Hagerman National Fish Hatchery Evaluation Team

Reducing Dissolved Nitrogen Gas from Water Supplied to a Single Bank of Rainbow Trout Raceways

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Abstract

The Hagerman National Fish Hatchery has experienced skin sloughing and associated secondary infections related to gas bubble disease for the past several brood years. In order to reduce gas bubble disease in the rainbow trout, the Hatchery crew installed a vacuum degas system on the intake pipelines to the rainbow trout. This degas system significantly reduced dissolved nitrogen from a mean of 103.8% to a mean of 97.5%. Trout grown in degassed water had no skin sloughing, tail rot, or secondary infections from skin lesions, better survival, and better feed conversion compared to the previous brood year. These results indicate that there is an advantage in degasing all production water at the Hagerman National Fish Hatchery.

Introduction

The Hagerman National Fish Hatchery (Hatchery) started to routinely monitor dissolved gas concentrations in its water supply in October 2007. This monitoring program was a direct result of a construction project that involved replacing the main spring intake structure. This construction project created a large plunge pool which entrained air into the water column as it was entering the piping system to the mixing chamber, which is the collection area for the entire Hatchery's spring water before entering the steelhead raceways. The results of this monitoring program indicate not only was the plunge pool creating gas supersaturation, but the water was coming out of the springs already supersaturated (HHET 2009). During the 2012 calendar year, the Hatchery observed total dissolved nitrogen gas levels ranging between 102% - 105% in both the main spring pool and spring 17. The mainspring pool is the primary water source for fish production at the Hatchery and spring 17 is only available to the rainbow trout raceways. Spring 17 is the sole source of production water for the rainbow trout until May when supplemental water from main spring is added to the rainbow trout raceways. Dissolved nitrogen gas should be <103% for optimal fish rearing conditions (Timmons and Ebeling 2010).

Shortly after the Hatchery started monitoring dissolved gasses in the spring water, skin sloughing was sporadically observed in the steelhead raceways. Skin sloughing is characterized as bluish hazy marks on the skin encircling the area below the dorsal fin. Within 4-5 days of the first observation, the skin begins to slough and exposes muscle tissue with mortality resulting several days thereafter. The Hatchery first observed this phenomenon in Brood Year (BY) 2009, and it has occurred every brood year since. Fish that exhibit this skin sloughing are almost always in raceways with first use water. Hagerman State Fish Hatchery also reported having fish with the same symptoms as the Hatchery was observing. The cause of the skin sloughing was never pinpointed, but florfenicol sensitivity, sunburn, steatitis, columnaris, supersaturated gas or a combination of the latter where all suggested as possible causative agents (HNFH 2010, HNFH 2011).

Although a definitive cause to the skin sloughing has never been confirmed in the steelhead, it is widely believed that chronic exposure to supersaturated dissolved nitrogen gas leads to a situation in which the fish are continually stressed and become immune incompetent. Although nothing has been found to prevent the skin sloughing, a three day

1.0 ppm drip treatment of potassium permanganate is used to treat secondary infections caused by the open wounds left by the sloughed skin. Prior to BY2011, skin sloughing was only observed in the steelhead. The rainbow trout broke in BY2011 with the same clinical signs of skin sloughing as which are normally seen in the steelhead (Figure 1). The Idaho Fish Health Center along with John Morrison, Olympia Fish Health Center, confirmed that this instance of skin sloughing was caused by gas bubbles through histological procedures (Figure 2).

Prior to ponding of BY2012 rainbow trout, a degas system was installed to treat the rainbow trout production water and to reduced nitrogen supersaturation levels down to <100%. The rainbow trout raceways were chosen for two reasons, one because of the smaller amount of flow that needs to be treated and two, because BY2012 rainbow trout were the first fish available to test prior to receiving BY2012 steelhead. At peak production, maximum flow to the rainbow trout raceways is 10 cfs, compared to 60 cfs that goes to the steelhead raceways. The objective of this project is to create a better rearing environment by reducing dissolved nitrogen to recommended levels and observe fish health. If the degas system provides the desired water quality and improved fish health, then it stands to reason the same system would be effective at treating the steelhead on a larger scale.

