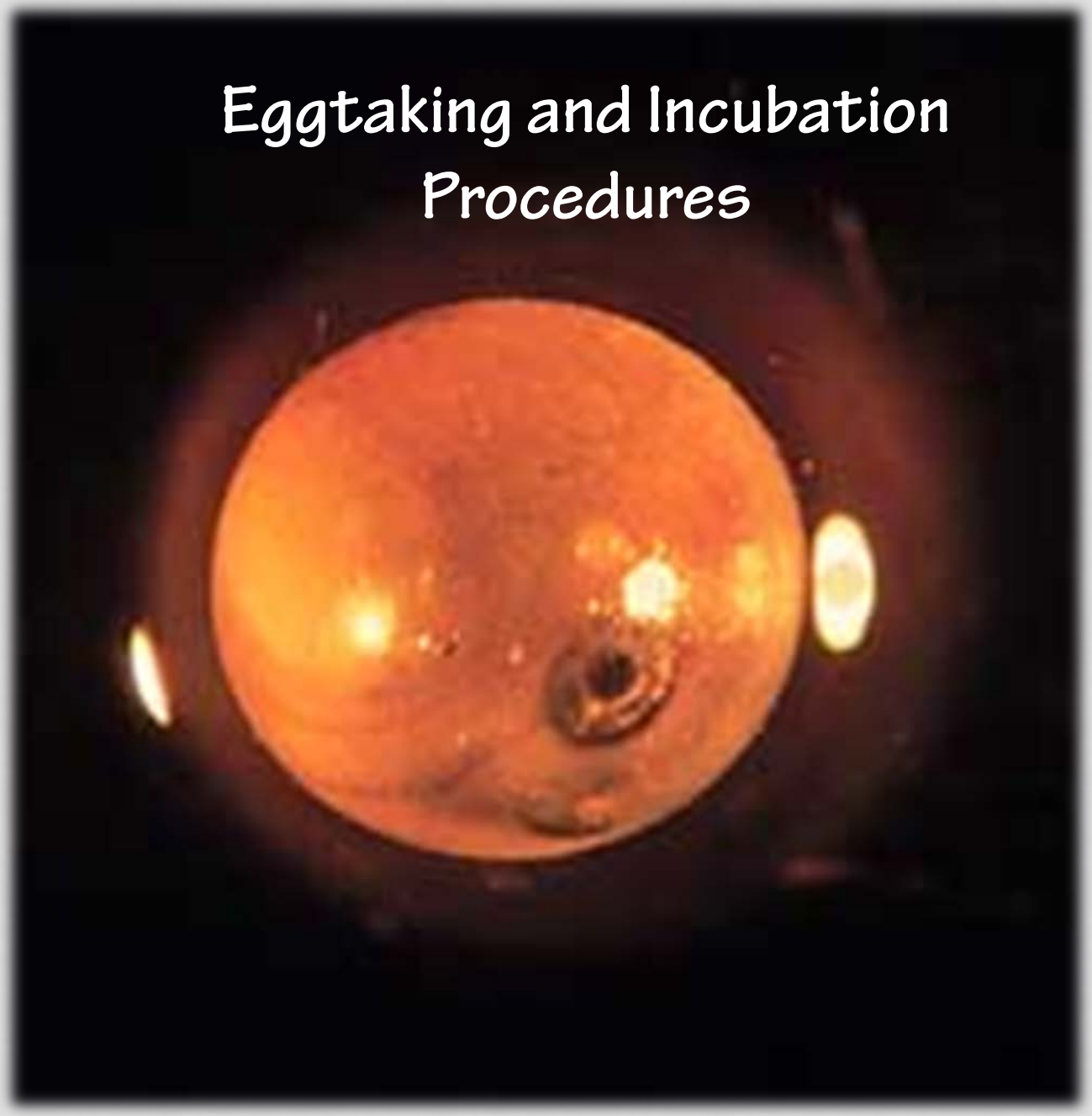
Eggtaking and Incubation Procedures



ASSIGNMENT DUE 10/13/14

- Read the chapter about Eggtaking Procedures in the Alaska Sockeye Salmon Culture Manual
  - Write a brief summary of your findings including any questions you may have about the information.
  - List some differences that exist for taking sockeye eggs vs. other species of salmon
- Read pages 21-29 in the ADFG Fish Culture Manual (related to eggtake procedures)
  - Write a brief summary of your findings – questions, inconsistencies with what you've learned

PART 1 – SETTING UP FOR EGGTAKE

Important aspects of this segment:

1. List the various types of anesthesia used during salmon eggtake operations
2. Describe a typical eggtake setup – be able to list the components

FISH RETURNING

- Broodstock collection methods vary
- Must have abundant water and holding space
- Amount of time they hold varies
- Most sites have a fish ladder

MEDVEJIE HATCHERY

- BARRIER NET
- FISH LADDER
- HOLDING RACEWAYS

BROOD HOLDING

Medvejie

Port Armstrong

SPAWNING SETUP

- Varies with site and production goals
- Varies with species and if "family tracking" is involved

SPAWNING METHODS STEP BY STEP

- ANESTHETISE FISH
- CHECK FOR RIPENESS
- KILL FISH (IF MATURE)
- GREEN EGG COLLECTION
- FERTILIZATION & SPERM ACTIVATION
- RINSING
- INCUBATOR LOADING
- WATER HARDENING
- EGG DISINFECTION
- FECUNDITY & GREEN EGG ENUMERATION

ADULT ANESTHESIA

- Electro – Anesthesia
- Carbon Dioxide
- MS 222 / Clove Oil
- Mechanical
- Manual

Sometimes you need to check fish prior to sacrificing them – sometimes you don't!

