

**PRELIMINARY RESEARCH PROPOSAL  
SUBMITTED TO THE U.S. ARMY CORPS OF ENGINEERS UNDER  
THE ANADROMOUS FISH EVALUATION PROGRAM  
2007 PROJECT YEAR**

**I. BASIC INFORMATION**

**A. TITLE OF PROJECT**

Effects of Total Dissolved Gas on Chum Fry

**B. PROJECT LEADER**

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**C. STUDY CODES**

SPE-P-06-1

**D. ANTICIPATED DURATION**

1 October 2006 – 30 September 2007

**E. DATE OF SUBMISSION**

August 2006

**II. PROJECT SUMMARY**

**A. GOALS**

1. Determine whether total dissolved gas (TDG) concentrations are elevated in chum salmon redds downstream of Bonneville Dam.
2. Assess the physiological signs of elevated TDG in chum salmon sac fry.

**B. OBJECTIVES**

1. Determine depth compensated TDG concentrations at chum salmon redd sites downstream from Bonneville Dam.
2. Conduct toxicity tests on the formation of gas bubble signs in chum salmon fry at TDG levels ranging up to 120% saturation.

**C. METHODOLOGY**

Two objectives were funded in FY 2006. The first objective was the review and synthesis of information related to the impacts of total dissolved gas supersaturation (TDGS) to fish species in the lower Columbia River downstream from Bonneville Dam. The product of the first objective was a report completed in early 2006 titled "Total Dissolved Gas Effects on Fishes of the Lower Columbia River" by K.E. McGrath,

E.M. Dawley, and D.R. Geist. In this report we determined that recent research supports previous findings, that short-term exposure up to 120% TDGS does not produce significant effects on migratory juvenile or adult salmonids when compensating water depths are available. However, we identified several TDG issues that may be of concern with respect to fishes in the lower Columbia River. One of these issues is the effect of TDG on sac fry (primarily chum salmon) incubating in hyporheic habitats downstream from Bonneville Dam. Although no work has been done with chum salmon, available literature on other salmonid species shows that exposure of sac fry to levels as low as 103% TDGS may increase mortality.

The second objective was the collection of empirical data on TDG from two chum salmon spawning sites downstream from Bonneville Dam that could be affected by spring spill operations at Bonneville Dam. To accomplish this objective we installed nine piezometers (i.e., screened well-points) into the riverbed near chum salmon redd sites at Ives Island (six) and Multnomah Falls (three) in February, 2006. The piezometers were screened at the depth of a chum salmon redd (~30 cm) and also at the riverbed-river interface. The piezometers were instrumented with TDG sensors (e.g., Hydrolab Minisonde 5) that monitored TDG, water level, temperature, and specific conductance at egg pocket depth and in the overlying water column. Some of the hydrolabs were also capable of monitoring dissolved oxygen (DO). We monitored TDG from February through the end of June, 2006. The original study plan proposed that sensors would be recovered and replaced with newly calibrated sensors approximately every two weeks. Because Columbia River flows were higher than average during 2006, the recovery and re-deployment occurred on 19 March, 21 April, 6-7 May, and 27-28 July. Because we were not able to follow our original recovery plan, some sensors lost battery power between recoveries. Further, initial calibration and testing of sensors revealed that some were not working within specifications and that circulators (i.e. stirrers) were needed to adequately sample the low flow environment of the riverbed. High Columbia River flows and sensor malfunction delayed deployment of sensors and limited the amount of data we were able to collect.

Even with the challenges from the high flows and sensor malfunction, we were successful in collecting data from both the hyporheic zone and the water column at the two chum salmon spawning sites during the period of spill at Bonneville Dam (spill began 10 April, 2006). Preliminary results showed that during the period 2 April 2006 to 1 June 2006, total dissolved gas levels at egg pocket depth within the Ives Island chum spawning area ranged from 100% to greater than 113.5%. Total dissolved gas frequently exceeded 103% at all three Ives Island locations that were monitored, and the lowest peak total dissolved gas value for a monitoring location was 106.5%. At the Multnomah Falls monitoring locations, total dissolved gas ranged from 94% to 103%, and was frequently less than 100%. These findings are preliminary and subject to change.

Based on the literature review and our preliminary review of the FY 2006 empirical data from the two spawning areas, we are proposing two objectives in FY 2007. The first objective is to repeat the field effort to collect empirical data on TDG from Multnomah Falls and Ives Island. During FY 2007, we will add sensors to new sample sites within the Ives Island and Multnomah Falls study areas. The sensor recovery and re-deployment will be more frequent than occurred in FY 2006, and will continue through the Bonneville Dam spill operations as was done in 2006.

The second objective is to conduct laboratory toxicity tests on hatchery chum salmon fry. We will evaluate lethal and sublethal effects of TDG on chum salmon embryos and sac fry at gas levels likely to occur downstream from Bonneville Dam, when available water depths (depth compensation) and temperatures that occur during spring spill operations are considered. We will examine the effects of two-week exposure to 100, 105, 110, or 115% TDG on direct mortality, sublethal tissue damage, delayed mortality due to gas bubble disease (GBD) injury incurred during exposure, and abnormal behavior. Two developmental stages will be tested: approximately two weeks pre-emergence and immediately prior to

emergence. These stages overlap with TDG exposure during spring spill. After the two-week exposure, some individuals will be sacrificed for histopathological examination and the remaining individuals will be held for 30 days at 100% TDG and examined for post-exposure mortality and abnormal behavior.