Materials and Methods

Rainbow Trout Production

The rainbow trout production plan at the Hatchery changes very little from brood year to brood year. The Hatchery normally receives 150,000 triploid eggs from the Hayspur State Fish Hatchery on the first week of January. These eggs are split evenly into 9 nursery tanks that are 15’ X 3’ X 2’. Fish are reared in these tanks until early March when fish are approximately 400 fpp with rearing conditions of a flow index of 0.50 and a density index of 0.42. Flow and density index is calculated based on the formulas provided by Piper et al. 1982. Once fish reach 400 fpp, they are moved to the outdoor rainbow trout rearing raceways which are 8’ X 70’ X 3’via a forklift hauling tank. At that time, fish are split evenly into 3 raceways with a total water flow of 3 cfs from spring 17. These fish are fed at a hatchery constant (Pier et at 1982) of 10.5. In early April, the feeding rate is increased to a hatchery constant of 12. In mid-April, flow is increased to a total of 6 cfs with an additional 3 cfs from main spring. In mid-May, 90,000 five inch fingerlings are released. Prior to release, rearing conditions are typically a density index of 0.30 and flow index of 0.60. The remaining trout are evenly split into four raceways. By mid-July the feeding rate is reduced to a hatchery constant of 6. The remaining 40,000 trout are released at nine inches in length on the first week of October when rearing conditions reach a density index of 0.30 and flow index of 0.60.

Degas System Design

The Hatchery, in collaboration with Jack Christiansen, Aquaculture Engineer, Dworshak National Fish Hatchery, installed vacuum degassing columns on the rainbow trout inlet water. These vacuum degassing columns work by creating a negative pressure difference to strip out dissolved gasses from the water. The top of these columns are sealed. Water enters the columns from the water intake pipe above the column through a flange connecting the intake pipe and column, then down into the column. A blower creates lower pressure in the column which causes the dissolved gasses to come out of solution from the water into the air.

The dimensions of the columns installed at the Hatchery are illustrated in Figure 3. One column was installed on the spring 17 inlet of the rainbow trout raceways. Water coming into the degasing column is regulated by a butterfly valve. A one inch stainless steel ball valve is used to maintain 50-55 inches of water within the column. A one horsepower regenerative blower is used to maintain the vacuum. The bottom 18 inches of the column is below the water line in the headbox creating a sealed unit. After water is degassed, it flows into the headbox.

A second series of three columns, as described above, were placed on the inlet of the main spring water into the rainbow trout headbox. Water to the columns was supplied through a manifold on the inlet pipe and controlled to each individual column by a butterfly valve.

Monitoring Parameters

The change in pressure (ΔP) levels in the rainbow trout production water, before and after degassing, was measured using an *In-Situ Inc*. Tensionometer 300E. The change in pressure measurement was then used to calculate percent dissolved nitrogen (Appendix 2). Dissolved oxygen readings were taken concurrently with the change in pressure readings with an *YSI* 550A meter. These measurements were taken weekly during the BY2012 production cycle.

The Idaho Fish Health Center sampled 10 fish monthly and 60 fish pre-release during both BY2011 and BY2012. Fish health professionals categorized the level of gas bubble presence and other fish health issues during both production seasons (Appendix 1).

Density index, flow index, monthly survival, and overall food conversion ratio were tracked during both brood years.

Statistical Analysis

Mean total dissolved nitrogen saturation levels, dissolved oxygen levels, density index, flow index, and survival were compared using an ANOVA. Survival data was arcsine transformed and dissolved nitrogen gas saturation data was logarithmic transformed before statistical analyses were run to form a normal distribution (Zar 1999).