ELECTRO-ANESTHESIA

EA-1000B Electroanesthesia System

Electroanesthesia as a technique for processing large numbers of fish offers a viable alternative to the use of chemicals. It is safe, fast, effective, and inexpensive to use - as compared to labor intensive handling protocols.

The Electroanesthesia System offers the operator the chance to select just the right settings to achieve the desired outcome in a

Starting at: $29,995.00

Price is for electroanesthesia pulse generator only. Tanks and electrodes are sold separately. Smith-Root can customize an electroanesthesia system to suit your current facility. Contact us for details or a quote.

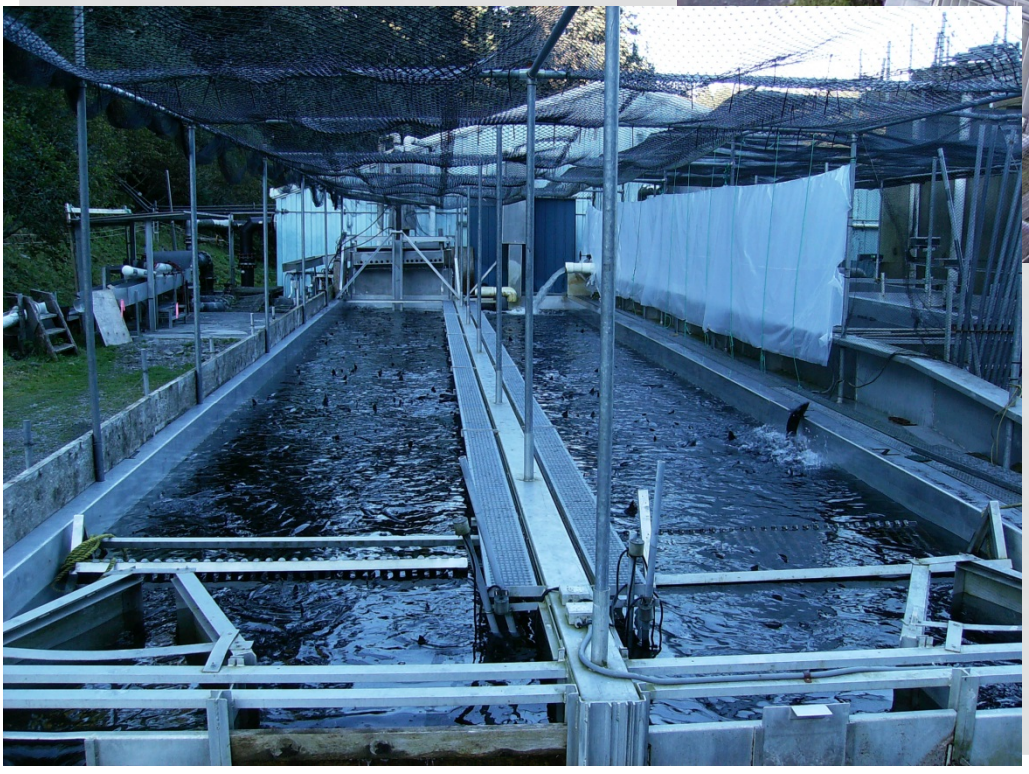
http://www.smith-root.com/electroanesthesia/ea-1000/

(some good videos here)

CROWDING MECHANISMS VARY

Port Armstrong

Kitoi Bay



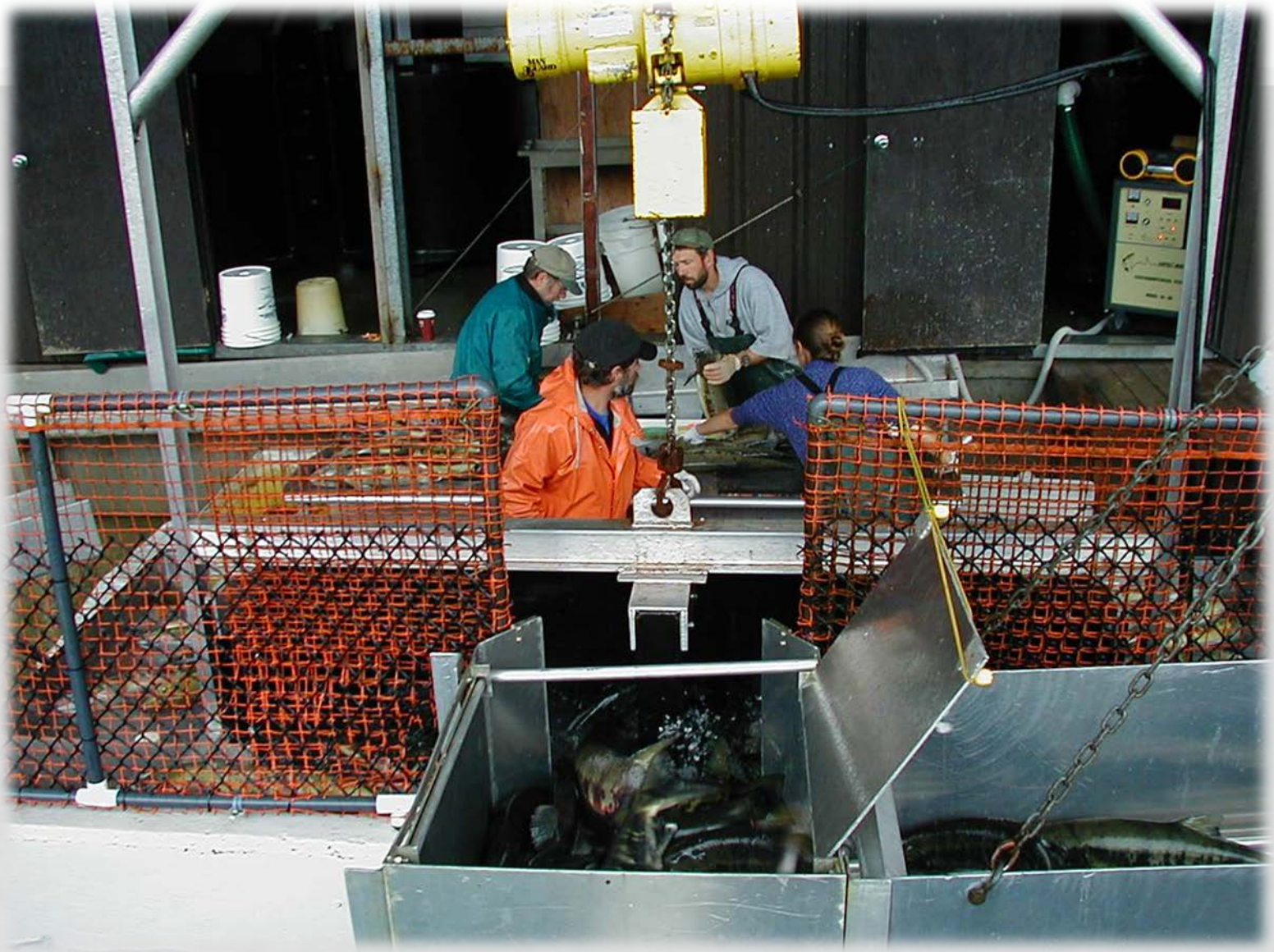
Sometimes you need to "prep" the spawning area before starting….

