



DIAGNOSTICS & HEALTH MONITORING

Carrie Barton & Christine Lieggi
5th Annual International Zebrafish Husbandry
Course



PART I

Bio-Security and Quarantine

BIO-SECURITY



Bio-security is the protection of animals or humans from any type of infectious agent -- viral, bacterial, fungal, or parasitic.

- **People** can spread diseases as they move within a facility and from one facility, room or system to another.
- **Animals or equipment** introduced into a facility can bring pathogens with them.
- Among the many bio-security procedures that can prevent these types of disease transmission are such measures as use of **protective clothing, waiting periods for new animals and visitors, and cleaning.**

BIO-SECURITY

Bio-security is the protection of animals or humans from any type of infectious agent -- viral, bacterial, fungal, or parasitic.

- **People** can spread diseases as they move within a facility and from one facility to another.

- Husbandry staff
- Research staff
- Facility Services
- Contractors
- Janitors



BIO-SECURITY

Bio-security is the protection of animals or humans from any type of infectious agent -- viral, bacterial, fungal, or parasitic.

- **Animals or equipment** introduced into a facility can bring pathogens with them.
- Incoming animal shipments
- Shared lab equipment
- Tools



BIO-SECURITY

Bio-security is the protection of animals or humans from any type of infectious agent -- viral, bacterial, fungal, or parasitic.

- Among the many bio-security procedures that can prevent these types of disease transmission are such measures as use of **protective clothing, waiting periods for new animals and visitors, and cleaning.**
 - Dedicated shoes, gloves, gowns
 - Quarantine protocols and testing guidelines
 - Facility entry guidelines
 - Sanitation SOP's



QUARANTINE

Goals

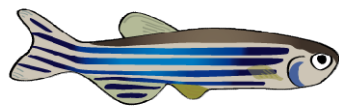
- Supply researchers with resources (Fish)
- Maintaining health of colony
- Prevent disease/parasite introduction
- As quickly as possible
- Quick detour vs. road block



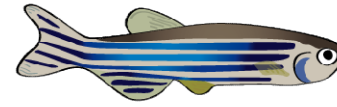
TRADITIONAL QUARANTINE PROTOCOL

- Import adult fish (no vet or health certificate required)
- Observe fish for two weeks for signs of illness
- Spawn fish, surface bleach embryos and raise in main facility

