Effects of gas supersaturation on migrating Atlantic salmon smolt (*Salmo salar*) in Evangervatnet

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Abstract

Total dissolved gas supersaturation (TDGS) downstream of power plants may cause harmful effects on the fauna in many rivers. The harmful effects of TDGS are known as gas bubble disease (GBD). TDGS has been proposed as one possible cause of mortality of migrating Atlantic salmon (*Salmo salar L.*) smolt in Evangervatnet, Norway. The present study investigated whether TDGS had negative effects on the survival of Atlantic salmon smolt downstream of the power plant in Evangervatnet.

Four complementary sub-studies were conducted from April to June 2016: (1) Atlantic salmon held in surface cages (0-1.15 m depth) and submerged cages (1.15-2.5 m depth) upstream and downstream the power plant for 4 and 14 days. (2) Electrofishing and snorkeling observations of wild fish. (3) Capture of Atlantic salmon smolt in traps. (4) Towing of Atlantic salmon smolt across Evangervatnet. The fish were examined for GBD by visual observations by use of a stereo microscope. The surface caged smolt were also examined for histology pathology and blood cortisol.

The smolt showed no mortality or signs of GBD during the field studies, suggesting that the smolt tolerated the TDGS levels in Evangervatnet. The TDGS levels at the cage locations ranged between 102.7 to 112.8% and medians between 105.6 to 107.5% (TDGS: lowest upstream the power plant and higher downstream the power plant). The density of salmon and trout (*Salmo trutta*) was similar at higher levels of TDGS close to the power plant as in the control area upstream, indicating that the fish distribution was not negatively affected by the power plant. The median cortisol levels in the smolt held in the cages were above the normal background levels at all sampling sites after both 4 days and 14 days of TDGS exposure, which indicates that the smolt were stressed. The high cortisol levels were found both at low and at higher levels of TDGS, suggesting that handling prior to blood sampling was the main contributor to the high cortisol levels. As a conclusion, TDGS values in Evangervatnet appeared to have no negative effects on the survival of Atlantic salmon smolt downstream of the power plant.

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1 Introduction

1.1 Background

Rivers are a natural part of the landscape and have influenced human settlement for centuries (Raven et al. 1998). Rivers provide benefits for human well-being, including water supply, hydroelectric energy, industrial use, recreational activities (e.g. rafting, fishing and hiking) and mitigate flooding events (Prideaux & Cooper 2009). However, rivers are not only important for humans, they are also an important habitat for many aquatic organisms. Aquatic organisms require specific water quality criteria, and deviation from the criteria can result in negative effects on the organisms (Andersen et al. 1997).

Air dissolved in water can result in high levels of gas supersaturation (Weitkamp & Parametrix 2008). Gas supersaturation can be harmful to aquatic organisms (Bouck 1980; Heggberget 1984; Nebeker, Baker & Weitz 1981; Nebeker 1976) and can cause acute mortality and sub-lethal effects (Weitkamp & Katz 1980; Weitkamp & Parametrix 2008). Gas supersaturation can occur both naturally in rivers during algal blooms and downstream from water falls, and artificially downstream from hydroelectric power plants (Marking 1987; Heggberget 1984). Hydroelectric installations have in many cases been linked to episodes of gas supersaturation with harmful outcome for aquatic organisms (Weitkamp & Parametrix 2008; Weitkamp & Katz 1980). The Atlantic salmon (*Salmo salar L.*) population in the Vosso watercourse (Norway) crashed in the late 1980s (Barlaup 2013, 2008). Since then, intensive abatement measures have been carried out to rescue the population. Gas supersaturation has been proposed as one possible cause of mortality (Haugen et al. 2016), and consequently the decline of the salmon population in Vosso watercourse.

1.2 Air dissolved in water

Air is a mixture of gas consisting mainly of nitrogen (about 78%) and oxygen (about 21%). In water, solubility of oxygen is twice as high as nitrogen (Weitkamp & Katz 1980). As a result, water contains about 35% oxygen and 65% nitrogen. The water is total dissolved gas supersaturated (TDGS) when total dissolved gas (TDG) in the water contains more dissolved gases than it would in equilibrium with the atmosphere at surrounding pressure and temperature (i.e. TDGS > 100%; Weitkamp 2000). The solubility of gas in water depends on the physical characteristics of the gas (the mass of the gas and the partial pressure in air), the particle content, atmospheric pressure and temperature (Harvey 1975). Of these factors, temperature and pressure are considered to be the most important for solubility in fresh waters (Harvey 1975). The gas solubility increases with increasing pressure and decreasing temperature. For example, the solubility decreases and TDG increases by approximately 2% for each rise of 1°C in water (Marking 1987). In addition, hydrostatic pressure in water increases the solubility of gas proportionally to the depth (Henry's law; Henry 1803) and results in reduced relative TDG-percentage, so the actual TDG-percentage the fish is exposed to is influenced by the depth occupied by the fish (Weitkamp & Parametrix 2008). Hydrostatic pressure in water decreases saturation by about 10% per m of depth, if other factors affecting the solubility and TDG are kept constant (Weitkamp & Parametrix 2008; Figure 1.1). Therefore, 110% TDG at water surface is approximately 100% TDG at 1 m depth, i.e. fish experiencing 110% TDG at surface can compensate for TDGS by staying at 1 m depth (Weitkamp & Parametrix 2008).

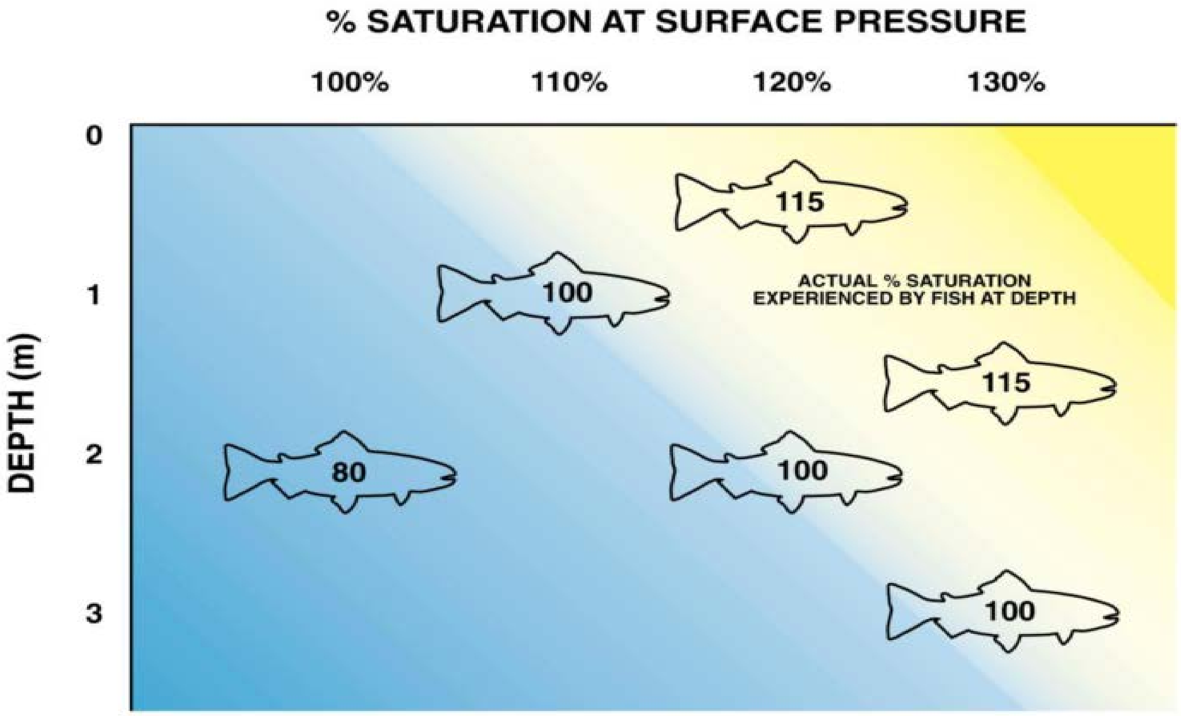

Figure 1.1: The relationship between surface gas saturation (%) and depth (m): TDGS is reduced by approximately 10% per m of depth (Adapted from Weitkamp & Parametrix 2008, p. 6).

1.3 Occurrence of gas supersaturation

Water can be TDGS if air and water are put under pressure greater than the local barometric pressure, followed by a decreased pressure or an increased temperature (Harvey 1975). In other words, increased pressure increases the solubility and the water will take up more gas. When the pressure is decreased, the solubility decreases and excess gas will cause TDGS.

TDGS can occur both naturally and artificially in rivers. Natural occurrence of gas supersaturated water can occur, for example downstream from waterfalls. This is because air in the water stream can become entrained in the water and transported under water with higher pressure. The air will then dissolve under pressure, and the water becomes TDGS (Marking 1987). Additionally, photosynthetic activity by the algae blooms producing O_2 can elevate the TDG value if dissolved under hydrostatic pressure (Weitkamp & Katz 1980). Furthermore, solar radiation heating the water can increase the level of TDG (Marking 1987).

Artificial occurrences of TDGS are often observed downstream of dams and outlets of hydroelectric installations (Weitkamp & Parametrix 2008). Artificial TDGS can occur in several situations. (1) TDGS can occur if air is entrained into pumps, pipes or turbines, where air dissolves in water at increased pressure. When the water is returned into atmospheric pressure, the pressure decreases, and causes TDGS (Marking 1987; Heggberget 1984). Air drawn into the pipes of hydroelectric installations is common when the pipes are connected to creek intakes. For example, creek intakes represent the most common cause of artificial TDGS in Norwegian rivers (Blindheim et al. 1984). However, entrainment of air into the pipes is greatly reduced by using proper design (a Pelton wheel will de-gas the water better than a Francis wheels) and maintenance of the creek grates at the inlets (Stokkebø et al. 1986). (2) Many rivers have experienced high levels of TDGS when water is released over dam spillways (Weitkamp & Parametrix 2008). The spillways are used to control flooding events, but they also provide fish passage (Weitkamp & Parametrix 2008). (3) Artificial TDGS can occur if mixing of water with different temperatures from the hydroelectric installation and the river are discharged into the river (Weitkamp & Parametrix 2008).

1.4 Biological effects of gas supersaturated water

Water supersaturated with atmospheric gases are known to be harmful to aquatic organisms (Weitkamp & Katz 1980). The fish gill is an effective gas exchange system because: (1) the fish gill provides a large contact area for gas exchange due to the primary and the secondary lamellae and the countercurrent exchange of blood and water (Kryvi & Totland 1994), (2) the cell layer between water and blood is thin (one-two cell layers thick), which results in a short distance for diffusion of gas between water and gills (Kryvi & Totland 1994), and (3) the saturation point of water and blood are similar, which makes gases in water easily absorbed into the bloodstream of fish (Marking 1987). Therefore, the blood of the fish will become gas supersaturated when the fish respire in supersaturated water. When the blood is gas supersaturated, excess gas can leave the blood and form bubbles, which induces a variety of lethal and sub-lethal effects, often referred to as gas bubble disease (GBD; Bouck 1980).

Signs of GBD in fish can be observed as gas bubbles in the tissue (emphysema) and in the blood (emboli). The gas bubbles commonly occur in the gill blood vessels tissue, eyes, mouth and organs, on the head and fins (Figure 1.2), along the lateral line, and sometimes the fish experience exophthalmia (“pop eyes”; Weitkamp & Katz 1980). Signs of GBD occur differently depending on the TDG level. High and acute exposure of TDG (about >125% TDG) is not correlated with externally visible signs of GBD (Weitkamp & Katz 1980). However, chronic exposure (about 105-125% TDG) and the extent of GBD are closely associated with the severity of the external bubbles (Weitkamp & Parametrix 2008; Mesa, Weiland & Maule 2000). Bouck (1980) described the development of GBD in three stages: Firstly, excess gas starts forming bubbles and the fish show sign of morbidity. Secondly, the mortality rate of the fish increases. Thirdly, the most tolerant fish that survive the first two phases have increased mortality.

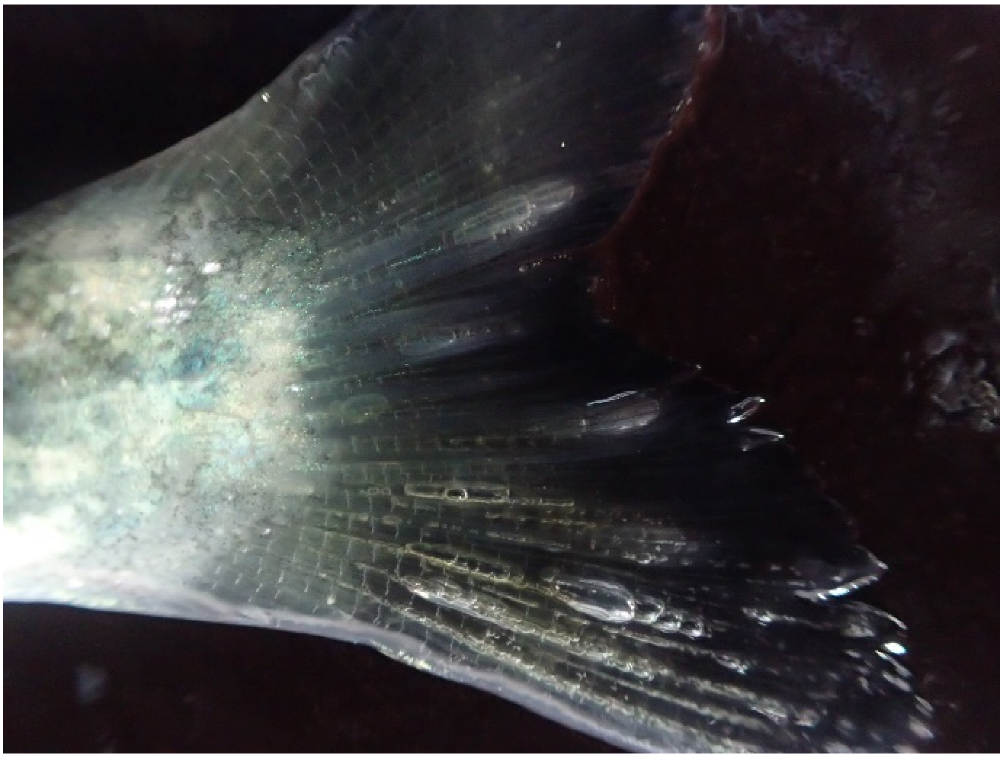

Figure 1.2: The caudal fin of *Salmo salar L.* from Otra above the Bygelands-fjord in Southern-Norway, with gas bubbles due to TDGS. Photo by: Sondre Kvalsvik Stenberg

Another potential effect of TDGS is change in blood chemistry (Newcomb 1974). Fish exposed to TDGS can become stressed (Weitkamp & Katz 1980). Stressed fish may have changes in cortisol levels due to stressors stimulates the hypothalamic-pituitary-interrenal axis, which controls the release of plasma cortisol (Wendelaar Bonga 1997). Stress can cause increased susceptibility to diseases (Barton 2002; Olsen, Falk & Reite 1992), decreased growth (Mommsen, Vijayan & Moon 1999), reduced sea water tolerance (Iversen, Finstad & Nilssen 1998) and mortality (Iversen, Finstad & Nilssen 1998; Iversen & Eliassen 2009). Nevertheless, caution should be taken when interpreting cortisol levels, as cortisol can be affected by natural background levels. Background cortisol levels can vary with annual variation (Thorpe et al. 1987), diel variation (Thorpe et al. 1987), life cycle (Thorpe et al. 1987; Langhorne & Simpson 1981) and individual variation (Olsen, Falk & Reite 1992; Sandodden & Iversen 2001; Iversen et al. 2003; Iversen, Finstad & Nilssen 1998).

The fish's natural behavior (depth distribution and avoidance response to TDGS) and the available depth will affect possible deleterious effects (Weitkamp & Parametrix 2008). The fish ability to compensate for TDG with increased depth is supported by studies where fish have been confined to different depths (Ebel 1969; Dawley et al. 1975; Weitkamp 1976; Heggberget 1984; Antcliffe, Fidler & Birtwell 2002). In addition, fish can recover from potentially high TDGS levels by swimming to greater depths (Knittel, Chapman & Garton 1980; Elston et al. 1997; Schiewe 1974).

1.5 Toxic levels of gas supersaturation

A few countries have enforced a TDG criterion to protect aquatic organisms. The US Environmental Protection Agency (EPA) developed a TDG criterion of maximum 110% TDG in 1972 (USEPA 1976). In addition, Canadian Council of Ministers of the Environment (1999) recommend a maximum of 103% TDG for shallow water less than 1 m deep. However, most countries lack TDG criterions for aquatic organisms.

TDG values exceeding the EPA criterion are considered harmful to aquatic organisms (Dawley & Ebel 1975; Nebeker et al. 1980; Jensen 1988). However, the decision resulting in the EPA criterion has been criticized for not having included studies that allow for hydrostatic compensation and chronic exposure of TDGS. The EPA criterion is mainly based on acute high levels of TDGS (usually > 115%) laboratory studies in shallow cages (30-100 cm deep) and some sentinel cage studies (smolt kept in cages in the river). The tests have normally been performed on Pacific salmon (*Salmo gairdneri*, *Oncorhynchus nerka*, *Oncorhynchus tshawytscha*; USEPA 1973). More recent studies have examined different levels of TDGS and its effects on salmonids in natural environments, with the possibility of hydrostatic compensation (Weitkamp & Parametrix 2008; Mcgrath, Dawley & Geist 2006; Heggberget 1984; Weitkamp et al. 2003). Based on these studies, several researchers have questioned the validity of the EPA criterion, and some have suggested that the criterion is too strict (Weitkamp & Parametrix 2008; Weitkamp 2000; Mesa, Weiland & Maule 2000). Nevertheless, salmonids exposed to 110% TDG have shown signs of gas bubbles (Newcomb 1974; Mesa, Weiland & Maule 2000; Antcliffe, Fidler & Birtwell 2002; Dawley & Ebel 1975) and sub-lethal effects at levels below 110% TDG (Schiewe 1974; Shrimpton, Randall & Fidler 1990; Dawley & Ebel 1975). Sub-lethal effects include weakening the swimming behavior (Schiewe 1974), inflation of the gas bladder (Shrimpton, Randall & Fidler 1990), decreased growth (Dawley & Ebel 1975) and increased susceptibility to predation (Mesa & Warren 1997). In spite of these findings, other studies examining the effects of chronic exposure at levels below 110% TDG found no support of sub-lethality and lethality responses in salmonids (Schisler, Bergersen & Walker 1999; Krise & Meade 1988; Geist et al. 2013). Despite the contradictory views of the validity of the criterion set by EPA, it remains as the applicable criterion of TDG in the US (Weitkamp & Parametrix 2008).

Heggberget (1984) suggested that all salmon species have equal tolerance to TDG, based on the notion of their physiological similarities (Heggberget 1984). However, newer studies have shown that the tolerance to TDG may differ among salmonids (*Oncorhynchus tshawytscha*, *Oncorhynchus mykiss*, *Oncorhynchus kisutch*, *Oncorhynchus nerka*, *Oncorhynchus tschawytscha* and *Salmo gairdneri*; Beeman & Maule 2006; Mesa, Weiland & Maule 2000; Stevens, Nebeker & Baker 1980; Weitkamp & Parametrix 2008). In addition, small differences in depth distribution among salmon species can constitute different biological effects as TDG will decrease with depth (Weitkamp & Katz 1980; Weitkamp & Parametrix 2008). The difference in tolerance to TDG among salmon species raises questions whether the most sensitive and vulnerable salmon species are affected by TDGS at lower levels than previously thought. Species specific tolerance to TDG should therefore be known when setting the criteria for maximum TDGS levels.

1.6 Toxic levels of gas supersaturation on Atlantic Salmon

The effects and the tolerances of TDGS on Atlantic salmon (*Salmo salar L.*) are poorly documented and have received far less attention than many other species (e.g. *Salmo trutta, Perca fluviatilis, Anguilla anguilla*; Blindheim et al. 1984; Golmen 1992; Heggberget 1984; Kroglund & Tjomsland 2003; Stokkebø et al. 1986; Thorstad et al. 1997). Blindheim et al. (1984) conducted cage experiments and a laboratory experiment on Atlantic salmon. They reported that Atlantic salmon exposed to 120% TDG likely suffer mortality, while values of 110 to 120% might result in harmful effects. Other studies of gas supersaturation on Atlantic salmon has focused on oxygen saturation (Kristensen et al. 2010; Espmark, Hjelde & Baeverfjord 2010). Espmark et al. (2010) observed gas bubbles in Atlantic salmon smolt exposed to water supersaturated with 160% oxygen after 14 days. However, salmonids (e.g. *Salmo salar*) exposed to water supersaturated with oxygen develop gas bubbles later compared to exposure of water supersaturated with air (Nebeker, Bouck & Stevens 1976; Espmark, Hjelde & Baeverfjord 2010). Krise and Herman (1991) showed that sub-yearling Atlantic salmon was less tolerant to TDGS than lake trout (*Salvelinus namaycush*), but the

TDGS values were not given. In addition, farmers of Atlantic salmon have reported production problems at 104 to 105% TDG, suggesting that Atlantic salmon are particularly sensitive to TDGS among the salmonids (life stage was not given; Marking 1987). Moreover, Atlantic salmon populations have been affected several km downstream hydroelectric installations and dams, and TDGS is not unusual in rivers with Atlantic salmon (Kroglund & Tjomsland 2003; Pulg, Gabrielsen & Normann 2013; Pulg, Barlaup & Normann 2014; Blindheim et al. 1984; Pulg et al. 2016). However, knowledge of the effects and the tolerances of TDGS on Atlantic salmon are largely lacking.

1.7 Potentially harmful gas supersaturation levels in Evangervatnet

The population of salmon in the Vosso watercourse crashed in the late 1980s. The population has not recovered, despite intensive abatement measures (Barlaup 2013, 2008). Annual releases of smolt and planting of eyed eggs, synchronized delousing and recapture of escaped farmed salmon have since 2000 been conducted in the watercourse. In addition, the discharge water from the power plant was limed to increase the pH and neutralize the acid water in 1994 (Barlaup 2013), and the water chemistry has been surveyed and a cage experiment has investigated survival rates of smolt in the Bolstadfjord (Bjerknes, Golmen & Åtland 1995). However, the main reason(s) for the collapse of salmon in Vosso watercourse and why it is slow to recover is still unknown.

The Atlantic salmon life cycle starts in fresh water, migrates as smolt to sea water in search for food and returns to fresh water to spawn (McDowall 1987). In order to track the migration of Vosso-smolt, Haugen et al. (2016) conducted an experiment with acoustic telemetry in Vosso watercourse. The analysis of the migration pattern indicated that the smolt experienced a mortality of about 90% when swimming through Evangervatnet, a lake in the Vosso watercourse (Figure 2.1). TDGS was suggested as a possible source for the high mortality (Haugen et al. 2016), and thus has been covered extensively in the media ('Evanger kraftverk under lupen etter at over 90 prosent av Vosso-smolten døde' 2016; Sado, Løland & Pettersen 2016; 'Vurderer å stenge evanger kraftverk for vossolaksen' 2016). Monitoring of TDGS in Evangervatnet has been conducted for several years (Table 1.1). TDGS occurs naturally at Vosso River (<107%). Nevertheless, TDGS at the power plant is on average some percent higher than at the Vosso River (Pulg, Gabrielsen & Normann 2013). Examining the effects and the tolerances of TDGS on Vosso-smolt are important to avoid conflicts, accusations and especially to reduce potential threats. Moreover, a clarification of these issues will be of great importance, as the information may aid in the management of rivers where TDGS can be a problem for the Atlantic salmon.

Table 1.1: Median and the minimum to maximum (Min-max) of total dissolved gas (TDG (%)) in the Vosso watercourse previous years (1992, 2012-2013 and 2015).

Location (year)	Median TDG (%)	Min-max TDG (%)	References
Power plant (23.06 1992 and 29.06.1992)	NA	104.6-105	(Golmen 1992)
Power plant (2012-2013)	104-106	99-110	(Pulg, Gabrielsen & Normann 2013)
Power plant (15-31 May 2015)	106.2	100-110	(Ulrich Pulg, Uni ResearchMiljø, pers. comm.)
Vosso River (2012-2013)	NA	NA-107	(Pulg, Gabrielsen & Normann 2013)

1.8 Benefit to society

Increased understanding about the effects and the tolerances towards TDGS in Atlantic salmon may be beneficial for both the society and the environment. The pressure on Atlantic salmon stock has increased, mainly because of human impacts. This has caused declines and extinctions in many rivers (Anon 2015; Mills et al. 2013; Dempson et al. 2011; ICES 2013). About one third of the world Atlantic salmon population is found in Norway. Therefore, Norway has taken on a special responsibility to preserve the Atlantic salmon (Regjeringen.no n.d.). Atlantic salmon fishing has long traditions in Norway, providing recreational opportunities and with an important employment and income source for many districts (Rieber-Mohn et al. 1999).