EA UNIT

Controls for:

- Amps
- Volts
- Cycles
- Time

CHUM EGGTAKE FACILITY USING EA UNIT



CARBON DIOXIDE

Sheldon Jackson Hatchery uses carbon dioxide

CHEMICAL ANESTHETICS: MS 222 / CLOVE OIL

- Useful for smaller quantity eggtakes
- Can be used as backup for other techniques such as EA and carbon dioxide
- Makes fish unusable as food fish after eggtake
- Might be more expensive to use overall
- Used at Sheldon Jackson Hatchery

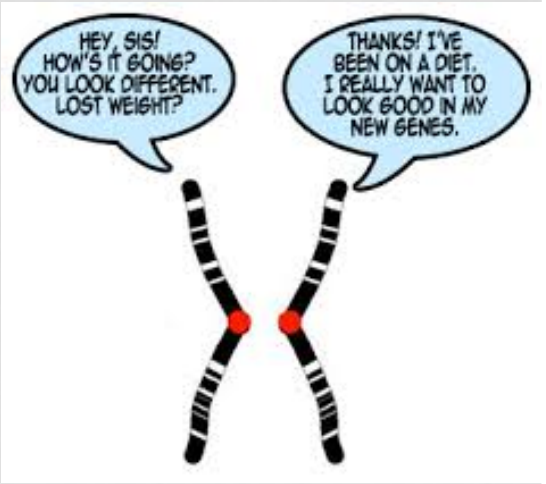
MANUAL ANESTHESIA – OLD SCHOOL BUT STILL EFFECTIVE IN MANY SITUATIONS

SPAWNING TO MAINTAIN GENETIC DIVERSITY

- How do you think this can be done?

GENETIC DIVERSITY

- Important to provide maximum contribution to the gene pool
- Eggtake should cover the entire length of the run
- Do not show favor to smaller / larger fish – keep a blind eye toward individual traits
- Pool gametes whenever possible