#### **D. RELEVANCE TO THE BIOLOGICAL OPINION**

The objectives of this project are consistent with the hydrosystem targets included in the FCRPS Action Agencies' 2005-2007 Implementation Plan (IP). Specifically, hydrosystem sub-strategy 1.3 of the UPA identifies that measures are needed to monitor TDG in mainstem spawning habitat. This proposed research would have addressed RPA Action 131 under the NOAA Fisheries 2000 Biological Opinion and contributes to the ESA commitments made by the Action Agencies under NOAA Fisheries' revised 2004 BiOp.

### **III. PROJECT DESCRIPTION**

#### **A. BACKGROUND**

##### **A.1 PROBLEM DESCRIPTION**

There are several spill operations which occur in the early spring at Bonneville Dam during the time when chum salmon sac fry are still present in the gravel. Spill occurs during March for the Spring Creek hatchery release and during April for juvenile migration needs at Bonneville Dam and in the lower river. The guidance that managers have used to provide protection for pre-emergent chum salmon fry has been to limit TDG to 105% after allowing for depth compensation. During adequate water years, water depths over chum salmon redds are sufficient to provide the depth compensation necessary for chum salmon sac fry to avoid the effects of elevated TDG (provided surface water TDG levels do not exceed 120% as per the current guidelines). However, during low water years, concerns about the effects of TDG on pre-emergent chum salmon fry have forced operators to choose between providing spill to improve juvenile fish passage or limit spill to protect incubating chum salmon. Few data have been collected to evaluate the effects of TDG on chum salmon fry, and we were unable to locate any previous research evaluating exposure of salmonid fry to TDG within spawning gravels (McGrath et al. 2006). Because chum salmon are spawning in environments that are very different than habitats previously studied, and because the presumed effects of elevated TDG on chum salmon sac fry are impacting spring spill management decisions at Bonneville Dam, field-determined TDG concentrations and the chum salmon fry's physiological response to them are needed. TDG effects on incubating fry may include behavioral effects, internal tissue damage, and delayed mortality. Testing TDG effects on sac fry under controlled laboratory conditions is necessary to fully evaluate effects of TDG exposure on chum salmon sac fry downstream from Bonneville Dam. Most studies identifying effects on incubating salmonids at low TDG levels are relatively old and methods used to quantify gas levels and GBD symptoms have advanced considerably since that time. In addition, studies conducted on larval fish did not include temperature, exposure duration, and TDG levels relevant to conditions occurring downstream from Bonneville Dam.

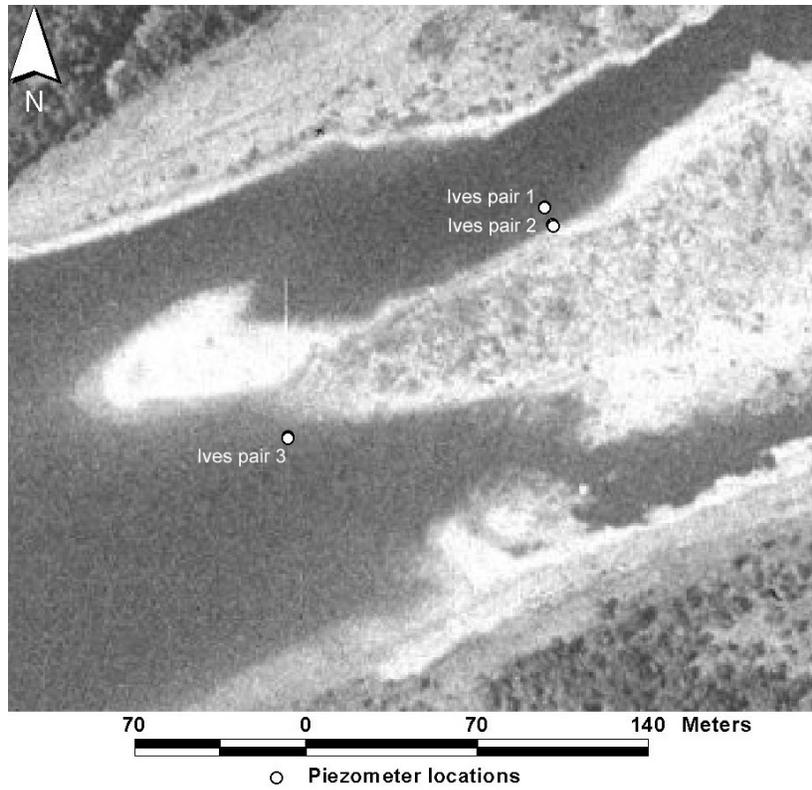
##### **A.2 LITERATURE REVIEW**

Chum salmon spawning and incubating downstream from Bonneville Dam near Ives Island and an associated site near Multnomah Falls collectively represent one of two remaining populations of the Lower Columbia River ESU listed under the Endangered Species Act. Spring spill from Bonneville Dam for the facilitation of downstream migrating salmonids, which produces gas supersaturation conditions, may be negatively impacting chum salmon incubating downstream from Bonneville Dam.