1.9 Benefit to hydroelectric installations

TDGS does not only have ecological impacts. Air entrained into the hydroelectric installations can under certain conditions result in a blow-out. Blow-outs have potential health risk for people close by the pipe inlet and the they can be harmful to the turbine runners (Stokkebø et al. 1986). In addition, entrained air is associated with turbulent water flow into the pipe (Jenssen et al. 2006). Turbulent water flow has high rotation speed and low pressure that produce cavitation (Jenssen et al. 2006). Cavitation damages the turbine runner due to formation of cavities on the metallic surface (Kumar & Saini 2010). Moreover, if TDG values are high, hydroelectric operators can be enforced to invest in innovative technology to prevent or reduce problems with TDGS (*Norges vassdrags- og energidirektorat* n.d.). In the worst case, hydroelectric operators can be forced to close their facilities (*Norges vassdrags- og energidirektorat* n.d.). Enforced TDGS regulations may cause costly investments (Weitkamp 2000). Summarized, hydroelectric facilities should aim to minimize air entrained into the pipes to reduce potential chances of health risk and maintenance of the turbine runners. In addition, clear guidelines could be set with increased understanding on the effects and the tolerances of TDG on the biota.

1.10 Aims and objectives

The aim of this study is to determine whether existing TDG values have negative effects on survival of Atlantic salmon smolt downstream of the power plant in Evangervatnet. The objectives of the present study were to examine potentially: (1) salmon smolt mortality caused by TDGS. (2) Signs of GBD on salmon smolt. (3) Elevated stress levels in salmon smolt. (4) Effects on the fish distribution downstream the power plant. The objectives were addressed by four field studies: (1) salmon smolt held in sentinel cages held at fixed positions in different distances to the power plant. (2) Electrofishing and snorkeling observations of wild fish. (3) Capture of salmon smolt in traps in the Vosso watercourse (4) Towing of smolt across Evangervatnet. TDG was monitored and smolt were examined for gas bubbles, histology pathology (heart, brain and gill) and blood cortisol. It was hypothesized that: (1) TDG could explain the high mortality observed in earlier studies. (2) Hydrostatic pressure could compensate for TDG and its effects. (3) Increased TDGS would be related with stress indicators and mortality. (4) Fish avoid areas close to the power plant.

2 Methods

2.1 Total dissolved gas monitoring

Total dissolved gas (TDG) was monitored by Total Gas Analyzer 3.0 (Fisch- und. Wassertechnik, Pulg 2015b, 2015a). The logging is based on a Weiss-Saturometer connected with an additional atmospheric pressure sensor. The saturometer has an accuracy of ±10 hPa, which is approximately 1% TDG. The TDG measured with 30 min increment by the logger, is always set in relation to atmospheric pressure at the surface. The value measured represents therefore the maximum TDG saturation the water would have at the surface. Four TDG monitors were situated in the Vosso watercourse from 01 January 2016 to 06 June 2016 (Figure 2.1). The TDG loggers in Evangervatnet were located at the power plant outlet, the Vosso River and the lake outlet (Figure 2.3, Figure 2.4). In addition, a TDG monitor was located in Bolstadhølen in Bolstad River (Figure 2.8).

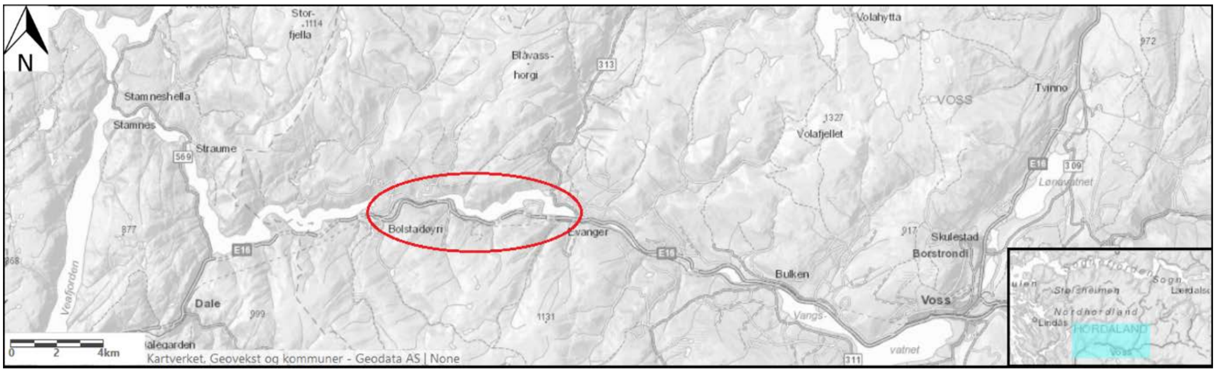

Figure 2.1: Red circle indicates an overview of the study area, which includes Evangervatnet and Bolstad River. Map data: *NVE atlas 3.0* n.d..

2.2 Water discharge

Water discharge in Vosso watercourse was obtained to examine potentially patterns between discharge and TDG supersaturation (TDGS). Hourly mean discharge at Bulken from 23 April 2016 to 02 June 2016 was obtained from NVE (*Vannføring for Bulken (Vangsvatnet)* n.d.). Bulken is at the outlet of Vangsvatnet approximately 7.6 km upstream the Vosso River outlet (Skog og Landskap, Statens Vegvesen & Statens Kartverk 2014). Daily mean operational discharge from the power plant was obtained from 1 January 2016 to 15 June 2016.

2.3 Fish origin

The 250 Atlantic salmon (*Salmo salar L.*) smolt used for the field experiments were obtained from the Haukvik gene bank as eyed eggs early spring 2015. The eggs were hatched and reared at Voss Klekkeri. The fish were fed with Skretting nutra Olympic. Light conditions were regulated by natural photoperiod from 15 November 2016 to 01 Mars 2016. Thereafter, the fish were reared with 24 hours day light until they were transported from Voss Klekkeri to a hatchery cage (4 m × 4 m × 4 m) in Evangervatnet 21 April 2016. The fish was moved from Voss Klekkeri to Evangervatnet to acclimatize the fish and to reduce the stress of handling during field studies. At Evangervatnet, the fish were fed with a 24 hours clock machine. Veterinary reports confirmed that the experimental fish was in good condition (A.1, Appendix), and the field experiments were conducted with permission from the Norwegian Food Safety Authority (application ID 8505).

2.4 Field studies in Vosso watercourse

The study included four sub-studies that were conducted in the Vosso watercourse, Norway (Figure 2.1): (1) sentinel cages, (2) electrofishing and snorkeling observations, (3) traps catching naturally migrating salmon smolt and (4) smolt tow. These methods were complimentary and give a multifaceted view on the effects of gas supersaturation on Atlantic salmon smolt in the Vosso watercourse. The peak salmon smolt migrating through Vosso watercourse occurs during mid-May (Barlaup 2008). The field studies were conducted when salmon smolt likely migrated through Evangervatnet (Figure 2.2)

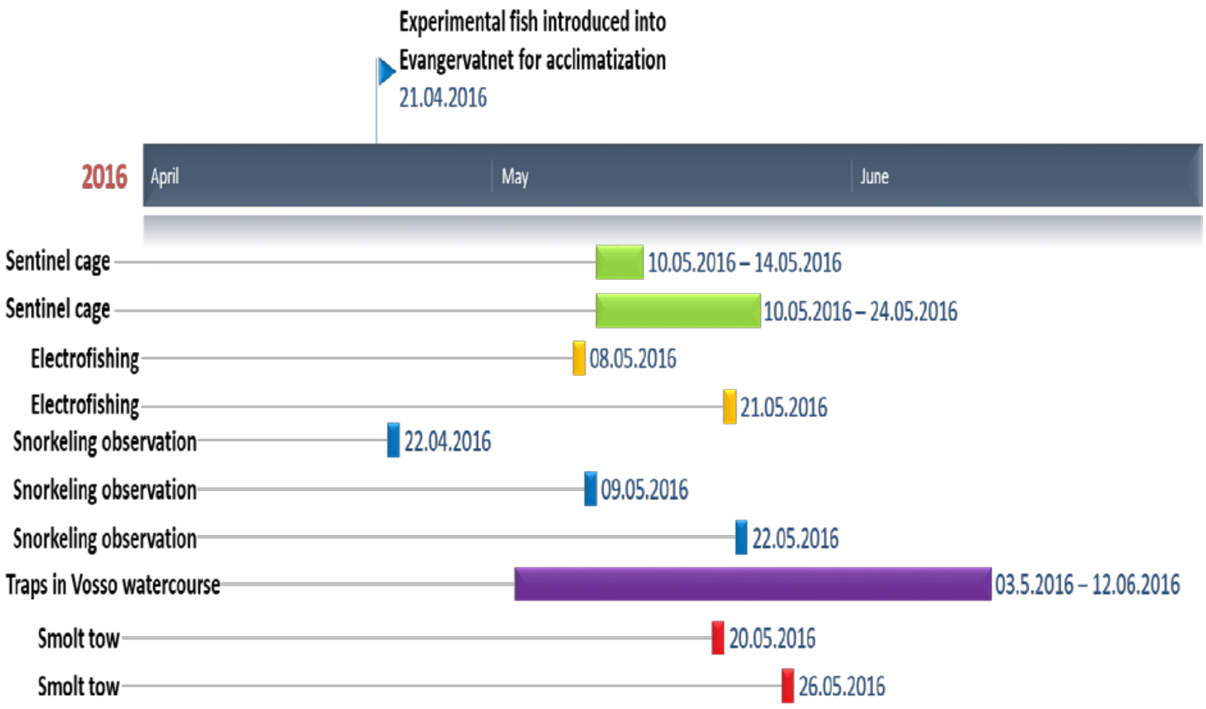

Figure 2.2: Overview of the periods with field studies in Vosso watercourse, April to June 2016. The field studies listed are: sentinel cages, electrofishing, snorkeling observation, traps in Vosso watercourse and smolt tow. The blue flag at the top of the time line represents the time fish were introduced into Evangervatnet for acclimation.

2.5 Field study 1: Sentinel cages

2.5.1 Study area

In this experiment, salmon smolt were kept in four fixed locations (Figure 2.3) at different distances from the power plant to study potential correlation among TDG, mortality, signs of gas bubbles disease (GBD) and elevated levels of stress.

The site at the lake inlet was located upstream the power plant close to the Vosso River (Figure 2.4). The site at the power plant outlet was located south of the water discharge from the power plant. Site F.H. strait was positioned in the strait between Fadnes and Hernes (Figure 2.4). F.H. strait is close to the power plant outlet and smolt migrating from Vosso River passes through this narrowing in the lake. The site at the outlet of Evangervatnet was positioned about 4.7 km downstream the site at F.H. strait (Figure 2.4).

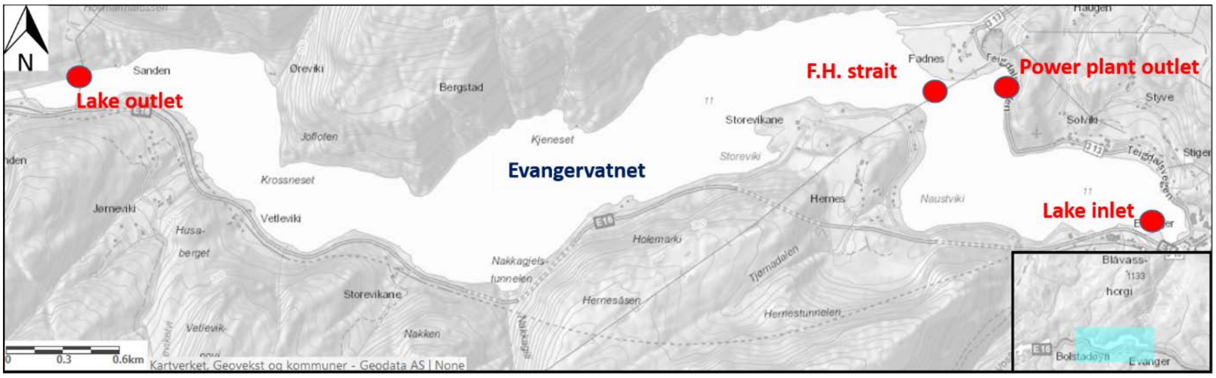

Figure 2.3: Overview of the sentinel cage sites (red circles) in *Evangervatnet*: the power plant outlet, the lake inlet, the F.H. strait (Fadnes Hernes) and the lake outlet. The direction of water flow runs from the lake inlet to the lake outlet. Map data: *NVE atlas 3.0* n.d..

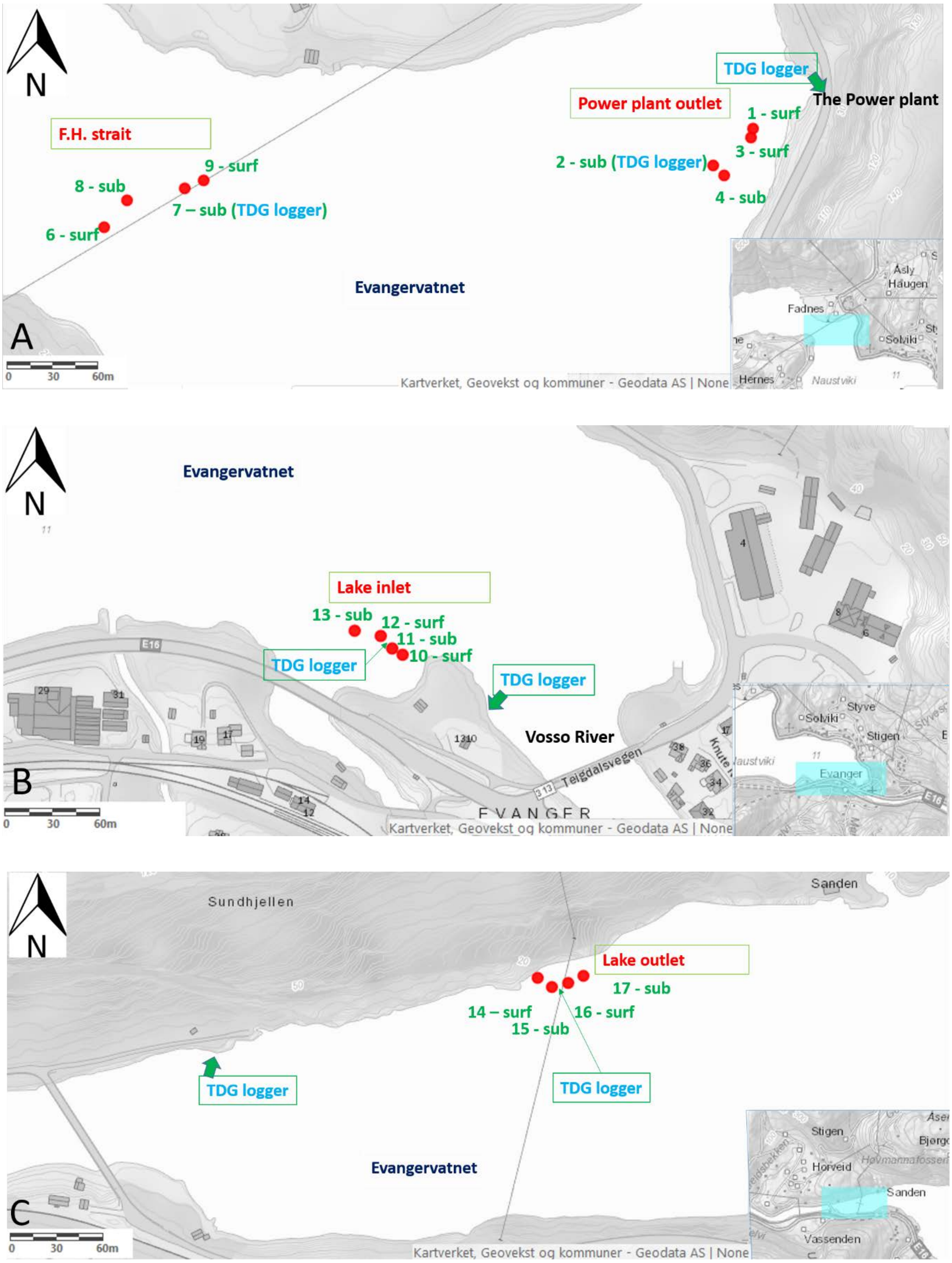

Figure 2.4: Location of the cages at the power plant outlet (A; cage 1-4), F.H strait (A; cage 6-9), the lake inlet (B; cage 10-13) and the lake outlet (C; cage 14-17). Surf and sub indicates whether it was a surface cage or a submerged cage, respectively, and the position of the TDG loggers are marked as "TDG logger". Map data: NVE atlas 3.0 n.d.

2.5.2 Cages

Two surface cages (held at 0-1.15 m depth) and two submerged cages (held at 1.15-2.5 m depth) were placed at each site in Evangervatnet (Figure 2.5). The purpose of the surface cage was to test the effects of TDGS among smolt located near the surface. The purpose of the submerged cages was to test the effect of increased hydrostatic pressure, as the levels of TDGS in Evangervatnet were expected to be compensated at 1 to 2 m depth.

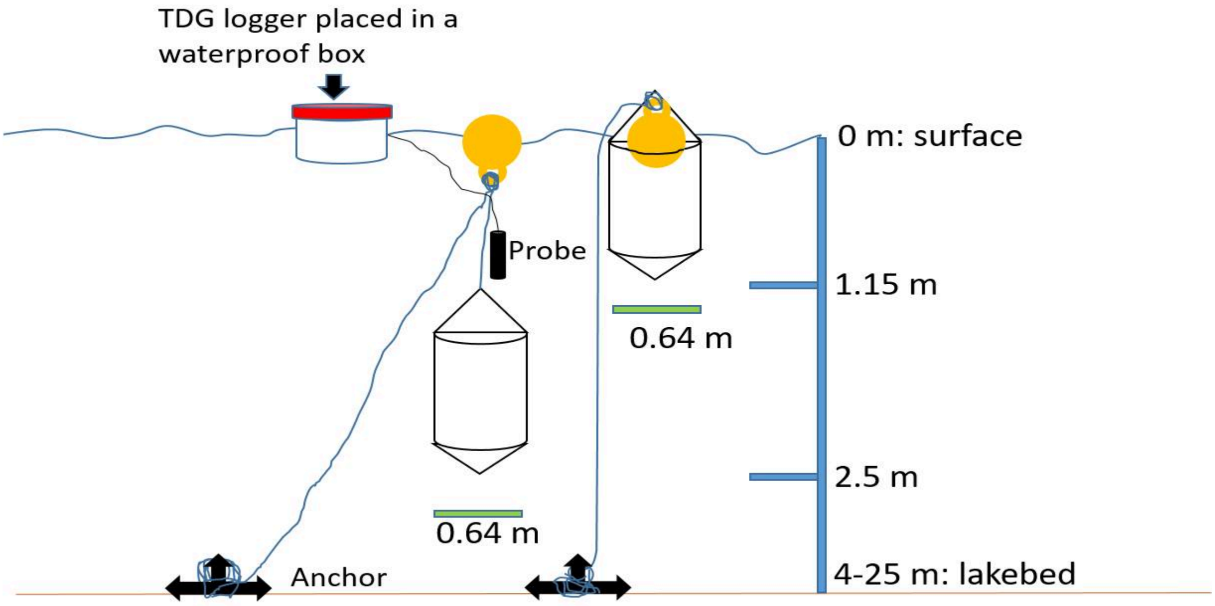

Figure 2.5: Two cage designs were used for the sentinel cage experiment. To the left is a submerged cage (1.15–2.5 m depth) and to the right is a surface cage (0–1.15 m depth). The float chamber (orange) is placed inside the net of the surface cage and outside the cage of the submerged cage. A lead rope (1.5kg) was placed at the bottom of each cage to ensure stability. The TDG logger was placed in a waterproof box attached to the submerged cage and the TDG probe (black cylinder) was attached to the float chamber.

All cages were anchored to the lake bottom allowing the cage to remain in a fixed position, without being stranded or hitting the bottom (Figure 2.4). The cages at the lake inlet and the lake outlet were placed in a row, by attaching the cages to a rope anchored at shore and in the lake. To avoid displacement of the cages by the current, the rope length from floater to the anchor was the sum of the depth plus 1/3 depth. The distance between surface cages at F.H. strait and the power plant varied during the sentinel cage period, while the distance was more or less constant at the lake outlet and the lake inlet. The cages at the power plant and the lake inlet were placed next to the outlets of the power plant and the Vosso River to avoid stressing the fish by exposing them directly to the strong outlet current (Figure 2.4). The distance between the cages (minimum 7 m and maximum 73 m) was minimized to ensure equal TDG exposure and low between-cage effects, and an operational distance between the cages was necessary to allow ready and easy operational access to each cage. The cages were placed in the water 15 days prior to the experiment to check that they remained stable on the same spot.

2.5.3 Fish

A total of 15 and 10 smolt were maintained in each surface cage and submerged cage, respectively. The group sizes were selected because this was believed to provide sufficient robustness of random mortality.

The fish were handled as gently as possible to reduce the chances of mortality. To reduce stress and potential mortality due to handling the smolt were gently placed into the cages from a tub in a motor boat. The smolt remained calm during transportation. A knot free dip net was used to allocate the smolt into the cages. The cages were inspected for placement stability every day. The inspections occurred with minimum 30 m distance from the cages to minimize stressing the fish. When debris was attached to a cage, it was gently removed.

The behavior of the smolt in cage 6 at F.H. strait and cage 12 at the lake inlet were observed by a snorkeler 12 May 2016. The snorkeler approached the cages gently to minimize stress.

2.5.4 Exposure period

The smolt were held in two surface cages at each sentinel cage location for a 4-day and a 14-day period from 10 May 2016 (Figure 2.6). Five fish from each of the surface cages were examined for physiology (GBD, cortisol, gill metal and histology) after four days (a total of 40 smolt, Figure 2.6). Moreover, five fish from each surface cage were sampled for physiology after 14 days (a total of 40 smolt). The remaining five fish in the surface cages were killed and weighed in the lab. Smolt held in two submerged cages at each sentinel cage location were held for a 14-day period from 10 May 2016 (Figure 2.6). The short exposure period in Vosso watercourse is representative for the exposure time that migrating smolt are likely to experience in the eastern part of Evangervatnet. The long exposure period in Evangervatnet was expected to provide knowledge on the cumulative effects of long-term exposure to TDG.

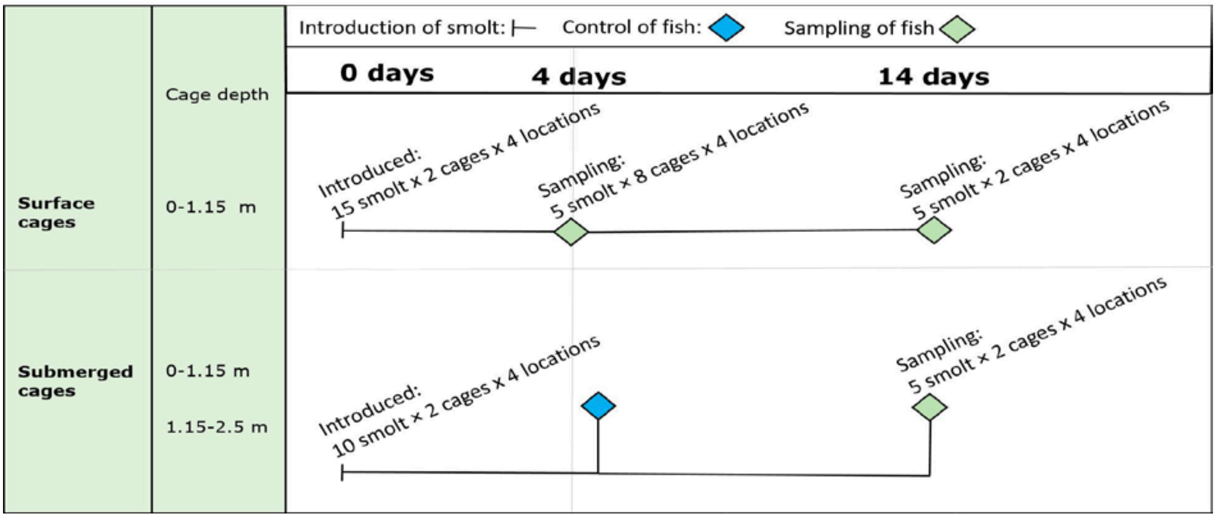

Figure 2.6: Experimental design: each of the four sampling sites contained four cages. Two surface cages (held at 0-1.15 m depth) with 15 smolt held in each cage and two submerged cages (held at 1.15-2.5 m depth) with 10 smolt kept in each cage. The green squares represent the exposure time to TDG before sampling of smolt (after four and 14 days). The submerged cages were raised to the surface for a control (blue square, day five).

2.5.5 Sampling procedures

Blood sampling procedures and analytic procedures

About 50 m from the cages, the boat engine was turned at low speed to reduce engine noise. At first sampling day, the surface cages were opened from the top, while still being in the water. The fish were netted from each cage with a knot free dip net and into a bucket with 20 l of water containing a lethal dose of clove oil (60 mg l^{-1}) source. All fish examined were overdosed consistently with clove oil. During the second sampling, the cages were lifted into the boat, and the fish were poured into the bucked of mixed water and anesthetic (clove oil 60 mg l^{-1}). Second sampling was different from first sampling to minimize the time from approaching the cages to the fish were put into clove oil. The time spent sampling fish at the surface cages were recorded (Table 2.2). The submerged cages were sampled consistently with the described sampling of day two.