Vertical vs. Horizontal Transmission



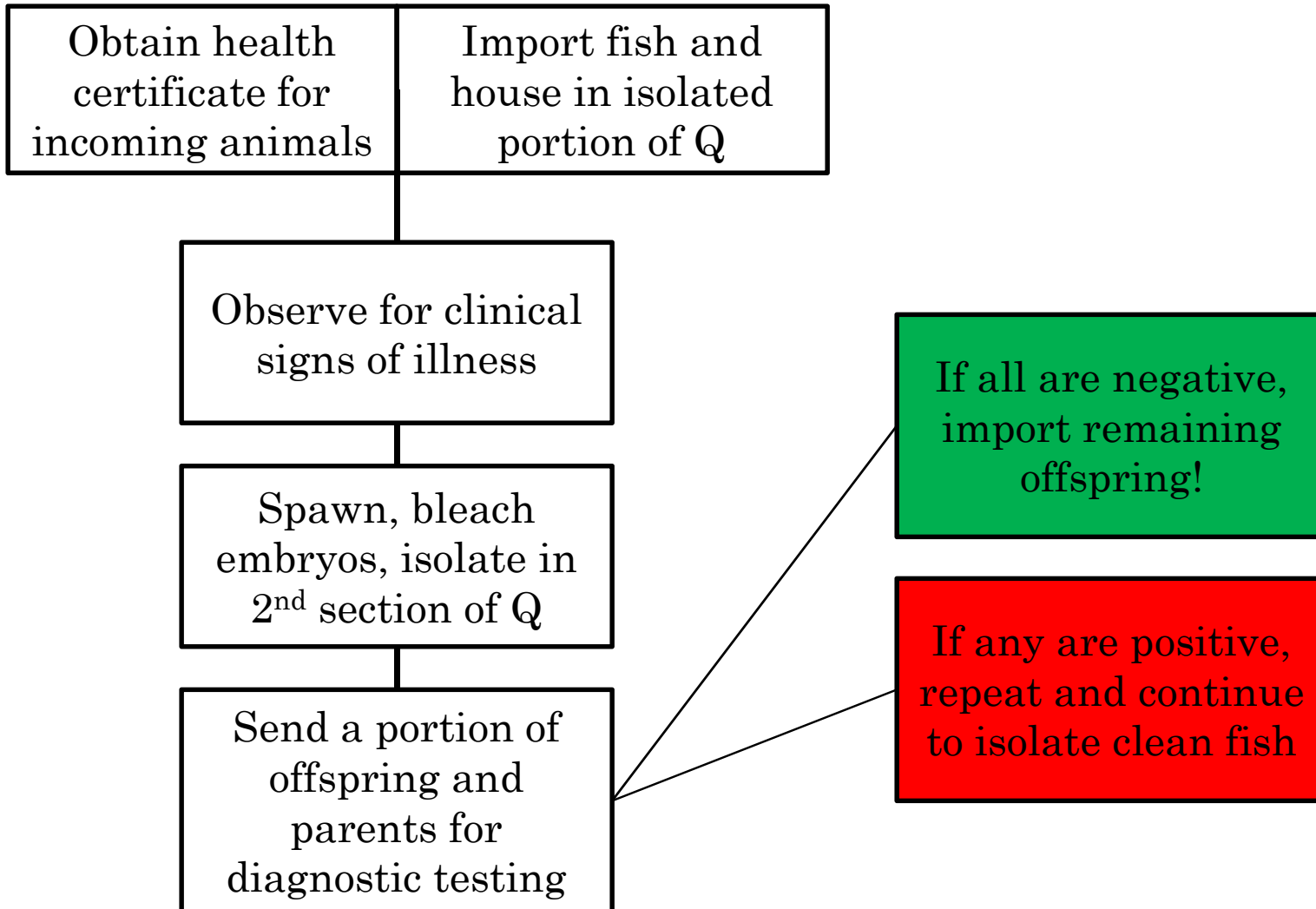
Tank Mates



Offspring

Concentrations of bleach safe for embryos are not effective against a number of pathogens and parasites

NEW QUARANTINE METHODOLOGY



IDEAL QUARANTINE SET-UP

- Separate from main facility
- As much separation of staff as possible
- Within Q, separation available for different groups of fish
- All supplies either disposable or can be autoclaved
- Easy access to sanitation equipment
- Disposable PPE (gowns, gloves, shoe covers)
- Flow through water or separate stand alone recirc. systems
- Diagnostic testing plan