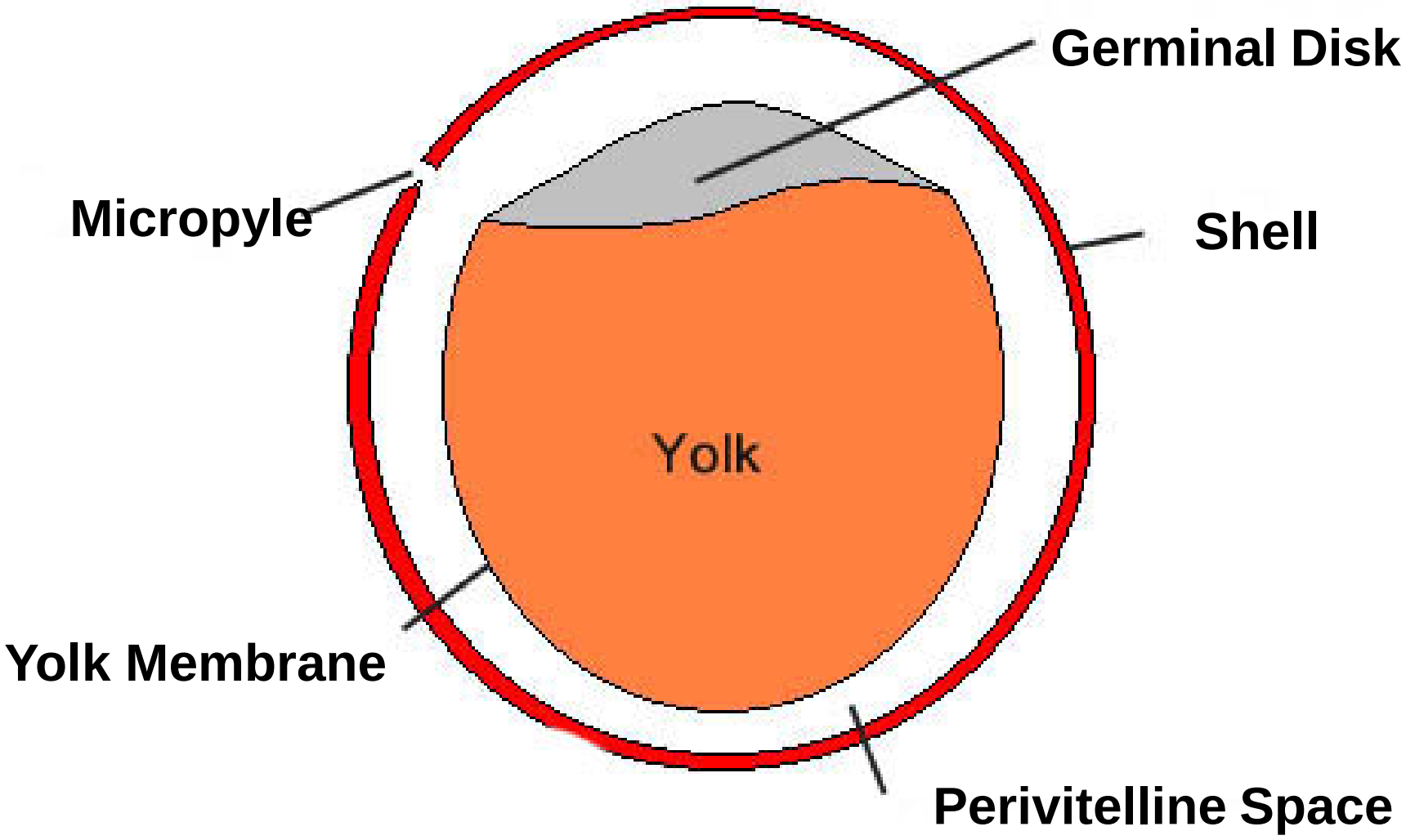
A SUCCESSFUL EGGTAKE REQUIRES LOTS OF "CONTEMPLATION"

PART 2 - EGGS!

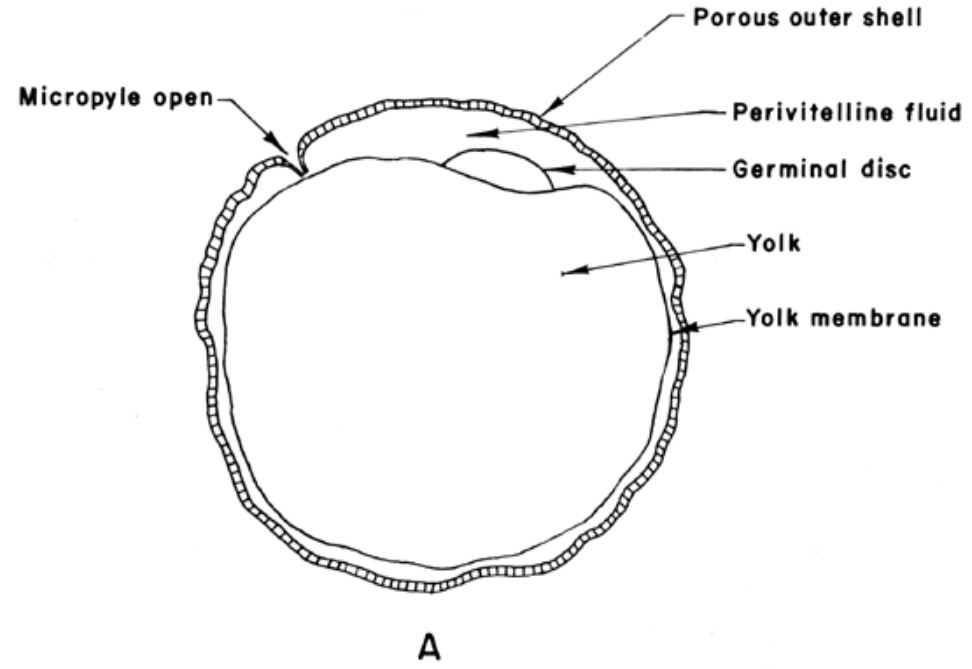
Important aspects of this segment:

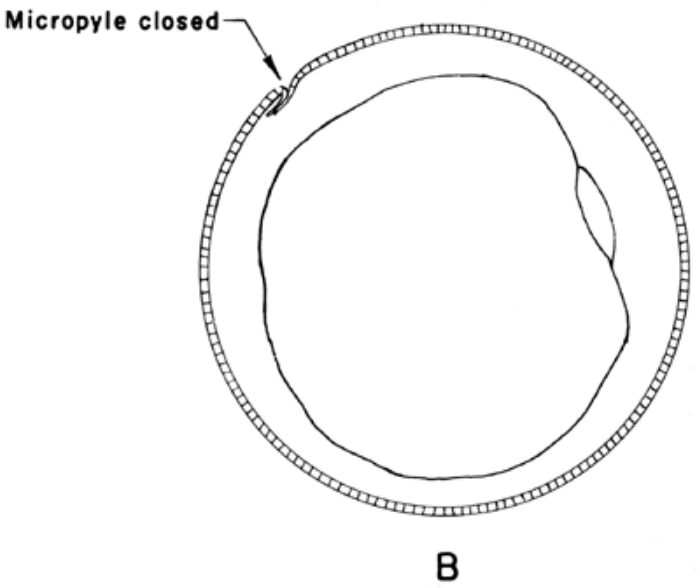
1. Describe the anatomy of a salmon egg – list the 5 key components
2. Explain why it is important to check for egg maturity in female salmon prior to spawning them.
3. Relate what "water hardened" eggs are and offer some reasons why this phenomenon happens.