Blood of surface caged smolt (n=80) were withdrawn from the caudal vein into a 1-ml sodium-heparinized syringe (Heparin LEO 5000 IE/ml Orifarm AS Oslo). The blood samples were stored on ice and brought to the lab at the Norwegian Institute for Water Research (NIVA) in Bergen. At the lab, the blood samples were centrifuged at 14000×g for 5 min. The plasma was decanted and stored at -20°C until analyzed. An ELISA kit (Enzo Life Sciences, Inc, Cortisol ELISA kit, AD-900-071, Farmingdale, NY and BioSource Porcine) was used to measure plasma cortisol, which is a competitive enzyme immunoassay. The procedure was performed according to the manufacturer's protocol (A.2.1, Appendix).

Table 2.2: Time from collecting fish from each cage, placed in sedative, first fish bled, last bled and last fish from each cage examined. Time indicates minutes and seconds.

Location	Cage - date	Exposed to clove oil	First bled	Last bled	Last dissected	Finish
Power plant	Cage 3 - 14.05.2016	2:03	7:55	16:15	30:23	38:00
	Cage 3 - 24.05.2016	0:30	6:40	23:04	27:04	27:04
	Cage 11 - 14.05.2016	1:55	8:30	25:25	29:04	36:00
	Cage 11 - 24.05.2016	0:36	8:01	21:39	29:05	29:05
Lake inlet	Cage - 12 14.05.2016	1:08	7:50	21:05	27:15	30:15
	Cage - 12 24.05.2016	0:28	9:55	23:21	29:09	29:09
	Cage - 10 14.05.2016	1:07	5:45	24:20	27:50	35:00
	Cage - 10 24.05.2016	0:28	9:15	24:25	30:31	30:31
F.H. strait	Cage 9 - 14.05.2016	1:10	8:00	22:00	25:30	31:08
	Cage 9 - 24.05.2016	0:33	8:34	23:39	31:04	31:04
	Cage 6 - 14.05.2016	2:00	9:20	27:00	30:10	NA
	Cage 6 - 24.05.2016	0:21	6:15	23:52	31:02	32:02
Lake outlet	Cage 14 - 14.05.2016	0:58	7:18	20:30	29:00	35:00
	Cage 14 - 24.05.2016	0:20	6:18	22:23	28:05	28:05
	Cage 16 - 14.05.2016	1:30	7:30	20:15	29:00	31:40
	Cage 16 - 24.05.2016	0:25	6:21	22:11	27:53	27:53

Gill metal

Gill metal was sampled to get closer to answer our research questions, as gill metal could potentially affect the salmon smolt. The first left gill arch of surface caged smolt (n=80) was sampled for metal analysis (iron, copper and aluminum). The gill arch was removed gently with forceps and placed on ice and frozen at -20°C. The gill metal samples were sent for analyses at the NIVA lab in Oslo, where the samples were lyophilized weighed and digested with 1% HNO_3 at 85°C. The metal ions were analyzed and measured with an ICP-MS.

Formalin-fixed paraffin embedded tissue samples

The second left gill arch, the brain and the heart of surface caged smolt (n=80) were excised and fixed in 10% natural buffered formalin. After a minimum of 48 h, the gills were embedded in paraffin wax and sectioned at 5 µm. The paraffin embedded tissues were stained with hematoxylin and eosin and reviewed for histological changes.

External examination and analysis of gas bubbles

Both submerged and surface caged smolt were: (1) visually observed for gas bubbles related to GBD. Fins, lateral line and eyes were carefully checked for the bubbles. (2) The smolt were external examined for gas bubbles using a stereo microscope (25 to 50 X magnification). In addition to the examination of the fins, the lateral line and the eyes; remaining gill arches were examined for GBD using the microscope.

2.5.6 TDG monitoring

TDG monitoring was recorded with 20 min increments monitored by Total Gas Analyzer 3.0 (Fisch- und. Wassertechnik, Pulg 2015b, 2015a), at each sentinel cage site from 10 to 24 May 2016 (Figure 2.3, Figure 2.4). TDG logging at F.H. strait continued until 02 June 2016. The TDG monitors at the sentinel cages consisted of two probes: high-and low pressure sensor. The low-pressure probe is more accurate to low levels of TDG (<124%) compared to the high-pressure probe. However, the low-pressure probe is poor at detecting high peaks of TDG (125-250%). The TDG meters at the sentinel cages were placed into 15 l barrels (Figure 2.5). The probe depth was 50 cm. Calibration of the TDG loggers and temperature loggers were conducted prior the field studies by the lake (Evangervatnet).

2.6 Field study 2: Electrofishing and snorkeling

Electrofishing and snorkeling were conducted to evaluate whether fish avoid areas close to the power plant and to examine wild fish for potentially signs of GBD. Electrofishing was conducted (08 May 2016 and 21 May 2016) at the transect sites M, N, O and P (Figure 2.7). Each electrofishing site was 30 m long and 3.5 m wide. The bottom of sites: N, O and P consisted of a shore slope with boulders. Site M consisted of fine sand and dead trees.

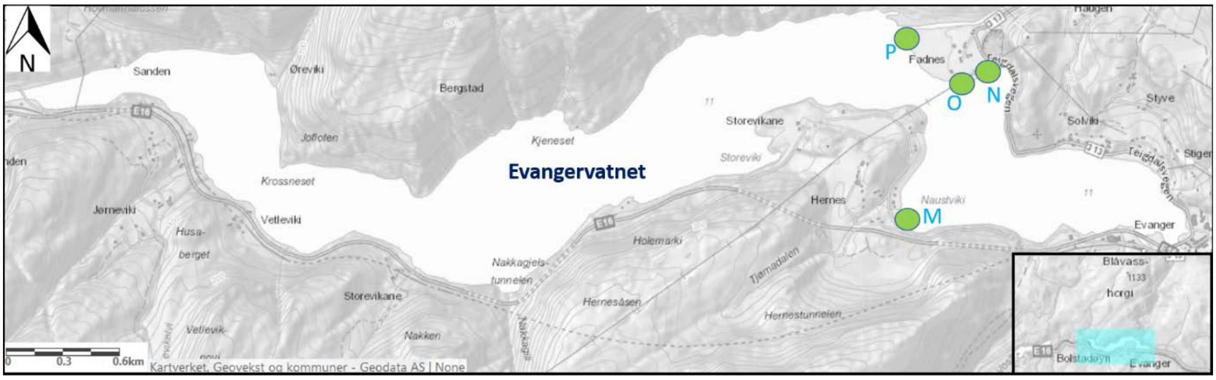

Figure 2.7: Electrofishing was conducted at site M, N, O and P. Snorkeling observations included site N, O and the part between the two sites (about 140 m distance). Closer overviews of the electrofishing sites and the snorkeling observations were found in the Appendix (A.3.). Map data: *NVE atlas 3.0* n.d..

Electrofishing was conducted according to the methods of Bohlin et al. 1989. Each transect site was repeatedly electrofished three times 08 May 2016 and 21 May 2016. The caught fish were sorted into buckets of water after which round they were caught. The fish were examined for gas bubbles accordance to section 2.5.5. The weather was overcast during electrofishing (08 May 2016 and 21 May 2016). At site N and M, it was windless. Site O and P were wind affected, with ripples on the clear water surface. The water was clear. The outer 0.5 m of the transects were difficult to electrofish due to higher water depth 21 May 2016.

From experience, high number of gas bubbles on the caudal fin and the anal fin can be observed without a light microscope. Pictures of fish were taken during the snorkeling observations. If GBD is present, then fish with gas bubbles would likely be detected in the pictures taken during diving. The pictures of the fish were enlarged and clear pictures displaying the fish fins were examined for gas bubbles. A transect of about 140 m close to land at the north side of the power plant outlet was snorkeled 25 April 2016, 09 May 2016 and 22 May 2016 (Figure 2.7). To conduct *in situ* observations of wild salmon and trout snorkeling were conducted along the shore from midnight to 02:00 am. The snorkeler was equipped with a flash light to see the fish and fish was registered. The total number of observed salmon and trout were recorded per date.

2.7 Field study 3: Traps in Vosso watercourse

Naturally migrating salmon smolt were trapped to examine whether the project was conducted during the migration time of salmon smolt and to potentially detect smolt with signs of GBD (Figure 2.8).

The trapping lasted from 25 April 2016 to 14 June 2016. Two types of trap were used; a trap net and a rotary screw trap. Trap nets used in Evangervatnet were similar to the trap nets developed by Barlaup et al. (2013). When fish hit the lead net of the trap, the fish were led into a residence cage (1.3 m deep) held at about 1.5 m depth (Figure 2.9). The fish were prevented from swimming around the trap entrance due to two wings (Figure 2.9), which lead the fish in the right direction. The rotary smolt screw trap was designed as a drum with 1.5 m in diameter entrance, whereof half of the drum was submerged (Figure 2.10). Fish that hit the entrance were led into a residence cage in the back of the rotary smolt screw trap (held at 0-0.4 m depth). Fish caught by the traps were examined for signs of GBD daily.

Figure 2.8: Overview of the traps in Vosso watercourse. The trap nets were located in Evangervatnet and the rotary smolt screw trap in Bolstadhølen in the Bolstad River. Map data: *NVE atlas 3.0* n.d..

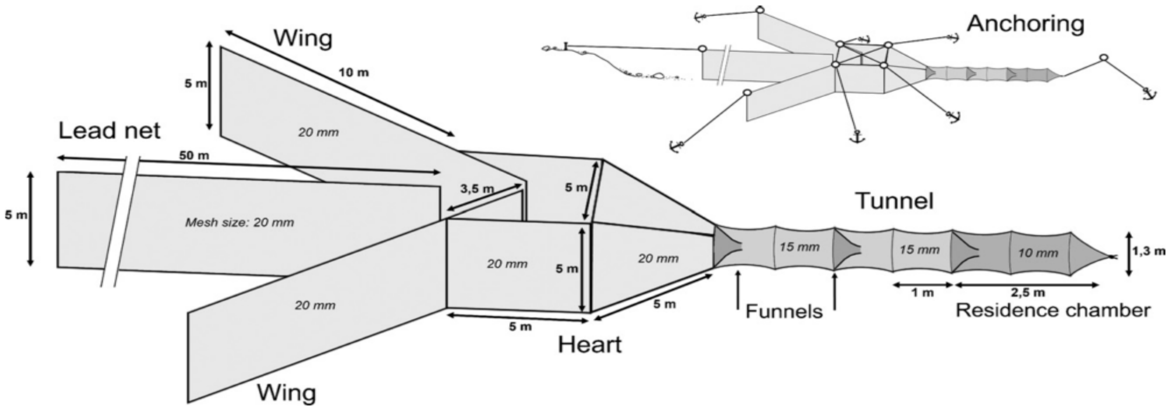

Figure 2.9: Trap net design: the lead net is 50 m long. The lead net, the wings and the heart is 5 m deep. The tunnel is 6 m long and 1.3 m deep, which includes the funnel and the residence chamber. The mesh size of the lead net, the wings and the heart is 20 mm. The funnels and the residence chamber have mesh sizes of 15 mm and 10 mm, respectively. Adapted from Barlaup et al. (2013).

Figure 2.10: Rotary smolt screw trap in the current of Bolstad River. Picture: Uni Research, Uni Environment, LFI.

2.8 Field study 4: Smolt tow

The towing of smolt was designed to provide information on the effects of TDGS on smolt that migrate through Evangervatnet. 10 smolt were towed across Evangervatnet at 20 May 2016 and at 26 May 2016 (Figure 2.11). The specially designed boat had a mesh-walled cage (0-1 m deep) that was lowered through and below the hull.

The towing boat was equipped with a TDG logger, a GPS and two cameras that filmed the smolt behavior. The GPS was used to trace the route by time. The data from the TDG logger were compared to the GPS track to find TDG values through the water. Although TDG logging while towing smolt only represent a snapshot of TDG at a given time, TDG logging in Evangervatnet could add information on the distribution of TDG in the lake. The TDG monitor measured partial pressure of O_2 and TDG every 1 min at 50 cm water depth. For calibration purpose, CO_2 was measured before the tow and remaining gases were set to one, which is an approximate measure of the remaining gases. With known partial pressures of O_2, CO_2, and remaining gases, partial pressures of N_2 were calculated. Low engine power and 70 m distance from the towed boat to the towing boat were used to minimize the chance of air bubbles from the boat engine to affecting the measured TDG values. The average speeds of the tows were 1.8 km/h 20 May 2016 and 2 km/h 26 May 2016.

The smolt behavior was filmed to potentially detect abnormal swimming behavior (e.g. loss of equilibrium) related to change in TDG level and to regulate the speed of the towing.

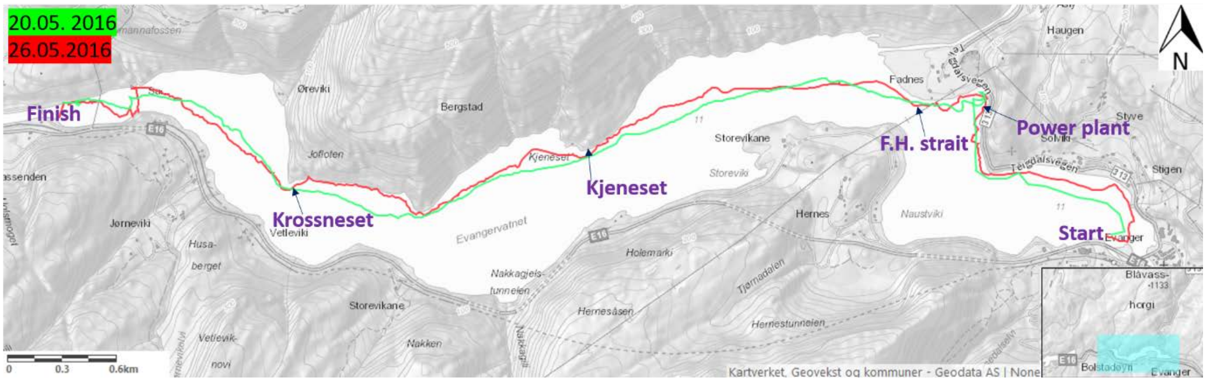

Figure 2.11: Overview of the path of the smolt tows 20 May 2016 (green) and 26 May 2016 (red). The smolt tows started (Start) at the lake inlet and finished (Finish) at the lake outlet. “Power plant (cage)”, “F.H. strait”, “Kjeneset”, “Krossneset”, represent different locations in the lake. Map data: *NVE atlas 3.0* n.d..

Smolt that showed abnormal swimming behavior during towing were removed from the cage with use of a small net. Smolt removed during towing were examined for gas bubbles in the towing boat (according to section 2.5.5) by use of a binocular microscope. Remaining smolt were examined for GBD at the lake outlet. High humidity and rain 20 May 2016 caused fogging of the microscope glass. Consequently, the fish was only visually examined on this date. Fish examined for sings of GBD 26 May 2016 was conducted by a similar procedure as that described in section 2.5.5.

2.9 Statistical analyses

To test the concentrations of metal on the gills and blood cortisol linear mixed effect models (LME) were used with site (power plant outlet, F.H. strait, lake outlet, lake inlet) and date (14 May 2016, 24 May 2016) and their interactions as predictor variables. In addition, TDG was tested among sites (power plant outlet, F.H. strait, lake outlet, lake inlet) with a LME. Specific cage as a random effect was applied to the metal data and the cortisol, and time as random effect for the TDG data. The lake inlet was used as the reference site as it was located upstream of the power plant. The response variables of the gill metal and TDG models were log-transformed because some values were high or low, and thus violating assumptions of normal-distribution. (A.2.2, Appendix)

Model selection was done by sequentially dropping variables and using a likelihood ratio test (A.4, Appendix). The models were also tested with a likelihood ratio test for temporal autocorrelation (A.4, Appendix). The P-value for statistical significance was set to P=0.05. The analysis was performed using the nlme library in R (version 3.2.0 (2015 April 2016)). Standard curves were made for each plate of blood cortisol being analysed with the ELISA kit (A.2.2, Appendix).

3 Results

3.1 Total dissolved gas measures

3.1.1 Long-term TDG monitoring

Total dissolved gas (TDG) values were generally higher at the power plant compared to TDG at the other TDG loggers from January to mid-April (Figure 3.1). The median (50th percentile) TDG values were 105.8% at the power plant, 102.1 at the lake outlet, 101.0% at the Vosso River and 100.1% at the Bolstadhølen (Table 3.1). The highest peaks of TDG measured during 01 January 2016 to 09 May 2016 occurred at the Vosso River (06 May 2016) and the power plant (08 May 2016) with TDG values of 109.6% (±1%) and 109.2% (±1%), respectively (Table 3.1).

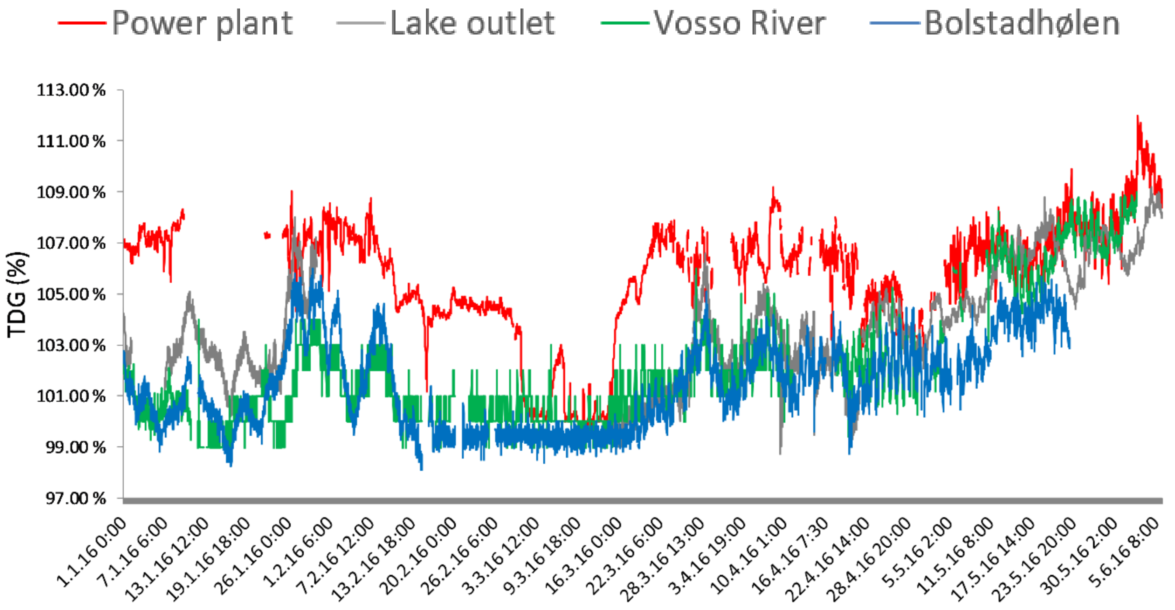

Figure 3.1: Recorded total dissolved gas (% TDG) levels at the power plant (red), the lake outlet (grey), the Vosso River (green) and Bolstadhølen (blue) from January to May 2016. Gaps in the graphs represent time periods with inoperative TDG logging.

Table 3.1: The 25th percentile, the median (50th percentile), the 75th percentile, the minimum value (Min) and the maximum value (Max) of total dissolved gas (%) from 01 January 2016 to 09 May 2016, 10 May 2016 to 14 May 2016, 10 May 2016 to 24 May 2016 and 25 May 2016 to 06 June 2016. Uncertainty of TDG is ±1%.

Date and location	**25th percentile TDG (%)**	**Median TDG (%)**	**75th percentile TDG (%)**	**Min TDG (%)**	**Max TDG (%)**
01.01-09.05.2016*					
Power plant	104.4	105.8	106.9	98.7	109.2
Vosso River	100.0	101.0	102.0	99.0	109.6
Lake outlet	100.6	102.1	103.7	98.7	108.0
Bolstadhølen	99.7	101.1	102.3	98.1	106.0
10.05-14.05.2016					
Power plant	106.3	106.8	107.2	104.7	108.4
Vosso River	106.1	106.5	107.0	103.2	108.3
Lake outlet	105.1	106.2	106.8	104.3	107.3
Bolstadhølen	103.0	103.9	104.4	101.6	105.4
10.05-24.05.2016					
Power plant	106.4	107.0	107.7	104.5	109.9
Vosso River	105.9	106.6	107.1	104.3	108.8
Lake outlet	105.9	106.4	107.3	103.2	108.8
Bolstadhølen**	103.6	104.1	104.5	101.6	105.7
25.05-06.06.2016					
Power plant	107.6	108.4	109.5	105.4	112.0
Vosso River***	107.1	107.9	108.4	106.1	109.0
Lake outlet	106.6	107.1	107.6	105.2	109.2
Bolstadhølen	Na	Na	Na	Na	Na

*The TDG loggers had several inoperative periods during 01.01-10.05.2016 (Figure 3.1).

** TDG logging: 10.05-24.05.2016

***TDG logging: 25.05.2016-02.06.2016

3.1.2 TDG monitoring at the sentinel cages

The Atlantic salmon *(Salmo salar L.*) smolt held in sentinel cages in Evangervatnet were exposed to diurnal fluctuating TDG values (Figure 3.2). The caged smolt were exposed to TDG levels that ranged from 102.9 to 108.8% during the 4-day TDG exposure period (10 to 14 May 2016) and 102.7 to 112.8% TDG during the 14-day TDG exposure period (10 to 24 May 2016; Table 3.2). The maximum TDG values during the 4-day TDG exposure did not differ by more than 1% among the cage locations, while the maximum TDG values during the 14-day TDG exposure were more varied among the cage locations (Table 3.2). Maximum values of TDG above 109% lasted for 20 min to five hours (with 20 min TDG monitoring increments). The diurnal TDG was highest at noon and night and lowest during midday (Figure 3.2). Two flooding events occurred in the Vosso watercourse during the TDG exposure experiment period (Figure 3.3). From the start of the second flooding event there were less diurnal TDG fluctuations (19 May 2016; Figure 3.3).

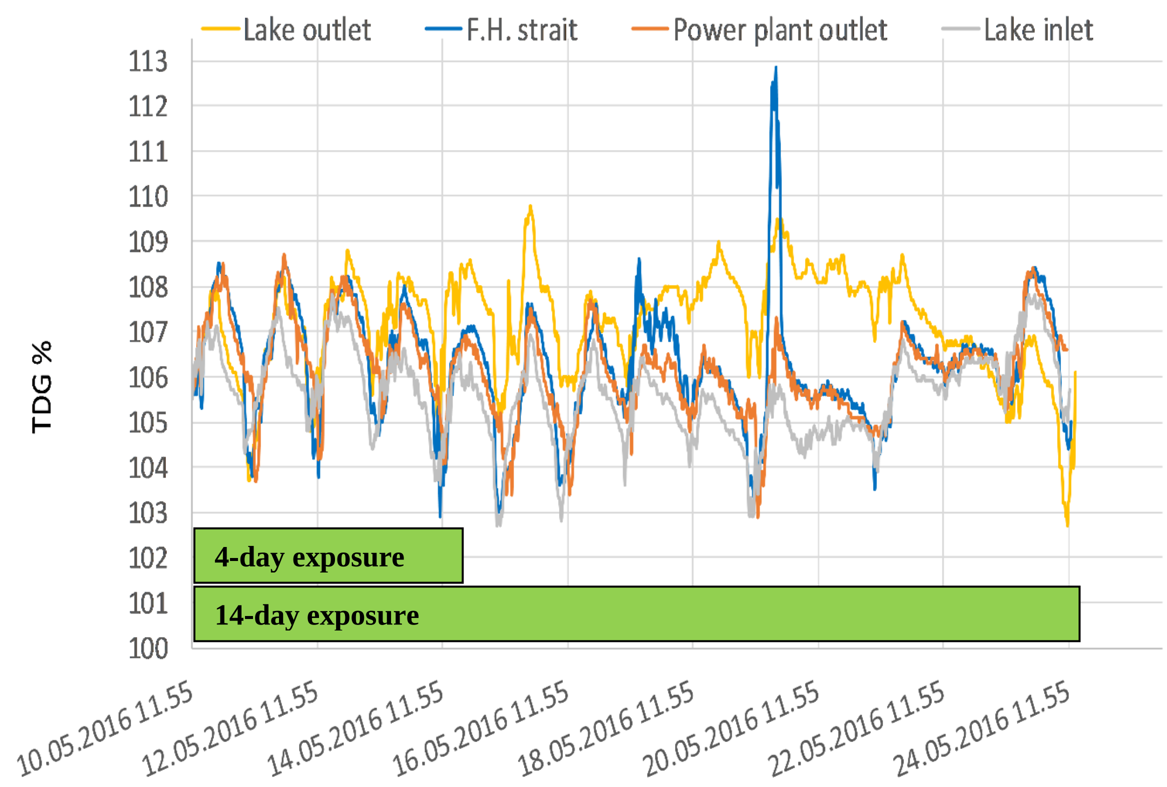

Figure 3.2: TDG (%) measured at the lake outlet (yellow), F.H. strait (blue), the power plant outlet (orange) and lake inlet (grey) from 10 May 2016 to 24 May 2016. The 4-day exposure and the 14-day exposure represent the periods with smolt held in sentinel cages.