EXAMPLE OF QUARANTINE PROGRAM

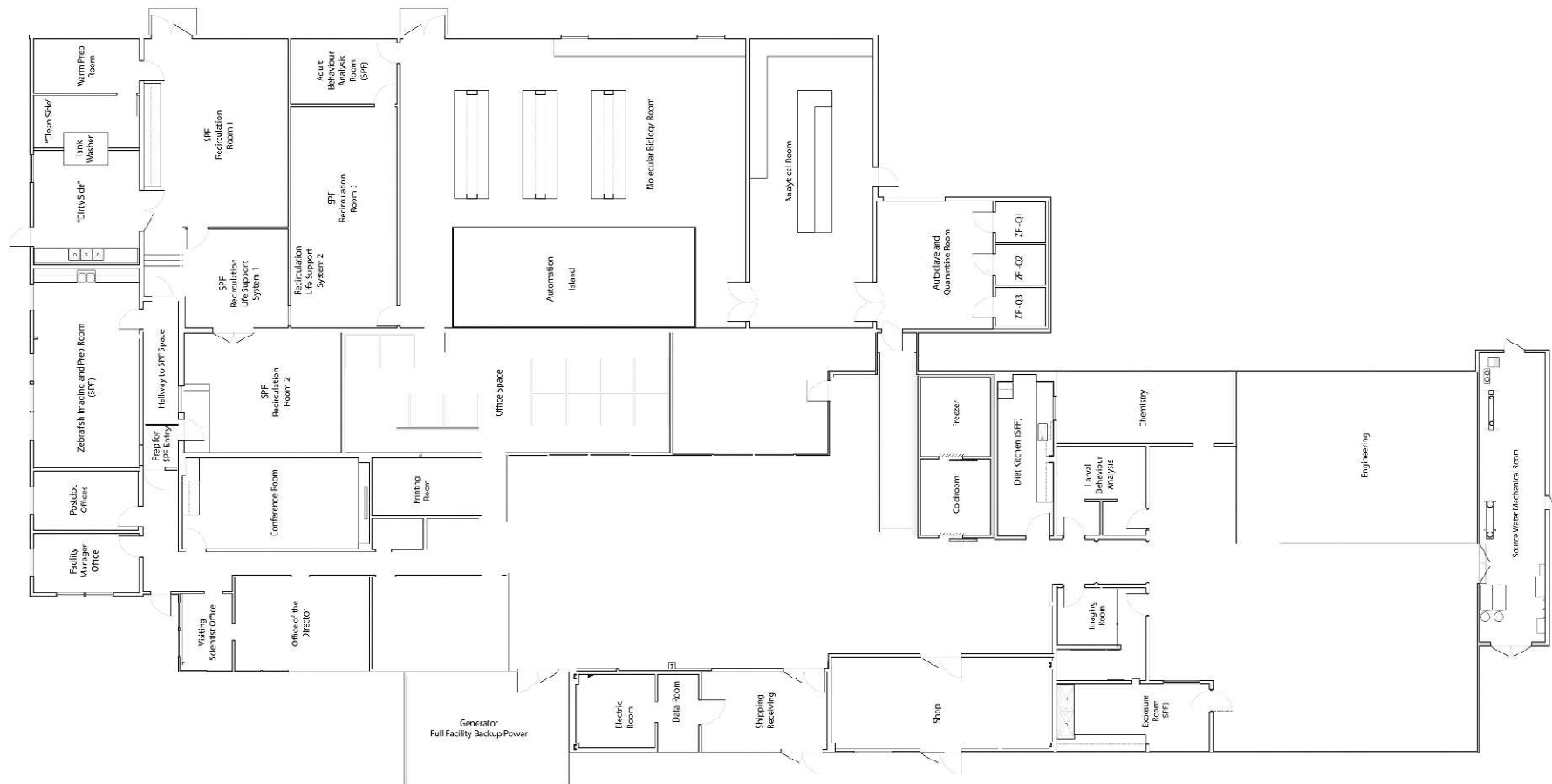
Inside Each Unit:

- rack w/ emergency air
- Independent light cycle, heat, and air processing
- Flow through water supply
- Work station w/sink for breeding, tank cleaning, etc.
- Storage for all unit specific supplies



QUARANTINE PROXIMITY WITHIN FACILITY

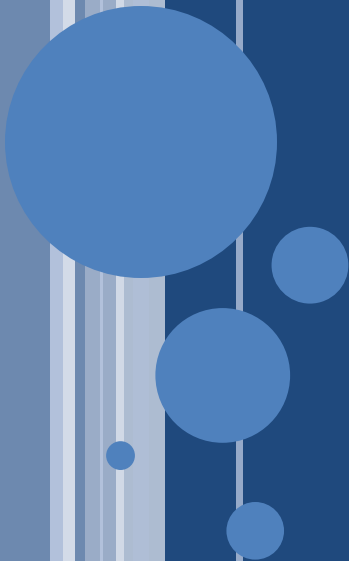
Maintain appropriate separation from colony



Maintain appropriate separation from colony



COLONY HEALTH MONITORING



INVEST IN YOUR STAFF



International
FishMed Conference
on Zebrafish Research

March 18-19, 2016, Warsaw, Poland
www.fishmed2016.pl

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OVPRED 500 - Zebrafish Online Husbandry
Education

Delivery Options: Totally online, instructor-led

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Animal Care and Use in Research and Education

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Animal Care and Use Courses

Working with Laboratory Zebrafish

Content: Concepts and procedures for working with laboratory zebrafish.

Audience: Technicians, researchers, and anyone who works with zebrafish in a research laboratory setting.



COMPONENTS OF COLONY HEALTH MONITORING

- Daily health monitoring
- Ongoing trend monitoring
 - Morbidity
 - Mortality
 - Reproduction
- Evaluation of sick fish
- Sentinel program
- Environmental Monitoring



DAILY MONITORING

- Daily observation of tanks
 - Check for sick & dead fish
 - Document location
 - Removal
- Identify tanks that need cleaning
- Check UV lamp!



