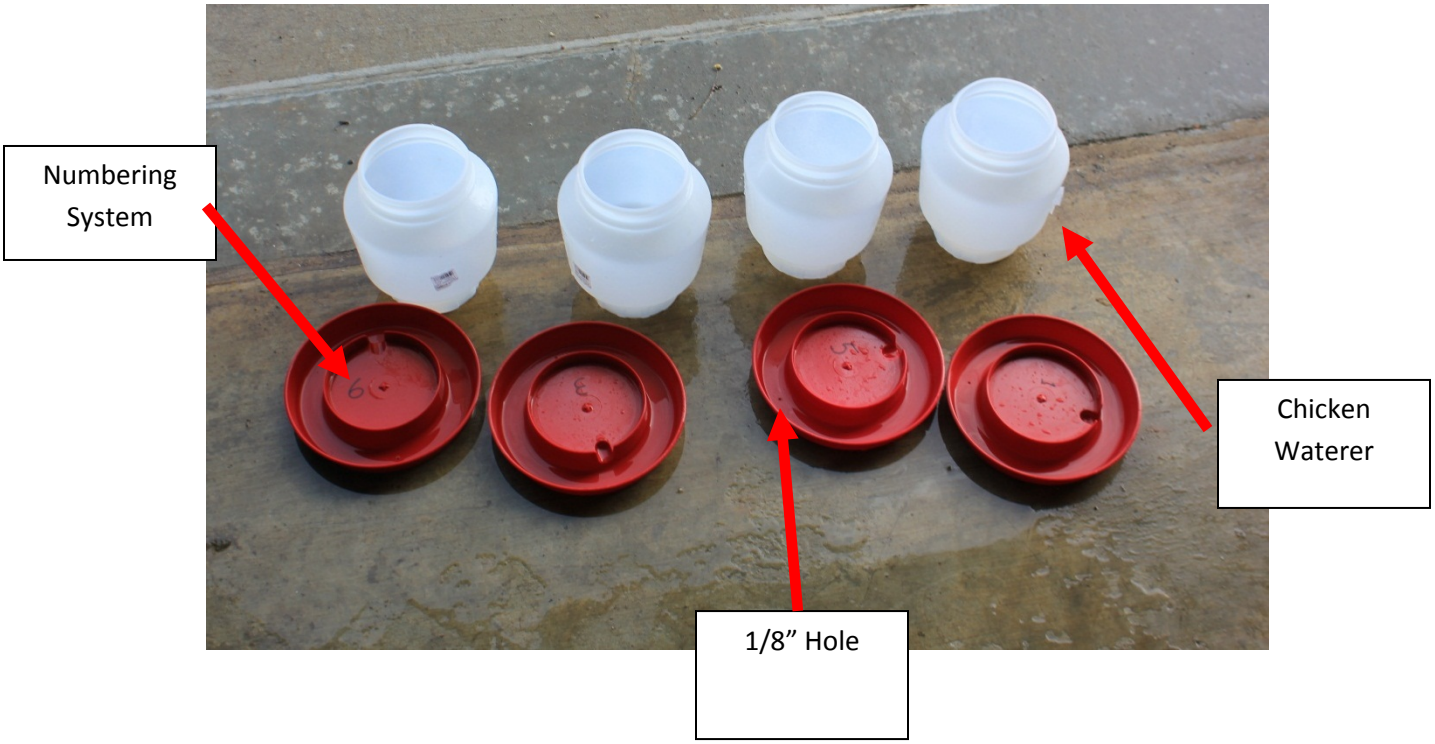
Formalin Egg Treatments

March 26, 2015

The Mora National Fish Hatchery (Hatchery) uses Heath-style egg incubation trays to incubate Gila trout eggs. Each tray is split into 4-6 compartments and can incubate up to 1,000 eggs per compartment (6,000 eggs max per tray). Each incubation stack is supplied with 5 gallons per minute of water. Generally, Formalin is administered as a flow-through treatment at 1667 ppm for 15 minutes every other day to control fungus growth (Mon, Wed, Fri). The formalin treatments can commence 1-2 days post-fertilization and must be suspended at hatching.

Formalin treatments are administered with a simple chicken-waterer with a 1/8” hole drilled in the watering trough. Both halves of each waterer are labeled (1,2,3 etc.). Keep these halves matched because small variances in the hole size can affect treatment timing.



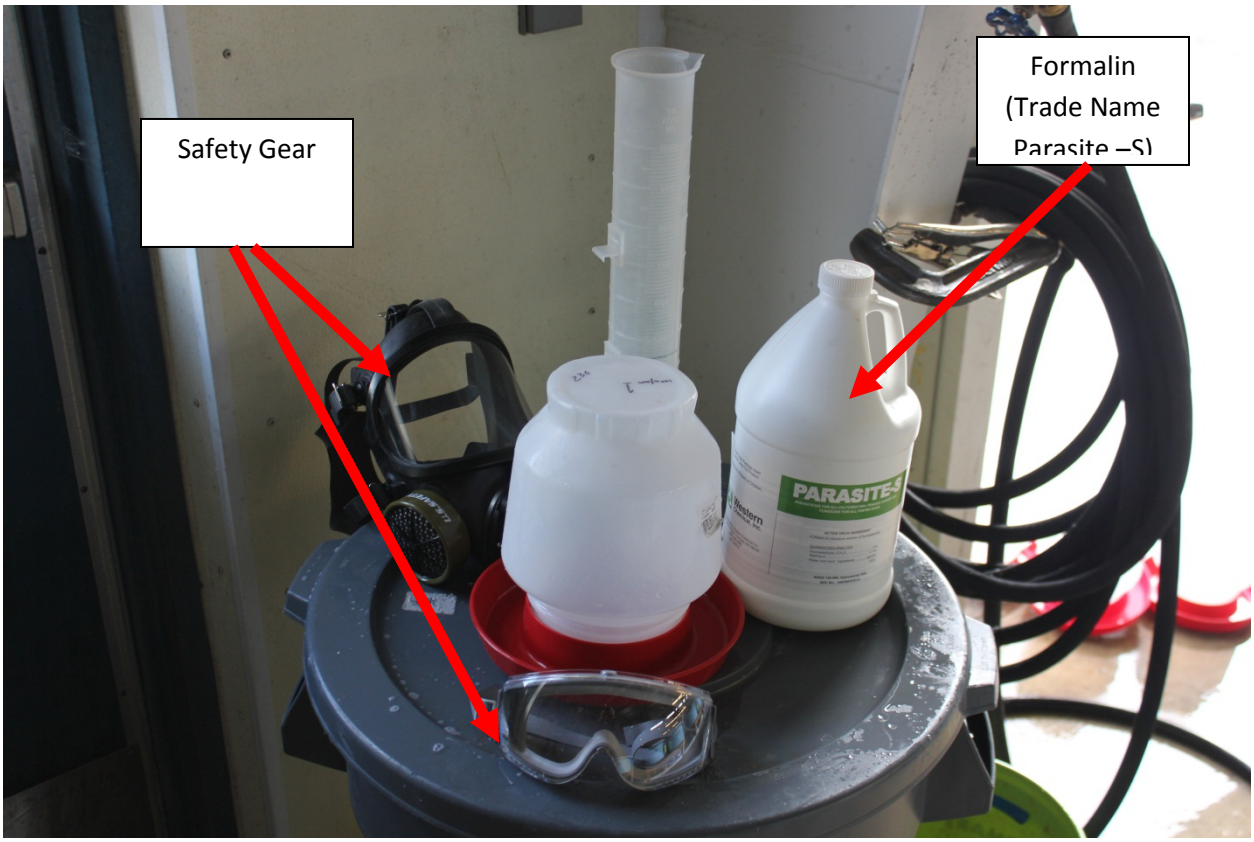
Formalin is a dangerous chemical! See attached memo for exposure concentrations and risks. Be sure to utilize a face shield or goggles, rubber gloves, apron, and rubber boots when dispensing chemical. Also, complete mixing near the open garage door to ensure good ventilation.

For 5 gpm per incubation stack, you'll add:

475 ml formalin

1,025 ml of water

This will give you 1500 ml of total solution. Since the 1/8" hole allows 100 ml of solution to escape per minute, you've now created a 1667 ppm treatment flowing for 15 minutes at 5 gallons per minute.



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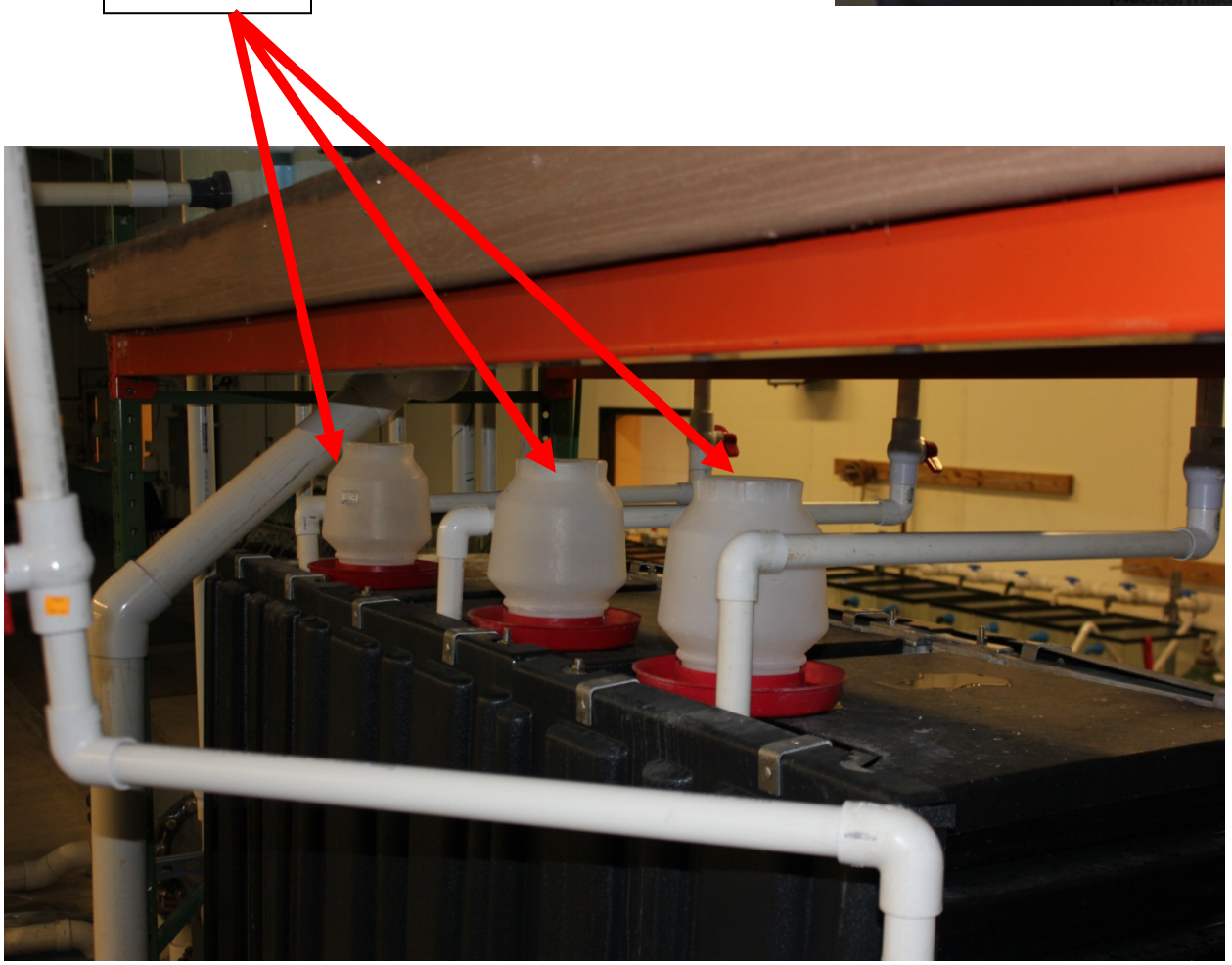
Once you've mixed the proper solution (475 ml formalin and 1025 ml water), carefully carry the inverted chicken waterer to the incubation stacks. When starting the treatment, take note of where the notch in the waterer is – point this up until you have flipped the waterer over to avoid splashing the formalin. Direct 1/8" hole to back of incubator next to incoming water supply to ensure mixing. Be sure to rinse down any spilled formalin so the fumes don't accumulate in the Hatchery building. Monitor treatment until it is finished and record chemical use on log for NPDES permit reporting.



United States Department of the Interior

FISH AND WILDLIFE SERVICE

Mountain-Prairie Region

IN REPLY REFER TO:

BA/SOH

Mail Stop 60184

MAILING ADDRESS:

Post Office Box 25486

Denver Federal Center

Denver, Colorado 80225-0486

STREET LOCATION:

134 Union Blvd.

Lakewood, Colorado 80228-1807

May 28, 2013

Memorandum

To: Jeffrey Powell, Project Leader, Mora National Fish Hatchery

From: Environmental Compliance Coordinator, Region 6

Subject: **Occupational Exposure to Formaldehyde**

This report is in response to a request from the Project Leader at the Mora National Fish Hatchery for assistance in evaluating exposure to formaldehyde among fish hatchery workers. An evaluation of the egg treatment process at the Mora National Fish Hatchery at Mora, New Mexico was conducted in April 2013. The results from this formaldehyde monitoring survey can be used to assess occupational exposure at other Service Fish Hatcheries doing similar type work.

Exposure levels measured from the breathing zones of one employee involved in the operation were below the regulatory limits set by the Occupational Safety and Health Administration (OSHA). However, to further minimize exposures the following recommendations should be considered.

Recommendations:

- continue use of personal protective equipment during chemical mixing and treating
- inform employees of these results

- train all employees at the time of initial job assignment and annually; thereafter, of the heath hazards associated to formaldehyde concentrations of 0.1 parts per million (ppm) or greater refer to Attachment 3
- keep mixing area well ventilated during dispensing either with fan or install a ventilation system
- facility plans to purchase smaller containers of formalin to help reduce exposure.

Process

Formaldehyde concentrations approximately 1667 ppm are used for treating the eggs of cold water species. Thirty-seven percent formaldehyde is stored in a 55-gallon drum and is pumped into a hand-held 6-gallon container, which is then carried to the treatment solution prep area where it is diluted to 1667 ppm one-gallon container. This solution is then transferred to the upper reservoir of the egg racks. The solution is allowed to trickle down through the eggs via gravity feed. The container filling process and transfer process lasted approximately 8 minutes. During the process, employees wore rubber gloves and aprons, and splash goggles. See pictures of the process in attachment 2.

The samples were collected from the employees' breathing zones on ChemDisk Personal Sampler. They were analyzed by the Occupational Safety and Health Administration (OSHA) laboratory in Salt Lake City, Utah using liquid chromatography according to OSHA Method 1007.