Results

Water Quality Parameters

Dissolved nitrogen trends before and after degassing throughout the production cycle during BY2012 are illustrated in Figure 4. The rainbow trout were exposed to a mean dissolved nitrogen level of 106.5% from January 3rd to March 9th while in the Hatchery 2 building. Once the trout were moved to the outdoor rearing raceways, mean dissolved nitrogen levels of incoming production water before degassing was 103.8%. The mean dissolved nitrogen levels after degassing into the headbox of the raceways was significantly reduced ($p < 0.05$) to 97.5%.

The mean dissolved oxygen level of incoming water before degasing was 8.55 ppm, compared to 8.22 ppm after degassing ($p < 0.05$).

Fish Health

A summary of monthly fish health diagnostics for BY2011 and BY2012 are in Table 1. During BY2011, the trout had high to moderate levels of gas bubbles throughout the production cycle, along with *Nucleospora salmonis* symptoms, skin sloughing, and fin erosion. During BY2011, a three day potassium permanganate treatment was administered for external *Columnaris* in mid-September. This treatment was a one hour, drip, flow thorough treatment every other day, starting with a concentration of 1.25 ppm followed by two treatments at a concentration of 1.75 ppm. The *Columnaris* was believed to be a secondary infection to tissue loss caused by gas bubbles.

Light gas bubbles, *Nucleospora salmonis*, and fin erosion were detected in the trout during BY2012. No skin sloughing or potassium permanganate treatments occurred during BY2012.

Fish Production

There was 12 cfs of water available for trout production in BY2011 compared to 7 cfs in BY2012. This resulted in an overall flow index of 0.31 for BY2011 which was significantly lower ($p < 0.05$) than the flow index of 0.50 in BY2012 (Table 2). There was no significant difference ($p > 0.0.5$) in density index between the two brood years. In BY2011 the density index was 0.22 compared 0.26 in BY2012.

A total of 124,814 trout totaling 22,900 pounds were released during BY2011. Average size stocked was 5.45 fish per pound (7.31 inches). Overall food conversion ratio for BY2011 was 1.04. A total of 128,851 trout totaling 24,810 pounds were released during BY2012. Average size stocked was 5.19 fish per pound (7.43 inches). Overall food conversion ratio for BY2012 was 0.94.

Overall survival for BY2011 was 84.49% compared to 93.95% in BY2012. Mean monthly survival was significantly lower ($p < 0.05$) at 91.97% for BY2011 compared to 94.52% during BY2012 (Table 3).

Discussion

The vacuum degas system installed by the Hatchery crew successfully reduced dissolved nitrogen gas from incoming rainbow trout production water to below 100% saturation. It is recommended that fish production water should be at or below 100% saturation to support good growth and survival (Piper et al 1982). Even though the raceway production water was degased, light gas bubbles were found in some of the fish during monthly fish health inspections. Gas bubbles can linger long after a supersaturation event since nitrogen can be inert, very stable, and it takes quite a bit of energy (increased pressure) to get it back into solution. Therefore, the Hatchery is not sure if the gas bubbles found in the sampled fish were remnant from being in the hatchery tanks or from an 8 hour power loss shutting down the degas system in early June of 2012.

The degas system significantly reduced DO by 0.33 ppm to the production water. This reduced the percent saturation from 94.9% to 91.2% (based on saturation level of 9.01 ppm – AFS 2012). Piper et al (1982) recommends a continual concentration of 80% saturation or more for optimal dissolved oxygen levels. However, the system can be tuned to reduce the amount of dissolved nitrogen being removed, which in turn, will reduce the amount of dissolved oxygen being removed.

Water available for production in BY2012 was 42% less than that of BY2011. Even with a significant cut in production water, fish grown in the degased water in BY2012 had over all better fish health, better survival, and a better food conversion ratio than the previous brood year.

