River Otra. Here, the smolts were sampled and examined after 3 and 8 days of exposure, except at Brokke + 0 km where one fish was sampled after 1.5,
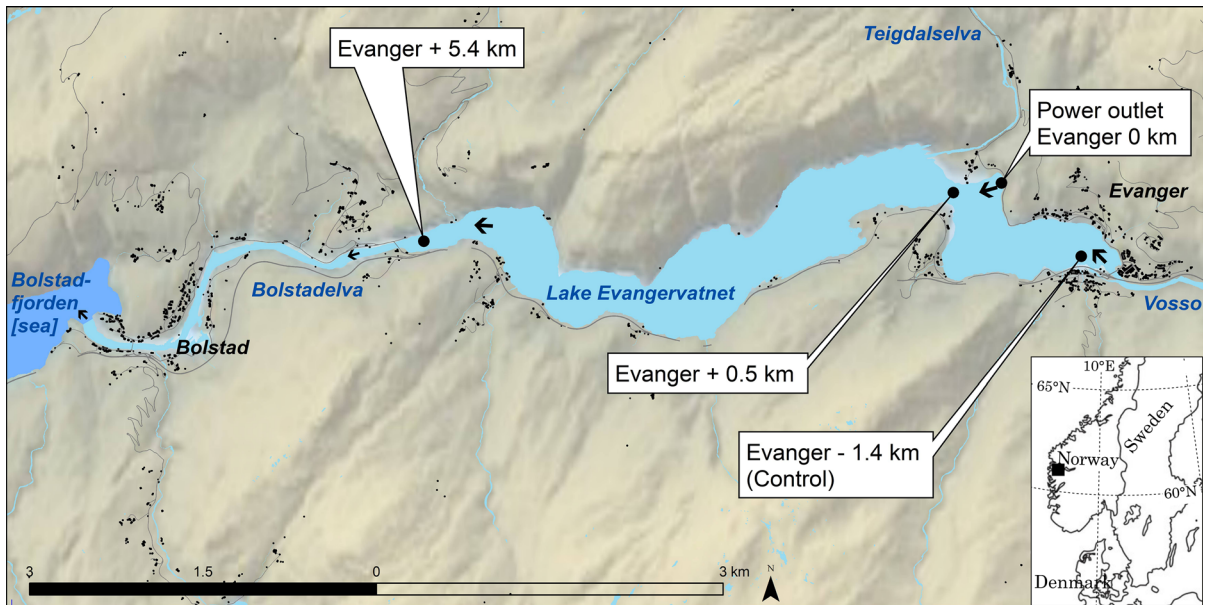
Fig. 2 Lake Evangervatnet in the Vosso River, western Norway, indicating the outlet of Evanger Power Plant and the study sites (black dots) at Evanger − 1.4 km (control site), Evanger + 0 km, Evanger + 0.5 km and Evanger + 5.4 km. Arrows indicate the direction of the water current

2, 3, and 4 h and the remaining fish were sampled after 12 h to ensure fish welfare since the fish experienced acute mortality. For animal welfare reasons, the number of fish at the start of the exposure at Brokke + 0 was still similar to the other sites. At the other sites in River Otra and Lake Evangervatnet, five fish from all cages were examined at each sampling until there were no fish left or until the termination date when the remaining fish were killed and examined. In Lake Evangervatnet, the experiment took place between May 10 and May 24 2016. The smolts from Lake Evangervatnet were sampled and examined after 4 and 14 days of exposure.

Daily inspections of the fish were conducted at all sites to record potential mortality. Inspections were done by a person wearing dive mask and snorkel that carefully approached the cages. Fish sampled for examination were netted with a knot free dip net and into a bucket with water from the site. Alive fish were euthanized with clove oil, while dead fish were placed in a separate bucked without clove oil. Immediately subsequent to death, the lateral line, the fins, the eyes and all the gill arches were carefully inspected for bubbles using a stereo microscope and 25 to 50 × magnification. The eyes were also examined for pop eyes or exophthalmia. During examination, the gill arches were removed from the fish and bathed in water from the site.