The exposure monitoring results are compared to the OSHA Short Term Exposure Limit (STEL) of 2.0 part per million. This is maximum allowable breathing zone concentration for a 15 minute exposure period. OSHA does have a training requirement for all employees exposed to formaldehyde concentrations of 1.0 ppm or greater. The National Institute for Occupational Safety and Health (NIOSH) and OSHA classify formaldehyde as a carcinogen and therefore NIOSH recommends that exposures be reduced to lowest possible levels. NIOSH has set a 15-minute ceiling concentration at 0.1 ppm.

Results:

Time	Location	STEL	Results	STEL Results*
2:08 - 2:16	Personal Sample	2.0	0.8821 ppm	0.47 ppm
2:07 - 2:16	Observer	2.0	0.4786 ppm	0.29 ppm
2:07 - 2:17	Area Sample		1.0070 ppm	0.60 ppm

*Zero exposure for the remaining time

Although these results are below the OSHA STEL of 2 ppm, the National Institute for Occupational Safety and Health recommends that exposures to formaldehyde be controlled to the lowest feasible concentrations. The container filling operation should be done in a well-ventilated area and the employees should continue to use the appropriate personal protective equipment. The mixing area

could be ventilated with a fan or exhaust system. The OSHA permissible exposure limit is 0.75 ppm measured as an 8-hour time weighted average (TWA) and the "action level is 0.5 ppm measured over 8 hours. NIOSH has a recommended exposure limit (REL) of 0.016 ppm as an 8-hour TWA. All of these regulatory limits were not exceeded.

The attachments contain the air sampling results that were collected on April 3, 2013. Refer to Attachment 3 for Health Hazard Information.

If I can be of additional assistance or you have any questions regarding this report, please call me at 303-236-4526.

Attachment 1 – Sampling Results

Attachment 2 – Photographs of Operation

Attachment 3 – Health Hazard Information

Attachment 3

Health Effects to Formaldehyde

Workers can inhale formaldehyde as a gas or vapor or absorb it through the skin as a liquid. Formaldehyde is a sensitizing agent that can cause an immune system response upon initial exposure. It is also a cancer hazard. Acute exposure is highly irritating to the eyes, nose, and throat and can make anyone exposed cough and wheeze. Subsequent exposure may cause severe allergic reactions of the skin, eyes and respiratory tract. Ingestion of formaldehyde can be fatal, and long-term exposure to low levels in the air or on the skin can cause asthma-like respiratory problems and skin irritation such as dermatitis and itching. Airborne concentrations of formaldehyde above 0.1 ppm can cause irritation of the respiratory tract. The severity of irritation intensifies as concentrations increase. Concentrations of 100 ppm are immediately dangerous to life and health (IDLH).

Generator Start-up

October 7, 2014

The Mora National Fish Hatchery (Hatchery) GenSet Generator provides back-up power to the Hatchery building to run the life-support systems for Gila Trout production. The generator system is tested weekly (currently not under load until transfer switch repairs). The key for the generator is located in the main office key box.

The Oil Pressure gauges, temp, etc. are marked with a black sharpie to indicate maximums. If these gauges are reading past the marks, the generator is not operating normally.

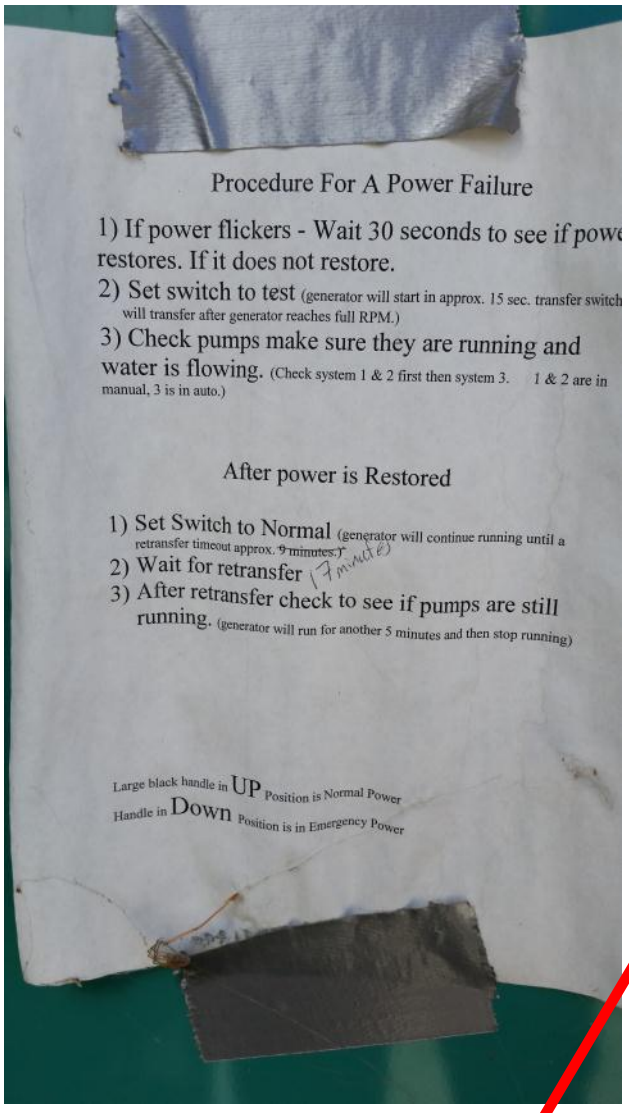
Generator Test Switch - return to "Auto" after test

During a power loss, the generator should start automatically. If the generator does not start, the first place to check is the transfer switch. The transfer switch safely switches power from commercial to back-up and protects both the Hatchery equipment and lineman working on the power outage.

Switch for Transfer Switch

The Transfer switch is marked by a “High Voltage” warning sign (the main breaker disconnect is also marked with a “High Voltage” sign).

If the power has not transferred, you can manually set the switch to test. Or try to re-set and then test . You may hear a large “klunk” as the transfer switch manually transfers from commercial to emergency power.



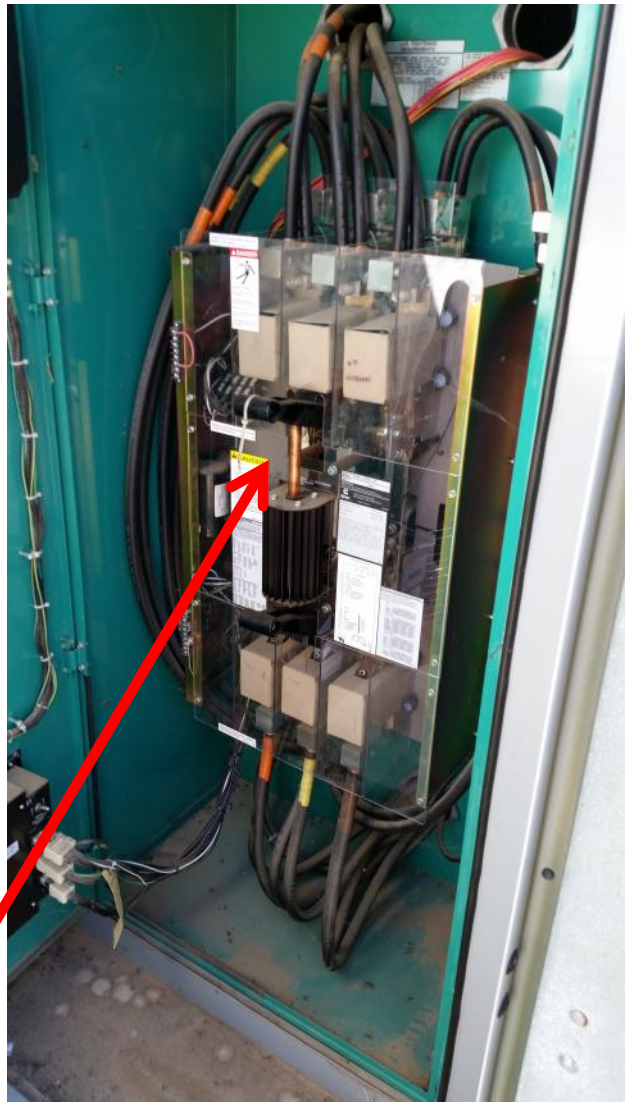
Procedure For A Power Failure

1) If power flickers - Wait 30 seconds to see if powe restores. If it does not restore.
2) Set switch to test (generator will start in approx. 15 sec. transfer switch will transfer after generator reaches full RPM.)
3) Check pumps make sure they are running and water is flowing. (Check system 1 & 2 first then system 3. 1 & 2 are in manual, 3 is in auto.)

After power is Restored

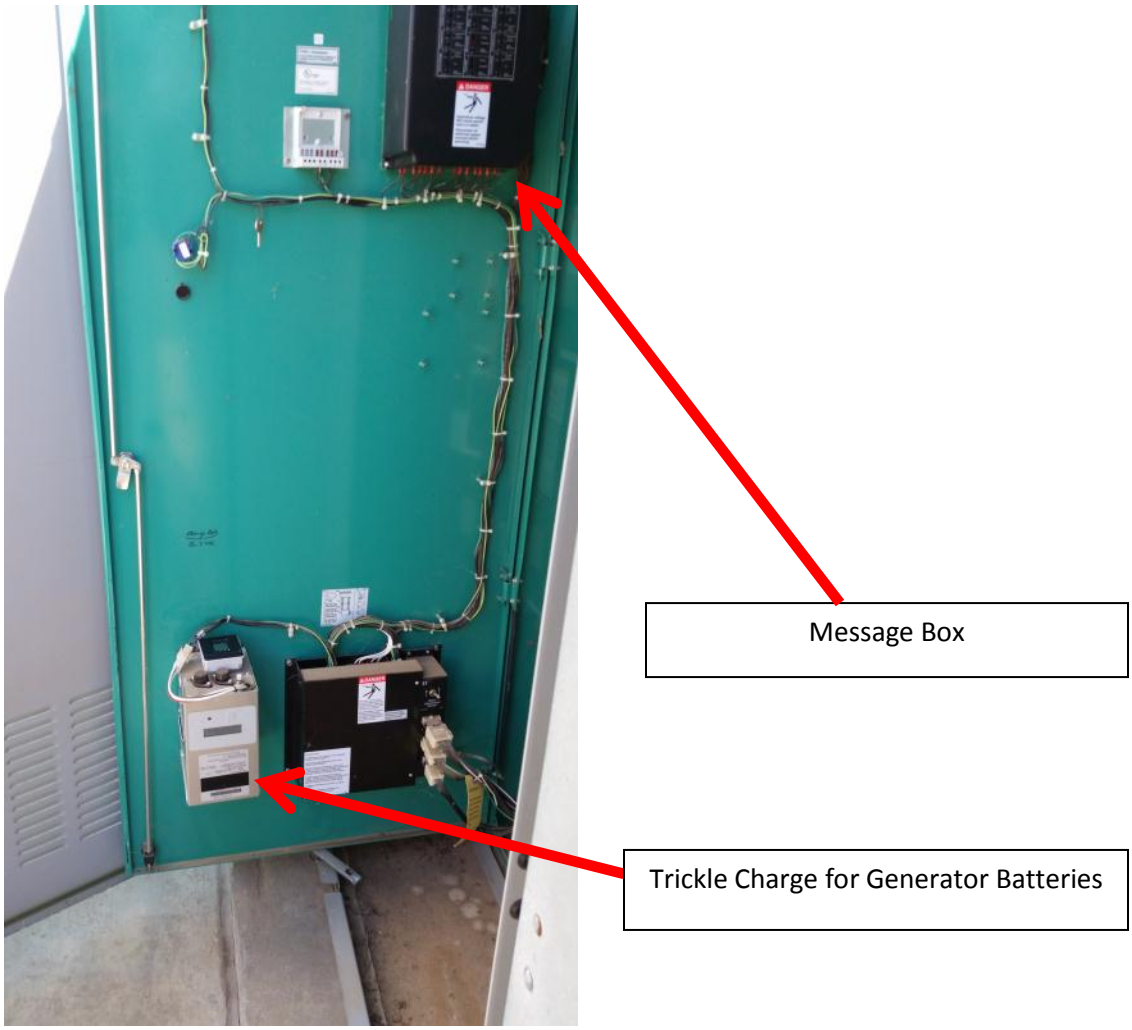
1) Set Switch to Normal (generator will continue running until a retransfer timeout approx. ~~9 minutes.~~)
2) Wait for retransfer (7 minutes)
3) After retransfer check to see if pumps are still running. (generator will run for another 5 minutes and then stop running)

Large black handle in UP Position is Normal Power
Handle in Down Position is in Emergency Power

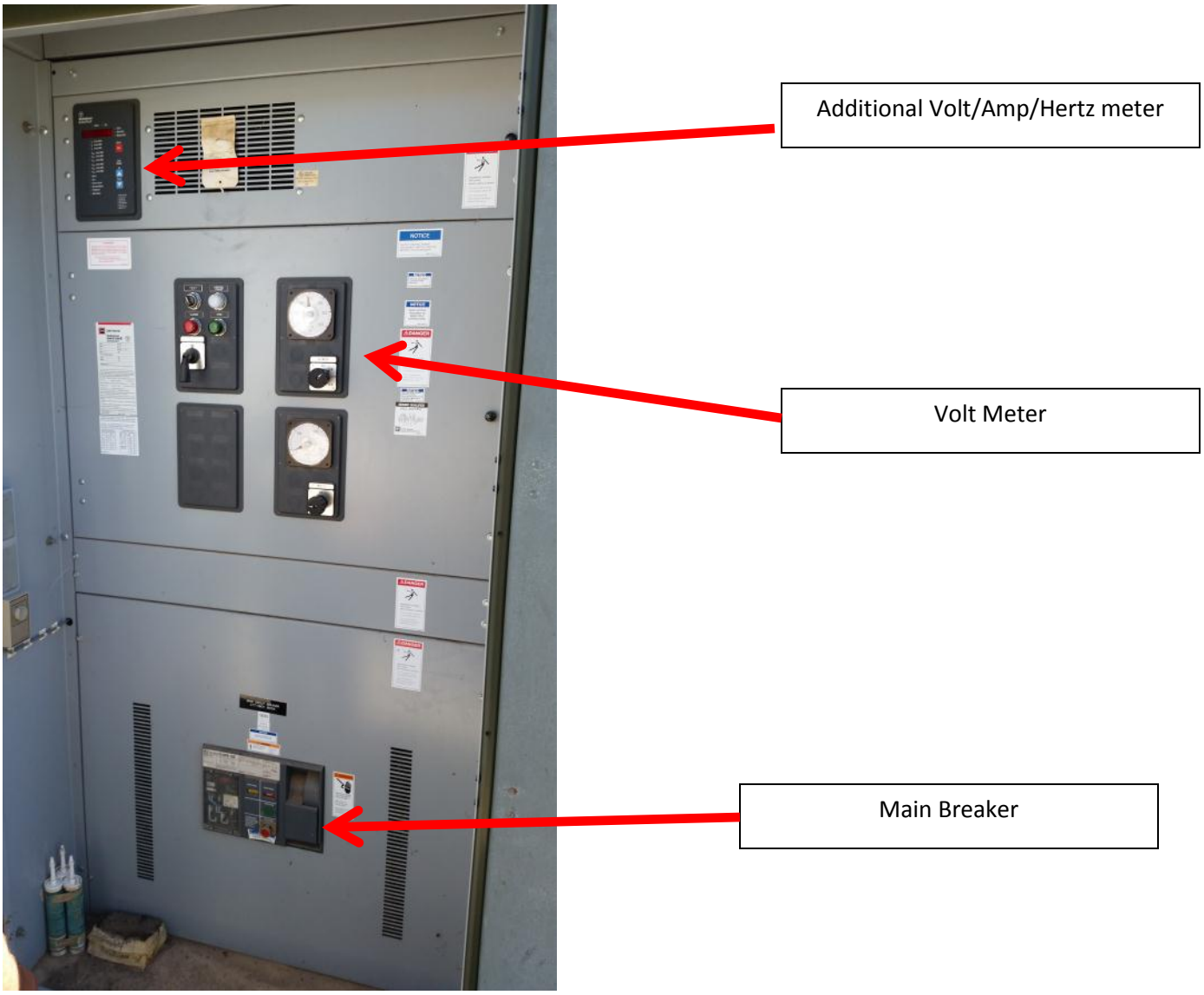


Switch is in Up position for “Normal Power”

On the inside door of the transfer switch, in the upper right corner, you'll see a black box that will give you messages about the status of the transfer switch. This box is useful for troubleshooting problems.