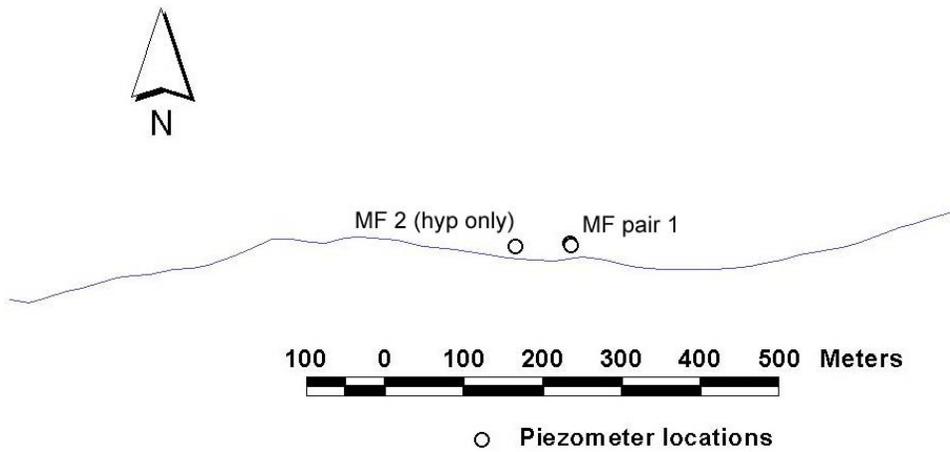
Gas supersaturation generated by spill from dams on the Columbia River was first acknowledged as an environmental concern in 1965 (Ebel and Raymond 1976). Following extensive assessment, the Environmental Protection Agency (EPA) adopted a nationwide standard of 110% TDG for the protection of aquatic life (NAS/NAE 1973). Beginning in the early 1990s, water quality agencies issued limited water quality waivers to facilitate spill for downstream juvenile salmonid migration. Existing empirical and modeling efforts reviewed in the Biological Opinions of 1995 and 2000 indicated that effects of TDGS levels between 110% and 120% had minimal impacts on aquatic biota in river environments (NOAA 1995, 2000). Waivers permitted up to 115% TDGS in downstream reaches where spill and powerhouse flows mixed and up to 120% TDGS in dam tailraces where flows from spillways were separated from those of powerhouse discharge (NOAA 1995).

Recently, gas supersaturation as a water quality issue has resurfaced (USACE et al. 2004), in particular regarding total dissolved gas levels in the incubation environment downstream from Bonneville Dam during spring spill. Elevated TDG levels within salmon redds may diminish survival of chum salmon progeny downstream from Bonneville Dam. Occurrence and effects of TDGS up to 120% supersaturation on naturally spawning (listed) chum salmon downstream from Bonneville Dam are uncertain.

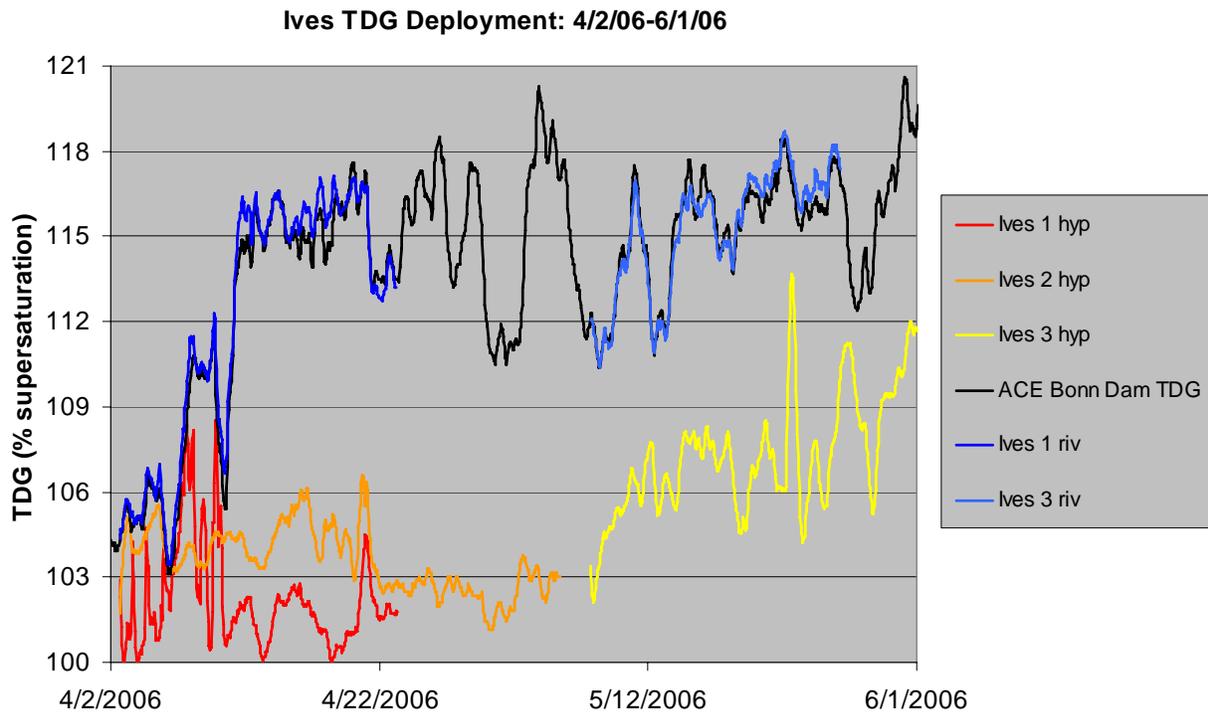
Field studies to measure TDG in hyporheic habitats have only recently been initiated. During the 2006 spring spill, the US Army Corps of Engineers (Corps) funded the Pacific Northwest National Laboratory (PNNL) to monitor hyporheic TDG levels in the mainstem Columbia River downstream from Bonneville Dam. Data from this effort are currently being analyzed (final sensor recovery occurred on 28 July, 2006). Preliminary results suggest that TDG levels in the Ives Island spawning area during spring spill operations exceeded 103%. TDG levels as low as 103% have been documented to cause mortality in sac fry (McGrath et al. 2006). From 2 April 2006 through 1 June, 2006, % TDG at egg pocket depth was monitored at three locations in the Ives Island Area (Figure 1) and at two locations at the Multnomah Falls site (Figure 2). At each site, surface water TDG was also monitored. In the Ives Area, two sensors (Ives 1 and Ives 2) recorded data during 2-22 April. TDG consistently exceeded 103%, with peak levels in excess of 108.5% (Figure 3). A third Ives Island sensor (Ives 3) recorded TDG from 7 May through 1 June. TDG at egg pocket depth consistently exceeded 107% with peak values greater than 113.5% (Figure 3). Surface water TDG in the Ives Area closely tracked TDG monitored by the Corps at Bonneville Dam (Figure 3). TDG was monitored at the Multnomah Falls site during 2 April through 1 June. TDG at egg pocket depth ranged from 94 to 103% and was generally lower than 100% (Figure 4). TDG in surface water at Multnomah Falls also tracked TDG at Bonneville Dam relatively closely (Figure 4).