STRUCTURE OF THE EGG



Within <u>minutes</u> of contact with water, the micropyle begins to close





Some definitions:

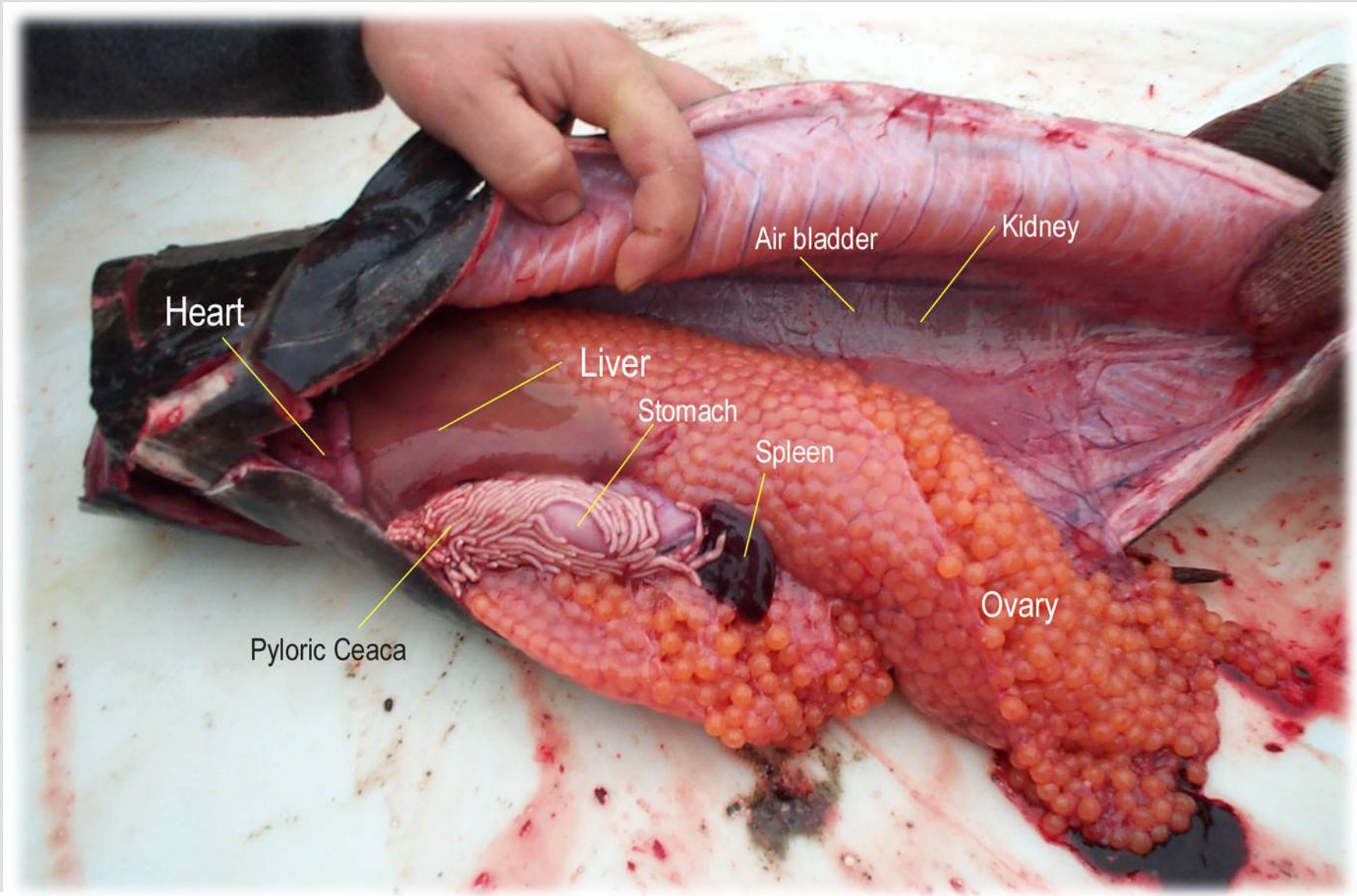
- **Micropyle** – Opening in the egg shell to permit entry of the sperm during fertilization. The sperm penetrates the yolk membrane to unite with the nucleus of the egg.
- **Shell** – Transparent and porous to allow exchange of gas, water and metabolites.
- **Germinal Disc** – Fertilization site
- **Perivitelline Space** – During water hardening process water enters through the shell and collects in the Perivitelline space making the egg rigid.
- **Yolk membrane** – Very thin and non-porous, when ruptured turns white indicating a dead egg.
- **Yolk** – Provides energy for the developing embryo.

IS THE FISH READY TO SPAWN?

Questions:
- what's wrong with having water in with the milt?
- How many sperm cells are there in milt?

- Males
  - In most cases males will be readily available and ripe in the presence of spawning females.
  - If scarce check for ripeness before killing. Males can also be used several times during a short period of time.
  - If scarce it also a good idea to pool the sperm in case a male is sterile.
  - It is good practice to expel a small bit of sperm before fertilizing eggs, it may include a small amount of water and bile.