Once the generator is on a load (meaning the pumps are running, etc.) you need to find out why the power failed. Before calling the electric Co-Op (575-387-2205 or 800-421-6773), it is a good idea to check the main breaker.



If the main breaker is tripped, you'll see a message of "Not Charged" and "Open" next to the Main Breaker. In this picture, the power is "normal" so you see "Charged" and "Closed" (Yellow and Red messages). If the main breaker is tripped, that is your power supply problem, not the Co-Op. There is a small red, reset button (behind Richie's hand in this pic), press the re-set button, then press the green "Push to Close" button.

If the main breaker is not tripped, check the voltage through the system. All three legs should be near 480 Volts. If one or more of the legs is showing a variation in voltage, you may have lost one leg of power (See partial power loss SOP). You should also see around 60 Hz on the system.

Voltage Meter – Switch through all three legs of power

Mora National Fish Hatchery

Gila Trout Recovery – Iron Creek Lineage Update – November 10, 2014

The Mora National Fish Hatchery has been holding 51 Iron Creek lineage Gila Trout since the Whitewater/Baldy Complex Fire Rescue on May 15, 2013. In the last several weeks we have removed five mortalities from this group. Four of these fish were large and robust (approximate 1 lb) males and appeared in good condition. This mortality is unusual, because until this point, the Hatchery has only lost five fish from this group after 1.5 years of holding. Overall survival is now at 80.4%

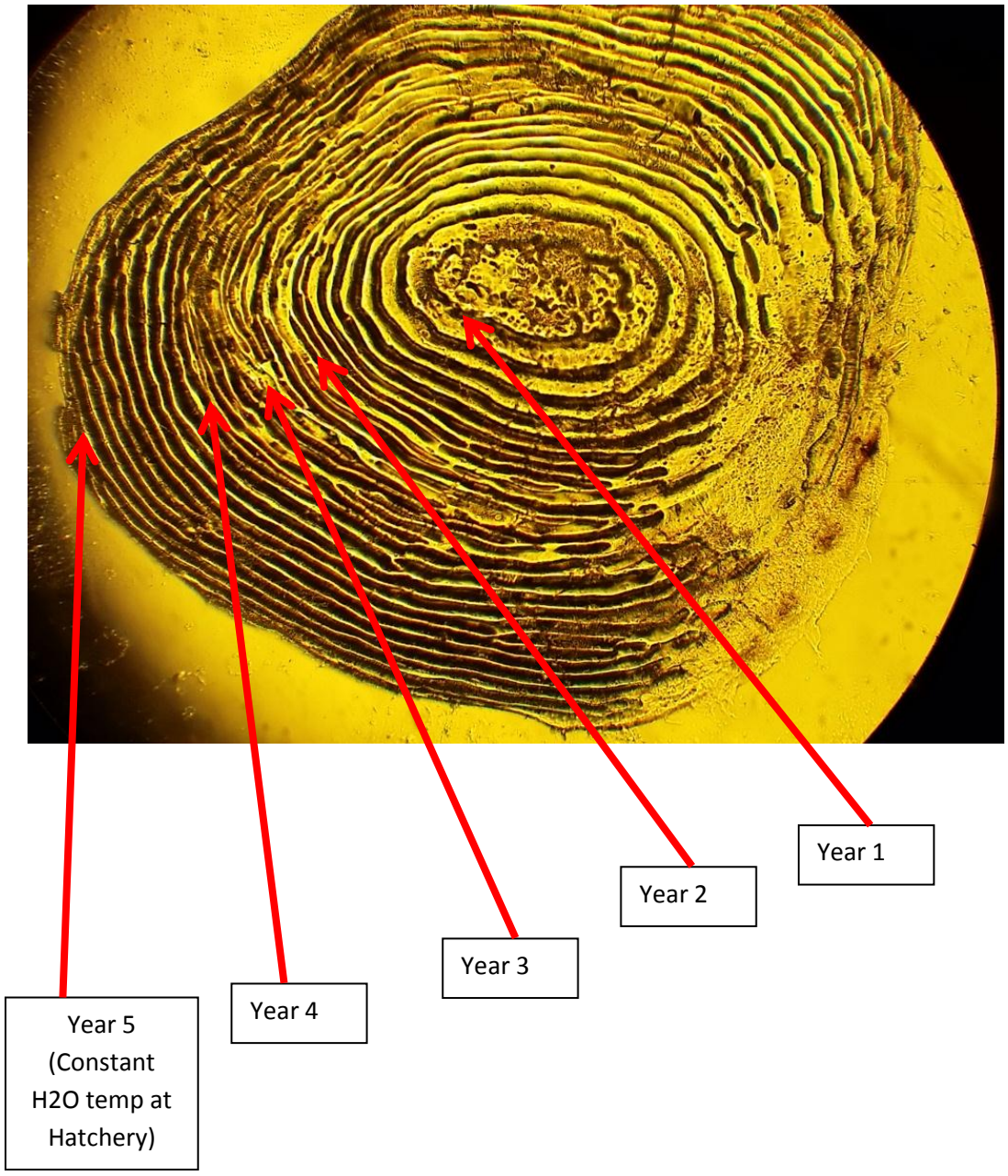
Teresa Lewis, Fish Health Unit Leader, examined several mortalities for necropsy and diagnostic testing on November 3, 2014. Teresa also examined two sacrificed South Diamond lineage fish on the same rearing system, as surrogates for the Iron Creek fish.

The Hatchery removed the fish from feed (they were actively eating) as a precautionary measure, increased the flow in the rearing units, and applied a 0.5% salt bath to the tanks and will continue the therapeutic salt treatment for three days. Current water quality parameters are listed below with preferred values in parentheses (from Fish Hatchery Management page 14-15). These values are all in the optimal range.

Nitrate = 1.6 mg/l (0-3.0 mg/l)
Nitrite = 0.007 mg/l (<0.2 mg/l)
Ammonia = 0.0 mg/l (<0.0125 mg/l)
CO2 = 8.75 mg/l (0-10 mg/l)
DO = 8.27 mg/l (>5.0 mg/l)
TGP = 98% (<100%)
N = ~97% (<100%)
Temp = 54.3 (<60)
pH = 7.2 (6.5-8.0)

Teresa's preliminary results suggested that the fish were relatively full of feed, but no cause of death was immediately apparent.

On November 9, 2014, the Hatchery lost an additional fish from the Iron Creek lineage. This fish was necropsied by the Hatchery staff. The fish had developing testes which suggests that pre-spawning stress may be an additional factor. Also, the Hatchery staff aged the fish at 5+ years of age, which suggests some of the fish may be reaching their life expectancy. The Hatchery is scheduled to spawn these Iron Creek fish in the spring of 2015.


Year 1
Year 2
Year 3
Year 4
Year 5 (Constant H2O temp at Hatchery)

Light Dimmers

January 7, 2015

The Mora National Fish Hatchery (Hatchery) utilizes timers to automatically control lighting in the fish production areas. These timers are important because light frequency can affect spawn timing and feeding patterns. This SOP reviews timer setting procedures.

The light timers cost approximately $20 each and have a life expectancy of **2 years**. The timers are plugged directly into an outlet and feed the 110 power to the light dimmers (2K Green Rimmir).

2K Green Rimmir Light Dimmer

Programming the Timer Switches

To program the timer switches, you must first press and hold the “reset” button with a paperclip or other fine wire object. Then use the “dial” and “ok” buttons to set the time, date, daylight savings, time zone, and location.

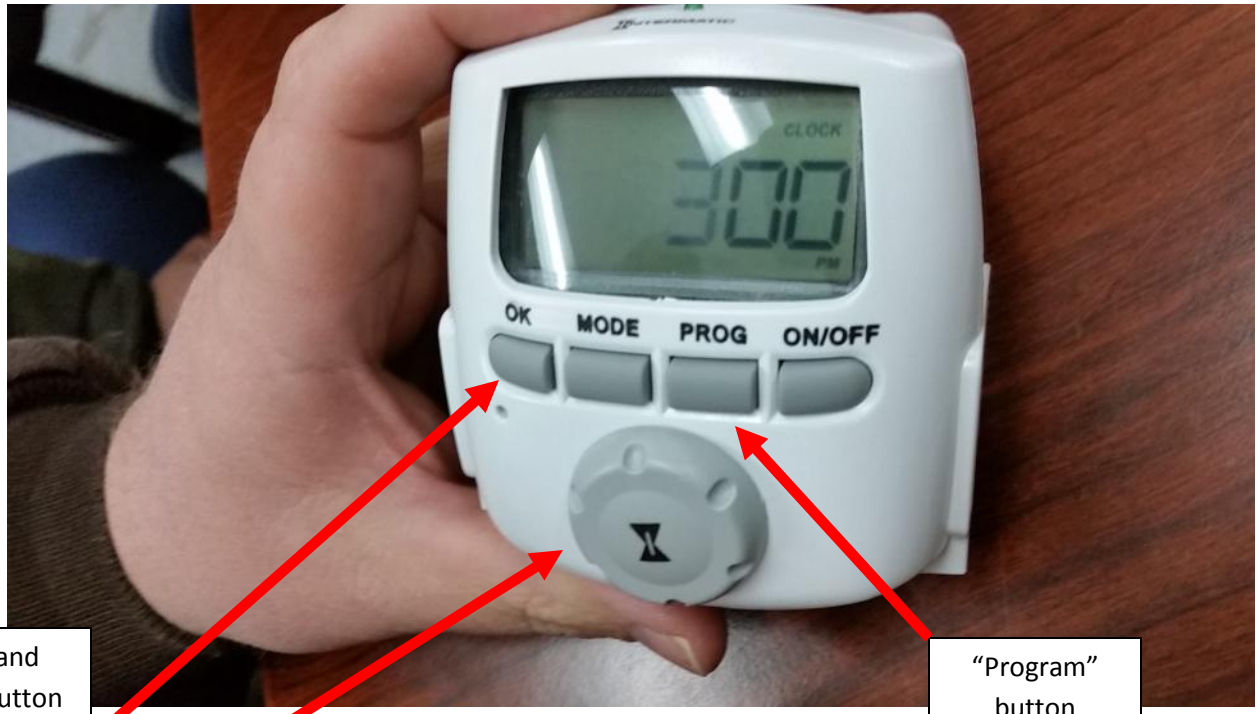
Time and Date = current time and date from cell phone

Daylight Savings = Auto

Time Zone = Mountain

Location = NM (New Mexico) and NE (North East)

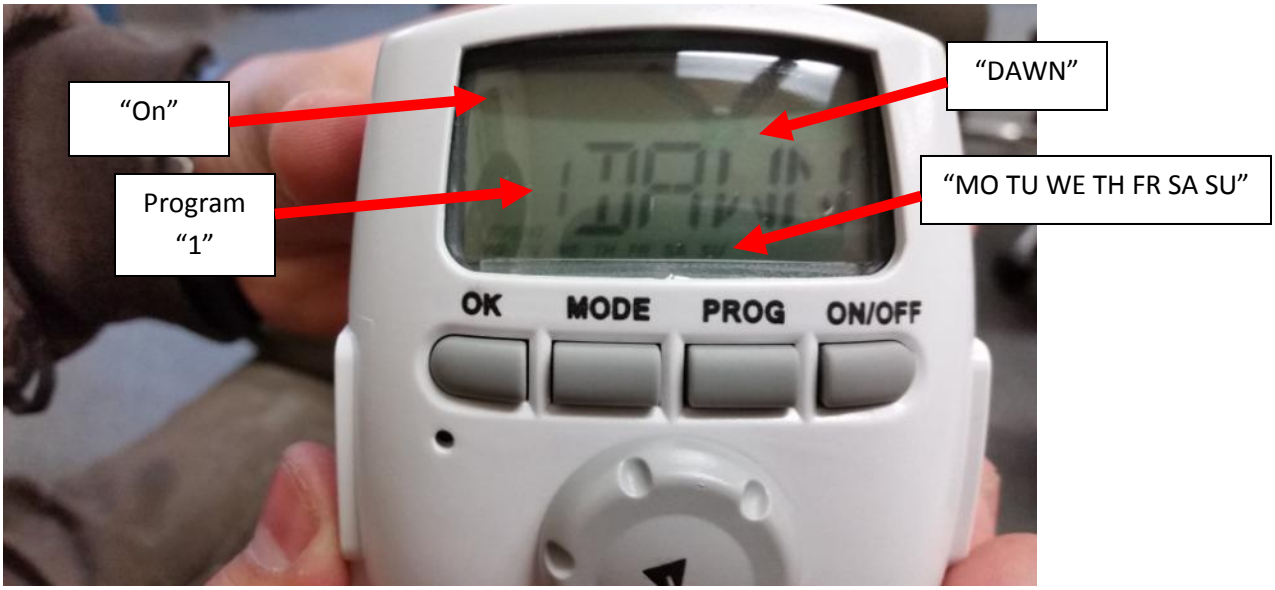
If you make a mistake setting these items, you’ll need to go back and re-set the device. If these settings were lost, the back-up battery in the unit may have died. If you re-set the device, the batteries are okay, and the device changes again, it likely needs to be replaced.