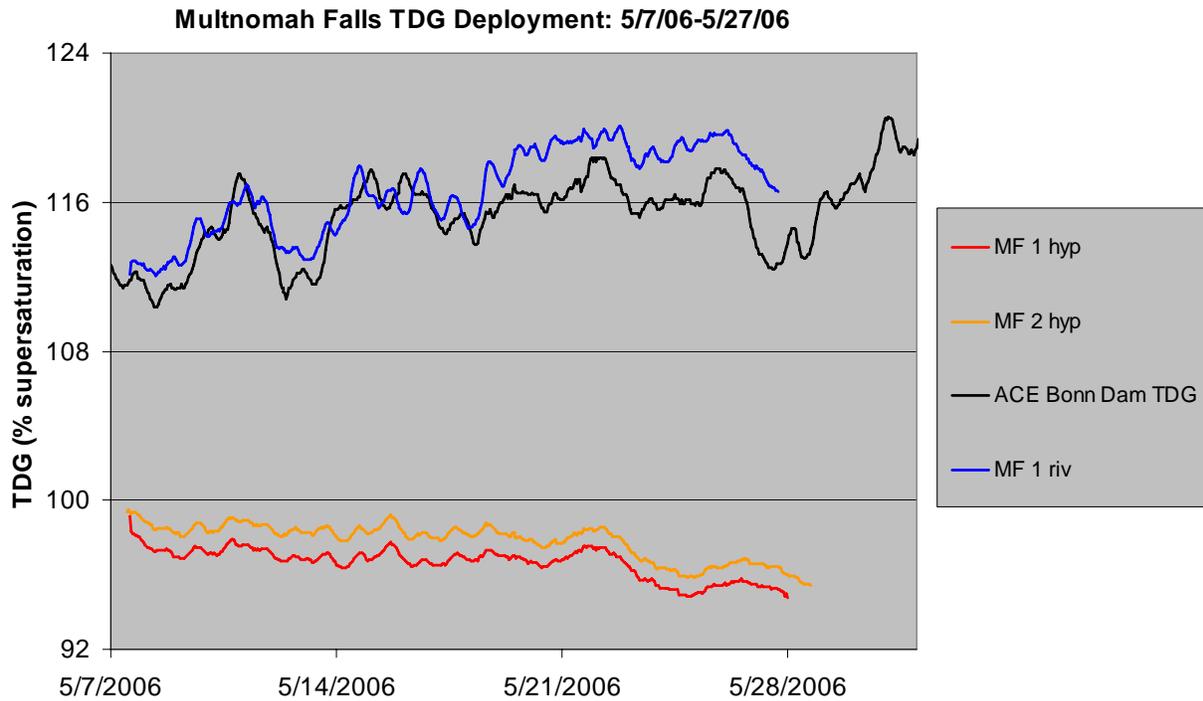
**Figure 1.** Location of TDG sensors installed in 2006 at Ives Island study area