Hello?



WATCH CLOSELY!



○ Behavior

- Swimming behavior
- Location within water column
- Flashing



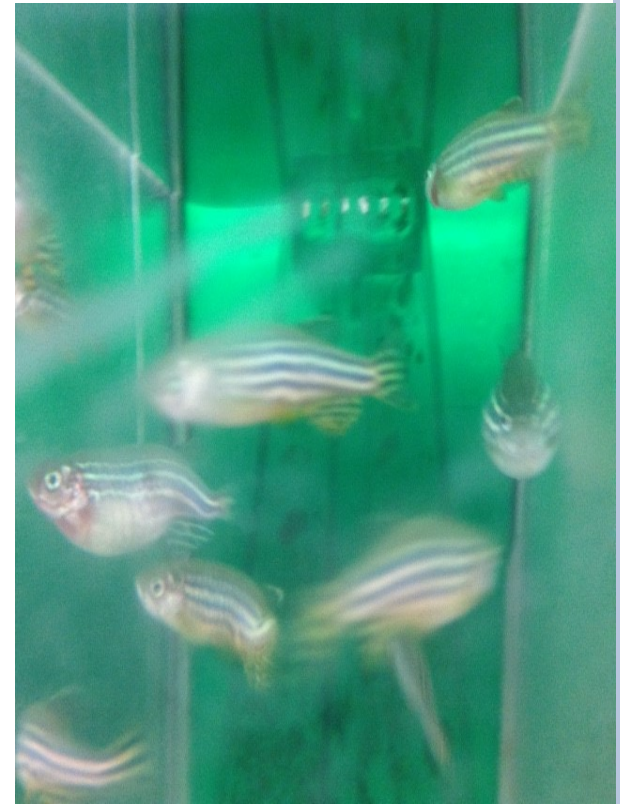
○ Body Condition

- Emaciation
- Deformities
- Swollen



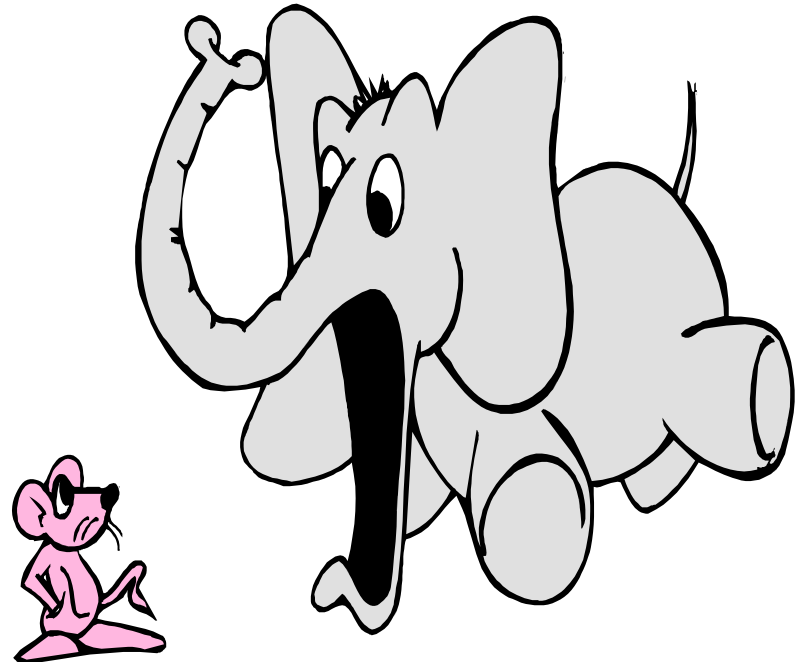
BODY SURFACES

- Discoloration
- Ulcerations
- Scales/fins



EVALUATION OF SICK FISH

- Remove from re-circulating system
 - Flow through tanks
 - Static tanks
 - Euthanasia
- What's the problem??
 - Infectious
 - Non-infectious causes
 - Diagnostics needed



To be continued.....