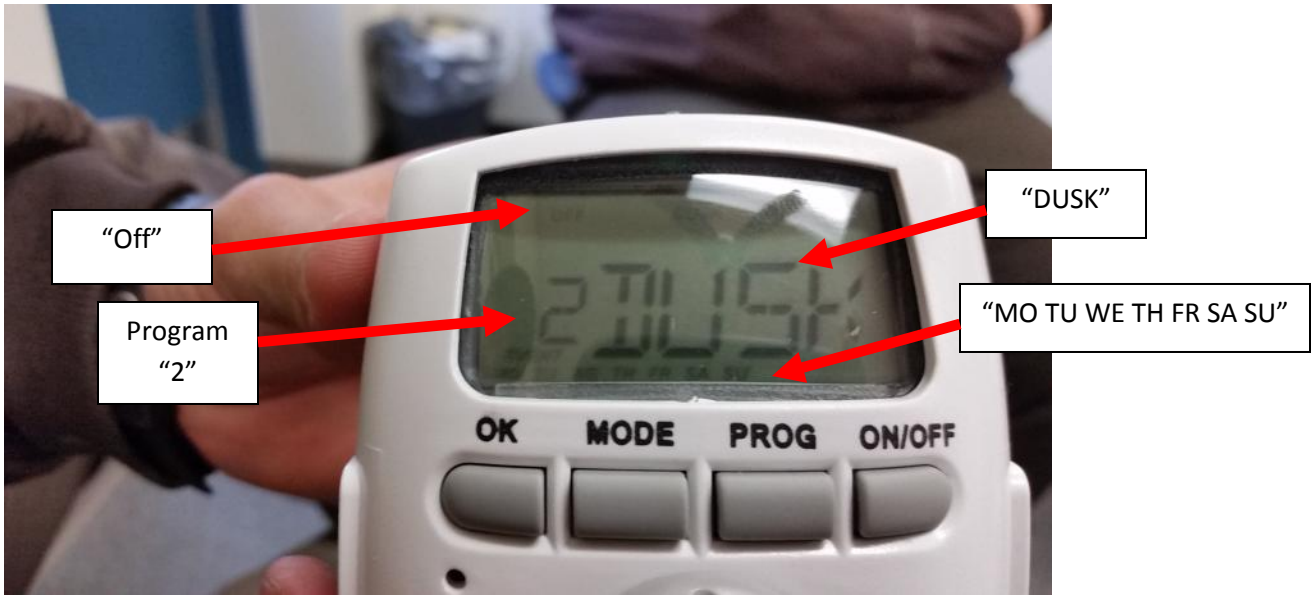
“Ok” and “dial” button needed for initial setting of time, date, etc.

“Program” button needed for setting dusk and dawn timers

To program the dusk and dawn settings use the “Program” and “Dial” buttons. There is no save button, after you use the “Program” and “Dial” buttons, use “OK” to exit the program menu. Hit “Program”, and a number 1 will show on the screen designating Program 1. Use the “Dial” button to choose Mon through Sun, DAWN, and “On”. When you are finished, it should look like this:



After setting Program 1 to “On at DAWN”, press the “Program” button again to enter Program 2. Set Program 2 to: “Off at Dusk”. When finished setting “Off at Dusk”, press the “OK” button. Program 2 should look like this:



Good luck!

Supplemental Live Diet SOP

October 9, 2015

The Mora NFH utilizes naturalistic rearing and poly-cultural systems along with live diets for Gila trout broodstock development. Naturalistic systems are designed to provide as much of a natural system as possible. Tanks are lined with rocky substrate, artificial cover, and fish that co-occur in the wild, like desert and Sonoran suckers. Supplemental live diets are used to stimulate feeding response, train fish on commercial diets, and ensure optimal nutrition for spawning success. Additionally, live diets are used to acclimate recovery fish for release to the wild.

The Hatchery currently utilizes crickets and mealworms for supplemental live diets. These are generally purchased from a commercial vendor as needed. Generally, crickets and mealworms are offered via belt feeder or delivered by hand periodically throughout the day. When transitioning fish onto commercial diets a technique called “shake and bake” is used. First chop mealworms and/or crickets into small pieces. Then, shake fish feed pellets in this chopped mixture to coat the pellets. This mixture is then feed immediately (that day). The scent and particles from the chopped live diets create a feeding frenzy in the fish and train them onto the commercial diets.

In 2015, the Hatchery will also utilize excess Gila trout fingerlings for supplemental live diets. Live fish are commonly utilized for other predatory species including tiger muskies, walleye, and northern pike. The Hatchery staff has anecdotally witnessed predatory behavior in Gila trout with Sonoran and desert suckers and cannibalistic behavior with small Gila trout. To minimize contamination risks, live fish diets will only be utilized in PIT tagged broodstocks (currently all broodstock are PIT tagged), and fish will be sorted at spawning to remove any un-tagged fish.

Supplemental Live Diet Care and Feeding

Crickets

General

- Crickets are kept in ten gallon glass aquariums. These tanks have a wire mesh cover that contains the crickets. The crickets should be kept on one of the heat pads, as the cold will kill them.
- Each aquarium can accommodate 500 crickets, so if there is a shipment of more than this, you will need to divide them up.
- After all the crickets have been fed out, clean the tank using a wet rag, or if there has been large cricket mortality, use a bleach solution. This must be done at least monthly.
- To transfer crickets from the shipment box to the aquariums, two methods are suggested. The first method, if a small box is sent, is to set the box into the aquarium on its short side. Carefully open up the box, lifting up the flaps. Remove packing material and gently tap off any insects. Repeat for all packing material. Then, rotate the shipping box inside the aquarium until it is upside down, and gently tap the box until all insects have come out. Rotate the box and look for any stragglers. Make sure that when you are doing this procedure that the crickets do not come around the sides, if they do gently brush them into the aquarium. After all the crickets are in the aquarium, place a couple of the egg crate packing material into the aquarium for them to hide in. The larger box can be difficult to contain all the insects. So, this is a method that I have found quite effective (via internet). Place the box into one of the large clear garbage bags. Open it and follow procedures above. After all the insects are out, get your holding tanks ready. The large boxes are usually for 1000+ crickets, so you will need at least two aquariums. Shake down any insects that are trying to escape and simply pour them into the aquariums. You may need to shake the bag slightly to get them all out.

Watering

- Crickets have a habit of drowning in a water bowl if provided, so I recommend a water gel product available through Flukers. The crickets will “suck” on this material to obtain their water.
- The water gel is usually good for two days if you completely cover the bottom of a disposable Petri dish. If the material becomes discolored or “disappears” add new gel .
- When the dish is refilled, it is important to wash it (removing excess feed and feces). If the dish becomes too bad, simply replace it.

- There are two alternative methods for watering the crickets. The first alternative method for watering was relayed to me from one of our suppliers. Use a dish of some sort and place rocks into the dish. This allows any crickets that fall in to get back out. The problem with this method is that you will still experience some mortality and the difficulty in sterilizing the rocks. The second alternative method is to use a few wet cotton balls. This will allow the crickets to suck at them without drowning.

Feeding

- There are two types of cricket feed commercially available. One is a dry feed, and the second is a moist orange cube that supplies both water and nutrition. In our experience, the crickets seem to like the dry diet better (Both are available from Flukers). Potato slices can also be used.
- Fill the other half of a disposable Petri dish with approximately two tablespoons of the dry diet. Before adding it, empty the container into the trash after all insects are out. Clean using the previous procedure. The feed should be replaced daily, as the insects will deposit feces in it.
- To use the orange cubes, use a clean Petri dish and cover the bottom like you do with the water gel.

Cleaning

- The aquariums must be cleaned out at least twice a week. The easiest way that I have found to do this is to remove the cover and all tank items. Gently tip the aquarium to a forty five degree angle. Then, using a piece of the cardboard packing material, gently push all material and crickets to the bottom corner. Replace one section of the egg packing material in the top corner and wait. Almost all of the crickets will head for the upper end. After this occurs, use your piece of cardboard and begin to scoop out the waste and mortalities. If the mortalities are fresh you can sift them out and feed them to the large brood fish. If they are “smelly” it is better to dispose of them into the trash can, or alternately outside. If you pick up any live crickets, gently brush them back into the tank. Replace substrate and refill all Petri dishes.

Cricket Sources

- Flukers.com: Provide a good product, low shipping (1-800-735-8537)
- Americancricketranch.com: Provide a good product, sent numbers questionable, high shipping (1-619-561-7327)
- Nyworms.com: Product hit or miss, reasonable shipping (no phone)
- Wormman.com: Product hit or miss, several dead shipments, reasonable shipping (no phone)

Mealworms

General

- Currently, we receive our mealworms from a supplier. The following procedures assume that the production methods work.
- There are currently three aquariums in active use. One contains the mealworms, one contains beetle eggs, and final contains a mixture of mealworm, beetles and eggs. One and possibly two other aquariums will be used to contain different sizes of mealworms.
- The mealworms should be kept on one of the reptile warming mats. This allows them to stay more active and develop quicker. However, during the summer, if the mealworms are molting or pupae are developing, you may need to unplug the heater.
- If mealworms are purchased, use the following procedure. Carefully open up the box, lifting up the flaps. Remove packing material and gently tap off any insects. Repeat for all packing material. Then, rotate the shipping box inside the aquarium until it is sideways, and gently tap the box until all insects have come out. Rotate the box and look for any stragglers which are usually found under the bottom flaps

Feeding and Watering

- Mealworms do not need much attention. The substrate that they are in contains their food. To supply water one can use two methods. The first is to lightly mist the surface of each aquarium every three days. Do not over water as this will cause the substrate to mold. The second method, which I find more effective, is to place half of an apple or potato into the tank, skin side down partially submerged. This will prevent the substrate from molding. Replace the apple half when it has been consumed.

Changing Substrate

- The night before you plan to sift the substrate, take a couple of buckets of bran out of the feed room freezer to warm. The bag is clearly marked “Bran”. Keeping the bran in the freezer will extend the life of it and keep other insects out.
- As the mealworms eat the substrate, a fine sand-like material will develop. This is called “frass” and is basically mealworm feces. If this is allowed to build up it will start turning the mealworms black, and will eventually kill them. To change the substrate you will need a container to sift

into and a container to receive any mealworms. Use a sifter of appropriate size to catch all the mealworms (sifters are part of a set of limnological substrate separators and are located above the mealworm containers). Sift the materials, as the mealworms cannot pass through. The frass makes an excellent fertilizer.

- Sift new material for larger particles that will not go through the screen and dispose of them.
- Put the mealworms into the new substrate.

Beetle Tank

- Every month, you will need to sift out the beetles and mealworms from the beetle tank. Using one of the sifters, go through all of the substrate. This separates all of the eggs from the other life stages that will cannibalize the eggs. After the other life stages are removed, date the tank and set aside.
- Then follow the procedures outlined above for changing substrate.
- As you place the mealworms and beetles into the fresh substrate, try to remove any dead beetles. To do this, use a chemical spoon that will be above the mealworms on the shelf. Discard the dead beetles.
- This provides future mealworms that are relatively the same size.
- If the beetle tank has too many mealworms, the apple half can be used to remove some. Withhold water or apple from the tank for several days. Then place the apple into the tank. Check periodically, as the mealworms will swarm the apple half. Lift up apple, gently brushing any beetles off, and deposit the mealworms into the feeder tank. Repeat as necessary.

Eggs

- According to the literature, it takes between 4 to 8 weeks for the eggs to develop into feedable mealworms. As with fish, this all depends on temperature. Keeping the egg tank on the warming pad will allow them to develop quicker.
- Mist substrate every couple of days, until the worms are visible, and then an apple slice may be used.
- During the cold months, several egg tanks may be in use at the same time due to lower temperatures.

Mealworm Sources

- Flukers.com: Provide a good product, low shipping (1-800-735-8537)
- Americancricketranch.com: Provide a good product, sent numbers questionable, high shipping (1-619-561-7327)
- Nyworms.com: Product hit or miss, reasonable shipping (no phone)
- Wormman.com: Product hit or miss, several dead shipments, reasonable shipping (no phone)

Incubation Degassing System

January 25, 2015

The Mora National Fish Hatchery (Hatchery) utilizes 10 incubation ½ stacks (8 trays each) to hatch 5 Gila Trout lineages under the Broodstock Management Plan. Each stack is supplied with 5 gpm of water. Each tray is divided into 4 or 6 compartments to keep lineages and individual female crosses (families) separated. Compartments are practical because average Gila Trout fecundities are only 600 eggs, essentially putting each tray capacity at 3600 eggs/tray. Many Trout and Salmon hatcheries routinely hatch 5,000 to 7,000 eggs per tray.

The incubation stacks have been moved several times. Recently they were installed over the Short Raceways so the water drained into the raceways. Unfortunately, it was difficult to supply the required 5 gpm because of the limited head pressure from the gas stripping towers. From the Short Raceways they were moved to the floor and re-plumbed to provide sufficient head pressure.

The Hatchery has experienced low eye-up rates for many years (range of 5-50% annually, average of 15% to 30%). During the Infrastructure and Operations audit in November 2014, conducted by Jack Christiansen, Aquaculture Engineer for the Lower Snake River Compensation Plan, the incubation water supply was identified as a possible place to improve water quality and hatch success. Historically, the incubation stacks have been supplied with water from the re-use system of System 3. This has several advantages:

1) Uses existing Infrastructure
2) Water is de-gassed by the System
3) Water is re-used

The main disadvantage of the System 3 re-use is that it contains more solids and ammonia than "fresh" water directly from the well supply. Since the eggs can act as a filtration medium, this can increase fungus rates, which in turn increases formalin treatment rates, and could potentially lower eye-up rates.