TDG monitoring

TDG was measured at every 10 min for each site in River Otra (1 min increments at Brokke + 0 km) and for every 20 min in Lake Evangervatnet. TDG was monitored by a Total Gas Analyzer 3.0 (Fisch- und. Wassertechnik; Pulg et al., 2016), which is based on the Weiss-saturometer principle (Weiss, 1970). A Total Gas Analyzer measures TDG pressure in a submerged gas permeable silicon hose connected to a pressure sensor and an atmospheric pressure sensor, which is at the water surface. The saturation is measured as the percent dissolvable air in the surface water (0 m depth) at the prevalent air pressure, and the saturometer has an accuracy of ± 10 hPa, which is approximately ± 1% TDG (pers. comm., manufacturer). We used new saturometers and atmospheric pressure sensors that were pre calibrated by the manufacturer in April 2016 at 1000 and 2000 hPa (approx. 100 and 200 % TDG at sea level). The dry saturometer pressure sensors were re-calibrated in the field 1 day prior to the onset of the experiment by synchronizing them to the atmospheric pressure

sensors. The saturometers were placed at 40 cm depth at the test sites. To avoid bubble formation in the silastic tubing of the saturometer, we knocked on the probes for a few minutes after submergence and ensured continuous water flow over the probes during the study to remove potential bubbles.

Numerical analyses

The fish survival distribution was tested using a Kaplan–Meier survival log-rank test (Kaplan & Meier, 1958). First, the survival curves were compared among sites. The log-rank test assumes that all survival time data is equally accurate, and the survival curves were considered statistically significant when $P < 0.001$. Second, we performed a pairwise multiple comparison analysis (Holm–Sidak method) to correct the P-value for multiple testing when the survival curves showed a significant difference among survival curves ($P < 0.05$) (Holm, 1979). The survival analysis was performed using Sigmaplot (version 13.0).

Results

Observed TDG levels

Average TDG varied from 105.5 to 111.6% and 105.6 to 107.2% in the River Otra and Lake Evangervatnet, respectively (Table 1). TDG saturation at Brokke + 0 km showed the highest maximum and average TDG. All sites in the River Otra had decreasing average and maximum TDG with increasing distance (0–30 km) from the power plant outlet, expect at the control site (Fig. 3). At Lake Evangervatnet, the average TDG supersaturation was relatively similar among the test sites (Fig. 4; Table 1). Here, the highest maximum TDG supersaturation occurred at Evanger + 0.5 km (112.8% TDG) (Fig. 4). The lowest average and maximum TDG were measured at the control site in both rivers (Table 1).

TDG saturation at both study sites showed a diurnal pattern with lowest TDG in the morning until noon and with increasing TDG saturation during the evening (Figs. 3 and 4). In addition, the average TDG in River Otra was higher during the first 3 days of the experiment than during the last 5 days (Table 1). There were no temporal differences in TDG levels in Lake Evangervatnet during the exposure period (Fig. 4).

Mortality

No Atlantic salmon died at the control site in the River Otra (Brokke + 11 km) during the 8 days of exposure. Here, the average TDG of the surface water was 105.5% (Table 1). The mortality probability at Brokke + 30 km and Brokke + 21 km were relatively small (10 and 25%, respectively), and the time until mortality was not significantly different than at the control site (Table 2; Fig. 3). First mortality at Brokke + 21 km occurred after 3 days of exposure at an average TDG of 108.3% (111.0% TDG maximum). While at Brokke + 30 km, first mortality occurred after 2 days of exposure at an average TDG of 109.6% (111.6% TDG maximum) (Table 1; Fig. 3).

There was a significantly different mortality time at Brokke + 17.5 km compared to the control site and Brokke + 30 km (Table 2). The salmon at Brokke + 17.5 km had a mortality probability of 55% after 3 days exposure at an average and maximum TDG of 110.2 and 111.8%, respectively (Table 1).

The mortality probability at Brokke + 0 km was 81% after 12 h exposure at an average and maximum TDG of 111.6 and 114.3%, respectively. Time to mortality was significantly faster at Brokke + 0 km than at the other sites (Table 2; Fig. 3).

We observed no mortality at the test sites in Lake Evangervatnet after 4 days or 14 days of exposure at an average TDG of 105.6 to 107.2% (Table 1; Fig. 4).

Gas bubble disease

We found gas bubbles on the fins and gills indicating GBD in fish at all study sites in River Otra, except at the control site (Table 1). No pop eyes or bubbles in the lateral line were observed. Of the fish sampled alive ($n = 64$), 22% had signs of GBD (Fig. 5). Of the fish sampled subsequent to death, 76% had bubbles on the caudal- and anal fins (Table 1). The first gas bubbles were observed after 12 h of exposure at Brokke + 0 km. Of the salmon sampled alive after 8 days of exposure at the sites Brokke + 11 km, Brokke + 21 km and Brokke + 30 km, we found gas bubbles only in fish from Brokke + 21 km. No fish in the Lake Evangervatnet had signs of GBD (Table 1).