TREND MONITORING

- Review morbidity logs
- Review mortality logs
- Regular review of DOF- What is your cull date?
 - Higher potential for pathogens
 - **Submit for evaluation if possible**

ZRC / MAIN Date: ~~2015~~ / 2015
JANUARY

SICK FISH ANIMAL LOG 1/2

LOCATION: SYSTEM 1 RACKS: 1-25

RACK	WEEK 1	WEEK 2	WEEK 3	WEEK 4	TOTAL
1		10, 11, 12, 13		21	4
2					
3					
4					
5		10, 11, 12, 13	14, 15, 16	17, 18, 19, 20	9
6	21, 22	23, 24	25		3
7	26, 27		28, 29	30	4
8					
9					
10				31	1
11					
12					
13					
14	1, 2		3, 4	5, 6	3
15			7, 8		1
16					
17					
18	9, 10				1
19					
20					
21		11, 12, 13	14		3
22					

BRB / MAIN Date: JUNE/2016

SICK FISH ANIMAL LOG 1/2

LOCATION: SYSTEM 1 RACKS: 1-25

RACK	WEEK 1	WEEK 2	WEEK 3	WEEK 4	TOTAL
1					
2					
3			1	12, 13, 14, 15	4
4					
5					
6			16, 17		1
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

COMMENTS:

FECUNDITY MONITORING

- Early indicator for problems
 - Water quality
 - Nutrition
 - Light pollution
 - Disease
 - Subclinical
 - Mild clinical signs

RARC BREEDING DATA CONTROL

% FERTILIZATION = is adjusted to the closest value in increments of 5%

When number of embryos count are =

< 100	< 100	The real value X is shown on tables
100	100	The real value X is between 100 and 150 (100 will be used as the average number).
150	150	The real value X is between 150 and 200 (150 will be used as the average number).
200	200	The real value X is more than 200 (200 will be used as the average number).

SYSTEMS = 2

PAIRS (TANKS) SET UP/SYSTEM = 5

BREEDING RATIO = 1:1

DATE	INITIALS	# TANKS WITH EGGS			# TOTAL COLLECTED EGGS			% FERTILIZATION				# EMBRYOS/ DAY	AVERAGE EMBRYOS/ FEMALE (>0% Fert ONLY)	AVERAGE EMBRYOS/ PAIR SET UP	COMMENTS
		S 1	S 2	TOTAL	SYSTEM 1	SYSTEM 2	AVERAGE EGGS/FEMALE	SYSTEM 1	SYSTEM 2	AVERAGE					
										ALL	>0% FERT. ONLY				
10/1/2015	RH	2	3	5	46	82	26	92.5%	76.7%	84.6%	83%	106	21	11	
10/6/2015	RH	2	2	4	83	156	60	50.0%	27.5%	38.8%	39%	89	22	9	
10/22/2015	RH	3	2	5	269	222	98	40.0%	80.0%	60.0%	56%	275	55	27	
11/6/2015	RH	0	2	2	0	34	17	0.0%	100.0%	50.0%	100%	34	17	3	New fish stock being used for first time
11/19/2015	RH	1	3	4	52	350	101	90.0%	90.0%	90.0%	90%	344	86	34	
11/27/2015	RH	1	0	0	16	0	16	100.0%	0.0%	50.0%	100%	16	16	2	
12/3/2015	RH	5	3	8	696	245	118	98.0%	100.0%	99.0%	99%	931	116	93	
12/17/2015	SH	5	4	9	715	500	135	80.0%	83.8%	81.9%	82%	944	105	94	
2/17/2016	SH	2	4	6	300	460	127	85.0%	75.5%	80.3%	79%	576	96	58	
2/24/2016	SH	3	3	6	420	420	140	97.0%	94.2%	95.6%	96%	787	131	79	
3/2/2016	SH	3	1	4	550	150	175	100.0%	66.6%	83.3%	92%	650	162	65	
3/30/2016	SH	4	3	7	550	550	157	94.4%	78.8%	86.6%	88%	951	136	95	
4/6/2016	SH	2	4	6	350	600	158	100.0%	99.6%	99.8%	100%	947	158	95	New fish were used for system 2
4/12/2016	SH	3	4	7	200	600	114	100.0%	95.8%	97.9%	98%	766	109	77	
4/19/2016	SH	5	5	10	750	1000	175	96.8%	92.6%	94.7%	95%	1654	165	165	
4/26/2016	SH	3	3	6	400	450	142	93.3%	98.3%	95.8%	96%	820	137	82	
5/6/2016	SH	4	5	9	500	750	139	95.8%	97.0%	96.4%	96%	1220	136	122	
5/11/2016	SH	4	4	8	590	600	149	92.9%	96.4%	94.6%	95%	1107	138	111	
5/17/2016	SH	3	4	7	550	800	193	93.3%	96.3%	94.8%	95%	1283	183	128	

WHAT ARE SENTINEL FISH?