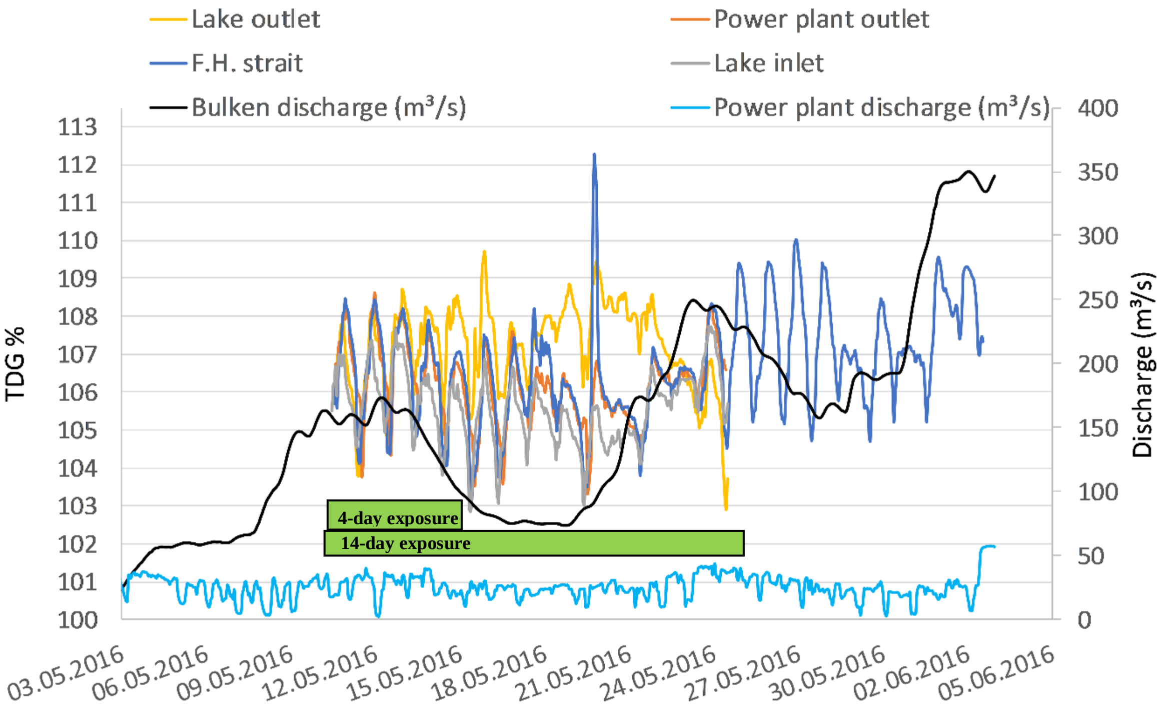

Figure 3.3: TDG (%) measured at the lake outlet (yellow), F.H. strait (blue), the power plant outlet (orange) and lake inlet (grey) from 10 May 2016 to 24 May 2016. The 4-day exposure and the 14-day exposure represent the periods with smolt held in sentinel cages. The secondary axis represents the water discharge at Bulken and the power plant.

TDG was generally highest at the lake outlet (downstream the power plant) and lowest at the lake inlet (upstream the power plant) for both exposure periods (Figure 3.4). For example, smolt held in cages were exposed to highest median (50th percentile) TDG at the lake outlet, while the lowest median TDG values occurred at the lake inlet. The caged smolt were exposed to median TDG values of 106.0 to 107.3% and 105.6 to 107.5% after the 4-day and 14-day TDG exposure, respectively (Table 3.2).

In addition, the LME model indicated that smolt at the lake inlet were exposed to significantly lower TDG values compared to the smolt at the other cage locations, both during the 4-day exposure and the 14-day exposure (Table 3.2).

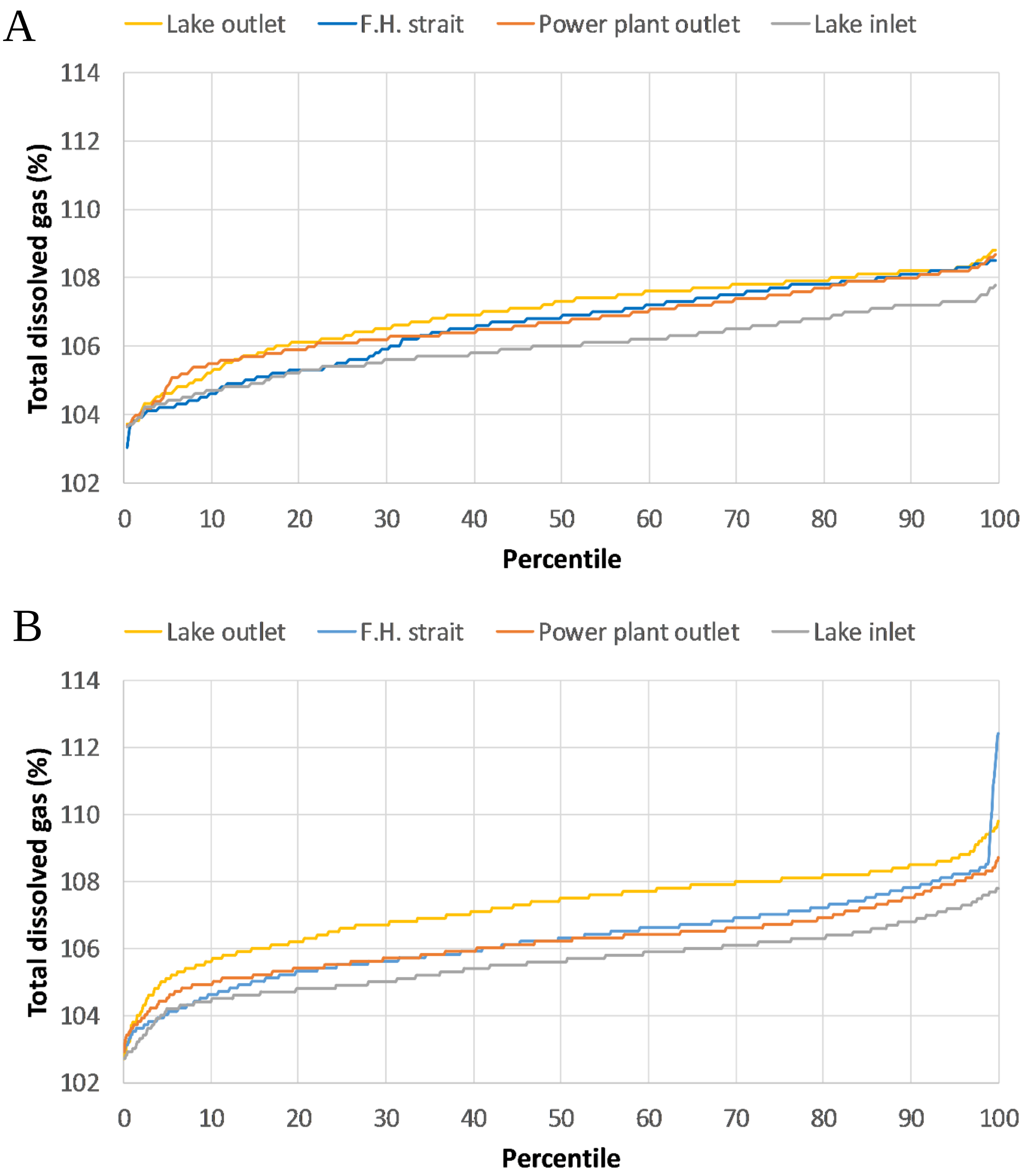

Figure 3.4: Total dissolved gas (%) 10 to 14 May 2016 (A, 4-day TDG exposure) and 10 to 24 May 2016 (B, 14-day TDG exposure) at the lake outlet (yellow), the F.H. strait (blue), the power plant (orange) and the lake inlet (grey) for different percentiles.

Table 3.2: The 25th percentile, the median (50th percentile), the 75th percentile, the minimum value (Min) and the maximum value (Max) of TDG (%) of the 4-day (10-14 May 2016) and 14-day (10-24 May 2016) TDG exposure. In addition, average temperature (°C) is given with ±95 confidence interval. TDG (%) uncertainty is ±1%. *, indicates that TDG was significantly higher than (P <0.001) at the lake inlet.

Exposure and location	**25th percentile TDG (%)**	**Median TDG (%)**	**75th percentile TDG (%)**	**Min TDG (%)**	**Max TDG (%)**	**Average °C ±95 CI**
4-day TDG exposure						
Lake outlet	106.2	107.3*	107.8	103.7	108.8	4.1±0.0
F.H. strait	105.5	106.9*	107.7	102.9	108.5	5.2±0.0
Power plant outlet	106.1	106.7*	107.5	103.7	108.7	5.0±0.0
Lake inlet	105.4	106.0	106.7	103.6	107.8	4.8±0.0
14-day TDG exposure						
Lake outlet	106.6	107.5*	108.0	102.7	109.8	5.0±0.0
F.H. strait	105.5	106.3*	107.0	102.9	112.8	5.6±0.0
Power plant outlet	105.5	106.2*	106.7	102.9	108.7	5.5±0.0
Lake inlet	104.9	105.6	106.2	102.7	107.8	5.3±0.0

Unlike any other TDG recordings at the cage locations, the TDG logger at the F.H. strait recorded TDG values greater than 110.0% TDG. TDG recordings ranged from 109.0 to 112.8% for four hours and 17 min on 19 May 2016 (TDG logging every 20 min). In the same period, TDG peaked at the power plant outlet (107.3%) and the lake inlet (105.8%, Figure3.2), and a rapid sharp peak in temperature occurred at the F.H. strait (Figure 3.5). Preceding the high peak at the F.H. strait (112.8% TDG), no similar recordings of TDG occurred at the power plant outlet and the lake inlet (Figure 3.2).

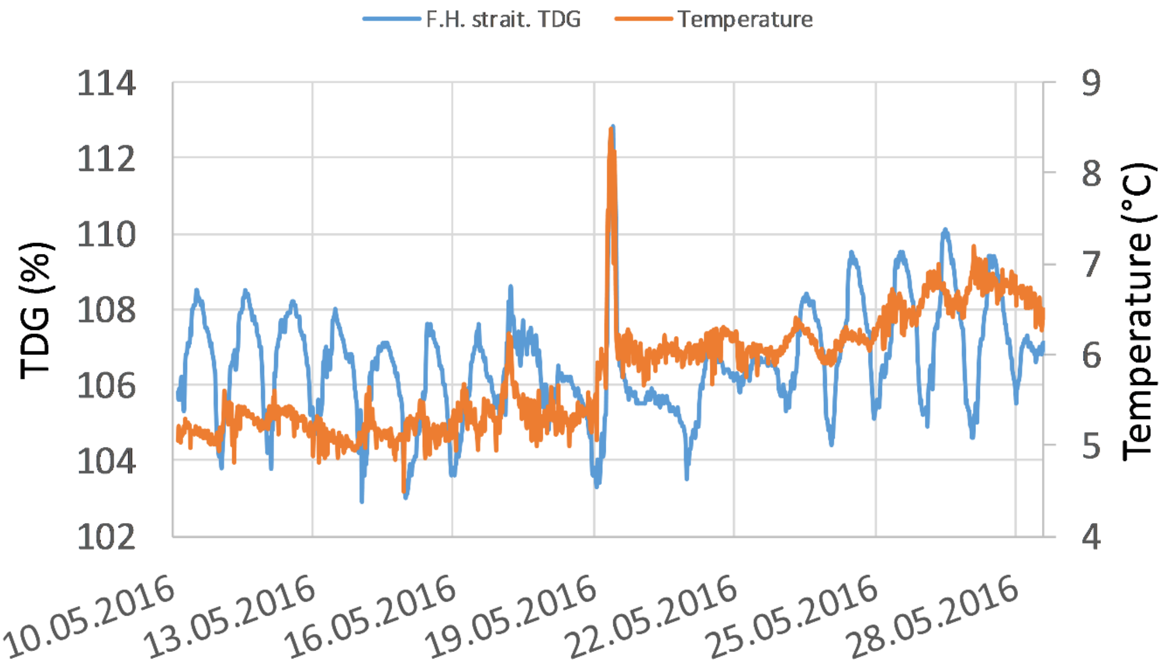

Figure 3.5: TDG (%, blue) and temperature (secondary axis, orange) at the F.H. strait 10 May 2016 to 02 June 2016.

TDG above 109.0% lasted 2.8% (maximum for 9.4 hours) of the time of the 14-day exposure period at the lake outlet and 1.1% (maximum for 3.7 hours) of the 14-day exposure period at F.H. strait (Figure 3.4). TDG values above 109% at the sentinel cages did not occur during the 4-day TDG exposure, and only a small fraction of the time during the 14-day TDG exposure.

Sampling of smolt was conducted during the period of the day with low TDG levels (Figure 3.2). 50% of the cages at the time of sampling (both 14 May 2016 and 24 May 2016) had TDG values below the 25th percentile of the exposure periods (Table 3.3).

Table 3.3: TDG (%) values at the time of sampling at each cage the day of sampling (Day 4, 14 May 2016; Day 14, 14 May 2016).

Sampling day and location	TDG (%) - cage	TDG (%) - cage
Day 4 (14.05.2016)		
Lake outlet	107.4 - cage 14	108.0 - cage 16
F.H. strait	103.6 - cage 9*	105.7 - cage 6
Power plant outlet	104.1 - cage 1*	105.5 - cage 3*
Lake inlet	104.4 - cage 12*	105.7 - cage 10
Day 14 (24.05.2016)		
Lake outlet	104.1 - cage 14**	106.1 - cage 16**
F.H. strait	104.9 - cage 9**	105.0 - cage 6**
Power plant outlet	106.6 - cage 1	106.9 - cage 3
Lake inlet	105.0 - cage 12	105.7 - cage 10

*TDG (%) below the 25th percentile for 10.-14.05.2016

** TDG (%) below the 25th percentile for 10.-24.05.2016

3.2 Field study 1: Sentinel cages

3.2.1 Surface cages

Mortality

The smolt held in surface cages suffered no mortality during neither the 4-day TDG exposure (120 smolt) nor the 14-day TDG exposure (80 smolt).

Plasma cortisol

The smolt (n=40) displayed median cortisol levels that ranged from 383.7 to 502.3 nM 14 May 2016. In contrast, median cortisol concentrations ranged from 578.0 to 771.4 nM 24 May 2016 (40 smolt). The cortisol levels were significantly higher 24 May 2016 (mean= 673.911, $F_{(1,\ 14)}$=36.810, P<0.001) compared with 14 May 2016 (Figure 3.6). No significant differences of cortisol were found among sites (A.4.1, Appendix).

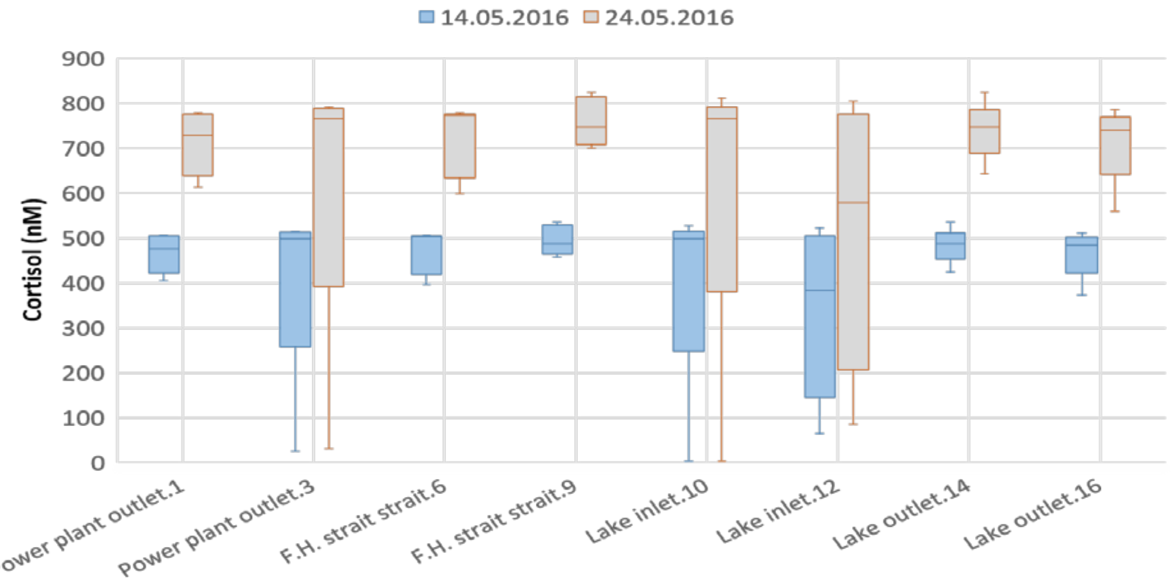

Figure 3.6: Boxplot (25th, 50th and 75th percentile) of cortisol levels (nM) May 14, 2016 (blue boxes) and 24 May 2016 (grey boxes) in salmon smolt (n=80) held in the surface cages at the power plant outlet (cage 1 and 3), the F.H. strait (cage 6 and 9), the lake inlet (cage 10 and 12) and the lake outlet (cage 14 and 16). Bars represent the maximum and the minimum values.

Gill metal

Gill metal levels were not significantly different among sites (A.4.3, Appendix). 85% of the gill aluminum (Al) samples (n=80) were below 30 µg/g. The median gill Al ranged from 3.6 to 10.4 µg/g dw 14 May 2016, while 24 May 2016 median gill Al ranged from 11.0 to 90.4 µg/g dw across the locations (Figure 3.7). Gill Al was significantly higher 24 May 2016 (mean = 10.000, $F(_{1,\ 14})$ = 21.509, P <0.001) than 14 May 2016. Gill Al values at the lake inlet, the power plant outlet and at F.H strait stand out by having high measured gill Al in one of the two cages on 24 May 2016 (Figure 3.7).

The median gill copper (Cu) ranged from 1.6 to 4.7 µg/g dw on 14 May 2016 and from 1.7 to 1.8 µg/g dw on 24 May 2016 (Figure 3.7). The highest gill Cu level was 8.7 µg/g dw (lake inlet, cage 10). Gill Cu was significantly higher 14 May 2016 than 24 May 2016 (Mean = 1.492, $F(_{1,\ 14})$ = 10.433, P< 0.01).

Median gill iron (Fe) ranged between 173.7 to 213.7 µg/g dw compared to 148.6 to 213.2 on 14 May 2016 (Figure 3.7). Maximum gill Fe was 346.71 µg/g dw (the power plant outlet, cage 1, Figure 3.7). Accumulation of gill Fe (µg/g dw) was significantly higher 24 May 2016 (mean =169.459, $F(_{1,\ 14})$= 6.460, P<0.01) than on 14 May 2016.

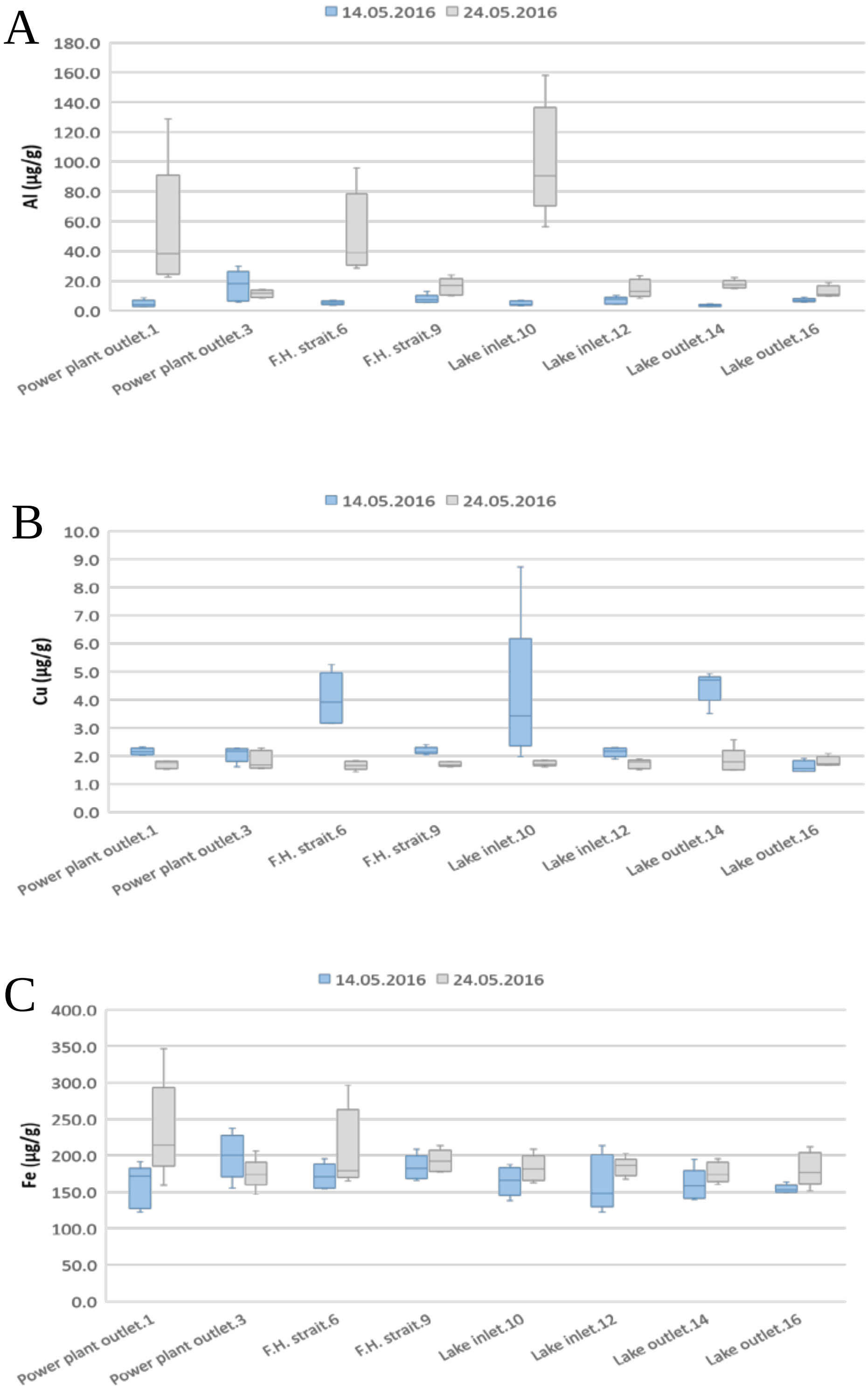

Figure 3.7: Boxplot (25th, 50th and 75th percentile) with bars representing the maximum and the minimum values of gill Al (µg/g dw, A), gill Cu (µg/g dw, B) and gill Fe (µg/g dw, C) across the sentinel cages (cage 1, 3, 6, 9, 10, 12, 14 and 16) at the different sites (the power plant out, the F.H. strait, the lake inlet and the lake outlet) 14 May 2016 (blue boxes) and 24 May 2016 (grey boxes).

Detection and analysis of gas bubbles

Neither the external examination (of fins, skin, ayes and gills) nor the examination of formalin-fixed paraffin embedded tissue samples (of gill, heart and gill) of surface caged smolt (14 May 2016, n=40; 24 May 2016, n=40; Figure 3.8) showed signs of gas bubble disease (GBD). A commensal parasite was found in 91.2% of the gills (Figure 3.8). 23.8% of the gills showed focal hyperplasia, clubbing and inflammation (Figure 3.9). Epithelial cell separation and odema occurred in 13.7% of the gills.