Results from this small scale degas system on the trout raceways indicates there would be distinct advantages to degassing the production water available to the steelhead raceways.

References

American Fisheries Society (AFS). 2012. Table 3. Dissolved Oxygen Spreadsheet (F). http://fisheries.org/hatchery

Hagerman National Fish Hatchery (HNFH). 2010. Hagerman National Fish Hatchery Fiscal Year 2010 Summary of Operations and Expenditures. U.S. Fish and Wildlife Service, Hagerman, Idaho.

Hagerman National Fish Hatchery (HNFH). 2011. Hagerman National Fish Hatchery Fiscal Year 2011 Summary of Operations and Expenditures. U.S. Fish and Wildlife Service, Hagerman, Idaho.

Hagerman Hatchery Evaluation Team (HHET). 2009. Overview of Total Gas Supersaturation at the Hagerman National Fish Hatchery. U.S. Fish and Wildlife Service, Hagerman, Idaho.

Piper, Robert, Ivan McElwain, Leo Orme, Joseph McCraren, Laurie Fowler, and John Leonard. 1982. Fish Hatchery Management. United States Department of the Interior, Washington, D.C.

Timmons, Michael and James Ebeling. 2010. Recirculating Aquaculture, 2nd Edition. Cayuga Aqua Ventures, Ithaca, New York.

Wedemeyer, Gary. 2001. Fish Hatchery Management, Second Edition. American Fisheries Society, Bethesda, Maryland.

Zar, Jerrold. 1999. Biostatistical Analysis, Fourth Edition. Prentice Hall, Upper Saddle River, New Jersey.

Tables and Figures

Figure 1. Rainbow trout exhibiting skin sloughing. Bottom fish has early symptoms of white patches on skin and opaque eye. Top fish has advanced symptoms of tail rot, exposed musculature, missing operculum, and blood seeping from eye.