- Fish placed on system to serve as indicators of disease
 - Exposed to system water
 - Sacrificed for diagnostic evaluation



WHY DO I NEED SENTINELS FOR HEALTH MONITORING?

- Early and Immediate Action
 - Determine presence of subclinical disease
 - Contain or control disease between fish
 - Limit transfer of fish to/from specific areas
 - Know about **zoonotic** diseases
- Monitor prevalence of known diseases
 - Researchers should report health conditions in publications
 - Impact on research
 - Important for fish sharing



HOW MANY FISH DO I NEED TO SUBMIT?

- Statistical formula assumes equal opportunity of infection for all fish
 - Various ages, genetic background, immune status, etc.

$$n = \left[1 - (1 - p)^{\frac{1}{d}} \right] \times \left[N - \left(\frac{d}{2} \right) \right] + 1$$

Table 1

Sample sizes required for a population (N) to detect at least one infected animal assuming a prevalence (P), with 95% confidence

N	P											
	0.5	0.4	0.3	0.25	0.2	0.15	0.1	0.05	0.02	0.01	0.005	0.001
10	4	5	6	7	8	9	10	10	10	10	10	10
20	5	6	8	9	10	13	16	20	20	20	20	20
50	5	7	9	10	13	16	22	35	48	50	50	50
100	5	7	9	11	14	18	26	45	78	96	100	100
200	5	7	9	11	14	19	27	51	105	156	191	200
300	5	7	9	11	14	19	28	54	118	190	260	300
400	5	7	9	11	14	19	28	55	125	211	311	400
500	5	7	9	11	14	19	29	56	129	225	349	500
600	5	7	9	11	14	19	29	57	132	236	379	597
700	5	7	9	11	14	19	29	57	134	243	403	691
800	5	7	9	11	14	19	29	57	136	250	422	782
900	5	7	9	11	14	19	29	58	138	255	437	868
1000	5	7	9	11	14	19	29	58	139	259	451	951



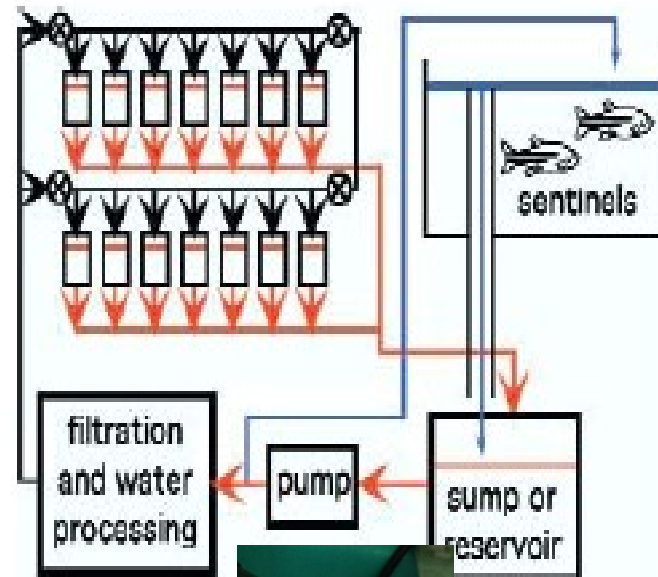
THAT'S A LOT OF FISH!

- # required for pathogens of low prevalence often impractical
- Use of pre-filtration sentinels increase chances of detecting infection
- # typically limited by financial constraints
- What do I do?