**Figure 2.** Location of TDG sensors installed in 2006 at Multnomah Falls study area

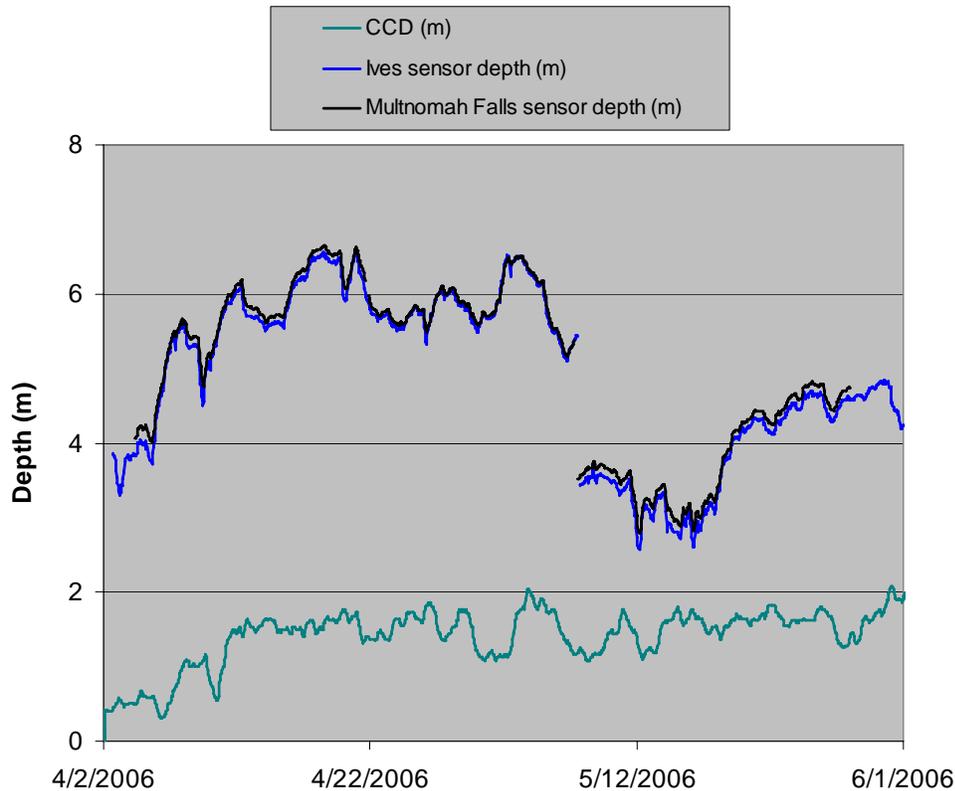


**Figure 3.** TDG levels at Ives Island from 2 April, 2006 through 1 June, 2006.



**Figure 4.** TDGS levels at the Multnomah Falls site from 7 May, 2006 through 28 May, 2006.

River levels during spring 2006 were sufficient to keep the TDG sensors below the calculated compensation depth (Figure 5), as expected during above average water years. However, TDG levels at egg pocket depth were sufficiently high that toxicity to sac fry might be expected during lower water years when compensation depths are not available. If water levels were lower than the compensation depth, there is a potential impact to chum salmon sac fry at these TDG levels. Previous data on water depths in the Ives Island area shows this occurs during normal to low water years.



**Figure 5.** Water depths at Ives and Multnomah TDG sensor locations and the calculated compensation depth (CCD) from 2 April, 2006 through 1 June, 2006.

Although considerable research has been conducted during the past 30 years on gas supersaturation effects on salmonids, primarily juveniles, relatively little attention has been given to other life stages, including incubating salmonids. Although direct and indirect effects of gas bubble disease have been documented in juvenile chum salmon (Birtwell et al. 2001; Greenbank et al. 2001), no information exists regarding the effects of gas supersaturation on incubating chum salmon. Studies of other salmonid species have only limited applicability because sensitivity to supersaturation varies among species within the salmonids (Weitkamp and Katz 1980).

Incubating salmonids are vulnerable to GBD, and hyporheic areas may present a special case of supersaturated TDG exposure. Total dissolved gas (TDG) toxicity and gas bubble disease (GBD) in alevins have been documented at TDG levels as low as 101-108% (Harvey and Cooper 1962; Wood 1968; Krise and Herman 1989). For example, Rucker and Kangas (1974) found 12 to 83% mortality in Chinook salmon fry from hatching to 50 days old in response to 112-128% TDGS. Sockeye salmon alevins experienced GBD and mortality at 108-110% TDGS (Harvey and Cooper 1962). Wood (1968) observed air bubbles and death in advanced yolk-sac and newly buttoned-up salmon fry at 103 to 104% TDGS. Krise and Herman (1989) found intracranial hemorrhaging and subcutaneous bubbles in lake trout sac fry after 15 days exposure to 101% TDGS and visible bubbles (intra-orbital, head, and abdomen) after 40 days exposure to 105% TDGS. Nebeker et al. (1978) reported mortality of steelhead yolk sac fry exposed to 115% TDGS beginning after 52 days of exposure and reaching 45% after 92 days of exposure. Montgomery and Becker (1980) found gas bubbles and some mortality of rainbow trout sac fry at 113% TDGS. Most studies identifying effects at low TDG levels are relatively old and methods used to

quantify gas levels and GBD symptoms have advanced considerably since that time. In addition, most studies conducted on larval fish did not include temperature, exposure duration, and TDG levels relevant to conditions occurring downstream from Bonneville Dam.

Gas bubble disease appears differently in larval fish than in juvenile or adult fish (Weitkamp and Katz 1980). Gas bubbles may appear in the gut or mouth, or exterior surface or yolk sac, causing fish to rise or swim abnormally or erratically (Wood 1968; Rucker and Kangas 1974). Birtwell et al. (2001) and Harvey and Cooper (1962) suggested that sublethal effects of gas bubble disease include impaired swimming performance and sensory capabilities, with affected individuals floating and/or swimming head or abdomen up. Cause of death can be due to bubbles in the buccal cavity, causing suffocation (Fidler 1988) or hemistasis (disruption of circulation; Bouck 1980; Counihan et al. 1998). Sublethal TDG exposure may produce tissue damage that results in infection and weakening of exposed fish, and may lead to increased indirect mortality (Lutz 1995; Toner and Dawley 1995).

Water depth and temperature affect toxicity of supersaturation (Weitkamp and Katz 1980). Depth is important since with each increase in depth of 1 m, gas solubility increases by approximately 10%. It is unclear whether salmonids are able to detect and avoid lethal gas concentrations by moving to deeper water (Ebel 1971; Weitkamp and Katz 1980) and the response may be species-specific. For example, Meekin and Turner (1974) showed that juvenile Chinook salmon successfully avoided supersaturated water whereas coho salmon did not, and Dawley et al. (1976) showed that Chinook salmon and steelhead that were able to move lower in the water column had higher survival rates. Salmonid alevins in the incubation environment may not have the ability to move to depth to avoid TDG exposure due to limited mobility. Temperature affects TDG exposure because dissolved gas solubility decreases with increasing temperature.