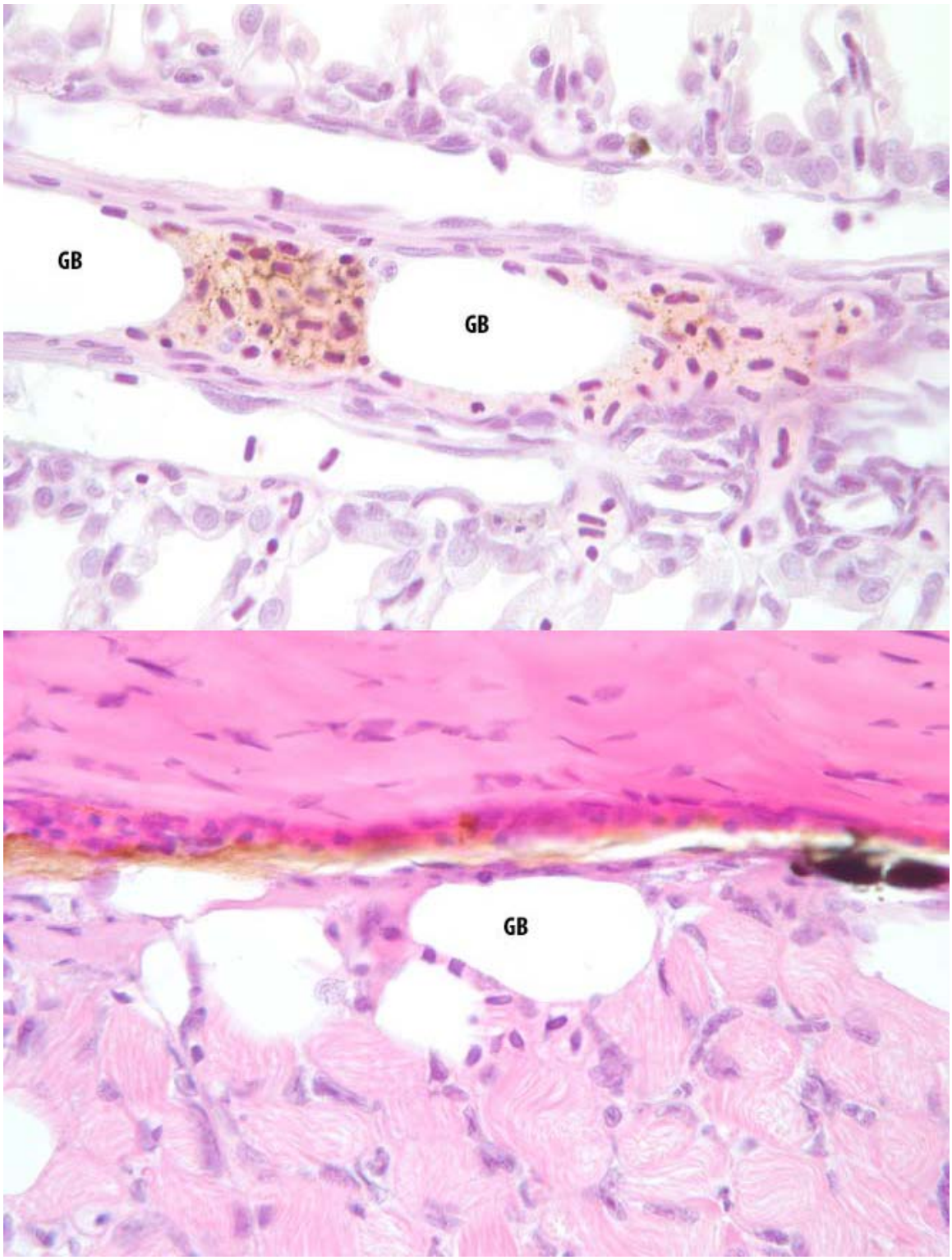

Figure 2. Histological samples taken from gill of rainbow trout BY2011. Top picture shows gas bubbles in the gill. Bottom picture shows subdermal gas bubbles. Pictures courtesy of John Morrison, Olympia Fish Health Center.