USE SENTINEL TANKS

- Pre-Filtration
 - Determine what is in your system
- Post-filtration
 - Determine effectiveness of filtration

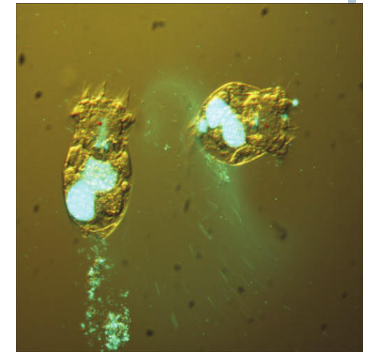


ZIRC



ENVIRONMENTAL MONITORING

- Contributes to overall colony health
- Live food cultures
 - Over 50% of microsporidia genera infect aquatic organisms
 - *Paramecium* and *Rotifers* ingest *Mycobacterium spp.*
- Dry food
 - Protein source is often fish meal
- Efficacy of disinfection procedures
 - Standard in rodent facilities

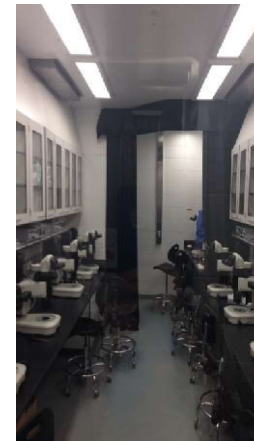
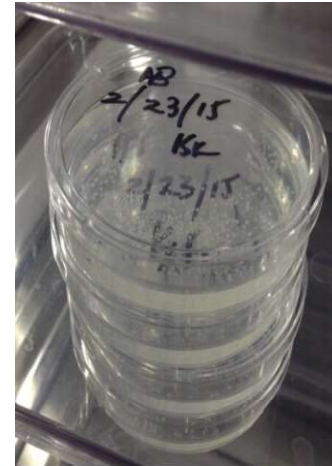




DIAGNOSTIC SAMPLES & TECHNIQUES

DIAGNOSTIC SAMPLES

- Fish
- Embryos
- Feces
- Water
- Equipment



DIRECT EVALUATION OF FISH



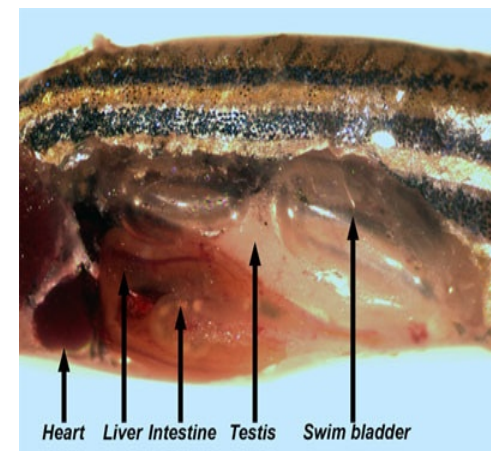
- Alive preferred for submission
- Moribund fish may have > value than sentinels
- Sentinels more value than healthy colony animals
- Older animals > likelihood of infectious/non-infectious disease
- Research colony animals
 - Only pathogens of high prevalence
 - Variable genetic background
 - Variable experimental manipulations



COMPLETE NECROPSY + HISTOPATHOLOGY + PCR



- Gross external exam
 - Parasite evaluation (gill & fin clips)
- Sterile culture collection
- Gross internal examination
- Collection of organs
 - Half frozen half fixed*
 - Freeze abnormal tissues
 - PCR
- Histopathology with special stains



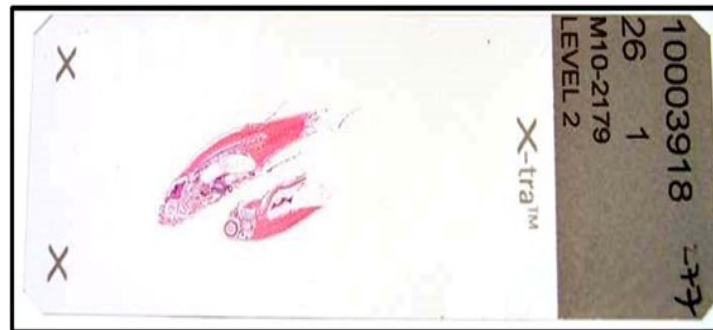
COMPLETE NECROPSY WITH HISTOPATHOLOGY VS PCR

Advantages

- Can look at entire animal
- Greatest sensitivity for widest range of diseases
- Potential to do follow-up PCR from blocks

Disadvantages

- **Can miss low burdens**
- **Lacks specificity**
- Long turn-around time
- Requires rapid evaluation upon death or euthanasia