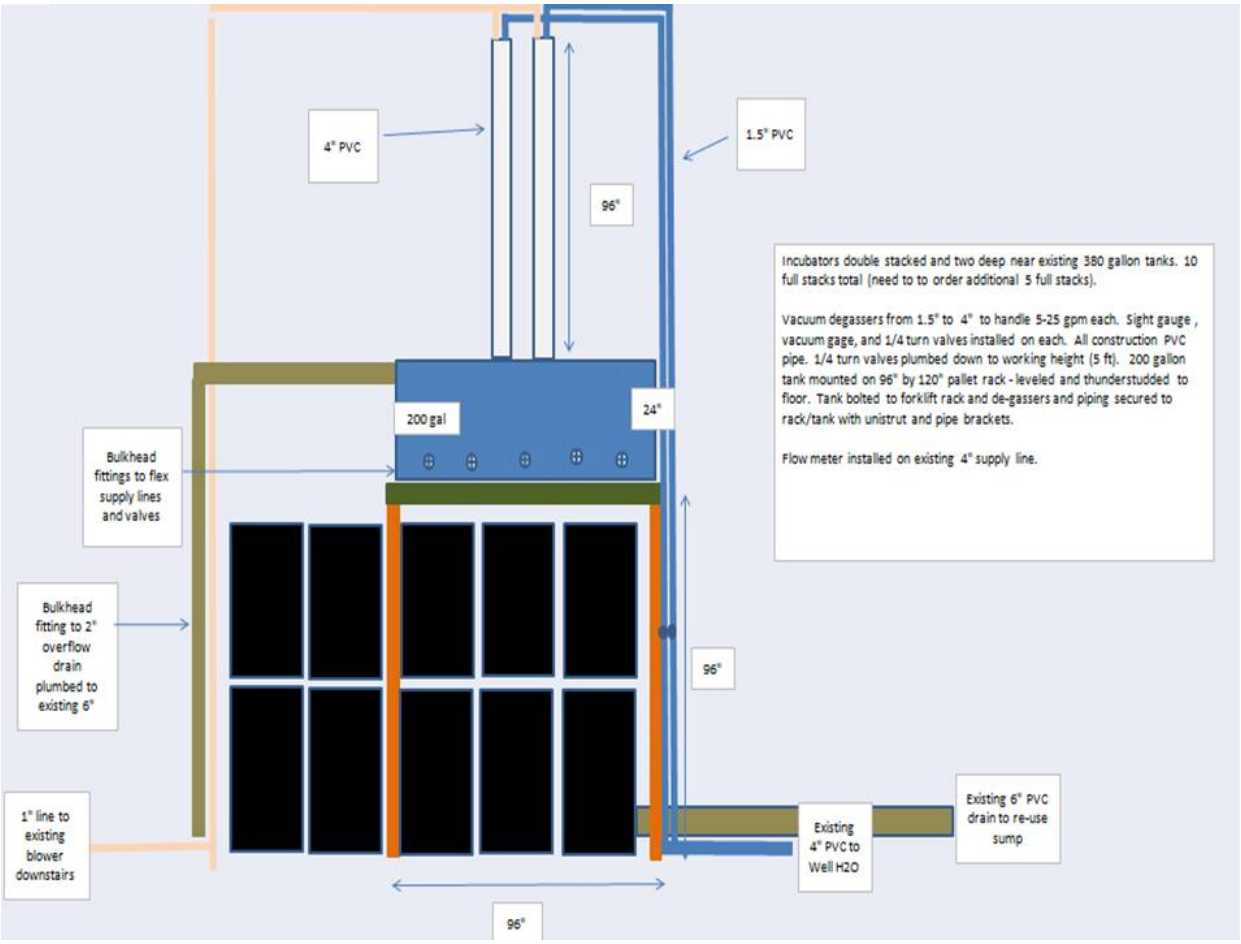
Utilizing well-water presents a challenge because the average Total Gas Pressure (TGP) is very high (114%) and only a limited amount of make-up (well water) is available during System operation. To overcome these challenges, the Hatchery staff constructed a de-gassing tower with an existing tank supported by pallet racking. Effluent from the incubation system was plumbed

to the re-use sump of System 3, which could effectively be used as the “make-up” water during System 3 operations. In addition, with the adequate head pressure, and very clean water, the stacks were double stacked (16 trays per stack) to essentially reduce the water needs by half. Double-stacking of incubation trays is very common in trout and salmon incubation systems using ultra-clean water sources.

Initial testing of the de-gassing system produced favorable results, lowering TGP from 114% to 98%. Dissolved oxygen rates were less than saturation (85%), so an O2 contactor was also installed for instances when 100% saturation is desirable. 100% saturation will likely be desirable just prior to and after hatching when O2 consumption rates increase.

The vacuum de-gassers should be operated at around -38 inches of head to effectively de-gas the untreated well-water. Manipulating head pressure is accomplished by adjusting blower vents and water supply valves to the de-gassing system head tank. The head tank should be maintained below the overflow at flow rates above 10 gpm (two stacks running). Flows below 10 gpm, may require spilling of 5 gpm to achieve desired TGP levels (below 100%).

Incubation Head Tank Design Plan



The initial design plan was put together for a flow rate of about 30-60 gpm and 10-incubation stacks (16 trays/stack). The Hatchery currently only has 5 incubation stacks. The Broodstock Management plan calls for a minimum of 80 successful pairs per lineage of Gila Trout with 50% wild broodstock influence. However, with the low eye-up rates and low availability of wild broodstock, genetic management would be better suited with 150 pairs of captive/wild fish per lineage until eye-up and wild fish availability increase (pers com. Wade Wilson, Dexter FTC). Current incubation stacks are capable of holding approximately 360 pairs (not all trays are divided in 6 sections). The addition of 5 more stacks, and conversion of all trays to 6 compartments, would increase family/lineage holding to 960 pairs. The resulting 10-stack system would require 50 gpm of well water during peak operation in April.

Plans are in place to possibly fund 5 additional incubation stacks and the Hatchery has secured 18 additional up-welling incubators on excess from the Dworshak National Fish hatchery. A combination of these incubation systems may be used to hold additional pairs/eggs.

9” Eagar Upwelling Jars

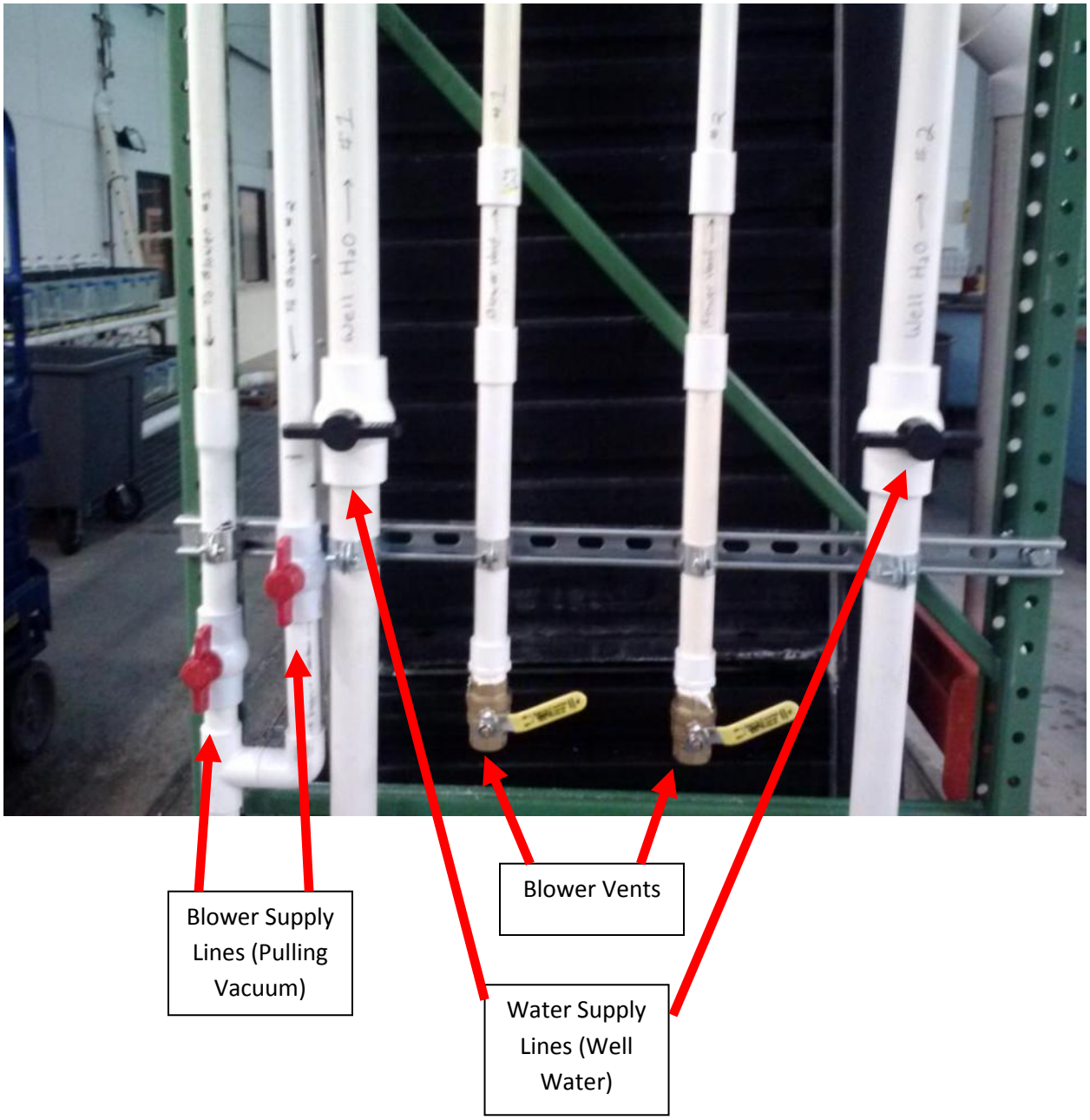
Eagar Upwelling Jars

These 18 jars were received on excess from Dworshak National Fish hatchery. New, they cost approximately $240 each. The jars could be plumbed into the incubation head tank, or alternatively plumbed into the Short Raceways. Their constant flow design generally eliminates the need for formalin treatments. Each incubator has a capacity of 30,000 eggs.

Hatchery Staff working on installation of Incubation de-gassing System.

Degassing System

Incubation System De-gassing Operation



Maintaining TGP can be accomplished by manipulating the water in the sight tubes of the de-gassers. Remember that you are creating a vacuum (trial runs indicated -38 inches working well). So, if you have TGP levels above 100%, you can either open (increase) the water supply valves (increasing vacuum), or close (decrease) the Blower Vents. In the test, we used about 15 gpm in both de-gassers; at lower flow rates, you may need to switch to only one de-gasser column. Also, do not close blower vents completely, the blower motor (under the steps between Systems 2 and 3) will likely suffer damage without some air input.

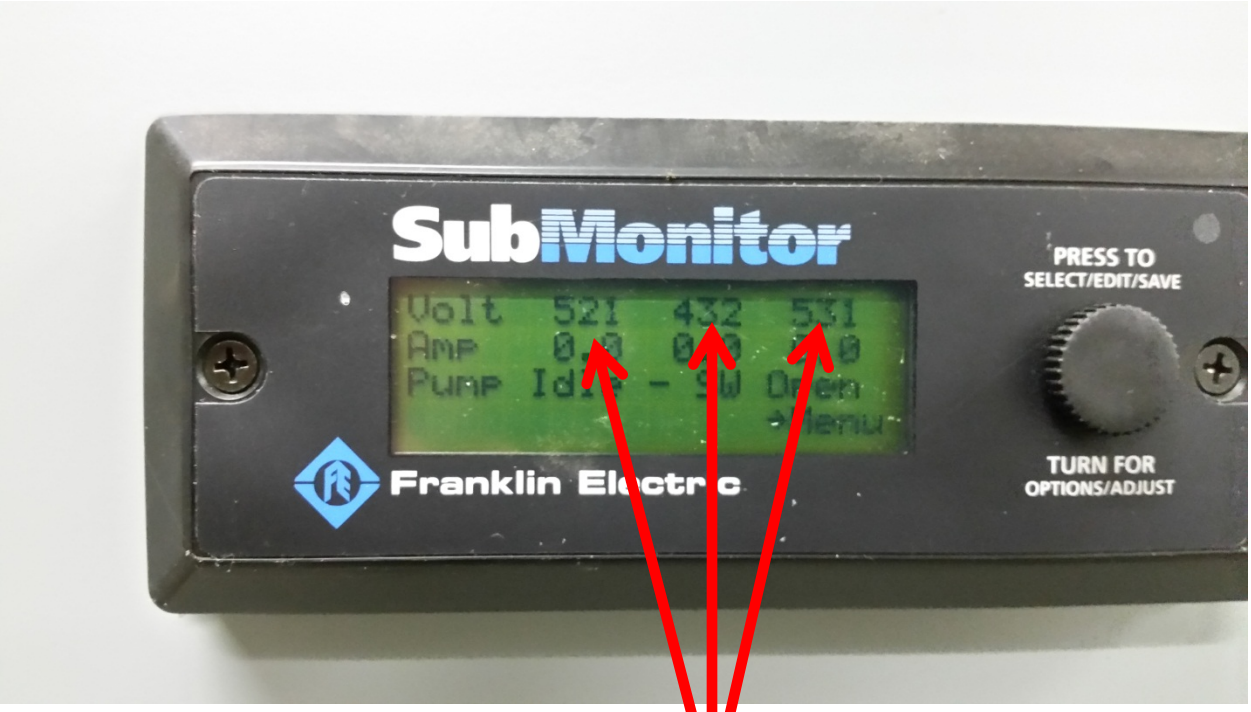
Well-Field Partial Power Outage

On October 3, 2014 at approximately 5:00 am, the Mora National Fish Hatchery (Hatchery) received a low reservoir tank alarm from the SCADA system. Upon inspecting the SCADA alarm system, all 3 operating wells were in "Fail" although they appeared to have power supply.

Staff responded to the well field and observed the following alarm panel lights on Wells 1-3. Well number 4 was out of service at the time of the alarm:

Power Monitor Alarm is "On"

The Hatchery staff attempted to reset the alarm panel and pumps, with no success at re-starting the well pumps. After closer inspection, it was apparent that the 3-legs of 480-voltage were not consistent on the SubMonitor:

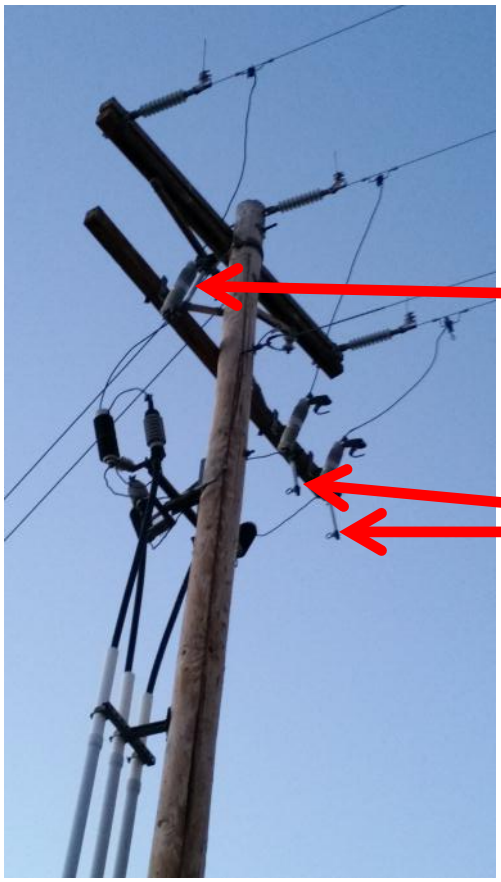


3-legs of 480 Voltage – Voltage should be consistent across legs

The Hatchery staff theorized that one or two legs of power had dropped from the 480 Voltage supply. The 120 Volt supplied lights, SCADA, etc., were still operational, but none of the Wells would turn on. To force the Wells on, the staff cut the power to Wells 2 and 3 to simulate a complete power outage. After cutting the power, the generator started and the transfer switches took the wells to emergency power. Water pumping was restored at approximately 6:20 am.