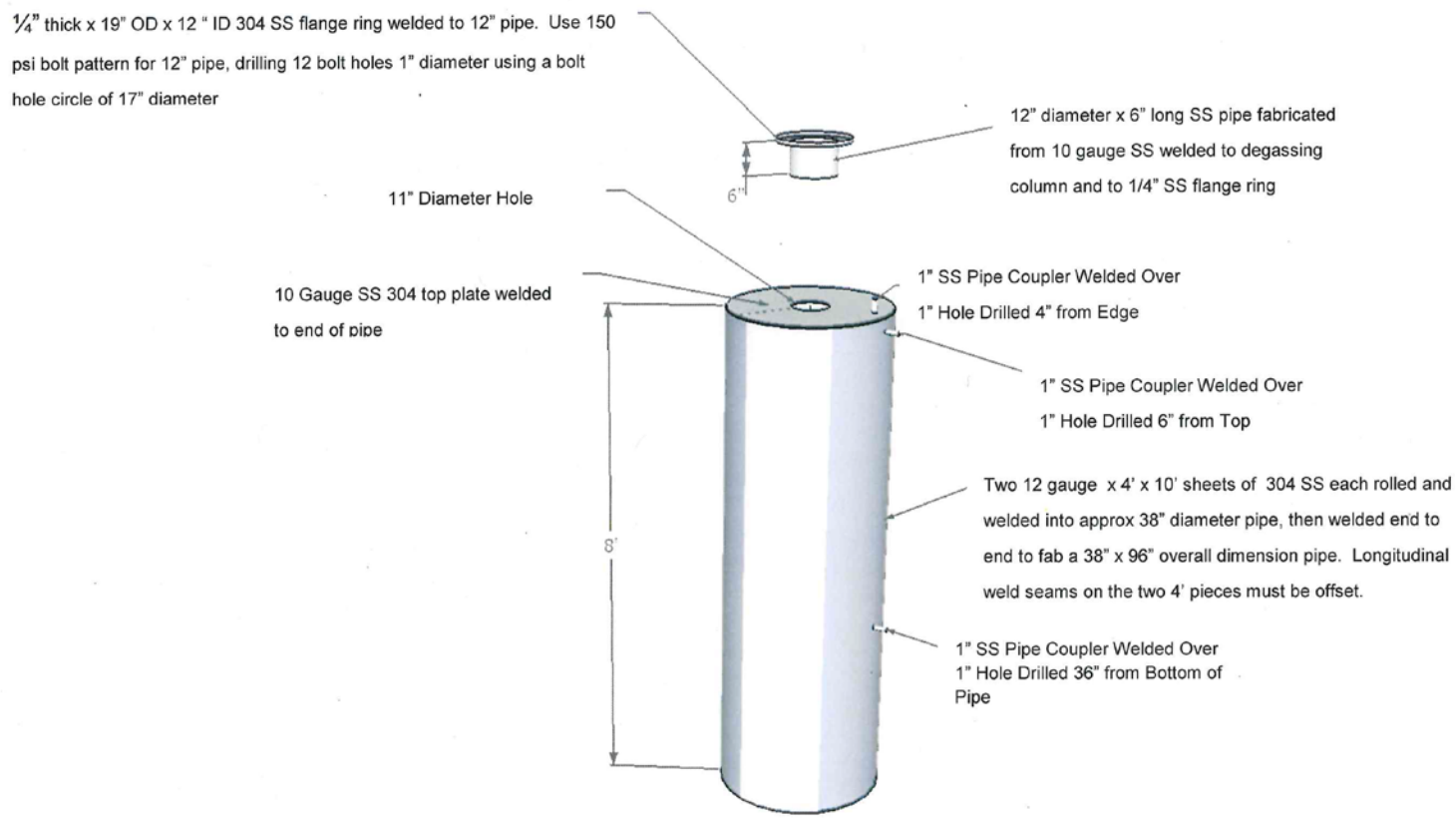

Figure 3. Vacuum degas column.