Green vs. Ripe



CHECKING FEMALES

- Pressure on the abdomen and expressing some eggs doesn't always work
- The best way to determine if a female is ready to spawn is to check the firmness of the abdomen.
- Any indication of firmness will usually indicate a female that is not mature (green)
- This is a job for experienced personnel – too much at stake
- This step can be skipped if 90%+ of fish encountered are ripe. Saves a lot of time.

FEMALES

- Pinks and chum in SE Alaska tend to ripen quickly in freshwater
- Coho are particularly hard to check (thick sidewalls!)
- If "green", return to holding area and check within 7 days – depending on water temp
- Handle females with care
- Holding females too long is a problem. Oviduct muscles weaken and allow water to enter abdominal cavity

FEMALE PINK SALMON READY TO SPAWN – NOTE EGG EMERGING FROM OVIDUCT

Mature eggs, but note some are "water-hardened"


Water-hardened eggs
This guy's hands are a bit "water-hardened as well……

PART 3 – EGGTAKE PROCESS

Important aspects of this segment:

1. List 3 factors which can affect fertilization of salmon eggs during the spawning process
2. Describe why some facilities utilize a 7ppt saline solution during the eggtake process.

FISH KILLED

- Determine if they are ripe.
- Euthanize using one of the methods mentioned
- If using a fish club, be careful about the hit
- Some techniques require that you bleed the fish prior to spawning
- Handle dead females with some degree of care

WHAT THE HECK IS A ZAK KNIFE?

THIS is a zak knife

THIS is a border collie

GREEN EGG COLLECTION

Before cutting the female:

- **Make sure spawning container is clean/dry**
- **Keep tail out of spawn container – no blood!**
- **Then:**

1. Insert zak knife in vent
2. Pull upward toward head
3. Go outside of pelvic fin
4. Then back into center
5. Up to about pectoral fins

Some places use a chute/others spawn into a container

ODDS AND ENDS

- Some fish will be "shakers"
- There are a variety of techniques that can be employed during an eggtake
- Discard all "bad" eggs: water-hardened, broken
- Protect eggs from rain and sunlight.

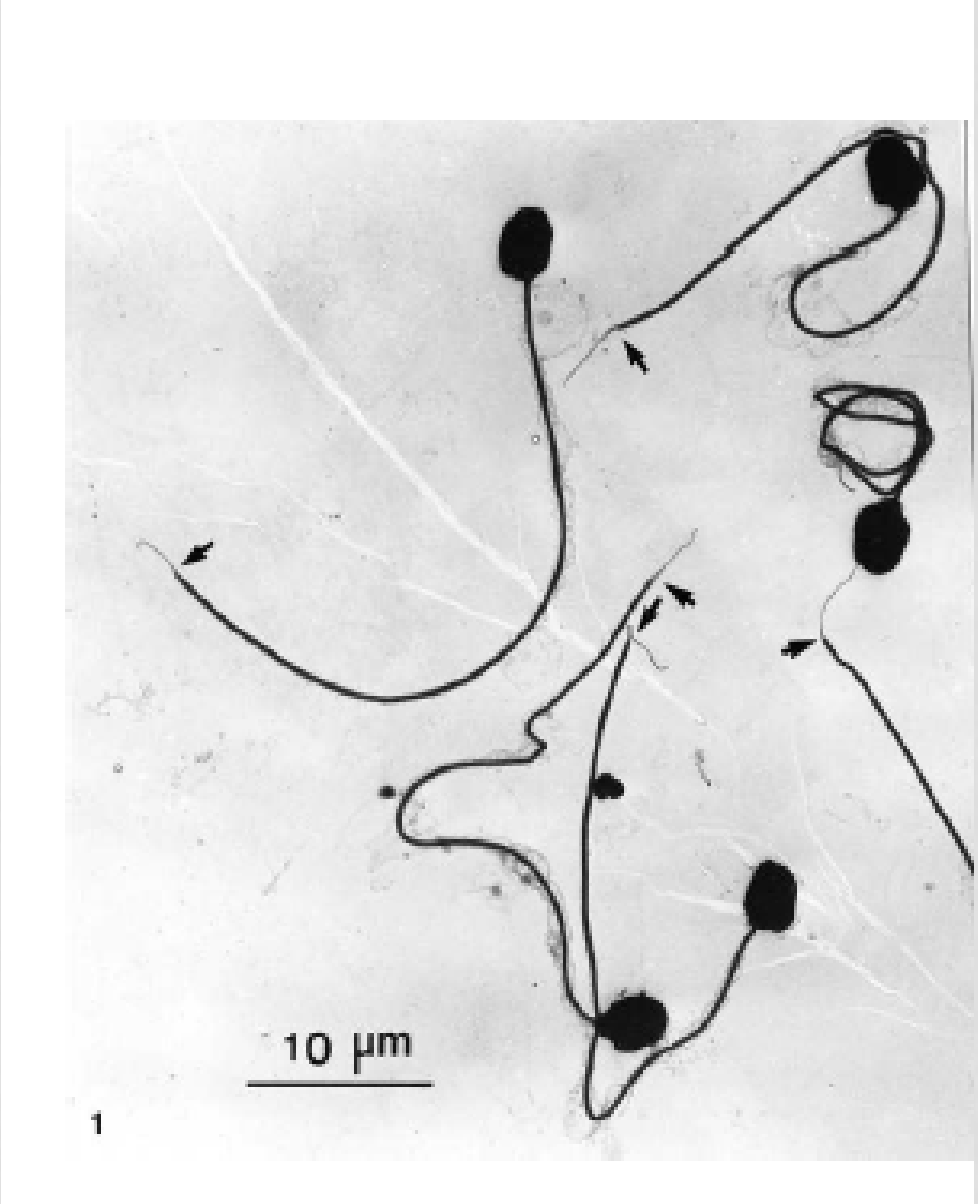
Family Tracking = treating each fish as an individual / isolating each "family", taking kidney tissue for ADFG pathology. Eggs are kept separate until results are in. Some extra steps:

- Disinfect hands and equipment between females during family tracking operations
- The ventral side of females should be wiped down with an iodine solution (1:100) to kill any virus or bacteria and prevent it from contaminating the spawned eggs.
- Lots of caution to insure against cross-contamination

WHAT HAPPENS WHEN WATER COME INTO CONTACT WITH SALMON GAMETES?

1. Magic!
2. A puff of smoke and then…nothing
3. It rains cats and dogs, not fish
4. Something else

Image of pacific salmon sperm cells. About 10k per ml (drop)



FERTILIZATION

- There are two methods of fertilizing eggs, dry and wet.
- Using the dry method sperm is added directly to eggs and mixed thoroughly to insure fertilization occurs. Often associated with remote eggtakes
- Using the wet method sperm is added to the eggs and immediately water or an activator is added to the mix and stirred gently. The added water activates the sperm which is very short lived in water.
- Male salmon are stripped by holding the fish head up tail down and stripped from the top of the body cavity to the vent expressing the sperm into the container of eggs.
- The male:female ration varies – generally speaking 1:1 is best for genetic diversity

ADDING MILT

Some facilities use a 7ppt saline solution during this process

FERTILIZATION

SPERM ACTIVATION

Use of 0.7% saline solution

FACTORS AFFECTING FERTILIZATION

- Exposure of eggs or sperm to water.
- Once the egg starts to absorb water the micropyle begins to close.
- Coagulated blood blocks the micropyle.
- Broken eggs.
- Presence of blood
- Poor technique, always mix well.
- Overripe females and males in poor condition.
- Warm water, 16 degrees C or above.

RINSING

- Need to remove all non-egg organics
- Most facilities will disinfect the eggs with iodophor.
- Rinse technique varies with eggtake style
- Get eggs into incubator fairly quickly

RINSING PINK SALMON EGGS

PART 4 – POST FERTILIZATION

Important aspects of this segment:

1. Understand what "iodophor" is and be able to calculate proper dosages for disinfecting eggs
2. Define "fecundity" and relate why it is an important aspect of the spawning process
3. Be able to enumerate total eggs taken during the course of an eggtake session

INCUBATOR LOADING

- Once again <u>be gentle</u>
- If possible, do a water to water transfer.
- Do not allow eggs to free fall through air into incubator.
- Some facilities disinfect newly fertilized eggs with iodine

Iodophor – your best friend……..

ARGENT Chemical Laboratories

ARGENTYNE

Buffered PVP Iodine Disinfectant

Argent invented Argentyne in 1978 to serve the growing demand for a high quality pH 7 buffered salmonid egg disinfectant. Today, Argentyne is preferred by hatchery managers nationwide for its high potency USP Grade PVP Iodine that keeps killing after other brands stop.

Contains 10% PVP Iodine equal to 1% free iodine. Argentyne may be diluted 1:100 for a 100-ppm free iodine solution.

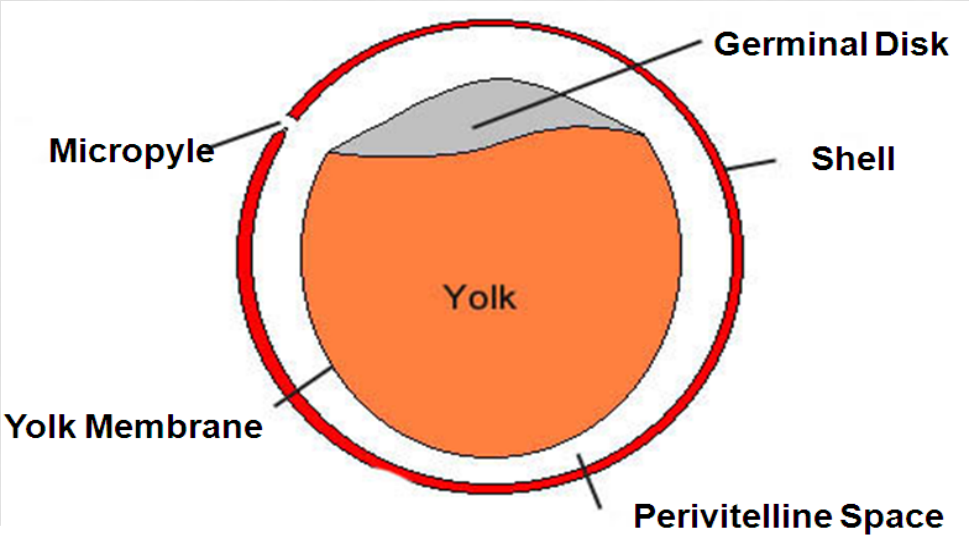
Packing	List Price	**SALE Price**
6 x 1 gal/case	$132.00/case	**$118.00/cs**
55 gal/drum	$990.00/drum	**$825.00/dr**

Special Offer: 12 cases $109.00/cs

2014 pricing:
$188/6 gal

WATER HARDENING

- Water on contact with the egg slowly enters the egg through the shell into the perivitellene space.
- Process takes anywhere from 30 – 90 minutes depending on temperature.
- As the egg absorbs water the micropyle will begin to close, once closed it is not possible for the egg to be fertilized.
- Eggs are considered safe for handling after 60 minutes and can be shipped at this time.
- During the water hardening process eggs are very tender and susceptible to physical shock resulting in mortality.
- Eggs swell and increase in diameter by about 25% - leave room for this!