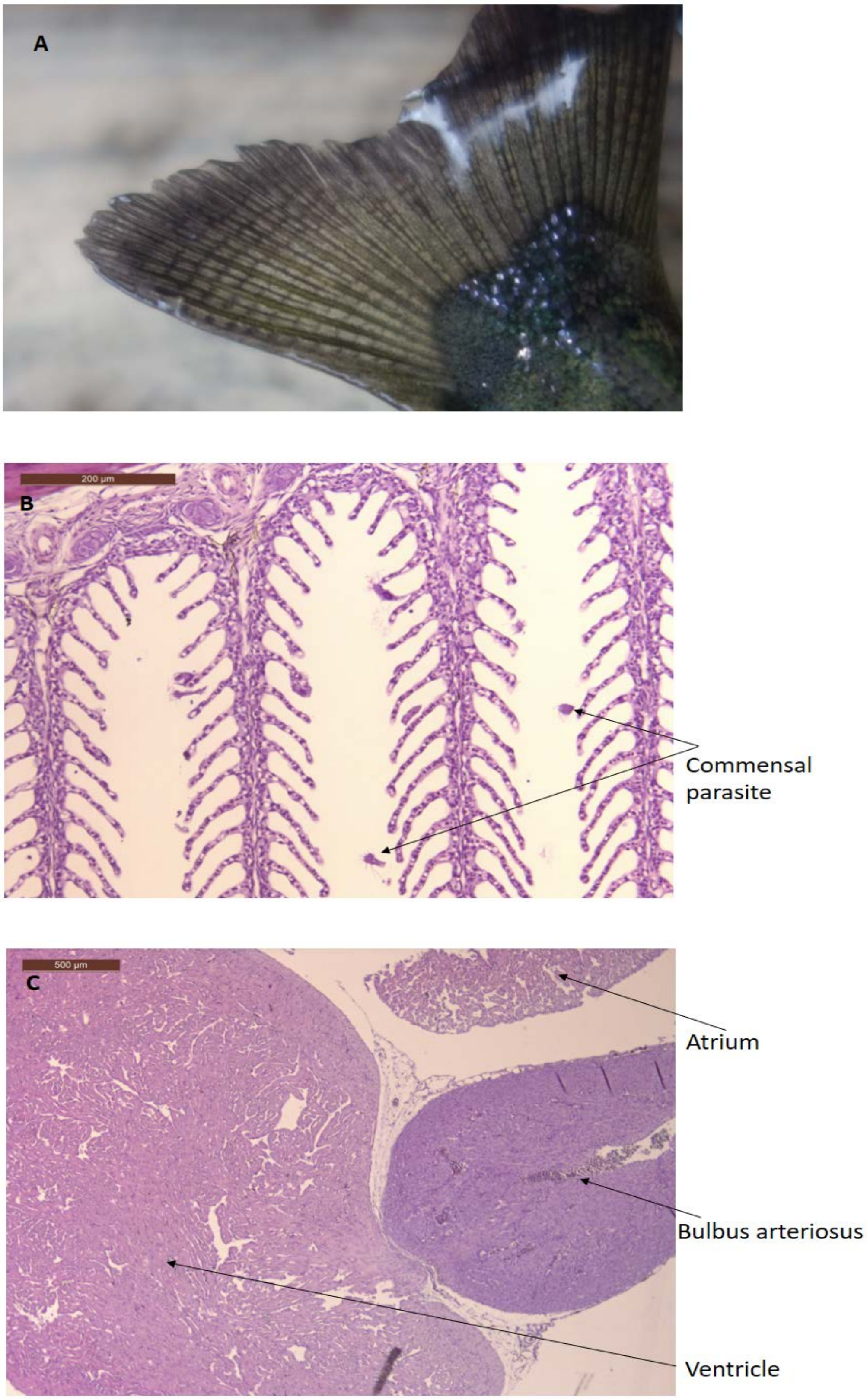

Figure 3.8: No signs of gas bubbles were found in the fish samples: A caudal fin (A), a gill with the commensal parasite (B), heart with arrows showing the atrium, bulbus arteriosus and ventricle (C). Photo A by: Sondre Kvalsvik Stenberg. Photo B and C by: Mark Powell, Norwegian Institute for Water Research (NIVA).

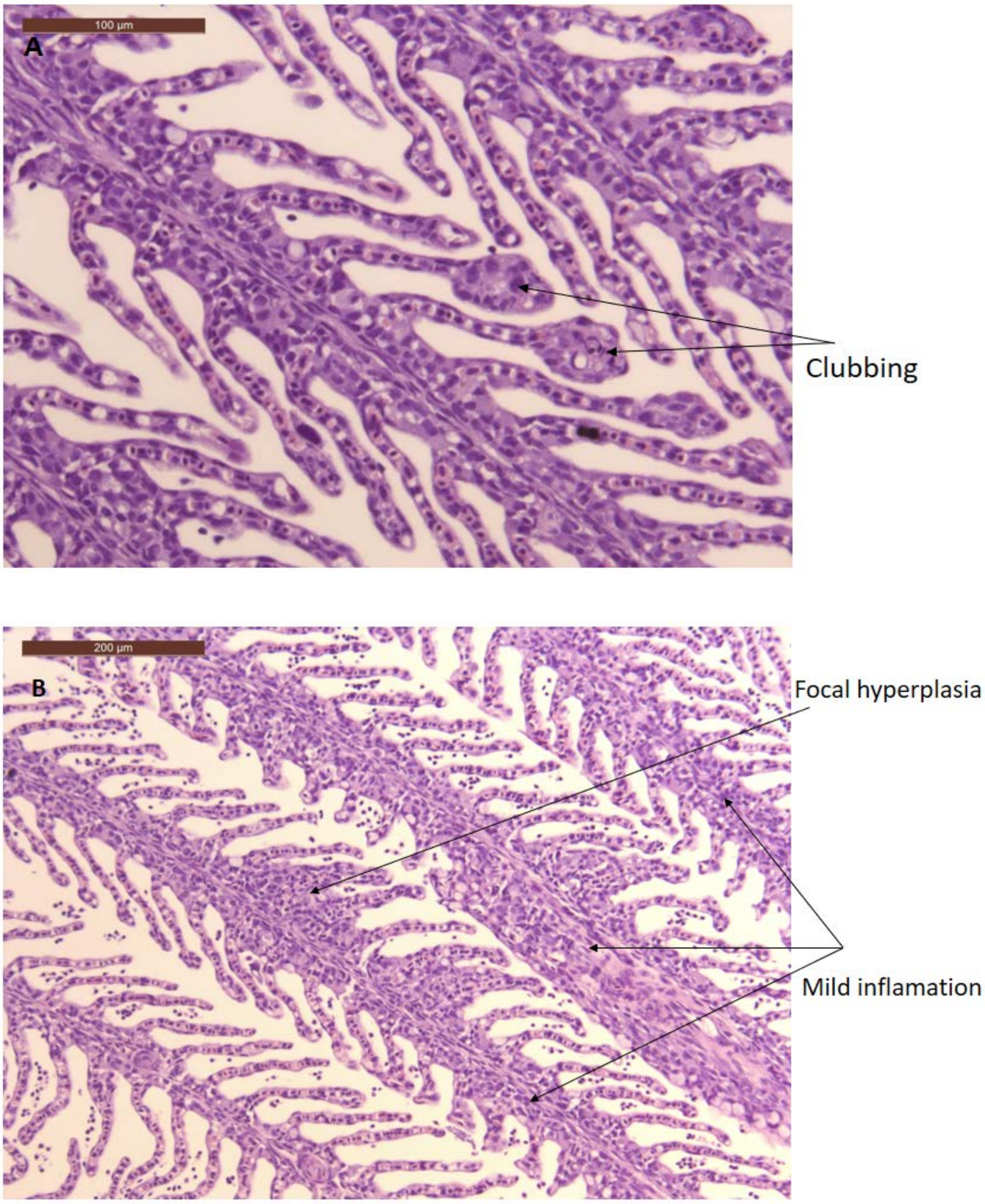

Figure 3.9: Smolt gill with clubbing of lamellae with inflammatory cells (A), mild inflammation in filamental epithelium (B) and hyperplastic focus (B). Photos by: Mark Powell, Norwegian Institute for Water Research (NIVA).

3.2.2 Submerged cages

Smolt held in submerged cages showed no signs of GBD (n=40) or mortality (n=80) during the 14-day sentinel cage experiment.

3.3 Field study 2: Electrofishing and snorkeling observations

Electrofishing

Both Atlantic salmon and trout (*Salmo trutta*) were caught during electrofish at sites closest to the power plant (sites N and O) on 09 May 2016 (salmon n=2, trout n=15) and on 21 May 2016 (salmon n=2, trout n=9). No salmon or trout were caught at the site M south of the power plant and site P downstream the power plant. The fish showed no signs of GBD.

Snorkeling observations

Both salmon and brown trout fry (0+), and juvenile and adult (>0+) were observed during snorkeling at the shoreline of the power plant (Figure 3.10). The fish fins showed no signs of gas bubbles in pictures taken during snorkeling (Table 3.4, Figure 3.11).

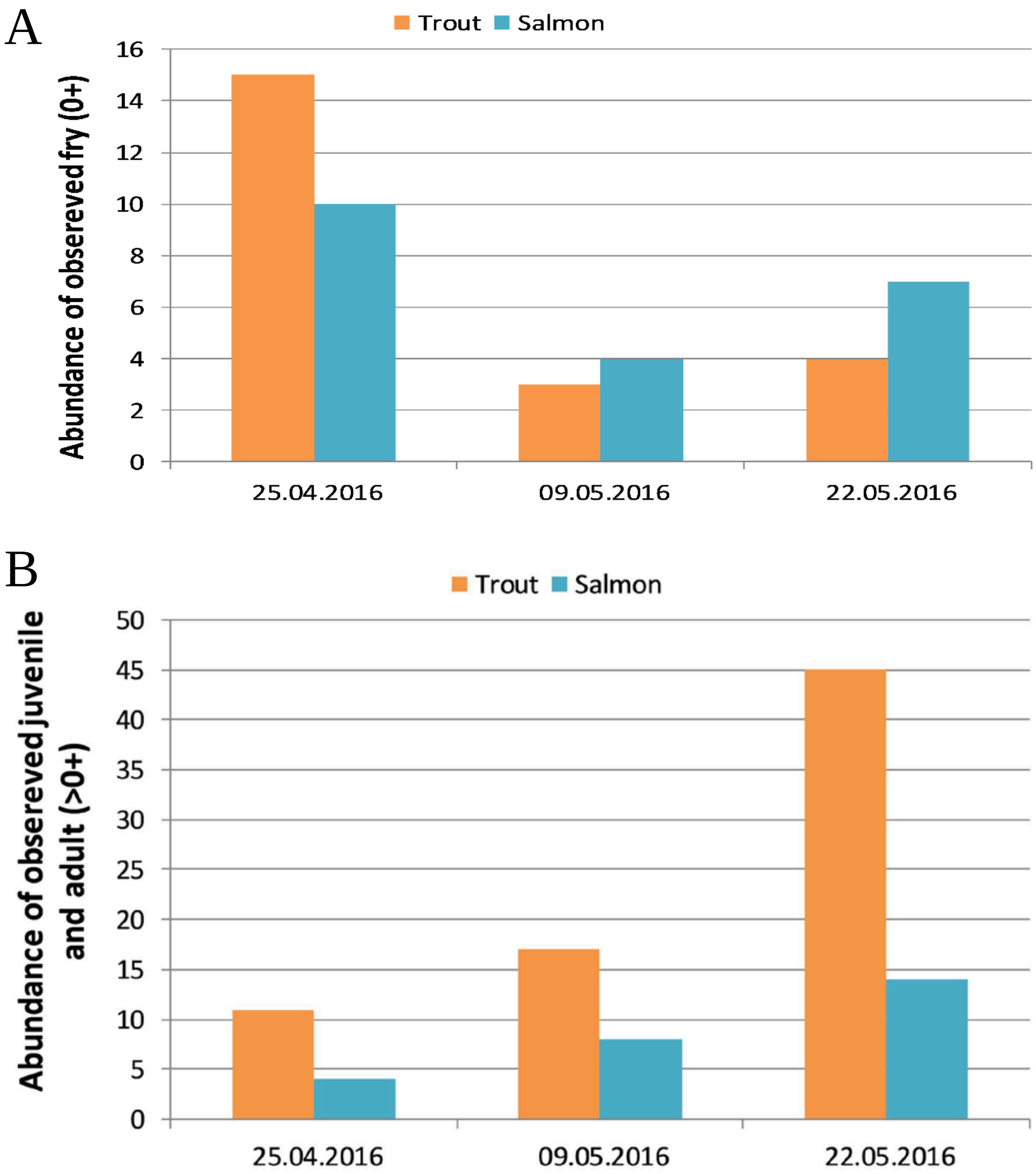

Figure 3.10: The abundance of brown trout (orange) and Atlantic salmon (blue) fry (0+, A), and juvenile and adult (>0+, B) observed during snorkeling on 25 April 2016, on 09 May 2016 and on 22 May 2016.

Table 3.4: Number of Atlantic salmon and brown trout fins (pectoral fin, dorsal fin, caudal fin, anal fin and pelvic fin) examined for gas bubbles in pictures taken during snorkeling and the number of fins with gas bubbles.

	Number of fish fins examined for gas bubbles					
Date and species	**Pectoral fin**	**Dorsal fin**	**Caudal fin**	**Anal fin**	**Pelvic fin**	**Fins with gas bubbles**
25.04.2016						
Salmon (n=3)	3	3	3	3	3	0
Trout (n=2)	2	2	1	0	2	0
10.05.2016						
Salmon (n=2)	2	2	1	2	2	0
Trout (n=12)	12	9	5	7	11	0
22.05.2016						
Salmon (n=9)	7	7	3	6	9	0
Trout (n=22)	17	18	10	13	17	0

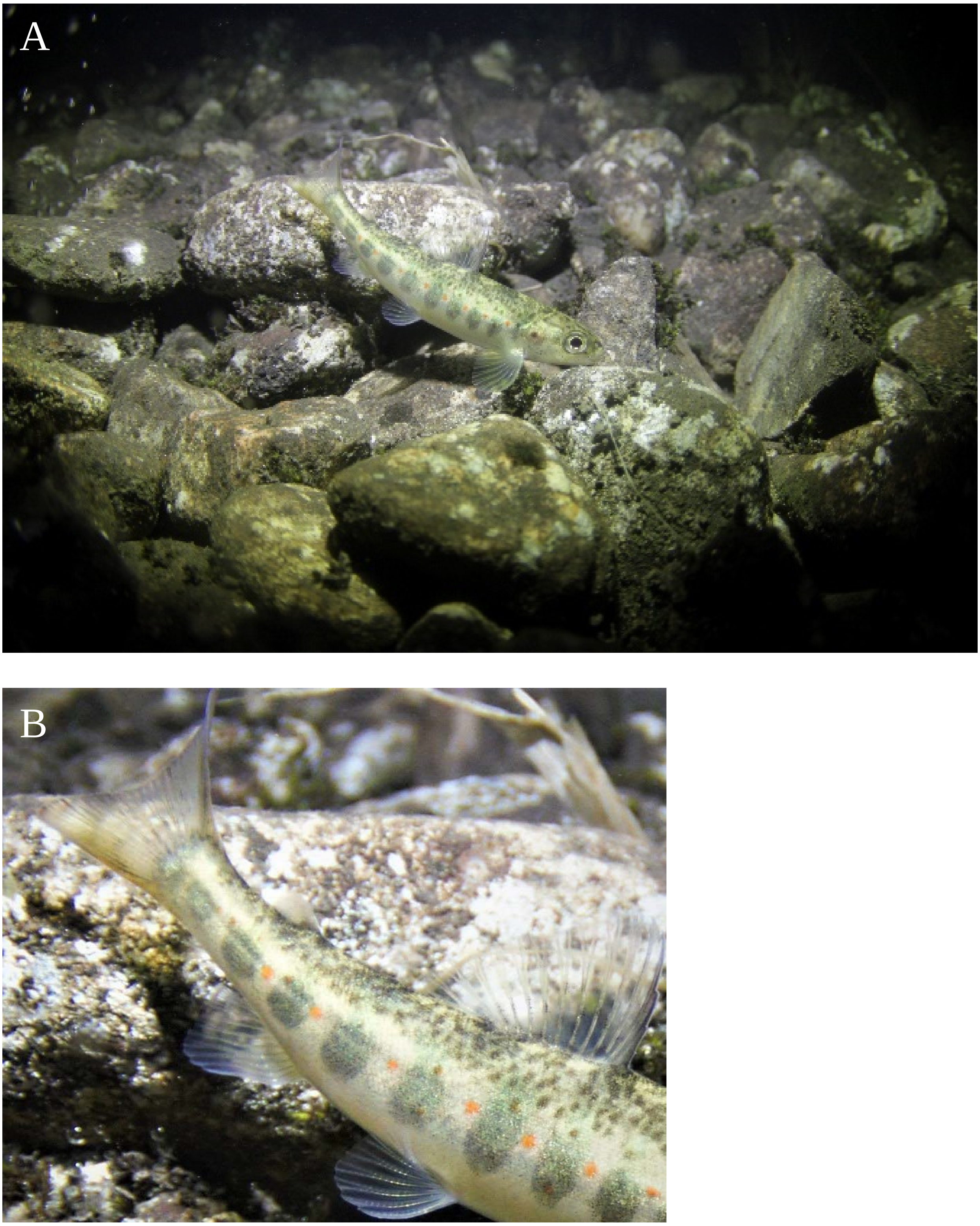

Figure 3.11: Original picture (A) and enlarged picture (B) of an Atlantic salmon smolt at the shoreline of the power plant showing no signs of GBD. Photos by: Bjørn Torgeir Barlaup, Uni Reseach, Uni Environment, LFI.

3.4 Field study 3: Traps in the Vosso watercourse

Migrating Atlantic salmon smolt were mainly caught 09 to 18 May at the trap in Evangervatnet (89% of the caught smolt) and 10 May to 01 June at the Bolstadhølen in Bolstad River (86% of the caught smolt; Figure 3.12). At Evangervatnet, peaks of migrating salmon smolt were found both 09 May and 11 May, with catches of 31% and 18% of the total amount of captured salmon smolt, respectively (Figure 3.12). The rotary smolt screw trap caught more salmon smolt than the trap nets in Evangervatnet (Figure 3.12).

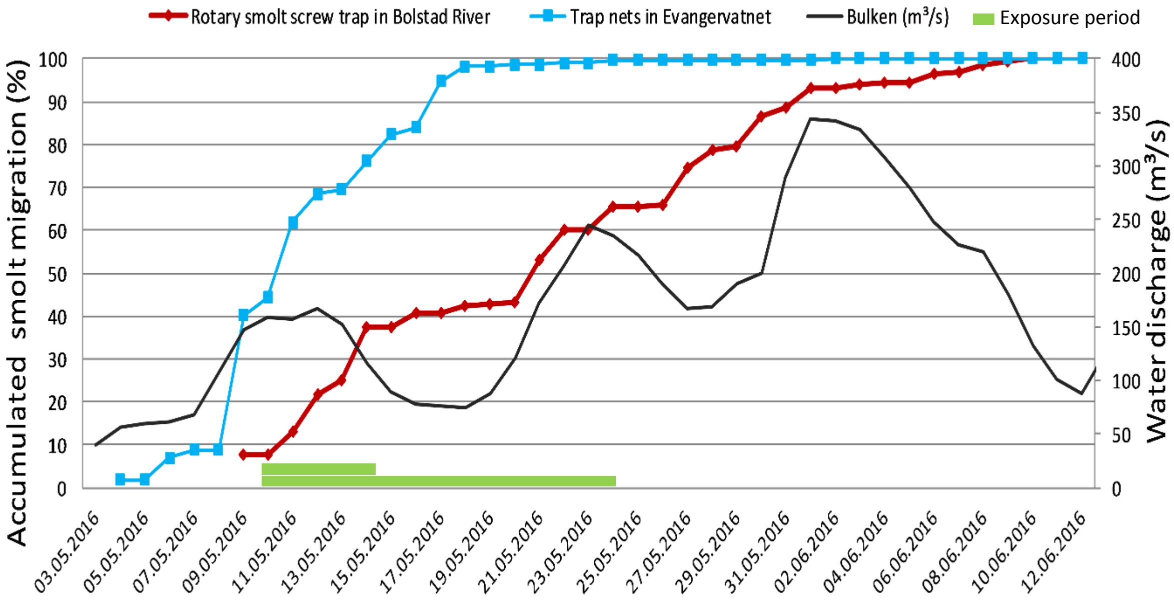

Figure 3.12: Accumulated smolt migration (%) in Vosso watercourse 3 May 2016 to 12 June 2016, by the rotary smolt screw trap in Bolstad River (burgundy) and the trap nets in Evangervatnet (blue). The green lines represent the 4-day exposure and the 14-day exposure of the smolt held in the sentinel cages. Secondary axis shows the water discharge at Bulken (m^3/s, black).

The Atlantic salmon smolt (n = 226) caught by the trap nets in Evangervatnet (n=226) by the rotary smolt screw trap in Bolstadhølen (n=399) showed no sign of external bubbles or loss of equilibrium. Five smolt were obtained by stomach rinsing of predators caught in the traps at Evangervatnet.

3.5 Field study 4: Smolt tow

The smolt towed across Evangervatnet showed no signs of GBD (n=19). 10 of 10 and nine of 10 towed smolt managed the towing 20 May 2016 and 26 May 2016, respectively. The smolt that did not manage to swim had a deformed caudal fin, which caused swimming problems. The tow took about 4 hours.

The TDG saturation generally increased during the first towing from the lake inlet to the lake outlet, while TDG fluctuated more across the lake during the second towing (Figure 3.13). Highest peak of TDG occurred right after Krossneset 20 May and just after Kjeneset 26 May (Figure 2.11). Temperature ranged between 3.6 to 7.0°C and 3.8 to 9.0°C during the smolt tow 20 May 2016 and 26 May 2016, respectively (Table 3.5).

Table 3.5: The minimum to maximum (Min-max) value of TDG (%), minimum to maximum nitrogen (N_2) saturation (%), minimum to maximum oxygen (O_2) saturation (%) and minimum and maximum temperature (°C) of towing smolt 20 May 2016 and 26 May 2016.

Date	Min-max TDG (%)	Min-max N_2 saturation (%)	Min-max O_2 saturation (%)	Min-max °C
20 May 2016	103.5-107.9	102.0-106.5	102.0-120.2	3.6-7.0
26 May 2016	104.1-107.2	101.5-104.0	105.2-126.1	3.8-9.0

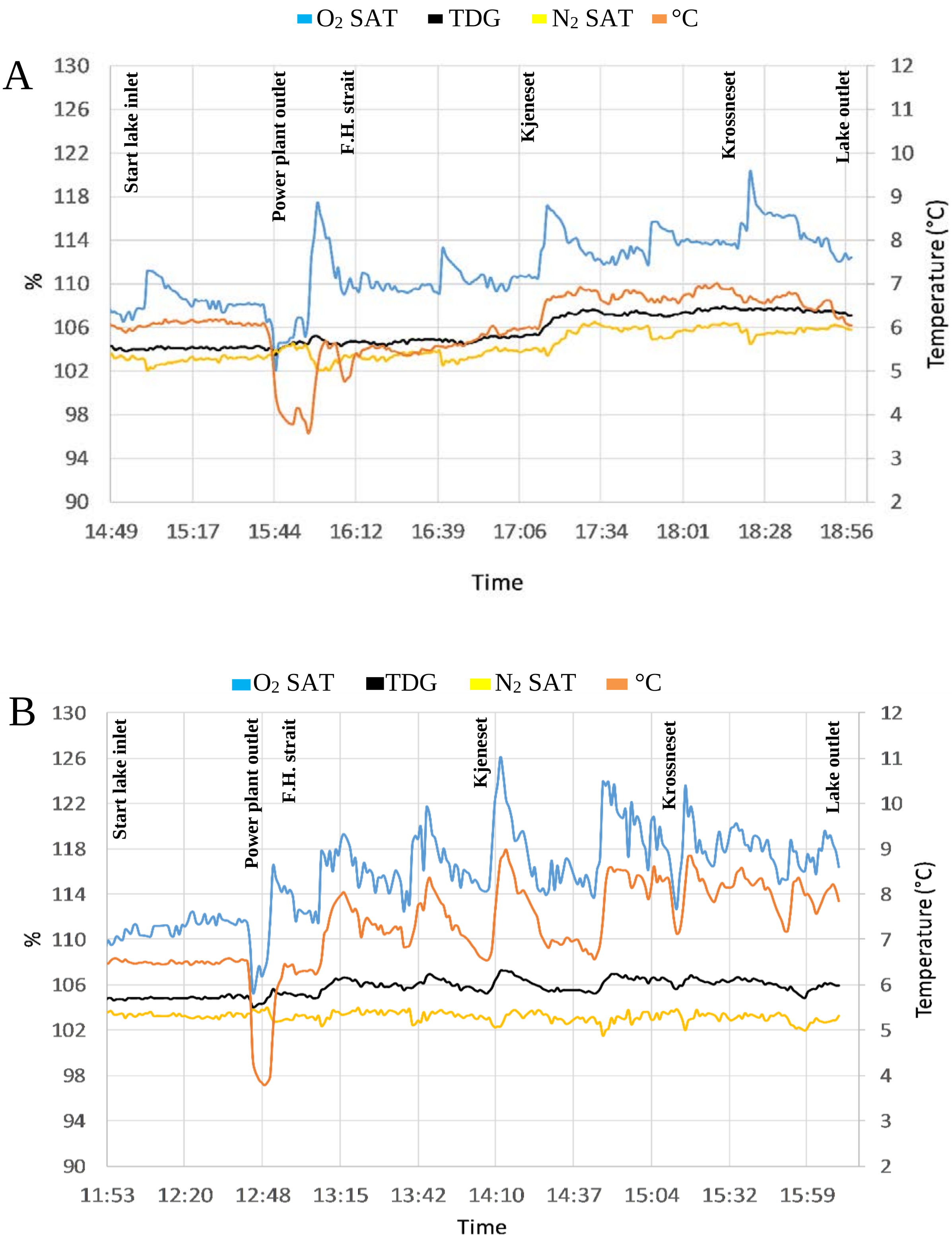

Figure 3.13: Time series of O_2 saturation (%, blue), TDG (%, black) N_2 saturation (%, yellow) and temperature (°C, orange) through Evangervatnet 20 May 2016 (A) and 26 May 2016 (B). "Start lake inlet, "Power plant (cages), "F.H. strait", "Kjeneset", "Krossneset" and "Lake outlet" represent different locations in Evangervatnet (See Figure 2.11 for a map).