### **A.3 RELATIONSHIP TO OTHER ONGOING RESEARCH**

Pacific Northwest National Laboratory (PNNL) has been involved in chum salmon research in the Ives Island area downstream of Bonneville Dam since 1999. We have been working with several state and federal agencies to implement BPA project 1999-003-01. The research proposed here is directly related to, and will be coordinated with, Project 1999-003.

## **B. OBJECTIVES**

1. Determine depth compensated TDG concentrations at chum salmon redd sites downstream from Bonneville Dam.
2. Conduct bioassays on the formation of gas bubble signs in chum salmon fry at TDG levels ranging up to 120% saturation.

## **C. METHODOLOGY**

### **C.1. DESCRIPTION OF PROPOSED STUDY**

The following activities are proposed to accomplish these objectives in FY 2007:

#### **Objective 1 - Determine depth compensated TDG concentrations at chum salmon redd sites downstream from Bonneville Dam**

Methods for monitoring empirical TDG levels in 2007 will be similar to methods used in 2006. In 2006, we deployed TDG sensors (Hydrolab Minisonde 5) to monitor water quality at egg pocket depth (30cm)

and within the water column at a study area near Ives Island and at a second study area near Multnomah Falls. The Ives Island site is located about 230 river kilometers (rkm) from the mouth of the Columbia River and 4.3 rkm downstream from Bonneville Dam. The Multnomah Falls site is located about 14.8 rkm downstream from Bonneville Dam and approximately 220 rkm from the mouth of the Columbia River. Chum salmon spawn at both areas and the sensors were located where annual surveys conducted since 1998 as part of Project 1999-003 indicated chum salmon consistently spawn.

Three pairs of piezometers were installed in the Ives Island study area (Figure 1), and one pair was installed at Multnomah Falls (Figure 2). Each piezometer pair consisted of one piezometer screened at egg pocket depth and one piezometer screened to the river (also referred to as a standpipe). Paired piezometers enabled simultaneous measurement of water quality in the hyporheic zone and water column. Piezometers were emplaced in the riverbed using a post-pounder or pneumatic hammer until the desired depth below the riverbed surface was achieved. This method is described in detail by Geist et al. (1998). Once the piezometers were installed, we developed them by removing fines with a hand pump. We also removed approximately 1 gallon samples of water from each piezometer for turbidity analysis. Finally, we recorded the locations of the piezometers with a global positioning system (GPS). We placed caps on piezometers that did not have sensors deployed in them to keep sediment from accumulating in the pipes. The same piezometers will be used in FY 2007.

We used Hydrolab Minisonde 5 multi-parameter water quality sensors to record water quality data in FY 2006; the same sensors will be used in FY 2007. Each Minisonde weighed 1.3 kg, measured 74.9 cm long, and had an outer diameter of 4.4cm. Seven of the Minisondes were equipped with sensors for measuring TDG, and DO, as well as conductivity, water level, and temperature. The additional water quality parameters will be used to determine the extent of groundwater – surface water mixing in the incubation environment. Groundwater could affect TDG levels either directly due to elevated concentrations of nitrogen, or by altering water temperatures which could decrease gas solubility in water and increase potential impacts of gas bubble trauma. We suspect this could be important to assessing potential impacts to chum salmon because chum salmon preferentially select groundwater upwelling sites to spawn (Geist et al. 2002).

Following field deployments, Minisonde sensors were downloaded and checked for accuracy using a side-by-side field test with a laboratory-calibrated sensor. We placed the lab-calibrated sensors and the recovered sensors in the river at an approximate depth of 90 cm for the side-by-side deployment. Following each recovery, all TDG membranes were exchanged for those previously tested for proper function in the lab. The used membranes were transported back to the lab and tested to confirm data integrity. Following each deployment, each TDG pressure sensor was tested for accuracy using a Druck® pressure calibrator at 100, 200, and 300 mmHG. If the pressure reading was off by more than the stated accuracy of  $\pm 0.01\%$  of the span (or 2 mmHG), we recalibrated the unit. After recalibration, the sensor was rechecked at all pressure levels. We calibrated the DO and depth sensors following procedures in the Minisonde user's manual. We then gave each Minisonde new batteries. Upon return to the laboratory, each membrane was tested for functionality before reuse.

Compensation depth influences TDG concentrations and therefore physiological effects on sac fry. We will compute the compensation depth using the equation of Tanner and Johnston (2000) modified from Colt (1984):

$$\text{Compensation Depth} = [\text{TDG Pressure (mmHg)} - \text{Barometric Pressure (mmHg)}] / 23$$

This is the method currently used by the Corps (i.e. the Corps online water quality data include compensation depth computed by this method).

In 2007, we will deploy sensors in all the same locations as in 2006, plus the following: (1) one pair of additional piezometers will be installed at Multnomah Falls. These piezometers will be used to rotate Minisonde sensors; (2) one additional river sensor will be installed at Multnomah Falls. This will ensure we have two pairs at this study site; and (3) 2 pairs of sensors will be installed at Ives Island within areas chum salmon spawn. Thus, a total of 5 additional sensors will be used in FY 2007. All sensors will be recovered and replaced with Minisonde 5 calibrated sensors using a sampling interval of 14 to 20 days during March through May. The calibration procedure will involve checking the operation of the TDG sensor in the field without disturbing it, replacing the field sensor with a laboratory-calibrated sensor, and verifying proper operation of the newly deployed field sensor. The laboratory calibration procedure will involve removing the TDG membrane, comparing pressure readings to barometric pressure, inserting the instrument into a pressure chamber to record pressure changes, and inserting it into water with high dissolved gas levels (soda water) to check gas concentrations.