Main Power Disconnect for Wells

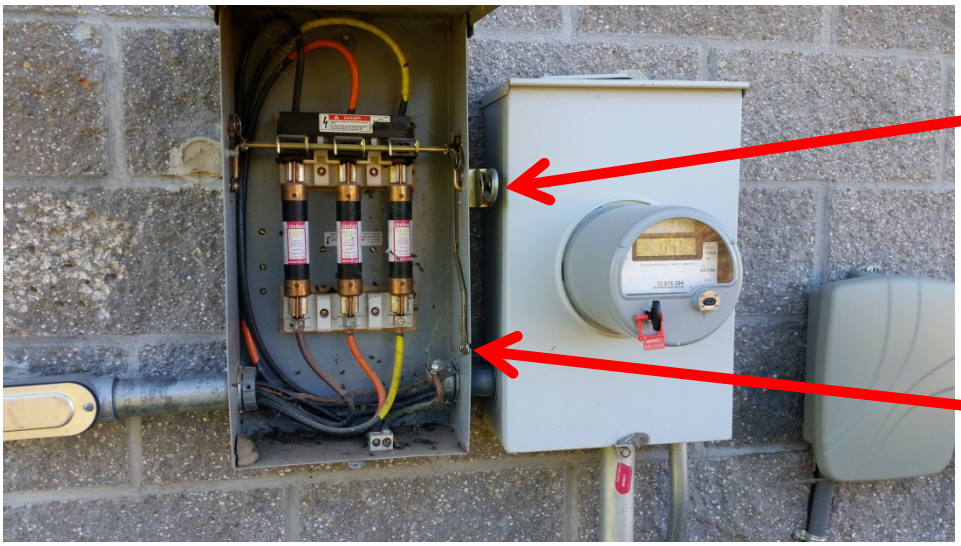
On the way back to the Hatchery, the staff stopped to look at the supply power pole just down the road from the Well Field. Sure enough, two legs of the 480V power had dropped. Of course, this was much easier to see, now that the sun had started to come up! The Co-op was contacted and power was restored around 9:30 am.



Intact Fuse Link

Blown Fuse Links

Once the power was restored, the staff manually restored the main power disconnects. To restore power at the main disconnect, the box must be opened and the safety trip on the right side of the box must be pushed up. Keep hands and face away from the inside of the box!



Main Disconnect
(Up to restore power)

Push this safety lever up to engage main disconnect

Once the power is restored, the transfer switches take about 5-10 minutes to engage the commercial power (normal rather than emergency). The lights on the transfer switches are notorious for burning out, so they may or may indicate the power transfer. You should be able to hear the transfer switch operating when it switches. After the transfer switch operates back to "normal", the generator will take 10-15 minutes to cool-down and shut off. Back to normal – Yipeeee!!!

Transfer switch lights indicating emergency or normal power

PIT Tagging Operations

August 28, 2015

The Mora National Fish Hatchery (Hatchery) annually Passive Integrated Transponder (PIT) tags 3-4,000 Gila trout for broodstock development purposes. Generally, the fish should be larger than 65 mm (150 fish per pound) for successful tagging. Additional tagging guidance is available in the online manual:

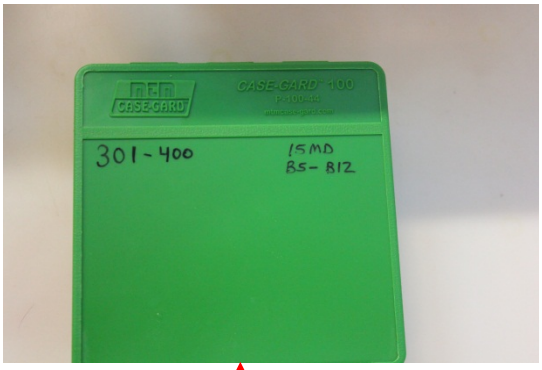
ftp://ftp.ptagis.org/Documents/PIT_Tag_Marking_Procedures_Manual.pdf

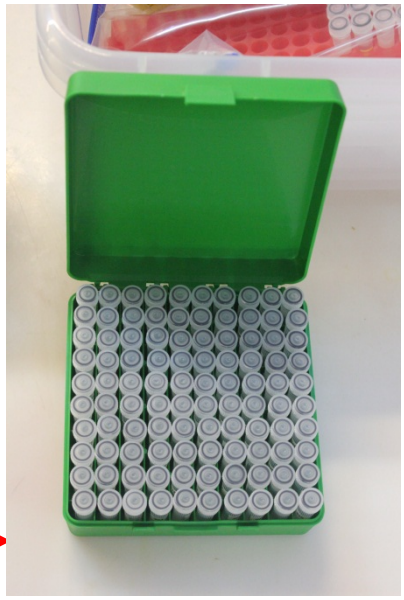
Gila trout are tagged dorsally (near the dorsal fin) to avoid tag loss during spawning operations. The Hatchery currently uses 8 mm PIT tags available from Biomark. Tags and needles can be purchased from Biomark.

Prior to tagging, vials must be prepared by filling with ethanol, labeling and organizing in cases.

2 ml microcentrifuge tubes from Fisherbrand with screw caps are generally the best for fin clip preservation. Current cost is about $90 per 500.

Labels are printed on the computer, cut apart and stuck on each vial



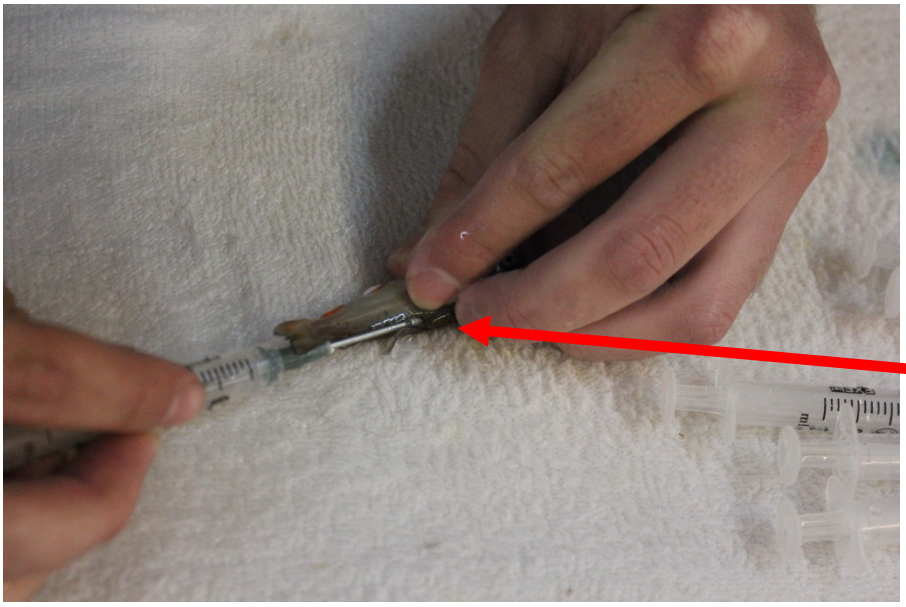


MTM Case-Gard 100 (P-100-44)
These are 44 Mag shell holders that fit 2 ml bottles well. Then can be bought directly from MTM for about $4.23 each

It will take about 15 labor hours per 1000 vials to fill, label, and organize. Once enough vials are prepared, needles are loaded with tags and fish are anesthitized with MS-222. Use 0.3 grams per gallon of MS-222 along with 1 gram per gallon of baking soda. Generally, the fish are small, so plastic totes with 2 to 4 gallons of water will suffice for anesthesia and rinsing. Work with small numbers of fish at a time, generally 3-5. Remember to group weigh each tank to deterimine a sample count (fpp) average for feeding purposes.

Rinse tote – change water often

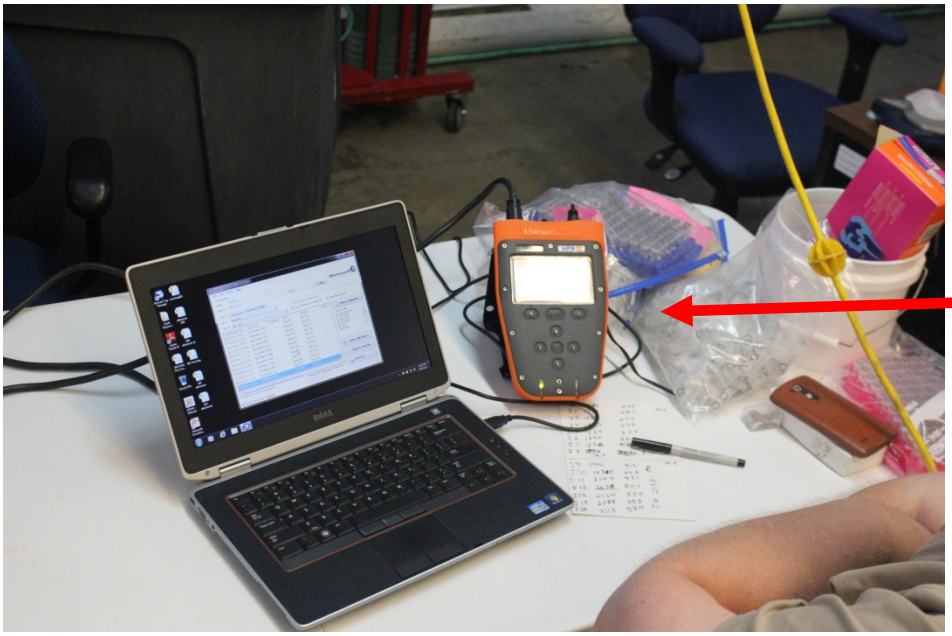
Anesthetic – MS-222 at 0.3 gr/gal



Tag Insertion Point – should be just under the skin about 1/8” past the insertion wound

Insert the tag dorsally. Slide the needle just under the skin and push the plunger to insert the tag. Ideally, the tag should rest at least 1/8” beyond the insertion wound so that it is less likely to fall out. Also, leaving a small portion of the needle exposed allows air from the plunger to escape and not be injected into the fish. Monitor fish in recovery bucket and dissect mortalities to see tag placement and adapt as needed. Needles generally last about 30-50 fish each before they must be replaced. Normal mortality for PIT tagging operations is between 1-3%.

Clip Adipose fin after PIT tagging and place fin in appropriate 2 ml ethanol vial.



PIT Tag reader and laptop for recording PIT tag information.

Be sure to record tank number in the comment section of the PIT tag program. Also, write down on scratch paper the start and stop vial number for each new tank. This will provide checkpoints when downloading and labeling the Excel file of PIT tags.

With a team of 5, you can expect to PIT tag and adipose clip about 1000 fish every 8-10 hours.

2015 Tagging Costs and consumable inventory

Item	Price each	2015 Tagging Number	Total Cost (3000 tags)	Inventory	Comment
Tags	$ 1.95	3,000	$ 5,850.00	5500	
Needles	$ 2.00	100	$ 200.00	100	
Vials	$ 0.18	3,000	$ 540.00	3,000	Flip Cap on-hand
Ammo Boxes	$ 4.23	30	$ 126.90	0	
Label Supplies	$ 15.00	8	$ 120.00	0	
Ethanol	$ 191.08	1	$ 191.08	3 gal	Per 5 gallons
MS-222	$ 671.00	1	$ 671.00	per kg	
Labor		195	$ -		Total Hours
		Total	$ 7,698.98		

Polishing Pond Maintenance

June 29, 2015

The Mora National Fish Hatchery (Hatchery) utilizes the polishing pond to further remove nutrients from the Hatchery effluents.

Both discharge from the settling ponds (sludge lines) and overflows (overflows at sumps) are discharged to the polishing pond. Sludge lines are settled at the settling pond prior to polishing pond discharge. Overflow lines go directly to the polishing pond.

In 2014, the Hatchery also plumbed the Visitor Center overflow directly to the polishing pond (3" PVC) since this line was running to the septic system. In addition, the staff lowered the standpipe height and drained the pond approximately 18 inches over the winter to "freeze-out" accumulated cattails and dry the banks for harvest.

In May 2015, Richie Garcia harvested cattails with the bobcat and mini-excavator attachment. In the future, an "Extend-a-Hoe" backhoe would be a superior piece of equipment for its reach.

The Hatchery plans on harvesting accumulated vegetation annually or semi-annually to promote healthy growth and remove as many nutrients as possible.

The Polishing pond has an internal and external Standpipe. The internal pipe is in the middle of the floating dock. The external standpipe is in the manhole just south of the floating dock. There is a valve that can be turned to bypass the external standpipe.

Harvesting Cattails from the Polishing Pond

Re-filling the Polishing pond in May 2015

Spawning

March 26, 2015

The Mora National Fish Hatchery (Hatchery) annually spawns up to 500 pairs of Gila trout. Each pairing is calculated to ensure the greatest genetic diversity possible by using pair-wise mating spreadsheets developed by Dr. Wade Wilson at the Dexter Fish Technology Center.

You'll need some equipment for spawning:

- 5 Spawning Tubs
- 4 Nets
- Oxygen Bottle, Regulator and stones
- PIT tag reader
- Laptop and associated spreadsheets
- Raceway crowders
- Datasheets and pencils
- Falcon tubes and pipettes for ovarian samples
- 1-gallon Zip-Lok bags and Black Sharpie markers
- Fertilization Solution and squirt bottle
- 3-4 staff with great attitudes ☺

Chemicals needed:

Anesthetic Tub

- 0.4 grams/gallon of MS-222 (20 grams for 50 gallon tub)
- 1 gram/gallon baking soda (50 grams for 50 gallon tub)
- 2.1 lbs salt for 50 gallon tub

Spawning Tub

- 2.1 lbs salt for 50 gallon tub (1% solution)

Fertilization Solution

- 0.17 lbs salt for 4 gallons (in 5-gallon bucket)

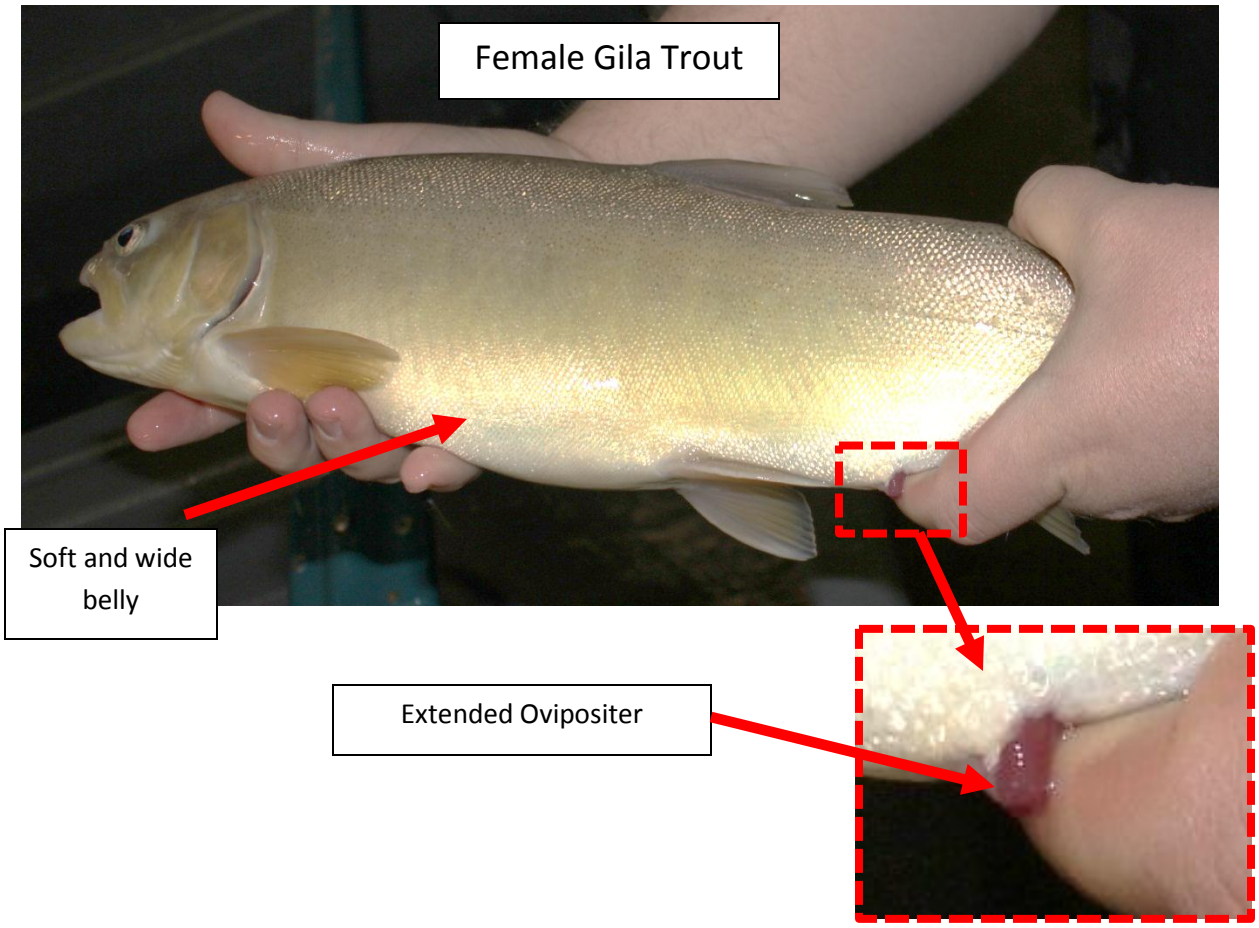
Typical spawning arrangement

Spawning generally starts around the 1st week of March. However, warmer winter water temps or altered photoperiod (besides natural photoperiod) can shift spawning earlier. Keep an eye out for spawning ques such as "going off feed", lining up around water inflows, etc. Once spawning starts, all broodstock are taken off feed for the duration of the spawning season.