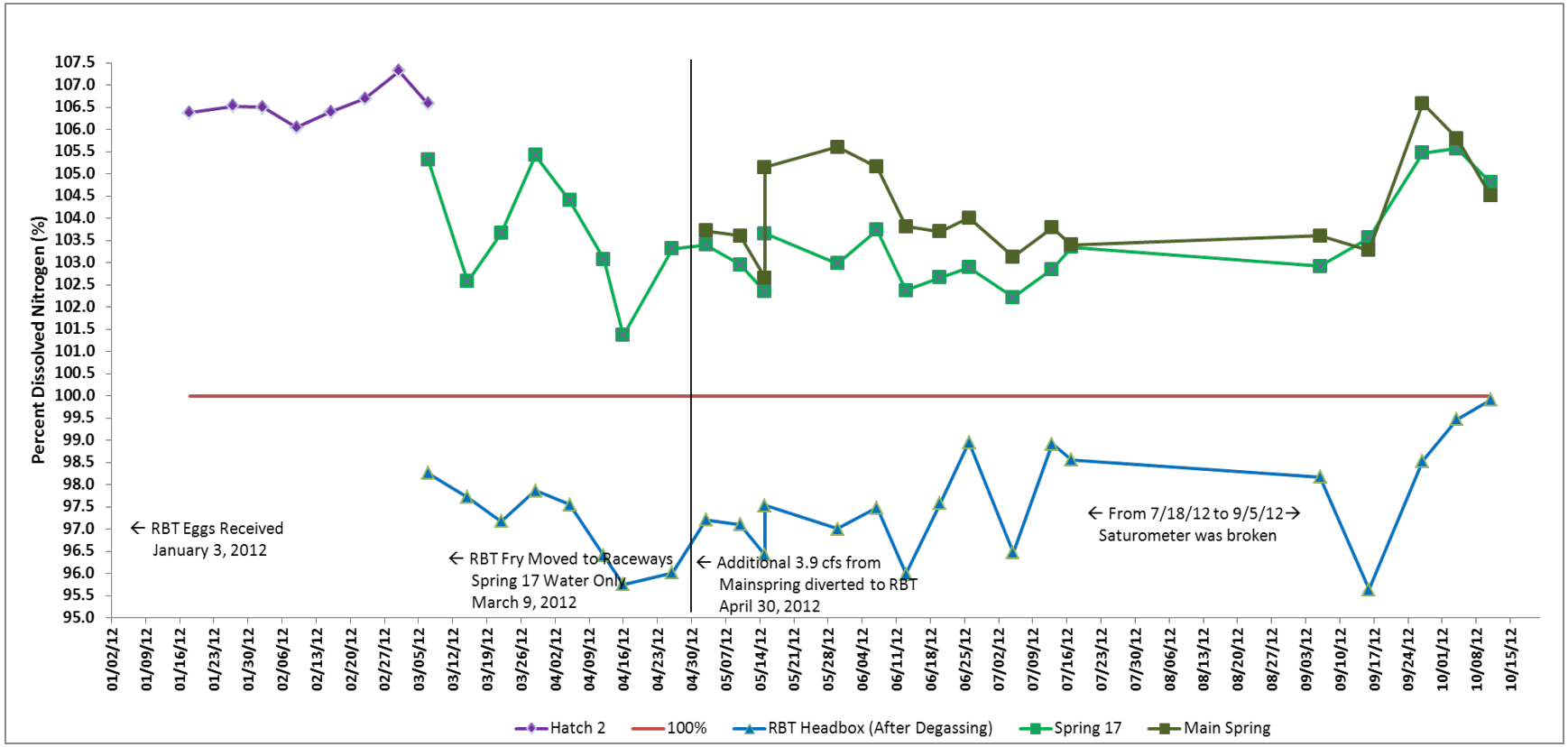

Figure 4. Percent Dissolved Nitrogen in Source and Production Water of Rainbow Trout at Hagerman NFH, BY2012.

Table 1. Summary of Idaho Fish Health Center monthly diagnostics for BY2011 and BY2012 rainbow trout.

	BY2011	BY2012
January	Only eggs present at Hatchery	Only eggs present at Hatchery
February	Report Unavailable	No gas bubble, no parasites, some remnant yolk
March	1 out of 11 had swollen kidney, yellow mucus filled intestine. Moderate fin erosion, gills lightly swollen, 3 out of 11 had light to moderate gas bubbles.	2 out of 5 had light gas bubble
April	Report Unavailable	1 out of 10 had low gas bubbles, low levels of *Sanguinicola*
May	5 out of 7 moderate to high gas bubbles 4 out of 7 had *Nucleospora salmonis* symptoms	6 out of 10 had yellow mucus filled intestine, moderate to severely eroded dorsal fins, no gas bubbles
June	4 out of 4 had *Nucleospora salmonis* symptoms, all severely eroded dorsal fins, no gas bubbles detected	1 out of 5 had light gas bubbles, 10 out of 10 had severely eroded fins and mucus filled intestines, *Nucleospora salmonis* detected
July	High levels of gas bubbles, severe tail rot	Frayed fins, 1 out of 10 caudal lesion, no external parasites, gills look good, *Nucleospora salmonis* detected, no gas bubbles
August	Heavy gas bubbles, opaque eyes, lateral line lesions, tail rot	Moderate to severe erosion of dorsal, pelvic, and pectoral fins, no parasites or gas bubbles observed, *Salmoninarum* and *Renibacterium* detected
September	Severe gas bubbles, multiple lesions along lateral line, severely eroded fins, *Columnaris* detected	No exam performed, fish in good health
October	Release of all rainbow trout on station	Release of all rainbow trout on station

Explanation of gas bubble rating can be found in Appendix 1

Table 2. Summary of rearing parameters for BY2011 and BY2012 rainbow trout.