If high water levels require SCUBA divers in order to recover and replace TDG sensors, PNNL will provide divers bi-weekly or as needed. Recovery and re-deployment will continue every 2-3 weeks until spill operations cease and flows are low enough to enable sensor recovery.

### **Objective 2 - Conduct bioassays on the formation of gas bubble signs in chum salmon fry at TDG levels ranging up to 120% saturation**

We will evaluate lethal and sublethal effects of total dissolved gas (TDG) on chum salmon embryos and alevins at gas levels likely to occur downstream from Bonneville Dam when available water depths (depth compensation) and temperatures that occur during spring spill operations are considered. In addition to direct mortality, indirect mortality from injury incurred during exposure and associated disease development has been documented (Harvey and Cooper 1962; Lutz 1995; Toner and Dawley 1995). We will test the hypotheses that these TDG levels do not cause:

- direct mortality,
- sublethal tissue damage,
- delayed mortality due to gas bubble disease (GBD) injury incurred during exposure, or
- abnormal behavior.

Larval chum salmon larvae will be exposed to TDG levels of 100, 105, 110, or 115% TDG for two weeks in shallow tanks at 10.5 °C. These conditions represent the range of conditions including depth compensation that chum salmon may experience late in incubation in the Ives Island area. A minimum of 100 individuals per treatment replicate will be tested to provide sufficient numbers of individuals for histopathological examination and lethal GBD examination, when expected mortality due to TDG exposure and random mortality are taken into account and to provide sufficient statistical power to detect differences if present among treatments. Two developmental stages will be tested: approximately 2 weeks pre-emergence and immediately prior to emergence. These stages overlap with TDG exposure during spring spill. Treatments will be examined 3 times/day and mortality and abnormal behavior will be recorded. All mortalities will be examined immediately for GBD symptoms. After the 2-week exposure period, up to 25 individuals total per treatment (25 individuals X 4 treatments X 2 life stages = 200) will be randomly selected, examined for GBD and preserved for histopathological examination (see below). Remaining individuals from all treatments will be carefully transferred to holding containers containing control (100% TDG) water and held for an additional 30 days. All treatments will be observed daily and abnormal swimming behavior and mortality will be quantified. Upon termination of the each test, subsamples of up to 25 individuals per replicate will be examined for GBD (emboli, hemistasis) and other disease (e.g. fungal) symptoms. Prior to testing with chum salmon, preliminary testing will be

conducted with larval hatchery steelhead or Chinook salmon to finalize testing and histopathological protocols.

Histopathologic analyses will be conducted under subcontract by Dr. Ralph Elston, Aqua-Technics, Inc. From chum salmon tests, approximately 200 (2 tests X 25 fish per treatment X 4 treatments) specimens will be randomly selected, preserved according to standard procedures and shipped to Dr. Elston. Tissue samples will be examined for damage due to gas bubble disease (GBD). Preliminary analyses will be conducted on approximately 20 specimens of steelhead or Chinook salmon from the preliminary toxicity test to develop the specialized methodologies necessary for examination of tissue damage associated with GBD in larval salmonids.

Chum salmon and steelhead used in the laboratory study will likely be obtained as eggs from a state or federal hatchery. We will obtain the necessary approvals for this transfer.

## **C.2. JUSTIFICATION**

Chum salmon are an ESA listed species, and there are currently two main spawning populations remaining in the lower Columbia River (Grays River and Ives Island Area). The Ives Island site is located approximately 4.1 rkm downstream from Bonneville Dam, where spring spill operations may be elevating TDG levels and negatively impacting chum salmon sac fry. We will monitor TDG levels in chum salmon spawning gravels utilizing sensors similar to past research evaluating TDG in surface water in the lower Columbia (Tanner and Johnston 2000; Tanner and Bragg 2001; Pickett 2002). Most studies identifying effects at low TDG levels are relatively old and methods used to quantify gas levels and GBD symptoms have advanced considerably since that time. In addition, most studies conducted on larval fish did not include temperature, exposure duration, and TDG levels relevant to conditions occurring downstream from Bonneville Dam. Toxicity testing will evaluate the range of potential TDG levels from Bonneville Dam spill, and will assess impacts including direct mortality, delayed mortality, abnormal behavior, and sublethal damage on larval chum salmon.

## **C.3. SAMPLE SIZE CONSIDERATIONS**

**HOURLY TDG AND OTHER WATER QUALITY DATA WILL BE COLLECTED DURING THE 2 MONTH STUDY PERIOD. OUR SAMPLING RATE WILL MATCH EXISTING USACE DATA COLLECTION EFFORTS MONITORING TDG, COMPENSATION DEPTH, AND TAILWATER ELEVATION NEAR BONNEVILLE DAM.**

Consistent with Backman et al. (2002), a minimum of 100 individuals per treatment replicate will be exposed in each toxicity test to provide sufficient statistical power to detect differences if present among treatments and to provide sufficient numbers of individuals for histopathological examination and lethal GBD examination when expected mortality due to TDG exposure and random mortality are taken into account and to. From chum salmon toxicity tests, approximately 200 (2 tests X 25 fish per treatment X 4 treatments) specimens will be randomly selected, preserved according to standard procedures and examined for TDG symptoms. An additional 20 steelhead or Chinook salmon individuals will be examined to establish histopathological protocols for GBD analysis.

## **C.4. LIMITATIONS/EXPECTED DIFFICULTIES**

We expect few major difficulties with completing the planned objectives. We have determined that access to the sensors for repair and calibration purposes will be necessary. Divers will be required to access the sensors because flows can be high during the spring spill period.