Table 1 Average ± s.d. (%) and range (%) of total dissolved gas in the surface water (0 m depth) with average temperature (°C) for each exposure time (days) at the study sites in River Otra and Lake Evangervatnet

Site	Days of exposure	Average water temp. °C	Average TDG (%) ± s.d (%) at the surface	Range of TDG (%) at the surface	Number of fish in the cage	Alive fish sampled and examined	Dead fish	Mortality probability (%) for each sampling	Mortality probability (%) at end of exposure	Bubbles on gills (% of fish)	Bubbles on fins (% of fish)
River Otra (0.1–0.55 m depth)											
Brokke + 0 km	0.0625	6.7	111.3 ± 0.4	110.0–112.4	20	1	0	0	0	0	0
	0.083	6.7	111.3 ± 0.4	110.0–112.4	19	1	0	0	0	0	0
	0.125	6.7	111.4 ± 0.4	110.0–112.4	18	1	0	0	0	0	0
	0.167	6.7	111.4 ± 0.4	110.0–112.4	17	1	0	0	0	0	0
	0.5	6.5	111.6 ± 1.1	109.0–114.3	16	3	13	81	81	66	66
Brokke + 17.5 km	2	8.3	110.2 ± 0.5	109.3–111.6	20	0	4	20	20	–	–
	3	8.2	110.2 ± 0.6	109.1–111.8	16	9	7	44	55	22	33
Brokke + 21 km	2	9.0	109.6 ± 1.2	105.4–111.6	20	0	3	15	15	–	–
	3	9.0	108.8 ± 1.6	105.4–111.6	17	10	2	12	25	20	30
	8	8.1	107.1 ± 2.2	103.4–111.6	5	5	0	0	25	20	0
Brokke + 30 km	2	11.1	108.7 ± 1.0	106.4–111.0	20	0	1	5	5	–	–
	3	11.0	108.3 ± 1.2	105.1–111.0	19	10	1	5	10	20	20
	8	9.9	106.4 ± 1.8	103.3–110.0	8	8	0	0	10	0	0
Brokke + 11 km (control)	3	9.4	106.1 ± 1.1	103.6–108.3	20	10	0	0	0	0	0
	8	8.3	105.5 ± 1.4	101.8–108.5	20	10	0	0	0	0	0
Evangervatnet (0–1.15 m depth)											
Evanger + 0 km	14	5.0	106.2 ± 0.1	102.9–108.7	30	30	0	0	0	0	0
Evanger + 0.5 km	14	5.2	106.3 ± 0.1	102.9–112.8	30	30	0	0	0	0	0
Evanger + 5.4 km	14	4.1	107.2 ± 0.1	102.7–109.8	30	30	0	0	0	0	0
Evanger – 1.4 km (control)	14	4.8	105.6 ± 0.1	102.7–107.8	30	30	0	0	0	0	0