Assuming some fish are ready to spawn, first fill five tubs with about 50 gallons of water each (see mark on the side of tubs). You can use a siphon tube or a 5-gallon buckets to fill the tubs. Generally, the MS-222 tub will be on one end of the arrangement followed by: ripe males, ripe females, unripe (both sexes), and spare. Check O2 bottle and system for pressure and leaks and put stones in each spawning tub.

Crowd the raceway towards the head-end (where the new water comes in) and place an additional screen/crowder in the empty section to split spawned and un-spawned fish. Once croweded, net 20-30 fish into the MS-222 tub. It should take 2-3 minutes for the fish to lose equilibrium. If you are faster than that, you may be dangerously close to overdosing the fish. Slower and you may want to add some additional MS-222.

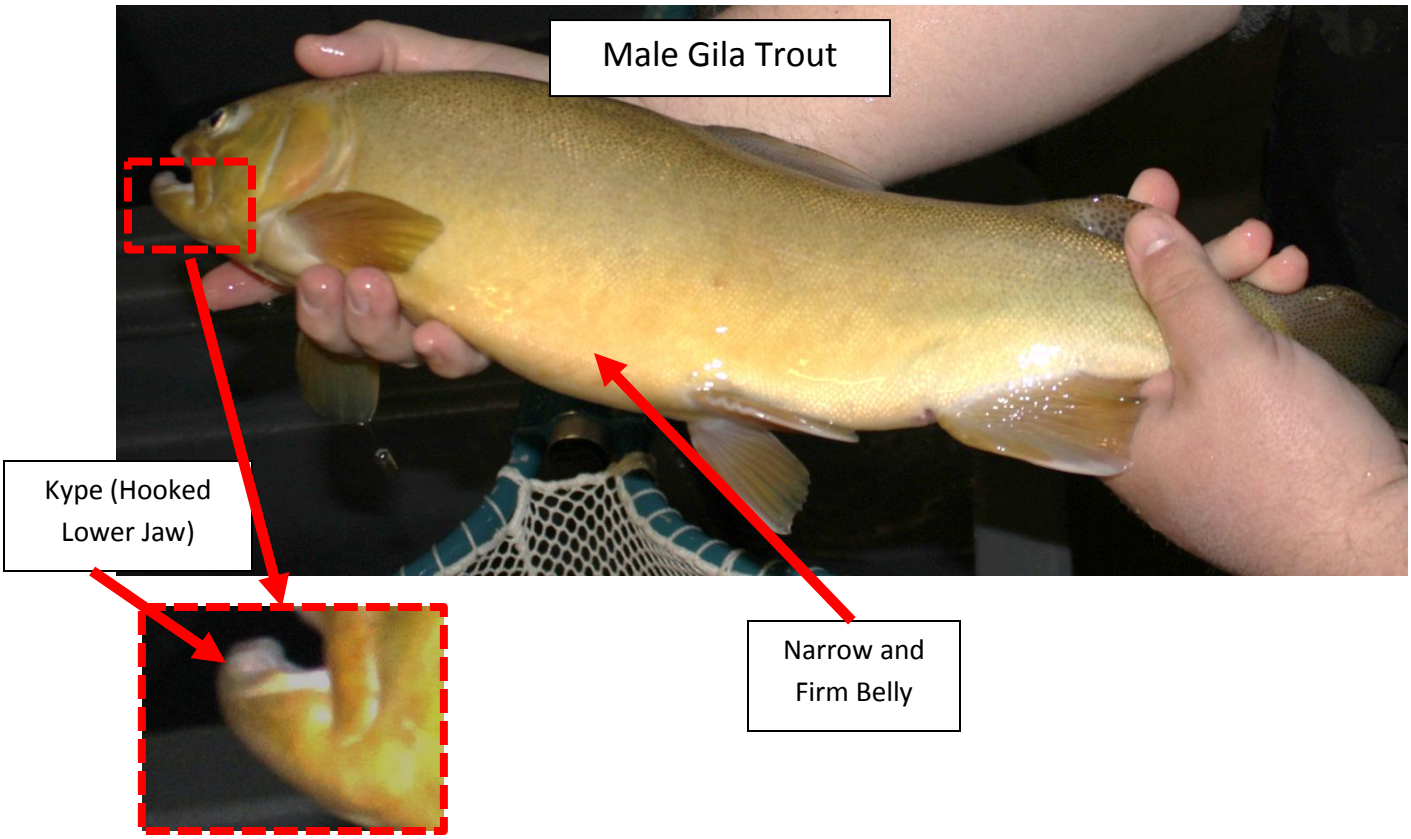
After the fish are anesthetized, place fish in net and rinse in the next tub. The fish can stay in this net as you sort through ripe males, ripe females, and unripe fish. Only anesthestize as many fish as you can sort in 2-3 minutes.



Ripe females will have an extended ovipositer, soft belly, wide frame, and slight pressure will expel eggs. With very ripe fish, be cautious not to hold the ovipositer lower than the rest of the body because gravity can expel the eggs as well.

The egg skein generally ripens from the tail end first. So when checking fish for ripeness, gentle pressure should be applied in the abdomen area, not near the tail. Otherwise, eggs can be expelled and only part of the egg skein has developed enough for fertilization.

When expelling eggs for fertiliztion, grasp the tail with one hand and use long gentle strokes of the abdomen to expel eggs (in contrast to fast short strokes). Hold the head and abdomen higher than the tail to allow gravity to help expel the eggs as well.



Male Gila trout are generally darker in color, have a pronounced kype, firm belly, narrow frame, and gentle pressure will expel some milt/seminal fluid.

Expelling milt is best accomplished with long even strokes of the abdomen. The testes extend almost the entire body cavity, so strokes only at the tail end will not produce as much milt as strokes across the entire body.

Again, use gravity to your advantage, grasp the tail and eleveate the head. It may also help to arch the tail towards the back of the fish as well. Be careful while arching the fish, it is possible to cause spinal damage if you bend too far.

Milt is activated by change in osmality i.e. drops of water or ovarian fluid. Once activated, milt is only viable (alive and able to fertilize an egg) for less than 60 seconds. Hence, be cautious to not drip excess water with the milt and fertilize (activate) immediately after expelling the milt onto the eggs.

If delayed fertilzation is being used (not current protocol), be sure to dry the vent area prior to collecting milt to avoid premature activation.

Sorting and Spawning Females

Sort fish until you find about 15 ripe females and as many males. Record PIT tags for each ripe fish and then find suitable crosses in the spawning matrix. Record female PIT tag number (first) than male PIT tag number on 1-gallon Zip-Lok bag. Only use the last 5 digits of the PIT tag number for labeling. Label each cross consecutively 1,2,3, etc. on the datasheet and bag. Each lineage will have a separate datasheet. Pull the first 7 to 10 ripe females and spawn them into their respective bags.

Hang or lay each bag in a cool, dark spot. Do not spawn more fish than you can complete in 10 to 20 minutes.

Collecting Ovarian Fluid Samples

Take ovarian fluid samples from each female. Try to get 1 ml of ovarian fluid per female. Combine fluid from 5 females into one falcon tube and label with lineage, broodyear, and wild/captive. Samples should be refrigerated as soon as possible and need to arrive at that lab within 48 hours. Generally, these are stored overnight in the refrigerator and shipped the next day via Priority Overnight FedEx delivery to Dexter Fish Health Lab. (Attention Teresa Lewis, Ashlie Peterson, or Jason Woodland)

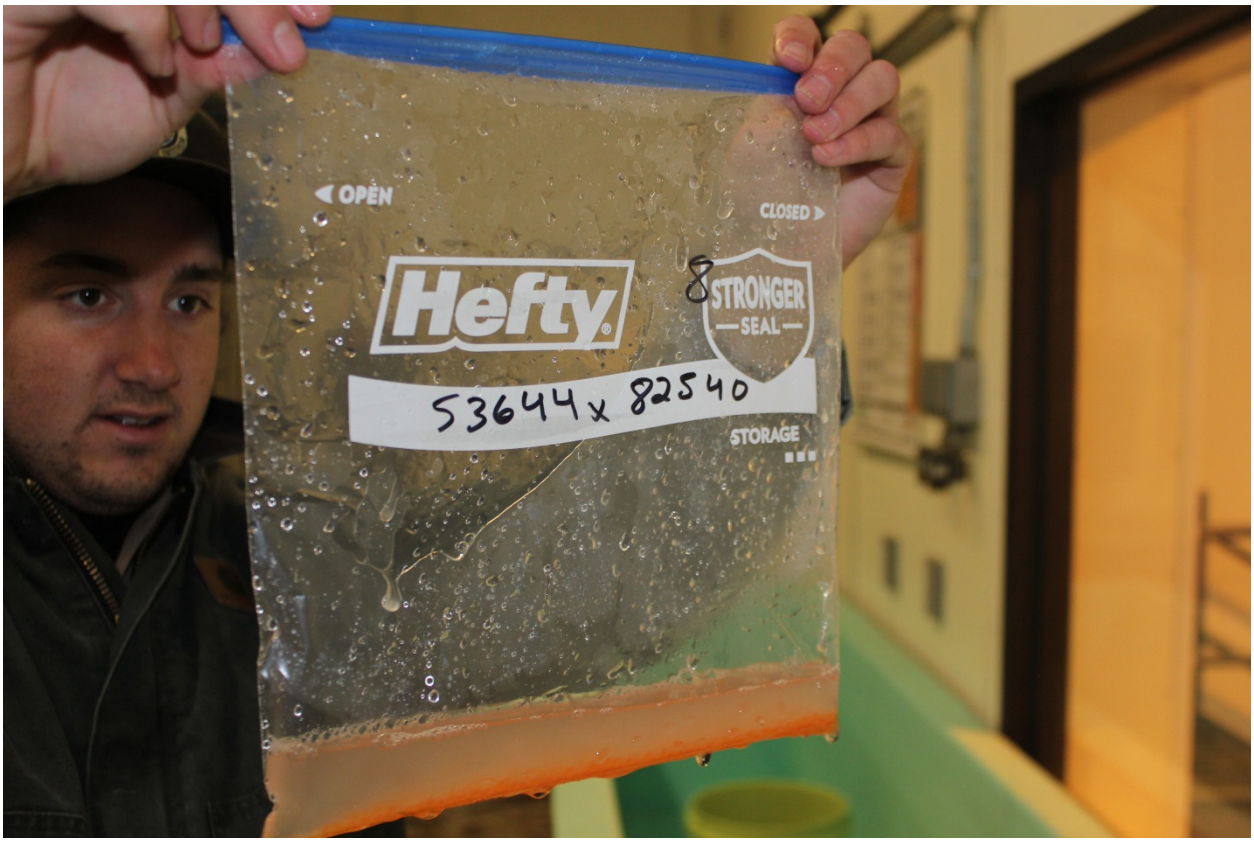
Collecting Ovarian Fluid

Fertilization

Once you have 7 to 10 bags of eggs, start sorting and anesthitizing males for fertilzation. It is very important to rinse the males because MS-222 is lethal to the sperm cells. Activate milt immediately following feriliztion with just enough activiation solution (1% salt solution) to cover the eggs. The less water you use, the higher the concentration of sperm cells per volume of water and the better chance of fertilization. Rock bag gently several times to ensure milt is dispersed, then hang bag for at least 2 minutes to ensure fertilization is complete.

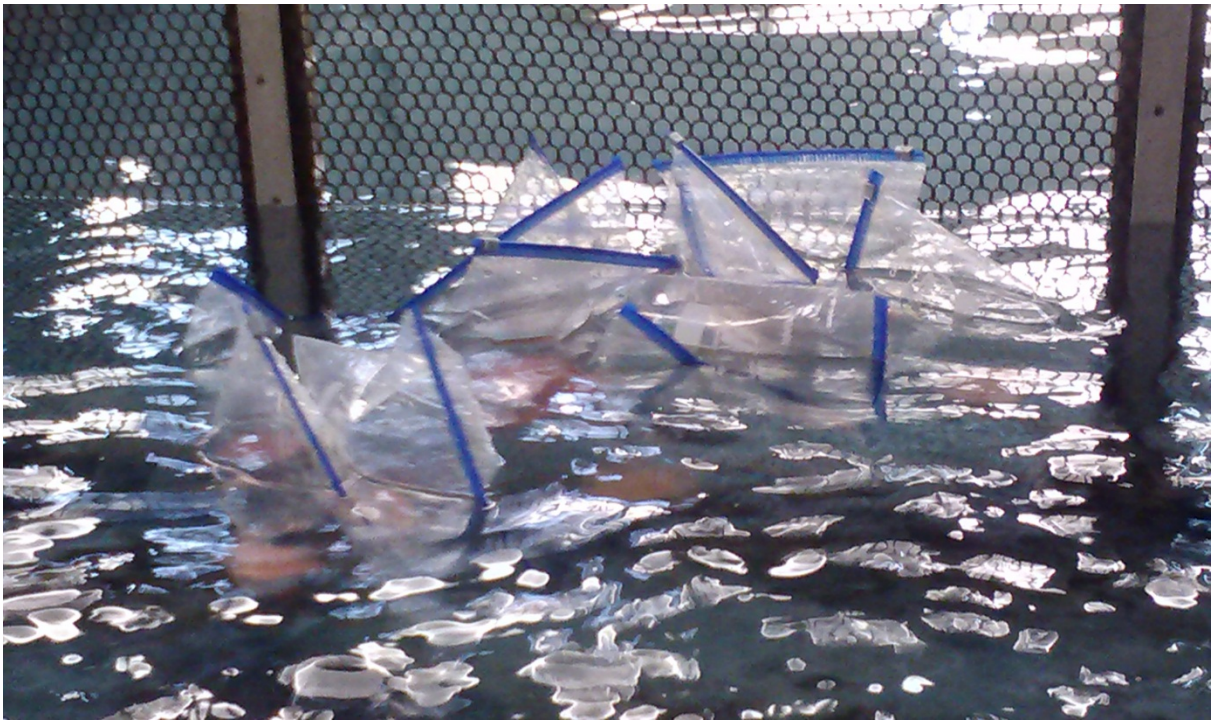
Rinsing Eggs

Bags can be rinsed in the raceway water if both parents orginated from that raceway. To ensure biosecurity, combinations of wild and captive broodstock must be rinsed at an isolated water source (laundry sink). Rinsing removes excess milt, blood, egg shells, old eggs, etc. to facilitate water hardening. Water hardening begins almost immediately after fertilzation and lasts about 1 hour.