4 Discussion

4.1 Smolt migration

Only a small proportion of the migrating smolt is caught by the trap nets in Evangervatnet and the rotary screw trap. If the trap efficiency is constant, then the number of fish caught should reflect the temporal pattern of smolt migration. Such traps have often been used to estimate the migration time of salmon smolt (Barlaup 2013; Hvidsted et al. 2002; Strand & Finstad 2004; Chaput & Jones 2004). Based on the catch data from the traps, this project was conducted during what appeared to be the peak migration time of Atlantic salmon (*Salmo salar L.*) smolt in Vosso watercourse. The fish caught by the rotary screw trap probably represent fish that migrated through Evangervatnet, and showed that smolt migrated through Evangervatnet during the period with field experiments. Of the smolt in the trap nets in Evangervatnet, 89% were caught between 09 and 18 May (Figure 3.12). Since the efficiency of the traps in Evangervatnet was reduced during the second flood event (19 May 2016), the main migration period was most likely spread over a longer period than indicated by the traps. Most of the field studies started 10 May. The highest peak of smolt caught in Evangervatnet was recorded at 09 May. Nevertheless, fish caught 09 May was probably still in the lake for several days according to recordings of time spent in Evangervatnet (Haugen et al. 2016). This suggests that the field studies were conducted during a relevant salmon smolt migration period.

4.2 Sentinel cages

The extent of GBD may relate to the prevalence and severity of gas bubbles (Weitkamp & Parametrix 2008; Mesa, Weiland & Maule 2000). For example, Bouck (1980) reported that the first stage in GBD is bubble formation in the blood and the gills. Thereafter, excess gas starts forming bubbles in the epithelia of the fish. Several studies have reported increased GBD mortality with increased level of TDGS (Newcomb 1974; Mesa, Weiland & Maule 2000; Antcliffe, Fidler & Birtwell 2002; Dawley & Ebel 1975) There were no signs of GBD or mortality of salmon smolt exposed to TDG in the cages in the present study, suggesting that the TDG levels are below the critical level for mortality and signs of GBD.

To date, no similar sentinel cage study has been performed with Atlantic salmon and TDG in a river setting. In contrast, laboratory and sentinel cage experiments have been performed with several species of salmonids at different life stages, confined to various depths and different TDG exposures (number of days and level; Antcliffe, Fidler & Birtwell 2002; Dawley & Ebel 1975; Geist et al. 2013; Heggberget 1984; Mesa, Weiland & Maule 2000; Shrimpton, Randal & Fidler 1990; Weitkamp & Parametrix 2008). The present study is supported by studies, which have observed no mortality of juvenile salmonids exposed to ≤110% TDG. For example, juveniles of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) suffered no mortality when exposed to 110% TDG at 0-28 cm depth for 22 and 11 days, respectively (Mesa, Weiland & Maule 2000; Antcliffe, Fidler & Birtwell 2002). Also, no mortality occurred during laboratory experiments with juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*, held in 23 cm and 25 cm deep tanks), which were exposed to 106% TDG for 35 days (Dawley & Ebel 1975; Newcomb 1974).

Unlike our study, these studies found several fish with gas bubbles in the lateral line and on the fins (Newcomb 1974; Mesa, Weiland & Maule 2000; Antcliffe, Fidler & Birtwell 2002; Dawley & Ebel 1975). However, at least four differences between these studies and the present sentinel cage study should be noted: Firstly, the smolt may have recovered from GBD by the time of sampling in the present study. Smolt held for recovery of GBD often developed fungal infections (Weitkamp 1976). However, the smolt held in the sentinel cages showed no signs of fungal infections. Secondly, species of salmon may have different tolerance to TDG. For example, juvenile Chinook salmon exposed to 130% TDG at 28 cm depth suffered 20% mortality after 3 to 6 h. When juvenile steelhead was tested for the same environmental conditions, a mortality of 20% was reached after 5 to 7 h (Mesa, Weiland & Maule 2000), suggesting that salmon species have different tolerance to TDG. Thirdly, most of the laboratory studies exposed the salmon for longer time (8-35 days) to TDGS compared with the present study, which increases the risk of GBD (Mesa, Weiland & Maule 2000). Fourthly, the salmon were kept closer to the surface in in the laboratory studies (< 30 cm depth) than in the present study. Fish that can compensate at a greater depth have reduced effects (e.g. mortality) from TDG compared to fish confined to shallow water (Ebel 1969; Dawley et al. 1975; Weitkamp 1976; Heggberget 1984). This is because TDG is reduced by approximately 10% for every m depth (Weitkamp & Parametrix 2008; Figure 1.1). For example, juvenile rainbow trout exposed to 122% TDG for 96 h experienced no mortality when held 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 (Antcliffe, Fidler & Birtwell 2002). In line with this, the differences between studies likely explain why some studies have observed fish with GBD exposed to ≤110% TDG.

Migrating salmon smolt behave differently than smolt held in cages. If wild migrating salmon smolt were swimming closer to the surface than caged smolt, then fish at the surface may experience harmful effects due to higher TDG levels closer to the surface (Weitdkamp & Parametrix 2008). To date, the depth distribution of migrating salmon smolt passing Evangervatnet is unknown.

Weitkamp and Parametrix (2008) suggested that cage studies and laboratory studies examine the worst-case scenarios due to limited available depth for fish in such studies. Fish's actual TDG exposure are often lower than TDG at the surface, because fish often swim at some distance from the surface (Weitkamp et al. 2003; Weitkamp 2000; Heggberget 1984). For example, radio tagged trout (*Oncorhynchus mykiss*, *Salmo trutta*, *O. clarki*, *Salvelinus confluentus*) occupied 1.3 m and greater median and average depth in the river (Weitkamp et al. 2003). Moreover, brown trout (*Salmo trutta*), perch (*Perca fluviatilis*) and eel *(Anguilla anguilla*) held near surface in cages resulted in high mortality compared to fish observed during diving and bottom gillnet fishing (Heggberget 1984). Based on these findings, Heggberget (1984) concluded that the resident fish did not suffer direct mortality from TDGS due to occupying deeper parts of the river than the fish held in the surface cages. Atlantic salmon were not present in Weitkamp et al. (2003) and Heggberget (1984). Differences in depth distribution among life stages and species are not unlikely (Weitkamp et al. 2003). Whether the findings in the present study apply to naturally migrating Atlantic salmon smolt is therefore unclear.

Some species of fish may have avoidance response to TDGS and therefore actively migrate from areas with high TDG (Beeman & Maule 2006; Stevens, Nebeker & Baker 1980). Stevens et al. (1980) tested avoidance response to TDGS in a tank with accessible compartments with smolt from salmon (*Oncorhynchus kisutch, Oncorhynchus gairdeneri* and *Oncorhynchus tschawytscha*) and rainbow trout (*Salmo gairdneri*). The salmon generally avoided the compartments with 145% and 125% TDG, while the rainbow trout showed no avoidance response (Stevens, Nebeker & Baker 1980). Little is known about avoidance response for Atlantic salmon to TDGS.

Atlantic salmon smolt held in sea cages often exhibit a diel swimming pattern (Fernö et al. 1995; Oppedal et al. 2001). The salmon often swim closer to the surface during night than during day (Fernö et al. 1995; Oppedal et al. 2001). However, feeding of fish held in sea cages is normally carried out at surface during the day, which motivates the salmon to swim closer to the surface during the day than at night. Migrating Atlantic salmon smolt have also shown a diel swimming pattern. Atlantic salmon smolt were mainly swimming at 1 to 3 m depth during the day and shallower than 0.5 m depth during night in Hardangerfjorden (Norway; Davidsen et al. 2008). The diel swimming pattern may relate to predator avoidance where swimming deeper during day minimizes avian predation from above, while swimming closer to the surface at night reduces fish predation from below (Reddin, Downton & Friedland 2006; Davidsen et al. 2008; Fernö et al. 1995). Like smolt migrating in the sea, smolt migrating in Evangervatnet were most likely predated at by both birds (e.g. *Mergus merganser*, *Mergus serrator* and *Phalacrocorax carbo sinensis*; Haugen et al. 2016) and fish (e.g. *Salmo trutta* and *Salvelinus alpinus*; Sægrov 1999). Migrating salmon smolt likely exhibit a similar diel swimming pattern when swimming in Evangervatnet. Then, smolt would be exposed to TDGS during the night and not be exposed during the day. It cannot be excluded that some fish swimming at the surface experience effects from TDG, but the number of fish affected and the effects were likely minor. This is because the smolt held in the sentinel cages showed no signs of GBD or mortality despite spending time above 0.5 m, which was observed during snorkeling inspections. Moreover, fish exposed to high TDG levels may recover from potentially high TDGS levels by swimming to greater depths (Knittel, Chapman & Garton 1980; Elston et al. 1997; Schiewe 1974). For example, sea-run rainbow trout (*Salmo gairdner*) held in cages (0.1 m, 0.5 m and 1 m deep) recovered from GBD when the fish was lowered to 3 m depth for two hours after a near-lethal TDG exposure (Knittel, Chapman & Garton 1980). Furthermore, migrating salmon smolt in Evangervatnet could usually migrate at depth and compensate for TDGS, as most of Evangervatnet is deeper than 10 m (Bjørklund & Brekke 2000).

4.3 Gill metal

Acidification can result in increased release of accumulative aluminum (Al), iron (Fe) and copper (Cu; Kroglund et al. 2007; Sandahl et al. 2007; Vuori 1995). Atlantic salmon can have reduced sea water tolerance (Monette, Björnsson & McCormick 2008) and reduced marine survival (Kroglund et al. 2007) with accumulation of gill Al. Fish mortality and reproduction can be affected with elevated gill Fe (Vuori 1995). Additionally, fish's ability to respond to chemical signals in the water could be reduced with increased gill Cu (Hansen et al. 1999; Saucier, Astic & Rioux 1991). Accumulation of gill metal can also cause acute mortality (Kroglund et al. 2007; Teien et al. 2008; Kristensen et al. 2009; Lyche Solheim et al. 2008). The Vosso-salmon have previously been considered negatively affected due to acid discharge water from the power plant in Evangervatnet (Barlaup 2013).

85% of the gill Al were within concentrations (<30 µg/g) considered as safe for Atlantic salmon smolt (Lyche Solheim et al. 2008; Figure 3.7). In comparison, 91% of gill Al from 2000 to 2012 at the power plant in Evangervatnet were below levels considered as detrimental (Barlaup 2013). The continued low gill Al levels (<30 µg/g dw) suggested that Al has limited effects on the migrating smolt. Several of the smolt groups held in the cages on 24 May had medians of gill Al at levels providing moderate effects (30-100 µg/g dw; Anne et al. 2008). This suggested that Al may have caused moderate effects on the salmon smolt examined 24 May. In addition, some of the smolt had gill Al values above 100 µg/g dw, indicating negative effects (Lyche Solheim et al. 2008). These guidelines are supported by Kroglund et al. (2007), who showed that marine survival of Atlantic salmon smolt was reduced by 20 to 50% when gill Al was between 25 to 60 µg/g dw. However, despite this, no mortality was observed in any of the fish used in the present study.

Gill Cu and gill Fe were below those normally considered to cause mortality (Kristensen et al. 2009; Teien et al. 2008).

4.4 Plasma cortisol

Fish mortality due to handling of the fish prior to- and during experiments have occurred in studies with caged fish (Heggberget 1984). Such stress may also bias the blood chemistry results. Therefore, the smolt were not measured or examined for swimming behavior to minimize stress of the salmon smolt prior to and during the sentinel cage experiment.

Plasma cortisol levels in the present study (medians ranged from 384-771 nM) were generally above normal background levels of Atlantic salmon (<50 nM ;Finstad, Iversen & Sandodden 2003; Iversen & Eliassen 2009; Iversen, Finstad & Nilssen 1998; Sandodden & Iversen 2001; Figure 3.6). Atlantic salmon smolt have a natural increase of cortisol in the parr-smolt transformation that occurs during spring. However, Atlantic salmon smolt late in the smolting period normally have cortisol levels of about 55 to 69 nM (Langhorne & Simpson 1981). This suggests that the smolt held in the cages may have been displaying cortisol levels consistent with elevated levels of stress.

The cortisol levels were not significantly different among the sites. Therefore, the difference in TDG at the locations upstream and downstream the power plant, appeared not to be related to the high cortisol levels.

The accumulated gill Al in some of the smolt may have contributed to the high cortisol levels. This is consistent with Ytrestoyl et. al (2001), who reported that sexually mature Atlantic salmon had higher cortisol levels when being exposed to acid water containing Al. However, the gill Al levels (217.7±112.3 µg/g dw) that affected the cortisol levels the study by Ytrestoyl et. al (2001) was higher compared with the gill Al accumulation in the smolt held in Evangervatnet.

The high cortisol levels in the smolt could also be a result of TDGS. Since the cortisol levels significantly increased from 14 to 24 May, which could be an increase in cortisol level due to chronic stress over time. In contrast to the present result, juvenile steelhead trout showed no change in blood chemistry when exposed to TDG below 110% for 35 days in shallow tanks (Newcomb 1974). This indicates that TDG was not a main contributor to the high cortisol levels in the present study. Stress-on-stress effects including gill metals and TDGS might have contributed to the high cortisol levels. Barton et al. (1986) reported that elevated cortisol levels coincided with multiple disturbances. However, little is known about stress-on-stress effects (Gale et al. 2004).

The cortisol levels in this study were similar to levels of acute stress occurring during short periods when for example transporting and netting salmon (Iversen, Finstad & Nilssen 1998; Iversen & Eliassen 2009). In contrast, chronic stress is usually lower than acute stress as salmonids usually starts adapting to stress (Pickering & Stewart 1984; Barton, Schreck & Barton 1986). In addition, stress related mortality has been reported at cortisol levels similar to the cortisol levels after the 4-day TDG exposure (Iversen & Eliassen 2009). The smolt held in the cages have therefore unlikely maintained these high cortisol levels throughout the exposure period without mortality. Although every effort was made to minimize stress due to handling, the most plausible explanation to why the smolt displayed high cortisol levels in this study is due to handling effect prior to blood sampling. Finstad et al. (2003) showed that

Atlantic salmon smolt moved directly into a tank of clove oil sedation (≥ 20 mg l^{-1}) would limit elevation of plasma cortisol for a minimum of 30 minutes. However, plasma cortisol will increase after a few minutes of stress, so the time spent while sampling the smolt is critical to prevent elevated plasma cortisol levels. The high cortisol levels may have occurred when smolt were sampled from nearby cages, or as a result of spending too long time from approaching the cages to the fish were put into sedation (Table 2.2).

The most plausible explanation to why the cortisol levels differed between dates is due to the observed difference between the standard curves (A.2.2, Appendix), and not caused by stress. This difference could be variation in the use of the pipettes during the different runs of the cortisol assays.

4.5 Observations of wild smolt

Both Atlantic salmon and brown trout at various sizes were caught by electrofishing and observed during night snorkeling (Figure 3.11). These fish mainly occupy shallow areas. The snorkeler could often approach the fish closely. Despite the short distance to the fish and good light conditions by use of a flash light, no signs of GBD were observed. In addition, the electrofished fish showed no signs of GBD. These data indicate that the fish exposed to the TDG levels during electrofishing and snorkeling observation may have been too low for clear symptoms of GBD.

Fish may experience sub-lethal effects due to TDG, for example inflation of the gas bladder (Shrimpton, Randall & Fidler 1990). Both Dawley and Ebel (1975) and Schiewe (1974) found no negative effects on juvenile salmonids (*Salmo gairdneri* and *Oncorhynchus tshawytscha*) when exposing the fish to TDG levels below 106%. However, at 106% TDG salmonids had reduced growth and swimming performance. The TDG values that resulted in the sub-lethal effects in these studies are at the measured median TDG values (Table 3.1, Table 3.2). Therefore, migrating smolt in Evangervatnet may have had reduced swimming performance and growth. However, reduced swimming performance and growth were likely limited, as migrating smolt may occupy greater depths and spend less time to corresponding TDGS levels compared to these studies, and fish showed no signs of reduced swimming performance in the present study.

Pulg et al. (2014) examined an area influenced by high TDG saturation and its effect on fish density at Brokke, Norway. The fish density in the area with highest TDGS (up to 173%) was low, with increasing fish density at decreasing levels of TDGS. In the present study, the fish density was not lower close to the power plant, fish clearly occupied areas close to the power plant and no effects of TDG were observed. Atlantic salmon and brown trout have habitat preferences (Einarsson, Mills & Johannsson 1990; Armstrong et al. 2003). The differences in catch among the electrofishing sites were therefore not likely caused by differences in TDGS, but due to differences in habitat. For example, site M south of the power plant was sandy and with submerged trees, while the other sites (N, O, P) consisted of sharper shore slopes and stones (Figure 2.7).

The migrating salmon smolt caught by the trap nets in Evangervatnet and the rotary smolt screw trap in the lower part of Bolstad River showed no signs of GBD. However, the trap net design was not ideal for detecting potentially signs of GBD. The reason is that the fish caught by the trap nets was confined to 1.5 to 2.8 m depth up to 24 hours prior to examination for GBD. The fish may have recovered from GBD by the time of examination if the fish stayed in the deeper part of the containment, resulting in underestimation of GBD. However, the examined smolt displayed no fungal infections, indicating that the trapped fish were not previously affected by GBD. Moreover, weakened fish with signs of GBD caught in the traps may have been an easy target for predators also caught by the traps. Regardless, only five predated smolt were obtained from stomach rinsing of the caught predators.

Another reason why wild fish lacked signs of GBD could be increased predation of sickened smolt. Fish exposed to TDGS may have had reduced swimming performance (Schiewe 1974), resulting in increased predation (Schiewe 1974; Shrimpton, Randall & Fidler 1990). Mesa and Warren (1997) showed that juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exposed to TDG above 130% (30 cm deep tank) had increased susceptibility to predation (for eight hours). However, juvenile salmon exposed to lower levels of TDG (120% for eight hours 112% for 13 days) were not more susceptible to predation (Mesa & Warren 1997). If these results also apply to Atlantic salmon, then salmon smolt in Evangervatnet were not likely more susceptible to predation. In addition, migrating smolt in Evangervatnet could compensate for TDG by migrating at greater depths, while fish in the study by Mesa and Warren (1997) were confined to shallow water. Moreover, if the migrating smolt had increased susceptibility to predation, then fish predators may also experience GBD. For example, the predators in the have had reduced food intake, which was reported for Northern pikeminnow (*Ptychocheilus oregonensis*; Bentley & Dawley 1981).

4.6 Smolt tows

No signs of GBD were observed for the smolt towed across Evangervatnet. The durations of the tows were around four hours. In contrast, wild salmon smolt spent on average nine and a half day to the middle of the Evangervatnet (Haugen et al. 2016). Despite a low number of smolt and high individual variation in time spent swimming across the lake in that study, this indicates that the migration period through Evangervatnet is considerably longer for migrating smolt than for the towed smolt. Hence, this indicates that the smolt tows represented a minimum exposure to TDGS compared to wild smolt. Nevertheless, natural migrating salmon smolt may have avoided areas with TDGS, while the towed smolt were forced at TDGS. Therefore, the towed smolt may have experienced higher TDGS than migrating smolt.

4.7 The representativeness of measured TDG

TDGS can be transported several km downstream from power plants (Pulg, Barlaup & Normann 2014; Pulg & Barlaup 2013; Pulg et al. 2016), and TDG from the power plant in Evangervatnet may have been transported across the lake (Pulg, Gabrielsen & Normann 2013). Blindheim et al. (1984) reported 107 to 108% TDG about 20 km from the outlet of the power plant in Tafjorden. Aeration of TDGS in lakes occurs slowly due to the slow water movement and slow diffusion process, and has been suggested to take years (Blindheim et al. 1984). Considering these reports, high TDG levels would likely have been detected by the monitors at the lower part of Evangervatnet, if the TDG levels were sufficiently high to be transported across the lake. However, elevated levels of TDG were not recorded at the lake outlet (Figure 3.1, Figure 3.2). Areas with potentially high TDG levels would also likely have been detected by the TDG monitoring during the smolt tows. However, the highest measured TDG value during the smolt tow on 20 May was comparable to measures at the lake outlet (Figure 3.13). In addition, the highest measured TDG values 26 May was only slightly higher than recorded at the lake outlet (Figure 3.13). This indicates that the measures of TDG were not underestimated or that large areas with high TDGS were missed.

The TDG monitors cover a small area of the lake and some aeration of TDG would occur. As a result, smaller areas might have had higher levels of TDG. For example, the highest level of

TDG this year peaked 112.8% at F.H strait, while no corresponding TDG values were recorded at the other TDG monitoring sites. TDG levels at the other stations were lower both during and prior to the peak of TDG (Figure 3.2, Figure 3.3). Therefore, the TDG peak at the F.H strait was not detected at the other monitoring stations. The most plausible explanation for the TDG peak at F.H was rapid heating of the water (Figure 3.5)Since an increase in temperature decreases the solubility of gases (Marking 1987). The TDG peak coincided with the second flood event and rainy weather (Figure 3.3). This indicates that mixing of cold melt water with warmer rain water may have resulted in the high TDG peak at the F.H. strait. However, other mechanisms could have contributed to the high TDG peak, including heating of the water by solar radiation (Marking 1987) and O_2 produced by algae (Weitkamp & Katz 1980). This indicates that TDG monitoring at the Vosso River and the power plant may not be adequate to detect all areas with high TDG levels in Evangervatnet.

TDG levels this year were generally at the same range of TDG as previous years (Table 1.1), suggesting that the effects from TDG on fish in 2016 are representative of the effects in previous years.

4.8 Further studies

Considerably more is to be learned about potential effects of TDG on Atlantic salmon smolt, on Atlantic salmon in general, as well as effects on other parts of the ecosystem. Future work on smolt should: (1) examine the critical TDG levels that negatively affect Atlantic salmon smolt since this level is largely unknown. An exposure experiment of smolt to different levels of TDGS in a shallow water tank could be conducted to provide an answer. (2) Examine depth distribution of Atlantic salmon smolt and avoidance response to TDG, due to the reduced effects from TDGS with hydrostatic pressure compensation (Weitkamp & Parametrix 2008; Weitkamp & Katz 1980). (3) Examine whether fish in Vosso River have adapted to live with naturally TDGS at Vosso River. (4) Evaluate the TDGS levels providing sub-lethal effects, since existing data are not sufficient to evaluate potentially sub-lethal effects. Sub-lethal effects may include increased infection rates (Weitkamp 1976), increased susceptibility to predation (Mesa & Warren 1997), reduced swimming performance (Dawley & Ebel 1975), reduced growth (Dawley & Ebel 1975), reduced sea survival. (5) Examine stress-on-stress effects. Stress-on-stress effects have been suggested to be a potentially source for the low population size of Atlantic salmon in Vosso watercourse (Barlaup 2008, 2013). For example, combined stress may be caused by TDGS, acid runoff, sea lice and interbreeding between farmed and wild salmon. Some of these potentially stress-on-stress effects could be examined by monitoring the return rate of one group of salmon exposed to stressors and one group not exposed to stressors.

Monitoring of TDG should continue until more of the potentially sub-lethal effects and potentially stress-on-stress effects are better understood. In addition, it is recommended to place a TDG logger in the middle of the lake (e.g. Kjeneset or Krossneset, Figure 2.11), due to higher TDG levels recorded in this area compared to TDG at the other sites.

4.9 Conclusions

The field studies were conducted during the peak migration periods of Atlantic salmon smolt. This suggests that the field experiments were conducted during a relevant period.

The salmon smolt in the present study showed no signs of GBD or mortality. This is valid for all smolt held in sentinel cages, caught by electrofishing, observed during snorkeling, caught in the traps and observed during towing. Despite some smolt may have swum at the surface and as a result experienced effects from TDG, the present study suggests that Atlantic salmon smolt were exposed to TDG levels below the critical levels for mortality and signs of GBD. The hypothesis that hydrostatic pressure could compensate for TDG and its effects remain unchanged, as fish held in the sentinel cages showed no signs of GBD.

High cortisol levels were found both in areas of high and low TDGS levels, indicating that the cortisol levels were not related to the TDG. The high cortisol values in the smolt most likely occurred due to the handling effect prior to blood sampling. As a result, the hypothesis that increased TDGS would be related with stress indicators and mortality remains unclear.

Fish clearly occupied areas close to the power plant, and the density of fish was not lower at the power plant. As a result, the fish distribution appeared not to be affected, which do not support the hypothesis that fish avoided the areas close to the power plant.