Also, number of fish of Atlantic salmon smolt in the cage prior to sampling, number of fish sampled, number of dead fish, mortality probability and relative abundance of fish with gas bubbles in gills or fins
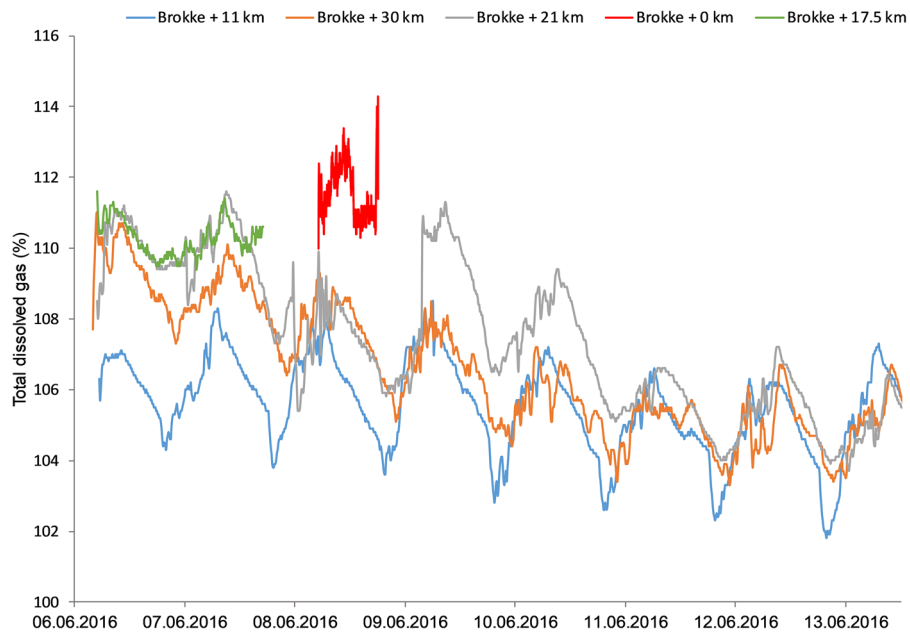
Fig. 3 Total dissolved gas (%) in the surface water (0 m depth) at the study sites (Brokke + 0 km (control), Brokke + 11 km, Brokke + 17.5 km, Brokke + 21 km and Brokke + 30 km) during the exposure period in River Otra
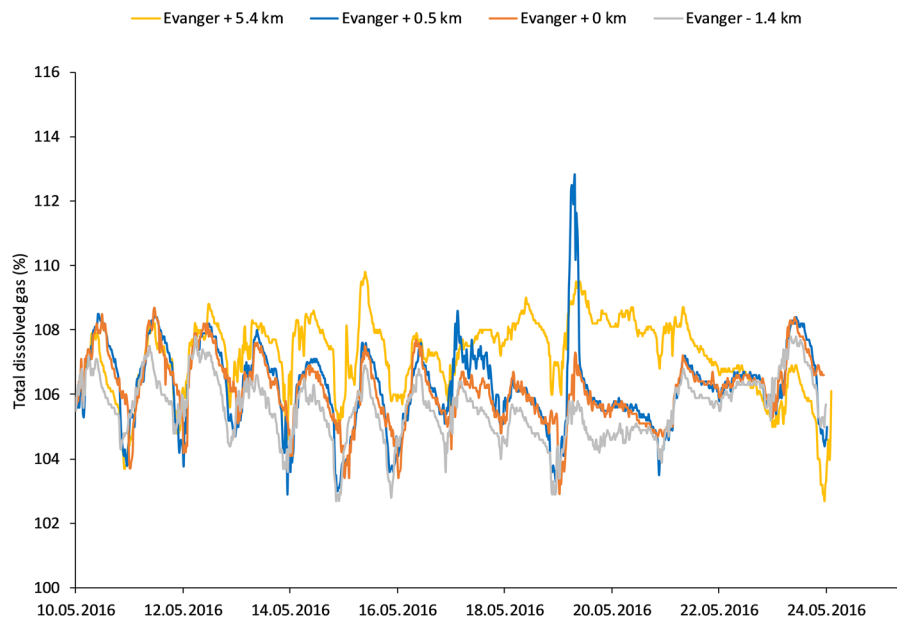

Fig. 4 Total dissolved gas (%) in the surface water (0 m depth) at the study sites (Evanger − 1.4 km (control), Evanger + 0 km, Evanger + 0.5 km and Evanger + 5.4 km) during the exposure period in Lake Evangervatnet

Table 2 Comparison of mortality of Atlantic salmon smolts among the sites Brokke + 0 km, Brokke + 11 km, Brokke 17.5 km, Brokke + 21 km and Brokke + 30 km

Comparison	t	P
Brokke + 17.5 km vs. Brokke + 0 km	24.728	< 0.01
Brokke + 21 km vs. Brokke + 0 km	24.728	< 0.01
Brokke + 30 km vs. Brokke + 0 km	24.728	< 0.01
Brokke + 11 km vs. Brokke + 0 km	24.728	< 0.01
Brokke + 17.5 km vs. Brokke + 11 km	14.725	< 0.01
Brokke + 30 km vs. Brokke + 17.5 km	8.692	0.016
Brokke + 21 km vs. Brokke + 11 km	5.577	0.071
Brokke + 21 km vs. Brokke +17.5 km	3.106	0.216
Brokke + 30 km vs. Brokke + 11 km	2.052	0.281
Brokke + 30 km vs. Brokke + 21 km	1.540	0.215