Eggs Ready for Rinsing (allow fertilization for at least 2 minutes)

Bags are filled with water at least twice and poured off to remove excess milt, dead eggs, etc. When finished, fill bag almost completely with water to facilitate water hardening of eggs.



Once rinsed, bags can be floated in the raceway until taken to incubation.

Transfer to Incubation and Disinfection

Anytime after rinsing, eggs can be transferred to incubation for disinfection and final water hardening. Be sure to record what tray each eggs were placed into on the datasheet. Decant off any excess water by pouring the eggs through the tray screen. Then disinfect in 75 ppm iodine for 30 minutes (each tray is approximately 2.5 gallons so 75 ml of iodine is about 75 ppm). The eggs can be left in the iodine up to 1 hour, if necessary. Leave tray approximately 4 inches out from normal position to bypass fresh water supply for 30 minute bath treatment.

Decanting Excess Water

Eggs are disinfected for 30 minutes in 75 ppm Ovadine.

Removing Dead Eggs

24 hours after water hardening, the dead eggs can be removed from the trays. Removing dead eggs reduces fungus loads. **DO NOT** move trays from 48 hours post fertilization to eye-up (eye-up occurs at 350 Temperature Units or about 3 weeks from fertilization). From the green egg stage (48 hours post fertilization) until eye-up, the eggs are very tender and any movement can kill viable eggs. Record date and dead eggs on the data sheets.

Notes from 2015 Spawning Season:

Recovery Spawning

Overall, the Hatchery had a successful 2015 spawning season. Main and South Diamond lineages had green egg to hatch rates of 48% and 49% for recovery fish. Whiskey Creek had a green egg to hatch rate of 34%. Unfortuntately, both Spruce and Iron lineages failed to produce viable crosses in 2015.

Gila Trout Recovery Spawning, Mora NFH 2015

Lineage	Crosses	Green Eggs	Eggs per Female	Hatch Rate %	Total Fry
Main	99	60,132	607	48%	28,709
South	115	61,387	534	49%	29,925
Whiskey	46	40,403	878	34%	13,881
Spruce	9	2,541	282	0%	-
Iron	5	683	136	0%	-
Total	274	165,146	603	44%	72,515

The Hatchery attributed successes to a couple of factors:

1. Direct fertilization rather than delayed
2. Genetically appropriate selected crosses
3. Inclusion of wild lineages in broodstock
4. Change to well-water for egg incubation

The failure of Spruce and Iron creek was attributed to low genetic diversity and underdeveloped eggs. In the case of Spruce creek, the fish were triggered to spawn in late 2014 and produced undeveloped eggs. Iron creek fish did not produce any viable crosses and interestingly the South Diamond wild females held in the same system (Shady Acres) also failed. Conversely, cryopreservation efforts of Iron showed good milt motility and South Diamond wild males produced viable crosses.

Experimental Crosses

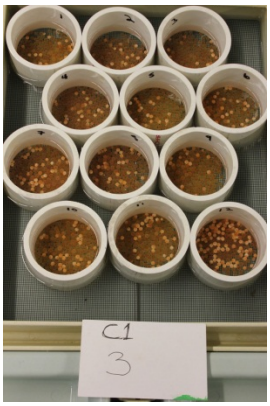
The Hatchery tried several different incubation systems and age-classes in 2015. Overall, Age-2 females and jar incubation (from green egg) had significantly lower hatch rates (15% and 14%) . However, Age-2 males and hatching directly in aquariums had no significant effect on hatch rate.

Cropreservation efforts utilized Age 2 and Age 4 females for milt viablitiy tests from South and Main Diamond lineages. Hatch rates matched controls (33% and 36%) using Glucose methanol extenders and were significantly better than balanced hank and cloud solution extenders (5%) that were used in previous studies.

Gila Trout Experimental Spawning, Mora NFH 2015, Main and South Diamond

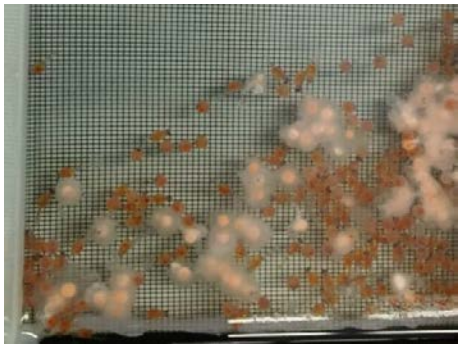
Lineage	Crosses	Green Eggs	Eggs per Female	Hatch Rate %	Total Fry
Upweller Jars	127	57,988	456	14%	8,013
Age 2 x Age 2	21	8,968	427	15%	1,364
Age 4 x Age 2	9	8,936	993	46%	4,104
Experimental Control	10	7,716	772	53%	4,059
Cryo Control	62*	2,791	-	36%	1,010
Cryo Hanks and Cloud	62*	8,284	-	5%	447
Cryo Glucose Methanol	62*	4,448	-	33%	1,479
Total	229	99,131	433	21%	20,476

*All females were mixed for Cryopreservation work



Incubation Strategies

Formalin for Fungus Control



The staff tried several methods of fungus control including picking eggs 24 hours post-fertilization and several formalin concentrations/dosages. Based on the trials, formalin treatments at 1667 ppm for 15 minutes adminstered 3x weekly are effective at reducing fungus loads. Eggs without formalin treatments had heavy fungus loading (see pic). Picking eggs 24-hours post fertilization had no effect on funus loads and the Hatchery will likely discard this practice in 2016.

Upweller Jars versus Trays

As mentioned above, upwelling jars from green to hatch had significantly lower hatch rates (14% to 53%). However, utlizing the upweller jars from eyed to hatch was anecdotally as good as heath-style trays. Additionally, the staff had success hatching eyed eggs for broodstock development (50 eggs) directly in 10-gallon aquariums. With these results, the Hatchery plans to use trays to isolate brood crosses and incubate from green to eyed stage. At the eyed stage, 50 eggs per cross will be hatched in an aquarium for brood development, 100-200 eggs from each cross will be combined into a upweller jar depending on Recovery stocking requests, and excess eggs will be combined in separate jar for additional recovery efforts.

To accommodate additional lineages coming on line, the Hatchery will be installing 5 more stacks of heath-style incubators for the 2016 spawning season. The dividers on the these new trays will be organized longitudanally rather than horizontally to improve water flow and reduce fungus loading.

Removing Dead Eggs

The Hatchery generally counts individual dead eggs with pipettes. In 2015, the staff attempted "salt floating" dead eggs with a salt solution (0.97 lb salt/ gallon H2O). The effort was marginally sucessfully at separating the dead eggs. Some obstacles included: multiple egg sizes and relatively low eye-up rate on the experimental group used.

Incubation De-gassing System

The incubation de-gassing system worked to reduce Nitrogen levels from 116% to around 100% saturation. However, by combining stacks, the Hatchery struggled in some instances to continue formalin treatments as early spawn takes hatched and later takes were still eyed (hatched alvins are susceptible to formalin toxicity). In 2016, the staff plans to install an additional head tank

with chiller units. These units can be used to manipulate eye-up and hatch timing to “match-up” multiple spawn takes.

2016 Spawning Plans

The Hatchery has several infrastructure/operations changes planned for the 2016 spawning season:

1. Move Iron Creek lineage to proven Visitor Center re-use system
2. Add 5-additional heath-style incubation stacks
3. Add 8 upweller jars on Short Raceways for eyed-egg incubation
4. Add additional head tank with chiller capacity to incubation system
5. Discontinue 24-hour post fertilization egg picking in favor of 3x weekly formalin treatments
6. Construct automated formalin delivery system for egg stacks to reduce staff exposure to formaldehyde fumes
7. Hatch broodstock development fry directly in isolation aquariums
8. Examine success ofAge-2 male with Age-3 female crosses to develop future broodstock holding strategies and reduce stratification of domestic brood lines
9. Add de-gassing capacity to start fry on well-water supplies rather than re-use system to reduce Bacterial Gill Disease susceptibility

Walk-Through SOP

08_31_15

Walk through should be completed each morning and each afternoon before leaving hatchery.

1. Start in Visitor center and work your way through each wild system, Shady Acres, Whiskey, Spruce. Check blowers and make sure they are blowing air, not sucking it in through vent **(Fig 1)**. Place hand on pumps to make sure they are warm (but not hot and overheating(**Fig 2**), Check oxygen levels on green bottles, if below 500, change out with new bottle. Spray off elliptical filter screens. Check for mortalities, if morts are present, read PIT tag number and record date and number. Put feed down on belt feeders, unless fish are off feed for stocking trip, etc. Check for wasted feed on bottom of tanks or note if tanks need siphoned. Observe fish for abnormal behavior (flashing, gulping air at top of tank, etc.)
2. After checking each wild system, check blowers on each re-use system. Check water levels on stripping tower sight tubes. (**Fig 3**). Check pressure of biofilters, if above 20 PSI then backwashing is necessary. Check level on liquid oxygen tank in cage outside. **(Fig 4)**
3. Check SCADA system for any alarms. If alarm is present record in alarm log, acknowledge alarm and troubleshoot problem. Record system temps and flows. Record data from Aqua manager program for wild systems.
4. Place meters in system and remember to record gas levels throughout the day, switching from system to system. (morning only, if oxygen levels in re use systems need adjusting, adjust on flow meters by stripping tower for respective system. If the oxygen level on big systems needs to get adjusted and the oxygen flow meters are already adjusted all the way down or adjusted all the way up, follow the oxygen line and there should be a check valve to adjust the oxygen level as needed. **(Fig 5)**
5. Walk down catwalks between raceways, observing fish behavior and looking for morts. Record mort PIT tag numbers and raceway where mort was found.
6. Check all rearing units 30s, 96s, 250s, SRs, etc. to ensure water is flowing and standpipes are in place.
7. Check all doors to ensure they are locked at end of day.

Fig 1. Blowers for Vacuum de-gassers

Listen for any abnormal sounds in pumps, blowers, etc. Look for leaks from tanks or water lines. Important to observe fish for abnormal behavior.

Fig 2. Check pumps to make sure they are running

Fig 3. Check water level in vacuum de-gasser/head tank.

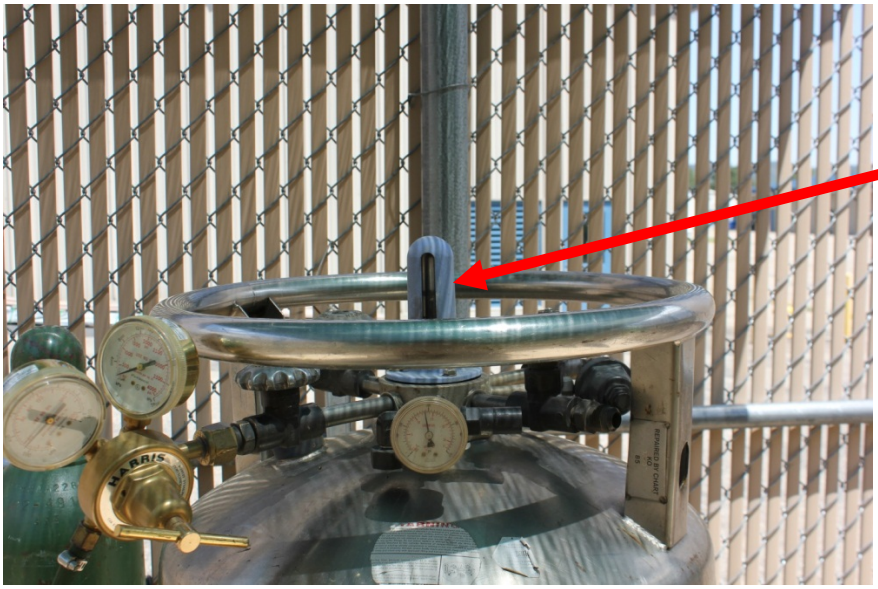


Fig 4. Check oxygen level in liquid bottle outside (O2 supply for systems)

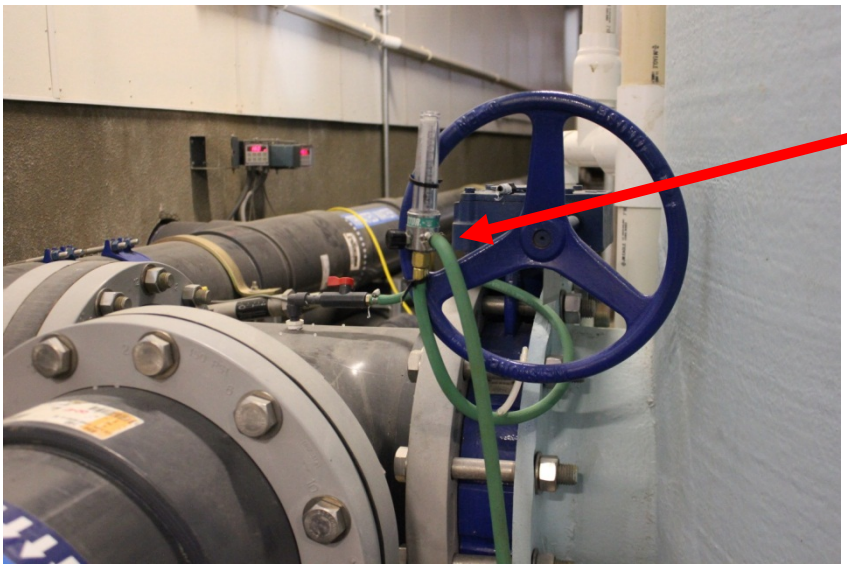


Fig 5. Adjust oxygen flow meters to Systems as needed.