TDG monitoring this year in Vosso watercourse seemed to represent the TDG levels across Evangervatnet. This is because large areas with high TDG levels would likely have been detected by the TDG monitoring, as aeration of gases in lakes occurs slowly (Blindheim et al. 1984; Pulg, Barlaup & Normann 2014; Pulg & Barlaup 2013; Pulg et al. 2016). However, the possibility that smaller areas of the lake potentially had higher levels of TDGS cannot be excluded.

Moreover, TDG levels seemed to be at the same range of TDG compared with previous years TDG monitoring, suggesting that the effects from TDG on fish in 2016 are representative of the effects in previous years.

Given the depth in Evangervatnet (Bjørklund & Brekke 2000), the TDG levels and the field studies, TDG appeared to have no negative effects on the survival of Atlantic salmon smolt downstream of the power plant. As a result, there is no support for the hypothesis that TDG may explain the high mortality observed in earlier studies.

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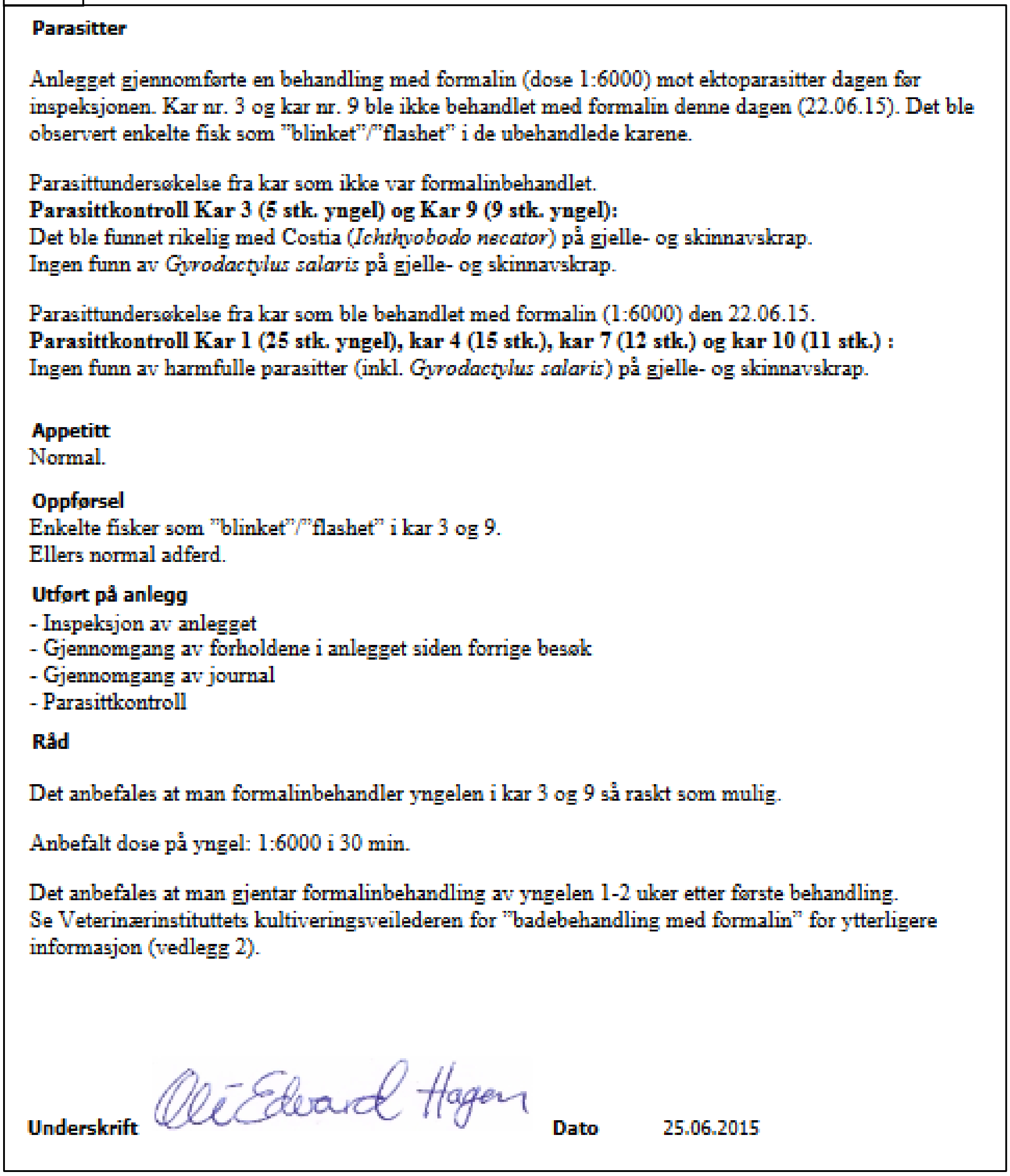
A. Appendix

A.1 Veterinary reports

Veterinary reports of the smolt from Voss Klekkeri (Figure A.1) suggest that that the smolt were in good condition.

A



Parasitter

Anlegget gjennomførte en behandling med formalin (dose 1:6000) mot ektoparasitter dagen før inspeksjonen. Kar nr. 3 og kar nr. 9 ble ikke behandlet med formalin denne dagen (22.06.15). Det ble observert enkelte fisk som "blinket"/"flashet" i de ubehandlede karene.

Parasittundersøkelse fra kar som ikke var formalinbehandlet.
Parasittkontroll Kar 3 (5 stk. yngel) og Kar 9 (9 stk. yngel):
Det ble funnet rikelig med Costia (*Ichthyobodo necator*) på gjelle- og skinnavskrap.
Ingen funn av *Gyrodactylus salaris* på gjelle- og skinnavskrap.

Parasittundersøkelse fra kar som ble behandlet med formalin (1:6000) den 22.06.15.
Parasittkontroll Kar 1 (25 stk. yngel), kar 4 (15 stk.), kar 7 (12 stk.) og kar 10 (11 stk.) :
Ingen funn av harmfulle parasitter (inkl. *Gyrodactylus salaris*) på gjelle- og skinnavskrap.

Appetitt
Normal.

Oppførsel
Enkelte fisker som "blinket"/"flashet" i kar 3 og 9.
Ellers normal adferd.

Utført på anlegg
- Inspeksjon av anlegget
- Gjennomgang av forholdene i anlegget siden forrige besøk
- Gjennomgang av journal
- Parasittkontroll

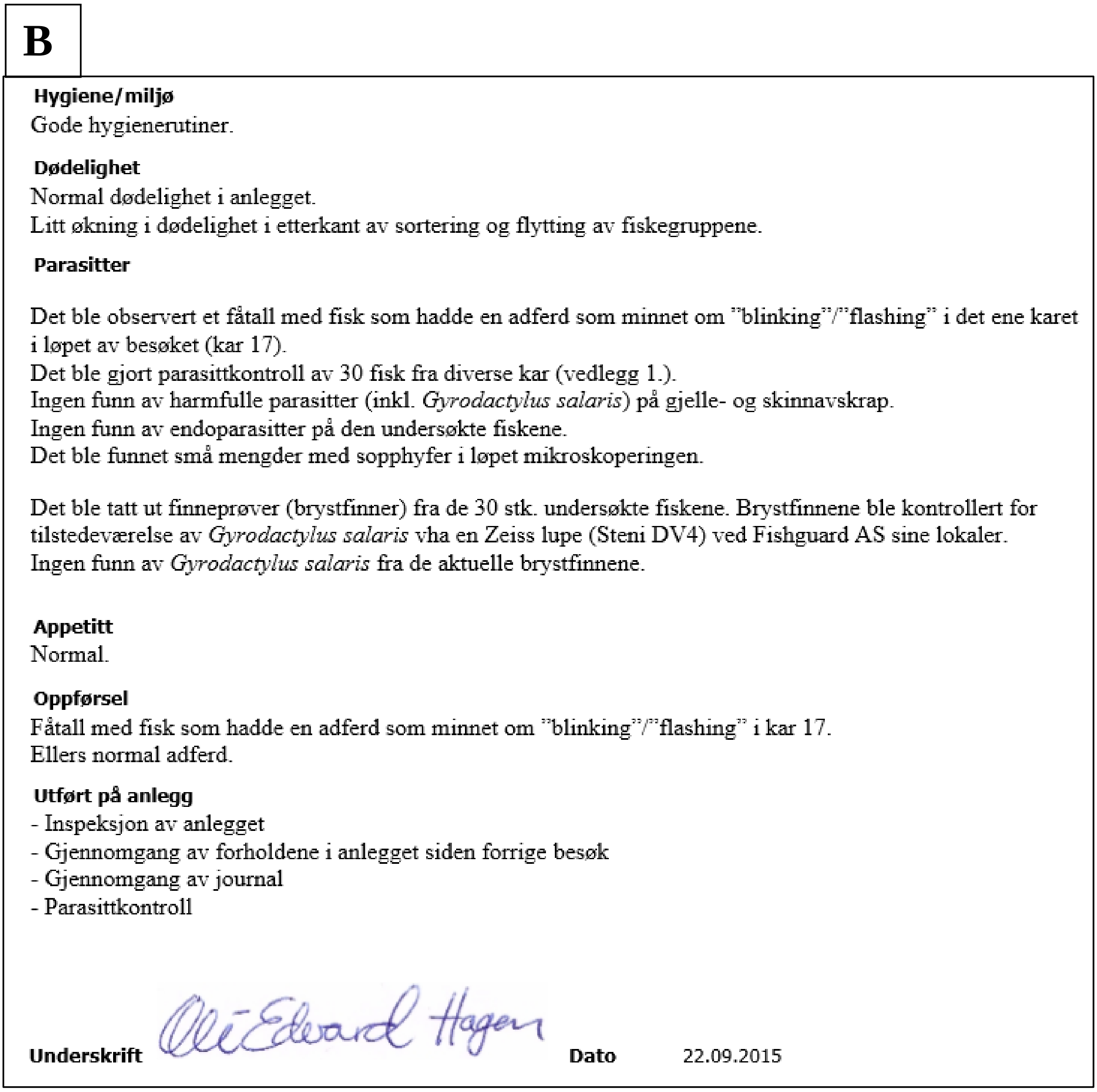
Råd

Det anbefales at man formalinbehandler yngelen i kar 3 og 9 så raskt som mulig.

Anbefalt dose på yngel: 1:6000 i 30 min.

Det anbefales at man gjentar formalinbehandling av yngelen 1-2 uker etter første behandling.
Se Veterinærinstituttets kultiveringsveilederen for "badebehandling med formalin" for ytterligere informasjon (vedlegg 2).

Underskrift Ole Edvard Hagen **Dato** 25.06.2015



B

Hygiene/miljø
Gode hygienerutiner.

Dødelighet
Normal dødelighet i anlegget.
Litt økning i dødelighet i etterkant av sortering og flytting av fiskegruppene.

Parasitter

Det ble observert et fåtall med fisk som hadde en adferd som minnet om "blinking"/"flashing" i det ene karet i løpet av besøket (kar 17).
Det ble gjort parasittkontroll av 30 fisk fra diverse kar (vedlegg 1.).
Ingen funn av harmfulle parasitter (inkl. *Gyrodactylus salaris*) på gjelle- og skinnavskrap.
Ingen funn av endoparasitter på den undersøkte fiskene.
Det ble funnet små mengder med sopphyfer i løpet mikroskoperingen.

Det ble tatt ut finneprøver (brystfinner) fra de 30 stk. undersøkte fiskene. Brystfinnene ble kontrollert for tilstedeværelse av *Gyrodactylus salaris* vha en Zeiss lupe (Steni DV4) ved Fishguard AS sine lokaler.
Ingen funn av *Gyrodactylus salaris* fra de aktuelle brystfinnene.

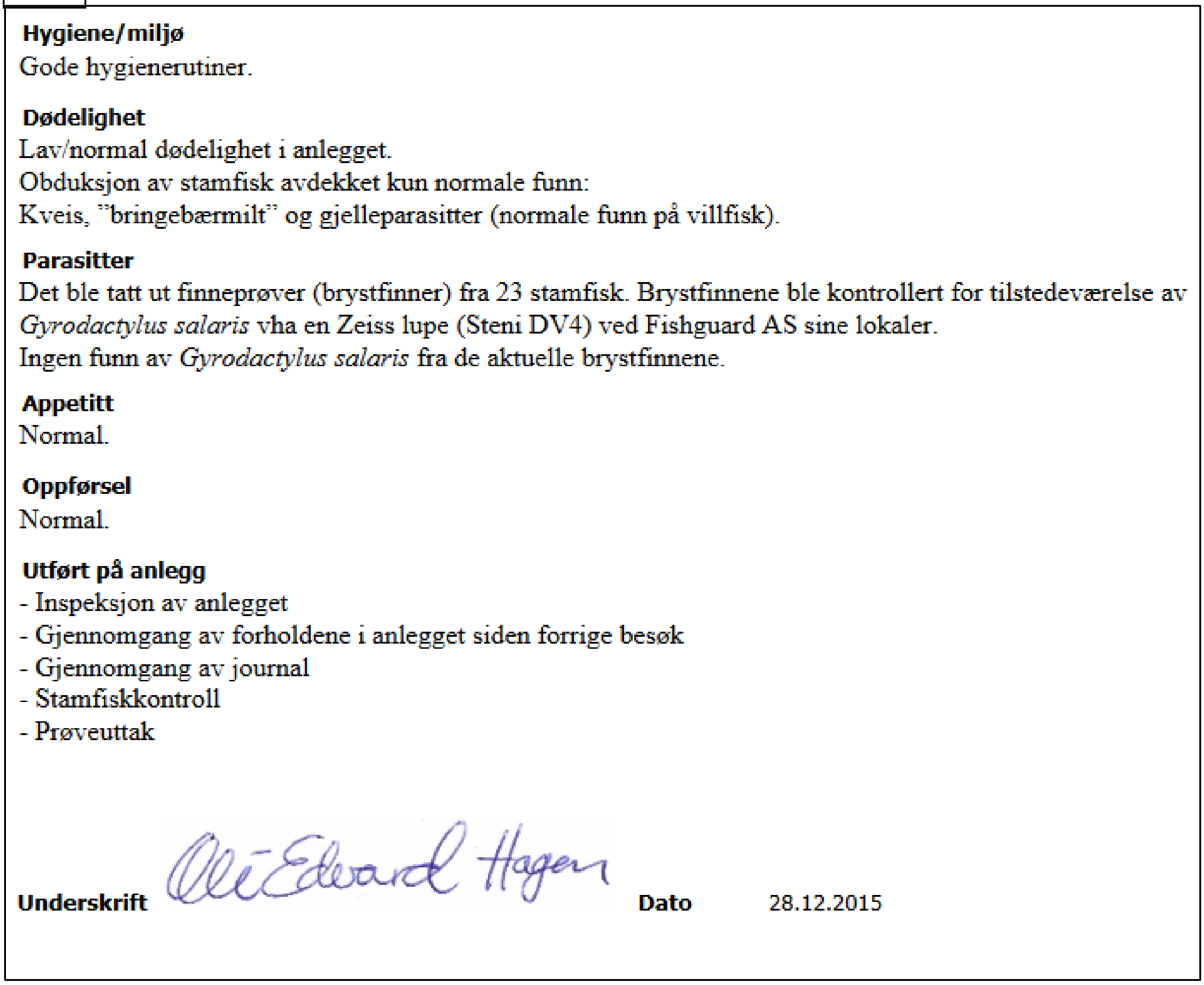
Appetitt
Normal.

Oppførsel
Fåtall med fisk som hadde en adferd som minnet om "blinking"/"flashing" i kar 17.
Ellers normal adferd.

Utført på anlegg
- Inspeksjon av anlegget
- Gjennomgang av forholdene i anlegget siden forrige besøk
- Gjennomgang av journal
- Parasittkontroll

Underskrift Ole Edvard Hagen **Dato** 22.09.2015

C



Hygiene/miljø

Gode hygienerutiner.

Dødelighet

Lav/normal dødelighet i anlegget.
Obduksjon av stamfisk avdekket kun normale funn:
Kveis, "bringebærmilt" og gjelleparasitter (normale funn på villfisk).

Parasitter

Det ble tatt ut finneprøver (brystfinner) fra 23 stamfisk. Brystfinnene ble kontrollert for tilstedeværelse av *Gyrodactylus salaris* vha en Zeiss lupe (Steni DV4) ved Fishguard AS sine lokaler.
Ingen funn av *Gyrodactylus salaris* fra de aktuelle brystfinnene.

Appetitt

Normal.

Oppførsel

Normal.

Utført på anlegg

- Inspeksjon av anlegget
- Gjennomgang av forholdene i anlegget siden forrige besøk
- Gjennomgang av journal
- Stamfiskkontroll
- Prøveuttak

Underskrift Ole Edward Hagen **Dato** 28.12.2015

D

Ingen mistanke om sykdom i anlegget.

Hygiene/miljø
Gode hygienerutiner.

Dødelighet
Lav dødelighet i anlegget

Appetitt
Normal.

Oppførsel
Normal.

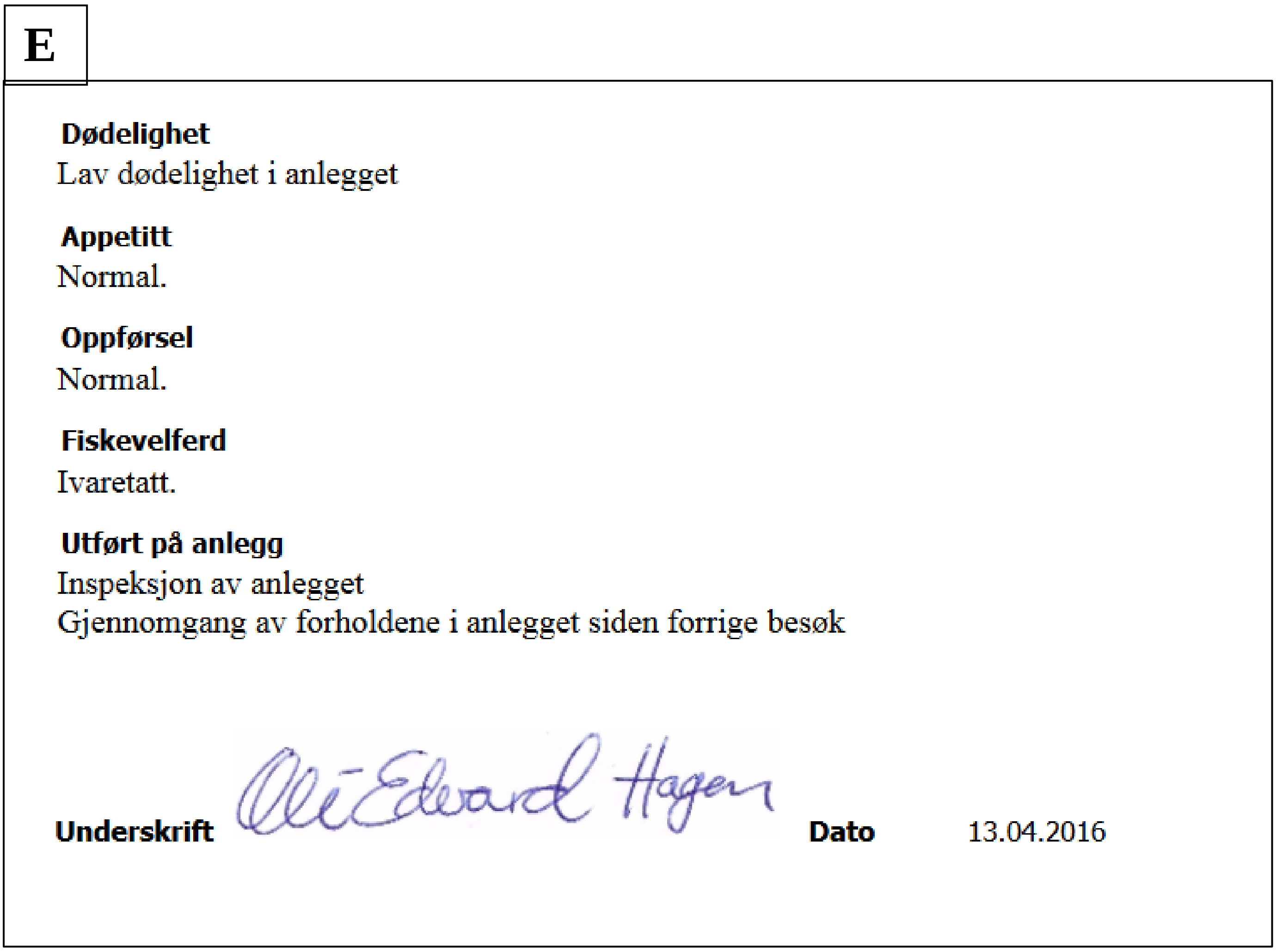
Utført på anlegg

Utskriftdato/tid	07.03.2016 08:55
Rapport	BR161860

1

- Inspeksjon av anlegget
- Gjennomgang av forholdene i anlegget siden forrige besøk

Underskrift Ole Edvard Hagen **Dato** 07.03.2016



E

Dødelighet
Lav dødelighet i anlegget

Appetitt
Normal.

Oppførsel
Normal.

Fiskevelferd
Ivaretatt.

Utført på anlegg
Inspeksjon av anlegget
Gjennomgang av forholdene i anlegget siden forrige besøk

Underskrift Ole Edward Hagen **Dato** 13.04.2016

Figure A.1: Veterinary reports 25 June 2015 (A), 22 September 2015 (B), 28 December 2015 (C), 07 March 2016 (D) and 13 April 2016 (E).

A.2 Cortisol

A.2.1 Protocol

Cortisol was analyzed by the Elisa kit (Enzo Life Sciences, Inc, Cortisol ELISA kit, AD-900-097, Farmingdale, NY and BioSource Porcine). The procedure was followed according to the manufacturer’s product manual:

The plasma samples were diluted by taking 10 µl of plasma and 90 µl of Assay Buffer. 10 µl of the sample was removed. 10 µl of Steroid Displacement Reagent was pipetted into the sample.

Copied from Enzo Life Sciences product manual:

ASSAY PROCEDURE:

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4°C.

2. Pipet 100 µL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0pg/ml Standard) wells.

3. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.

4. Pipet 100 µL of the Samples into the appropriate wells.

5. Pipet 50 µL of Assay Buffer into the NSB wells.

6. Pipet 50 µL of blue Conjugate into each well, except the Total

Total Activity (TA) and Blank wells.

7. Pipet 50 µL of yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

8. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.

9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 Washes.

After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

11. Add 5 µL of the blue Conjugate to the TA wells. 12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.

13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.

14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm.

If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

A.2.2 Standard curves

98.8% and 97.5% of the observed variation in mean absorbance (OD) could be explained by the cortisol levels 14 May 2016 and 24 May 2016, respectively (Figure A.2).

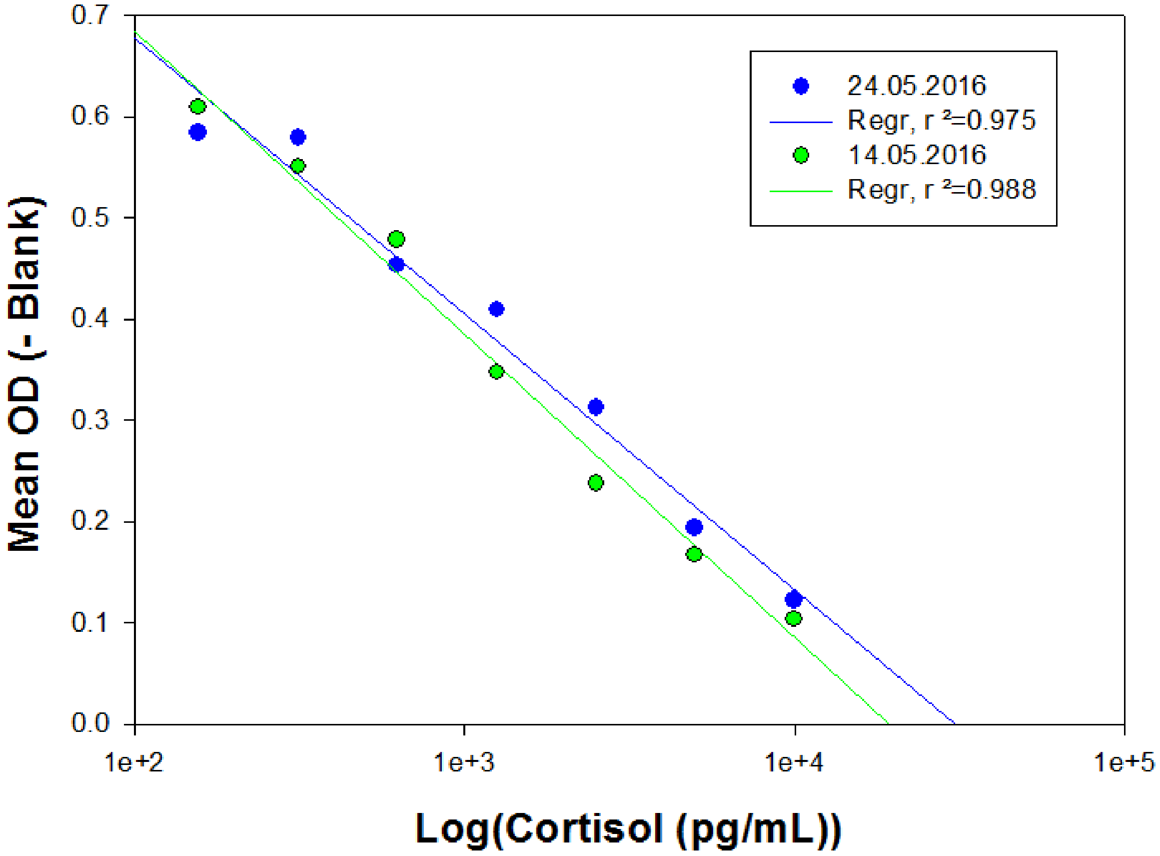


Figure A.2: Mean OD (-Blank) for log(Cortisol (pg/mL)). The straight lines indicate the standard curves for cortisol levels 14 May 2016 (green) and 24 May 2016 (blue).