The overall significance level was 0.05
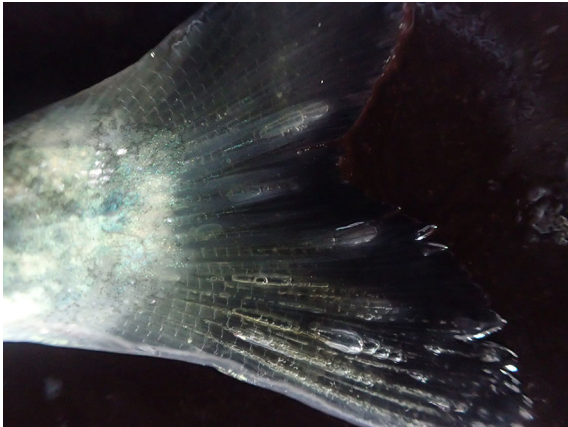
Fig. 5 A caudal fin of an Atlantic salmon (*S. salar* ssp. Bleke) with gas bubbles. The fish was held for 3 days at Brokke + 30 km in River Otra

Discussion

No mortality was observed in the Evangervatnet trials. In the River Otra trials, however, GBD and mortality occurred. The mortality probability was larger than 50% after exposure to an average of 110.2% TDG (maximum of 111.8%) for 3 days in the River Otra. The lowest average TDG level at which mortality occurred was 108.3 % TDG (maximum of 111%). We observed that fish mainly used the lower part (ca. 0.3–0.55 m depth) of the cages in the River Otra and middle part (ca. 0.3–0.9 m depth) of the cages in the Lake Evangervatnet. When compensating for pressure

(Pleizier et al., 2020), an average TDG of 110.2 and 108.3% in the surface water provides compensation to ca 107 and 105% TDG at 0.3 m water depth and 105 and 103% TDG at 0.9 m depth at the bottom of the cages. In the Lake Evangervatnet, an average TDG of 107.2% did not cause mortality. A TDG of 107.2% at the water surface provide compensation to 104 and 98% TDG at 0.3 m and 0.9 m water depths, respectively. Thus, the higher mortality in Otra than at Evanger can be explained by the higher TDG levels in Otra and better depth compensation in Evanger. After compensating for depth, the results indicate a gradually increasing risk of GBD in Atlantic salmon smolt above 105% TDG.

Tolerance toward TDG differs among species (Mesa et al., 2000) and life stages (Geist et al., 2013). Most studies of Pacific salmonids indicate increased mortality and GBD above 115% TDG (Weitkamp, 2008; Geist et al., 2013; Brosnan et al., 2016). Several studies have also found GBD and mortality at lower TDG than 115%. Mesa et al. (2000), Antcliffe et al. (2002) and Dawley & Ebel (1975) showed that gas bubbles in juvenile Chinook salmon and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) can occur from about 106% to 110% TDG, which corresponds to our results. In addition, salmonids have shown low mortality rate (0.0–3.3%) at 106% TDG in 25 cm deep tanks for 35 days (Dawley & Ebel, 1975). In contrast to our results, previous studies on salmonids found no significant mortality at 110% TDG (Newcomb, 1974; Mesa et al., 2000; Antcliffe et al., 2002) and TDG levels up to ca. 110% are referred to as "chronic" or "sub-lethal" for Pacific salmonids, while increasing levels may result in acute GBD (Alderdice & Jensen, 1985; Antcliffe et al., 2002). When compensating for depth, the threshold from which GBD commenced in Atlantic salmon in our study was approximately 2% lower than the threshold for Pacific salmonids (Alderdice & Jensen, 1985; Antcliffe et al., 2002). This may indicate a higher vulnerability of Atlantic salmon.

In a study where Atlantic salmon was exposed to TDG supersaturation at 15 cm water depth in laboratory settings, acute mortality occurred from 117% TDG and no mortality occurred at ≤ 110% TDG (Krise & Herman, 1991). There are at least six factors that may explain differences in mortality between our study and the study by Krise & Herman (1991). These factors may to some extent also be valid for other