Several permits will be required to accomplish the proposed project. We expect no difficulties in obtaining these permits:

- (1) Fish Transport Application from the Washington Department of Fish and Wildlife; and
- (2) Animal Care Use Protocol from PNNL's Institutional Animal Care and Use Committee (IACUC).
- (3) State (Oregon and Washington) and Corps of Engineers in-water work permits to install sensors.

### **C.5. EXPECTED RESULTS AND APPLICABILITY**

This project is expected to result in an assessment of the potential effects to chum salmon fry from TDG exposure downstream from Bonneville Dam. This information will assist managers that are faced with deciding between more spill to aid downstream migrants (at the potential expense of elevated intragravel TDG) versus the reduction in TDG with reduced spill (at the potential expense of downstream migrant passage).

The objectives of this project are consistent with the hydrosystem targets included in the FCRPS Action Agencies' 2005-2007 Implementation Plan (IP). Specifically, hydrosystem sub-strategy 1.3 of the UPA identifies that measures are needed to monitor TDG in mainstem spawning habitat. This proposed research would have addressed RPA Action 131 under the NOAA Fisheries 2000 Biological Opinion and contributes to the ESA commitments made by the Action Agencies under NOAA Fisheries' revised 2004 BiOp.

### **C.6. SCHEDULE**

Objective	Task	Schedule
1	Monitor TDG (includes bi-weekly site visits)	Mar. 2007-May 2007
2	Permit acquisition	Dec. 2006-Jan. 2007
2	Sac fry toxicity tests	Feb. 2007-Aug. 2007
2	Sac fry histopathological analyses	Mar. 2007-July 2007
1+2	Submit draft report to Corps	October 15, 2007
1+2	Submit final report to Corps	November 30, 2007

### **D. FACILITIES AND EQUIPMENT**

PNNL has extensive experience conducting aquatic research in the hyporheic zone of the Columbia and Snake River systems. We possess all the necessary equipment to successfully install and maintain the equipment that will be used in this study. Our office in North Bonneville is conveniently located near the study site and houses our data telemetry server. The primary investigator and his team have previously published literature summarizing water quality, physicochemical gradients, and groundwater – surface water mixing within the hyporheic zone of the Columbia and Snake rivers (Hanrahan et al. 2005; Moser et al. 2003; Geist et al. 2002; Geist 2000a,b). PNNL also has extensive laboratory capabilities to link empirical field data to simultaneous laboratory work. PNNL operates an aquatic facility that supports a variety of research on fish and other aquatic life, covering topics as diverse as toxicology, bioengineering, and biosensor development. Housed in PNNL's Life Sciences Laboratory 1 in Richland, Washington, the laboratory is operated for the U.S. Department of Energy. Waters from the Columbia River and a

groundwater well are delivered to the lab and conditioned to meet specific research needs. The laboratory is capable of producing water for fish exposures that can be supersaturated with dissolved nitrogen.

AquaTechnics Inc. will be used for histological analysis of tissue samples. AquaTechnics specializes in fish and shellfish health management and aquatic environmental assessment. Laboratory capabilities in fish health management include histological assessment of tissues, gross necropsy of fish, stereo, bright field and fluorescence microscopy, photomicroscopy, and virological, bacteriological, polymerase chain reaction (PCR) and other microbiological assays for fish and shellfish pathogens, and physiological assays of fish condition (e.g. plasma evaluation). AquaTechnics has both private and governmental clients in all Pacific coast states as well as in South America, Europe and Asia. AquaTechnics staff are experienced in team management, sample documentation, chain of custody tracking, quality assurance and quality control procedures, project management, and preparation of reports.

## **E. IMPACTS**

### **1. Other ongoing or proposed research**

The on-going BPA project will not be impacted by this study. In fact, there will be a net benefit to both projects through cost-sharing and collaboration.

### **2. Special operations**

There may be need to reduce flow at Bonneville Dam on weekends to accommodate access to the site. This was coordinated with the Corps in 2006 and has been coordinated with BPA in the past on BPA Project 1999-003. In both cases the agencies have been extremely cooperative and flow management has worked well to enable short-term access to monitoring stations. The use of SCUBA divers will also be used to reduce this impact.

## **F. COLLABORATIVE ARRANGEMENTS AND/OR SUB-CONTRACTS**

We will subcontract with Dr. Ralph Elston, Aqua-Technics, Inc. for histopathological analyses. Dr. Elston is a recognized expert in the field of histopathology and has considerable experience with gas bubble disease in salmonids (Elston et al. 1997a, b). Mr. Earl Dawley will be consulted for experimental design and data interpretation on field and laboratory studies.

## **IV. LIST OF KEY PERSONNEL AND PROJECT DUTIES**

Dr. David Geist	Project Manager
Dr. Kathleen McGrath	Senior Fish Biologist
Mr. Evan Arntzen	Field Lead
Dr. Ralph Elston	Sub-contractor, Aqua-Technics, Inc.
Mr. Earl Dawley	Sub-contractor
Mr. Scott Abernethy	Laboratory Manager

## **V. TECHNOLOGY TRANSFER**

Information acquired during the proposed work will be transferred in the form of written and oral research reports. A presentation will be made at the Corps' annual Anadromous Fish Evaluation Program Review. Technology transfer activities may also include presentation of research results at regional or national fisheries symposia, or publication of results in scientific journals.

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## **VII. BUDGET**

Detailed budget by PNNL will be provided under separate cover.