Egg Tenderness

Time 0

Very tender

1 – 2 hrs

Can be handled

48 hrs

Very tender

Visible eye

Durable

Oxygen Demand

Time 0

Increasing

Ponding

EGG DISINFECTION

- All eggs shipped to or from a hatchery need to be disinfected for (at least) 10 min. in a buffered iodophor solution of 1:100.
- Disinfection can be flow through or static bath.
- What is the difference?
- Disinfection is performed to prevent the spread of pathogens. Kills bacteria, viruses, fungi and mold.
- Chinook, Coho and Sockeye are normally disinfected while water hardening in a buffered iodophor solution for 60 minutes. Disinfect eggs in isolation from incubation areas.

EGGTAKE QUESTIONS

- Questions about sorting? Euthanasia?
- What do you do with the carcasses?
- Kidney tissue from both male and female?
- Other pathogens we sample for?
- Single male or multiple/female in tracking?
- Incubation criteria aside from good rinsing?
- Use of iodine by culturists
- Is water in the Heath trays controlled in any way?
- Why do we wipe down females with iodine?

METRIC SYSTEM 101 …..

- How many milliliters in a Liter?
- How many liters in a gallon?
- How many milliliters in a gallon?
- Displacement: converting solid measurements to liquid: 1 gram = ?

FIGURING OUT VOLUME

- How can we calculate rate of flow of water?

- If you now know the flow, how can you figure out a certain volume?

QUICK CALCULATION

- You have 10 liters of water
- Make an iodophor solution of 1:100
- What would you do?

IODOPHOR TREATMENT CALCULATIONS

Flow through 10 min treatment

- Incubator flow = 15 gpm = 56.8 liters (3.78L/gal)
- 56.8l = 56,800 ml/100 = 568 ml (1L = 1,000ml)
- 568 ml of concentrated, buffered iodophor x 10 min. = 5.68 liters per 10 min. treatment.
- Deliver 568 ml of iodophor/ minute of flow to incubator.

Static Bath

- Mix up a stock solution of 1:100 buffered iodophor.
- 35gal garbage can = 133,000 ml/100 = 1.33L of iodophor to make stock solution.

FECUNDITY – WHAT IS IT? HOW IS IT CALACULATED?

- What is it?

- How would you figure it out?

FECUNDITY

- Fecundity is the number of eggs per female.
- Divide total eggs taken/females spawned.
- Example: 1,880,000 eggs/800 females = fecundity of 2,350 (eggs per female)
- Averages for local stocks
  - Chinook 5,000 – 6,000 eggs
  - Coho 3,000 – 4,500 eggs
  - Summer Coho 1,800 – 2,200 eggs
  - Sockeye 2,800 – 3,500 eggs
  - Chum 1800 - 2500 eggs
  - Pink 1,500 – 2,000 eggs

GREEN EGG ENUMERATION

- Green eggs collected are estimated daily during the egg take to monitor progress to reaching egg goal.
- Three common methods for enumerating eggs are:
  - Volumetric
  - Displacement
  - Weight

VOLUMETRIC SAMPLES

- Eggs are spawned into a bucket of known volume. Markings should be obvious (like 1L lines)
- Count total # buckets in a day – get total number of liters of eggs taken
- At least 3 @ 100 ml samples of fertilized eggs should be collected randomly throughout the course of the egg take.
- Eggs in each sample are counted by hand using a tally whacker yielding number of eggs/ml.

EXAMPLE OF EGG ENUMERATION

- Total buckets = 120
- x 10L/bucket = ? liters
- Samples:
  - Sample 1: 100ml = 343 eggs = eggs/ml
  - Sample 2: 100ml = 321 eggs = eggs/ml
  - Sample 3: 100ml = 333 eggs = eggs/ml

- Total liters =
- Find total milliliters
- Find average eggs/ml from samples
- Find total number of eggs taken

A QUICK TOUR OF EGGTAKING OPS

CHUM EGGTAKE FACILITY

CHUM EGGTAKE FACILITY


Sheldon Jackson
Hatchery Pink salmon
eggtake

FAMILY TRACKING

- Sometimes facilities need to isolate individual fish for spawning
- Bacterial Kidney Disease
- How would this impact the eggtaking process?

CHINOOK EGGTAKE FACILITY

CHINOOK SPAWN - BLEEDING

CHINOOK EGGTAKE - IODOPHOR

CHINOOK SPAWN - ACTIVATOR

CHINOOK FEMALE SPAWN

CHINOOK SPAWN - MALE

CHINOOK SPAWN – CWT OPS

EGGS INTO INCUBATOR

CHUM EGGS TO INCUBATOR

CARCASS DISPOSAL – GOTTA DO SOMETHIN' WITH ALL THOSE BODIES

ASSIGNMENT 4: DUE 10/6/15

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  - Write a brief summary of your findings including any questions you may have about the information.
  - List some differences that exist for taking sockeye eggs vs. other species of salmon
- Read pages 21-29 in the ADFG Fish Culture Manual (related to eggtake procedures)
  - Write a brief summary of your findings – questions, inconsistencies with what you've learned