studies that find different susceptibility toward TDG supersaturation for single species. (1) The Atlantic salmon in River Otra is a relict landlocked population, whereas the Atlantic salmon from Lake Evangervatnet and in the laboratory study (Krise & Herman (1991) are anadromous populations. Although the landlocked and anadromous populations constitute the same species, physiological differences could potentially have formed over the 9000 years since the populations were separated (Hutchings et al., 2019). For example, the landlocked population smoltifies, but not to the same extent as anadromous salmon that undergo more extensive physiological adaptations to increased salinity. Therefore, we cannot rule out that the Bleke strain from River Otra is more vulnerable to TDG than other populations of Atlantic salmon. (2) Divergent results among studies can potentially be attributed to differing sensitivity toward TDG among populations, life stages and individuals of the same species (Weitkamp & Katz, 1980; Mesa et al., 2000). For example, Meekin & Turner (1974) found 8 to 100 % mortality among groups of juvenile Chinook salmon exposed to 114% TDG for 6 days in tanks with 20 cm water depth. (3) In contrast to the laboratory study by Krise & Herman (1991) with a fixed TDG concentration in the laboratory, there were fluctuating levels of TDG in our field study. These levels varied according to the production at the power stations and according to natural levels of water discharge. Brosnan et al. (2016) found that Chinook salmon smolts exposed periodically to an average of 132% TDG and chronic exposure to an average of 118% TDG had increased mortality compared to smolts exposed to only chronic 118% TDG. This suggests that multiple exposure to TDG supersaturated water can cause increased mortality. (4) The fish in our study were reared in hatcheries that have been operating for more than two decades without signs of GBD. Thus, TDG was not measured at the hatcheries prior to the study. However, if the fish were exposed to unknown supersaturated water prior the study, this could potentially cause an increased susceptibility to TDG. (5) Fish in the field may be more sensitive to TDG than fish in controlled laboratory settings. For example, stress caused by translocation to the field, diseases, potential predators, lack of food and water current in the field may induce increased sensitivity. (6) As in most field studies, limited sampling interval, number of fish, and measure accuracy ($\pm$ 1% TDG) may influence the results. The

results must be set into a larger context, preferably a meta-study, before generalizing.

The preference for depth varies among fish species and life stages. For example, radio tagged rainbow trout, cutthroat trout (*O. clarkii* Richardson, 1836), brown trout (*Salmo trutta* Linnaeus, 1758) and bull trout (*Salvelinus confluentus* Suckley, 1859) occupy median depths from 1.3 to 2 m in a river with 101% to 135% TDG (Weitkamp & Sullivan, 2003). The depth preference for fish is important because hydrostatic pressure protects fish from GBD by ca 10% per meter depth (Pleizier et al., 2020). Fish can compensate by swimming deeper, thereby reducing harmful effects from TDG compared to fish confined to shallow water (Ebel, 1969; Weitkamp, 1976; Heggberget, 1984; Cao et al., 2019; Pleizier et al., 2020). This was demonstrated by Antcliffe et al. (2002), who exposed juvenile rainbow trout to 122% TDG for 96 h in tanks of differing water depth. They found no mortality at 0 to 2.5 m depth, 22% mortality at 0 to 1 m depth and 90% mortality at 0 to 0.25 m depth. Field studies have also shown that the survival of fish exposed to TDG supersaturation is influenced by available depth (Cao et al., 2019; Heggberget, 1984). Brown trout, perch (*Perca fluviatilis* Linnaeus, 1758) and eel (*Anguilla Anguilla* Linnaeus, 1758) held in cages near the surface showed signs of GBD and higher mortality compared to fish at greater depth (Heggberget, 1984). Based on these findings, Heggberget (1984) concluded that resident fish that can occupy deeper water are less likely to suffer mortality from nitrogen supersaturation (100–200% N_2). Therefore, results from the current study are relevant for fish that occupy the shallow areas of the watercourse, which are the preferred habitat for juvenile Atlantic salmon. In addition, the results are relevant for salmon that occupy deeper parts and occasionally spend time closer to the surface. Even brief periods spent close to surface can cause GBD due to the combination of lower hydrostatic pressure at shallower depths and the effects of decompression (Weitkamp, 2008).

Some countries have national TDG guidelines to protect aquatic organisms. The US Environmental Protection Agency (EPA) enforces a TDG threshold at 110% TDG (USEPA, 1976). Despite the TDG criteria in the EPA guidelines, each US state can include exemptions to the guidelines with its own regulations (ex. maximum up to 125% TDG in Oregon). In Canada, the Canadian Council of Ministers of the

Environment (1999) recommend a maximum of 103% TDG for shallow water less than 1 m deep. Similar to the US, each province in Canada have separate TDG regulations. However, most countries lack TDG criteria for aquatic organisms. Future research should focus on five key themes to set robust management criteria for Atlantic salmon: (1) find critical TDG levels for mortality and sub-lethal effects for all life stages of Atlantic salmon, (2) examine the depth distribution and avoidance behavior of Atlantic salmon in areas with TDG supersaturation, (3) examine effects caused by repeated exposure, (4) examine stress-on-stress effects and (5) examine effects on biological production, including fish and benthic invertebrates.

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