A.3 Electrofishing and snorkeling

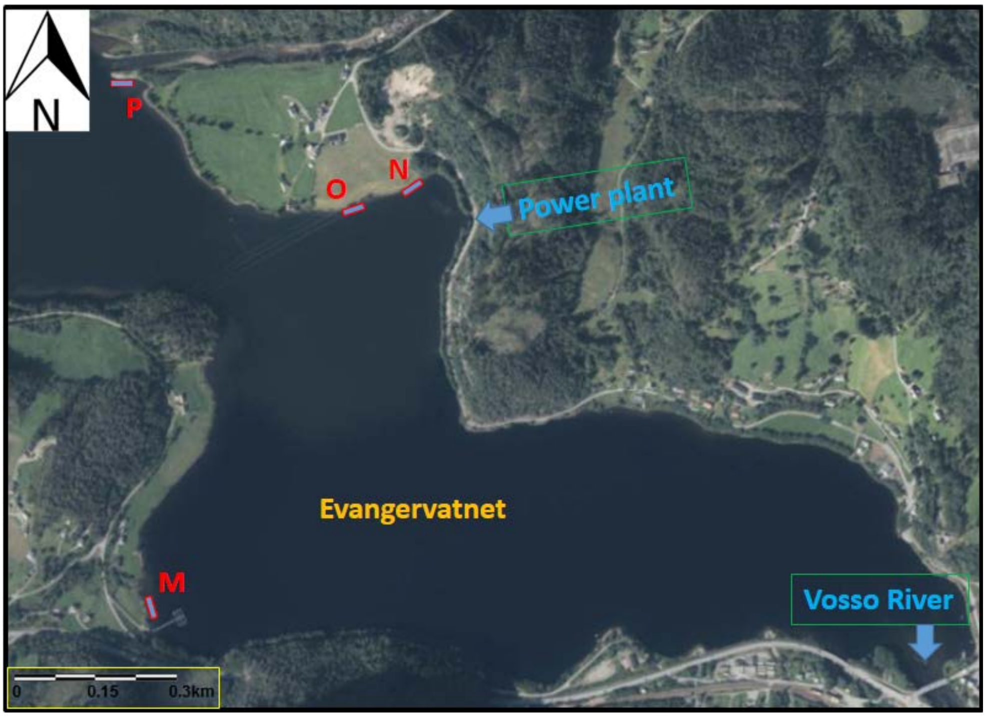


Figure A.3: Electrofish sites (M, N, O and P) in Evangervatnet. Map source: Skog og Landskap, Statens Vegvesen & Statens Kartverk 2014.

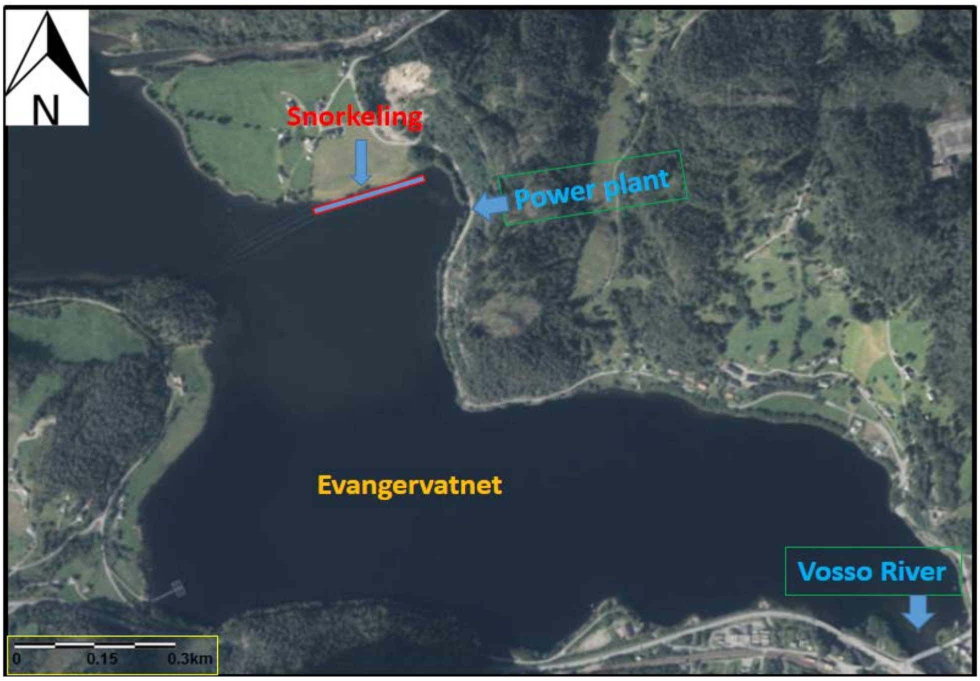


Figure A.4: Snorkeling area. Map source: Skog og Landskap, Statens Vegvesen & Statens Kartverk 2014.

A.4 Model selection

A.4.1 Cortisol

Likelihood ratio test were conducted by sequentially dropping variables to find the best model (Table A.1, Table A.2). The interaction term date:site in the cortisol model was not significant (F($_{3,\ 8}$) = 0.140, P = 0.934). The cortisol model was significantly improved when the main effects date was included (Table A.2). Autocorrelation did not significantly improve the model (Table A.2). The random cage effects were low (SD=0.018) compared with random effect of residual (SD=171.903).

Table A.1: Linear mixed effect models (LME) of cortisol tested for different variables that could have affected the cortisol. Green row represents the best fitted model.

Model	Cortisol
Mod1	lme((Cortisol)~Dato*Site, random=~1|Cagel, method='ML')
Mod2	lme((Cortisol)~Dato+Site, random=~1|Cage, method='ML')
Mod3	lme((Cortisol)~Dato, random=~1|Cage1, method='ML')
Mod4	lme((Cortisol)~Dato, random=~1|Cage, cor=corAR1(), method='ML')

Table A.2: Output from the likelihood ratio test when testing different models of cortisol: Df, AIC, BIC, logLik, Chisq, Chi Df and Pr(>Chisq).

Model	**Df**	**AIC**	**BIC**	**logLik**	**Chisq (χ^2)**	**Chi Df**	**Pr(>Chisq)**
Mod1	10	1058.789	1082.610	-519.395			
Mod2	7	1053.253	1069.927	-519.627	0.4636062	-3	0.9268
Mod2	7	1053.253	1069.927	-519.627			
Mod3	4	1056.514	1066.042	-524.257	9.26069	-3	<0.05
Mod 3	4	1056.514	1066.042	-524.257			
Mod4	5	1058.144	1070.054	-524.072	0.3696673	1	0. 5432

A.4.2 TDG

The likelihood ratio test for temporal autocorrelation significantly improved the models of both the 4-day TDG exposure and the 14-day TDG exposure (Table A.3, Table A.4). Temporal autocorrelation was therefore included to the models.

Table A.3: Linear mixed effect models (LME) of total dissolved gas (TDG) tested for different variables that could have affected the measured TDG. Green row represents the best fitted model.

Model and date	**TDG (Total dissolved gas)**
10-14 May 2016	
Mod3	lme(log(TDG)~Location, random=~1|Time.round, method='ML')
Mod4	lme(log(TDG)~Location, random=~1|Time.round, cor=corAR1(), method='ML')
10-24 May 2016	
Mod1	lme(log(TDG)~Location, random=~1|Time.round, method='ML')
Mod2	lme(log(TDG)~Location, random=~1|Time.round, cor=corAR1(), method='ML')

Table A.4: Output from the likelihood ratio test when testing different models of TDG 10 to 14 May 2016 and 10 to 24 May 2016: Df, AIC, BIC, logLik, Chisq, Chi Df and Pr(>Chisq).

Model and date	Df	AIC	BIC	logLik	Chisq (χ^2)	Chi Df	Pr(>Chisq)
10-14 May 2016							
Mod 3	6	-8253.115	-8222.757	4132.557			
Mod 4	7	-8447.352	-8411.935	4230.676	196.2375	1	<0.001
10-24 May 2016							
Mod1	6	-26902.970	-26865.180	13457.490			
Mod2	7	-27985.54	-27941.45	13999.770	1084.311	1	<0.001

A.4.3 Gill metal

Gill Al

The interaction term date:site was not significant for gill Al (µg/g dw; $F_{(3,\ 8)} = 0.523$, $P = 0.678$).

Site and date as main effects and site did not significantly improve the model (Table A.5, Table A.6). The likelihood ratio test showed that the best fitted model for gill Al (µg/g dw) included date as predictor (Table A.5, Table A.6). Because of the among-cage variance, the proportion of variation contributed from random effects are mostly from the cages (SD=0.555) compared to remaining residuals of random effects (SD=0.394). The variability of gill Al among individuals indicates that some smolt were exposed to higher concentrations of accumulative Al or had different affinity towards Al accumulation. This is supported by several studies, that have shown that gill Al can vary among the individual fish held in the same cage (Taylor et al. 2000; Marr et al. 1996). This results indicate that Vosso watercourse have sources with accumulative Al (e.g. downstream acid streams).

Table A.5: Linear mixed effect models (LME) of gill aluminum (Al) tested for different variables that could have affected the gill Al. Green row represents the best fitted model.

Model	**Aluminum (Al)**
Mod1	lme(log(Al)~Dato*Site, random=~1|Cage, method='ML')
Mod2	lme(log(Al)~Dato+Site, random=~1|Cage, method='ML')
Mod3	lme(log(Al)~Dato, random=~1|Cage, method='ML')
Mod4	lme(log(Al)~1, random=~1|Cage, method='ML')
Mod5	lme(log(Al)~Dato, random=~1|Cage, cor=corAR1(), method='ML')

Table A.6: Output from the likelihood ratio test when testing different models of gill Al: Df, AIC, BIC, logLik, Chisq, Chi Df and Pr(>Chisq).

Model	**Df**	**AIC**	**BIC**	**logLik**	**Chisq (χ^2)**	**Chi Df**	**Pr(>Chisq)**
Mod1	10	132.846	156.666	-56.423			
Mod2	7	128.501	145.176	-57.251	1.656	-3	0.647
Mod 2	7	128.501	145.176	-57.251			
Mod 3	4	124.379	133.907	-58.189	1.877	-3	0.598
Mod4	3	136.244	143.390	-65.122			
Mod3	4	124.379	133.907	-58.189	13.865	1	<0.001
Mod3	4	124.379	133.907	-58.189			
Mod5	5	125.553	137.464	-57.778	0.8253	1	0.364

Gill Cu

The interaction term date:site ($F_{(3, 8)}$ = 0.447, P = 0.726), date and site as main effects and site were not significant when being included in the model of gill Cu (µg/g dw; Table A.7, Table A.8). The best fitted model of gill Cu (µg/g dw) included date as the main effect (Table A.7, Table A.8). Difference in measured gill Cu among the cages resulted in higher cages variance (SD = 0.231) than the residuals variance (SD = 0.183).

Table A.7: Linear mixed effect models (LME) of gill copper (Cu) tested for different variables that could have affected the gill Cu. Green row represents the best fitted model.

<table>
<tr><th>Model</th><th>Copper (Cu)</th></tr>
<tr><td>Mod1</td><td>lme(log(Cu)~Dato*Site, random=~1|Cage, method='ML')</td></tr>
<tr><td>Mod2</td><td>lme(log(Cu)~Dato+Site, random=~1|Cage, method='ML')</td></tr>
<tr><td>Mod3</td><td>lme(log(Cu)~Dato, random=~1|Cage, method='ML')</td></tr>
<tr><td>Mod4</td><td>lme(log(Cu)~1, random=~1|Cage, method='ML')</td></tr>
<tr><td>Mod5</td><td>lme(log(Cu)~Dato, random=~1|Cage, cor=corAR1(), method='ML')</td></tr>
</table>

Table A.8: Output from the likelihood ratio test when testing different models of gill Cu: Df, AIC, BIC, logLik, Chisq, Chi Df and Pr(>Chisq).

Model	Df	AIC	BIC	logLik	Chisq (χ^2)	Chi Df	Pr(>Chisq)
Mod1	10	8.001	31.830	5.995			
Mod2	7	3.434	20.108	5.283	424053	-3	0.620
Mod2	7	3.434	20.108	5.283			
Mod3	4	-1.584	7.944	4.792	0.982	-3	0.806
Mod4	3	4.610	11.756	0.595			
Mod3	4	-1.584	7.944	4.792	8.194	1	<0.05
Mod3	4	-1.584	7.944	4.792			
Mod4	5	-0.111	11.799	5.056	0.528	1	0.468

Gill Fe

The interaction term date:site in the gill Fe model was not significant (F($_{3,\ 8}$) = 0.01, P = 0.998). Temporal autocorrelation was included to the gill Fe-model (Table A.9, Table A.10). Difference in measured gill Fe among the cages resulted in lower cages variance (SD =0.000) than the residuals variance (SD = 0.160).

Table A.9: Linear mixed effect models (LME) of gill iron (Fe) tested for different variables that could have affected the gill Fe. Green row represents the best fitted model.

Model	Fe (iron)
Mod1	lme(log(Fe)~Dato*Site, random=~1|Cage, method='ML')
Mod2	lme(log(Fe)~Dato+Site, random=~1|Cage, method='ML')
Mod3	lme(log(Iron)~Dato, random=~1|Cage1, method='ML')
Mod4	lme(log(Fe)~Dato+Site, random=~1|Cage, cor=corAR1(), method='ML')

Table A.10: Output from the likelihood ratio test when testing different models of gill Fe: Df, AIC, BIC, logLik, Chisq, Chi Df and Pr(>Chisq).

Model	Df	AIC	BIC	logLik	Chisq (χ^2)	Chi Df	Pr(>Chisq)
Mod1	10	-56.889	-33.069	38.445			
Mod2	7	-62.845	-46.171	38.423	0.04416055	-3	0.9976
Mod2	7	-62.845	-46.171	38.423			
Mod3	4	-61.406	-51.878	34.700	439671	-3	0.0591
Mod3	4	-61.406	-51.878	34.703			
Mod4	8	-63.903	-44.847	39.952	10.49777	4	<0.05

A.5 Pilot cage

A cage similar to the sentinel cages was placed in the current of the power plant (Figure A.5). The purpose of the pilot cage was to investigate weather more directly exposure of the outlet water from the power plant showed any signs of GBD in the fish. High water current at the pilot cage made the pilot cage collapse and the cage was tilting in the water. The pilot cage was inspected (10-,12- and 18-May 2016) by a diver to monitor the smolt condition. Three TDG exposure periods were conducted: (1) Surface cage (fish held at 0-1.15 m): 10 of 10 smolt were sampled after the exposure period 10 to 14 May 2016. Sampling was performed before sampling of fish at the surface cages to test the sampling procedure and the planned examination of fish. (2) 10 new smolt were placed in the surface cage from 14 to 21 May 2016. The cage was lowered (at 1.15-2.5 m depth) for 25 hours at 20 May 2016 11:35 AM, and then lifted to the surface (0-1.15 m depth) for 4.5 hours. Lowering and rising of the cage was conducted to check whether reduced hydrostatic pressure conditions tended to potentially increase incidence of GBD. (3) 10 smolt were exposed to TDG in the surface cage (fish held at 0-1.15 m) from 21 May 2016 to 02 June 2016. This exposure period was outside the sentinel cages period. Therefore, this period was conducted to possibly detect GBD in the watercourse after the sentinel cage experiment finished.

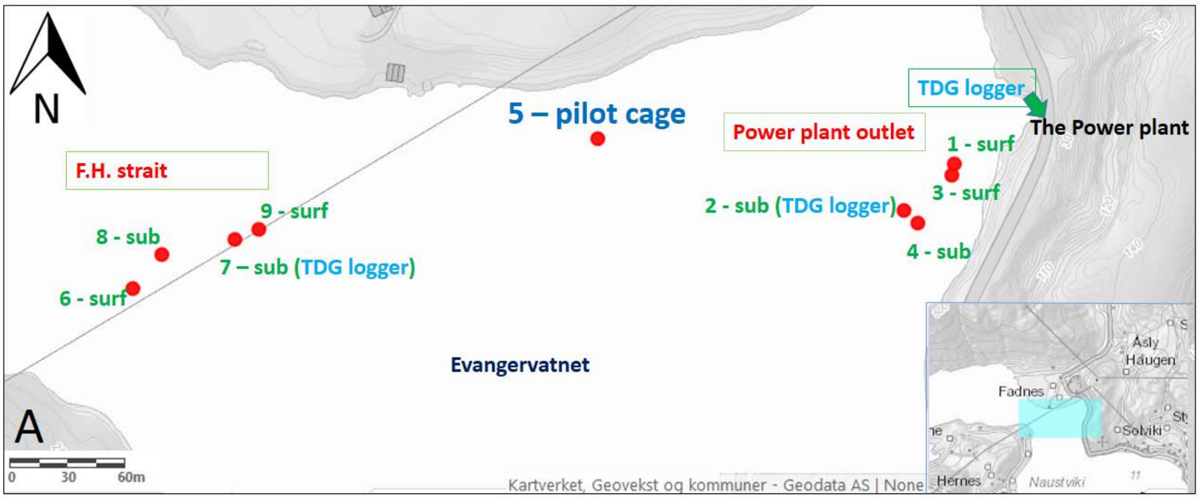


Figure A.5: Overview of the cages at the power plant outlet (cage 1- surf, 2- sub, 3-surf, 4-sub), the pilot cage (5-pilot cage), and the F.H. strait (cage 6-surf, 7-sub, 8-sub, 9-surf) in Evangervatnet. “surf” and “sub” indicate surface cage and submerged cage, respectively. “TDG logger” represents the locations of TDG monitoring.

All the smolt were examined for GBD according to section 2.5.5.

No mortality occurred and no signs of GBD were observed during the pilot cage experiments. TDG peaked 112% TDG the day of sampling 02 June 2016. The fish were hiding in-between the lead rope on the bottom of the cage during snorkeling inspections 10 May 2016 and 12 May 2016. Although the cage collapsed, the remaining space in the cage seemed enough for the fish to swim freely. 18 May 2016 most of the smolt were hiding under the lead rope. However, two of the smolt were pushed into the net with the current hitting the side of the fish.

A.6 Cortisol vs sampling time

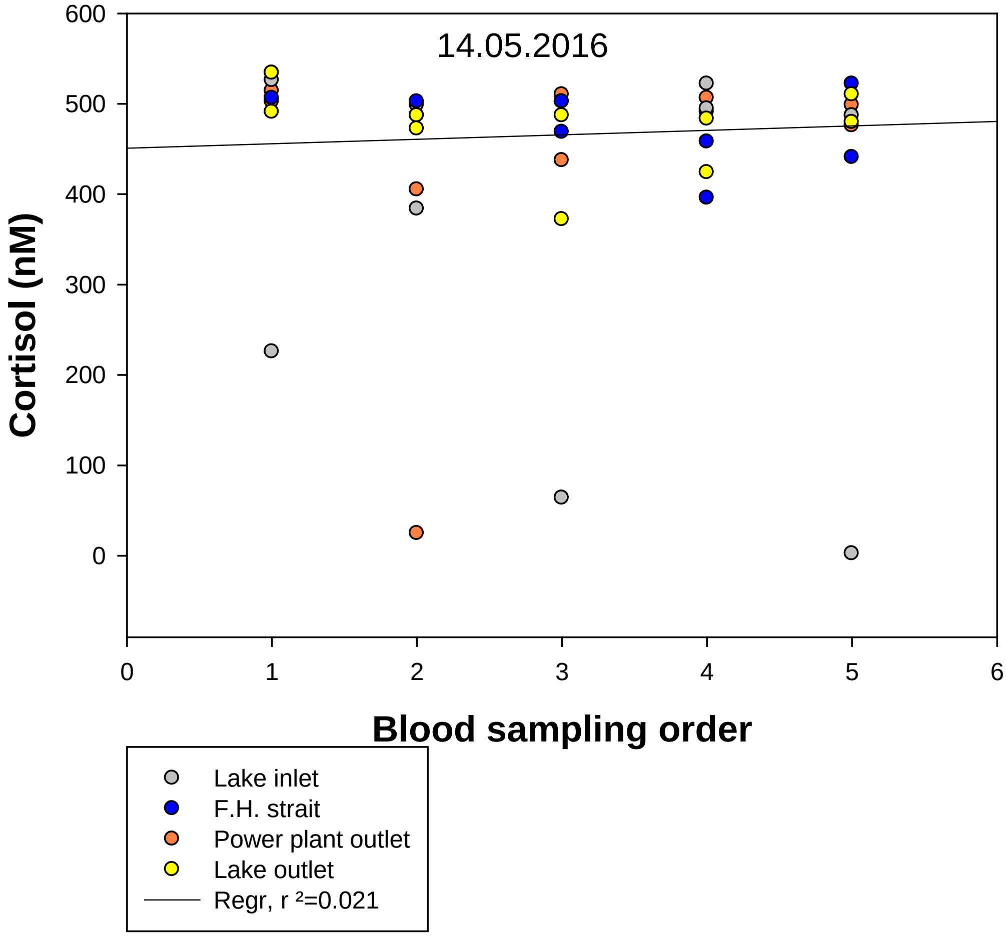


Figure A.6: Cortisol (nM) concentrations in the order blood were sampled 14 May 2016.

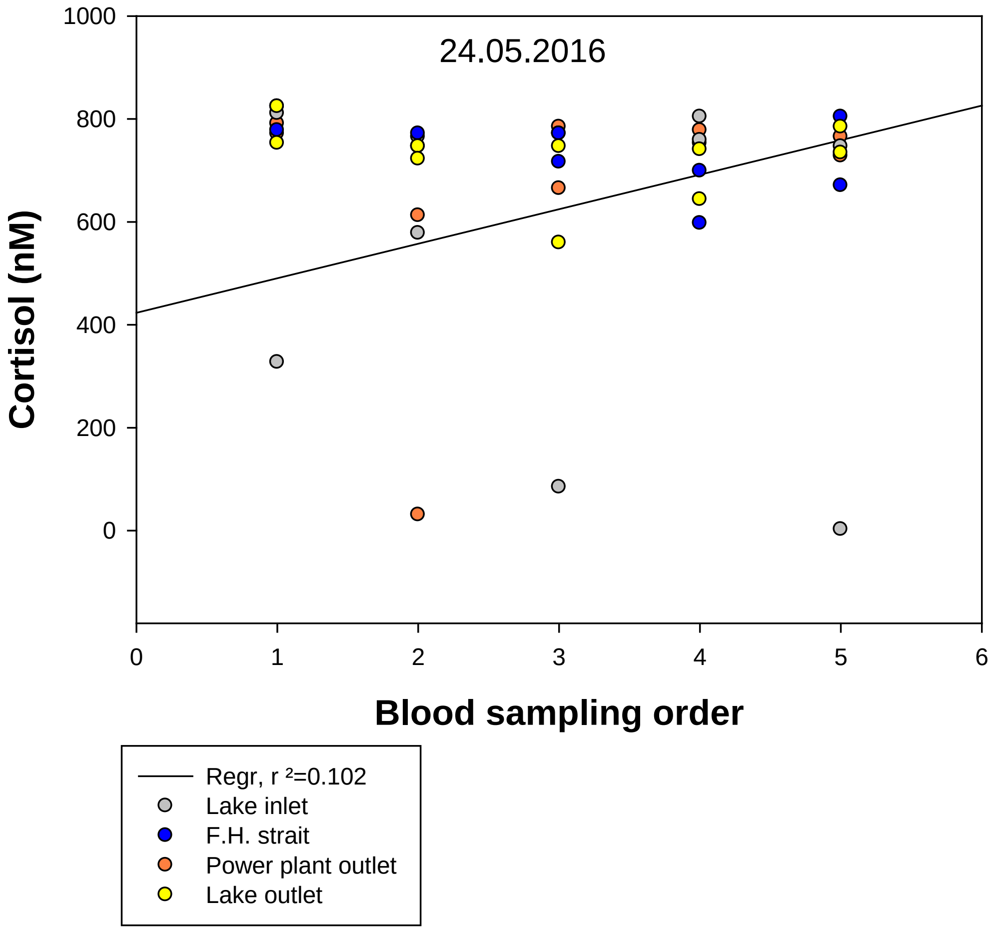


Figure A.7: Cortisol (nM) concentrations in the order blood were sampled 24 May 2016.