	Density Index		Flow Index	
	BY 2011	BY 2012	BY 2011	BY 2012
January	0.09	0.10	0.13	0.15
February	0.06	0.39	0.19	0.56
March	0.15	0.17	0.47	0.52
April	0.29	0.31	0.42	0.96
May	0.27	0.13	0.35	0.23
June	0.17	0.19	0.16	0.33
July	0.23	0.27	0.28	0.47
August	0.29	0.31	0.34	0.55
September	0.32	0.35	0.38	0.62
October	0.32	0.36	0.38	0.64
Mean	0.22[a]	0.26[a]	0.31[a]	0.50[b]

Density Index and Flow Index where calculated using methods described by Piper et al 1982.

Available water for RBT production in BY 2010 was 12 cfs and in BY 2012 was 7 cfs

[a] Different letters indicate a significant difference of p value < 0.05

Table 3. Monthly summary of survival for BY2011 and BY2012 rainbow trout.

	BY 2011	BY 2012
January	94.68	96.27
February	93.84	95.42
March	93.30	94.81
April	92.85	94.40
May	91.96	94.16
June	91.61	94.13
July	91.43	94.07
August	90.94	94.01
September	89.56	93.97
October	89.49	93.95
Mean	91.97[a]	94.52[b]

[a] Different letters indicate a signi
difference of p value < 0.05

Appendix 1. Gas Bubble Level Standardization

Idaho Fish Health Center Gas Bubble Level Standardization

Sample size should be at least 5 fish. It may be desirable to look at one fish at a time if looking to document changes in the gas bubble size.

Remove the fish's first gill layer. Place on slide and remove the gill arch. Cover gill with Distilled water and a cover slip.

Assigning levels:

Low: One or two small bubbles in the gill filaments of less than half of the fish examined.

Moderate: Small bubbles (1-2) in majority of the fish examined. OR Numerous (3-5) small bubbles in at least half of the fish examined. Bubbles occurring in filaments only. Any bubbles seen in lateral line or fins in addition to bubbles in gills.

Severe: Any bubbles in eyes and mouth, or bubbles in fin and lateral line. OR Any bubbles seen in both filaments and lamellae of random grab fish.

Individual fish levels may be given. Note moribund fish tend to have more bubbles. This should be noted when assigning a population a level

Appendix 2. Dissolved Nitrogen Gas Calculation

The following link is for a gas supersaturation calculator from Wedemeyer (2001): http://www.fisheries.org/afs/docs/pub_gas_super.xls

However, if this website is not available, the following readings and calculations can be used to get percent nitrogen saturation:

1. Change in Pressure (DP) = Reading off of the tensionometer; mm of Hg

2. Dissolved Oxygen; mg/L

3. Temperature; degrees C

4. Barometric Pressure; mm of Hg (from airport or website)

5. Bunsen Coefficient; L/L-atm

=0.00099902*EXP((9.7265-5268.95/(Temperature+273.15)+1004170/((Temperature+273.15)*(Temperature+273.15))))

6. Vapor Pressure of Water; mm of Hg

=760*EXP(11.8571-3840.7/(Temperature+273.15)-216961/((Temperature+273.15)*(Temperature+273.15)))

7. Total Gas Pressure; %

=(Barometric Pressure + Change in Pressure/Change in Pressure)*100

8. O_2; mm HG

=(Dissolved Oxygen/Bunsen Coefficient)*0.5318

9. N_2; mm HG

=Barometric Pressure + Change in Pressure – O_2 – Vapor Pressure of Water

10. Nitrogen Saturation; %

=(N_2 /(0.7902*(Barometric Pressure – Vapor Pressure of Water)))*100