PCR VS HISTOPATHOLOGY

Advantages

- Sensitive and Specific
- Fast turn-around time
- Wide variety of samples
 - Whole fish
 - Embryos
 - Water
 - Live feeds
- Can be evaluated at later time
- Can pool fish samples to decrease cost

Disadvantages

- Frozen samples give best results
 - Need to plan ahead
- Testing for specific pathogens only
- Only detects nucleic acid
- Need to evaluate appropriate samples
- Non-infectious conditions not detected



ENVIRONMENTAL SAMPLES

- Tank detritus- non-lethal pathogen testing
 - Useful for pathogens spread in feces or found in biofilms
 - Microscopic evaluation
 - PCR
 - *P. tomentosa*
 - *Mycobacterium spp.* and other bacteria
- Food- pathogen screening
 - PCR
 - Live food
 - Dry food

Caution with negative results!



WATER SAMPLES

- Culture

- Fungal
- Bacterial

- PCR

- Submission of filters
- Effective for detection of *Mycobacterium* spp. and other bacteria
- Spawning water for detection of *P. neurophilia*
- Not equally effective for all pathogens

- Water Quality tests

- Cause for illness may not be infectious



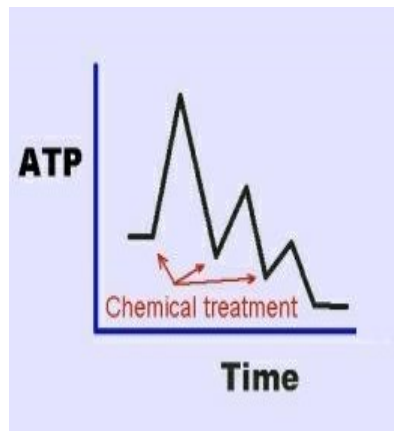
SO WHAT SHOULD I DO FOR MY SENTINEL PROGRAM?

- Customize to your program
- Determine current health status
- Determine your pathogens of concern
 - What do you want to keep out?
 - What requires immediate treatment/action?
 - What is the timeline of infection?
- Determine budget
- Combination of techniques & frequency of testing
 - If you already have it, do you need to test for it frequently?
 - PCR or Histo or both?

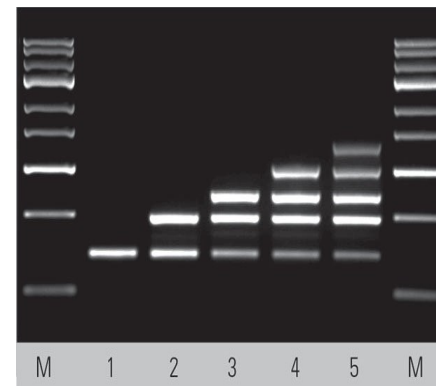


ENVIRONMENTAL MONITORING OF EQUIPMENT

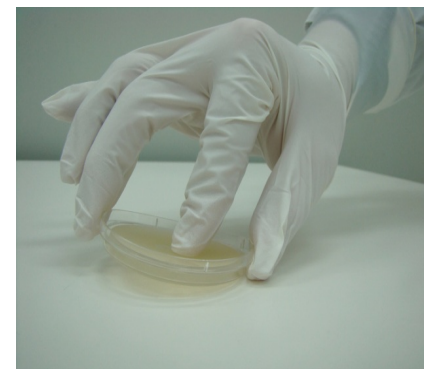
- Housing tanks
- Breeding tanks/devices
- Laboratory equipment



PCR



RODAC PLATE



Infectious diseases aren't only cause for sickness!

- Water Quality
- Toxin screening- water, fish, embryos
 - Chlorine
 - Heavy metals
 - Plasticizers
- Environmental conditions
 - Light cycle
 - Vibrations
- Genetics
 - Inbreeding



ENVIRONMENTAL MONITORING

<i>Sample site</i>	<i>Mycobacterium spp.</i>
Biobed	+
Buffer tanks	+
Computer keyboard in fish housing room	+
Embryo collection egg strainer	–
Euthanasia chamber: interior	+
Euthanasia chamber: top and handle	–
Floor: dirty side washroom	–
Floor: facility entrance PPE station	–
Floor: under rotifer cultures	+
Footwear: bottom of facility-provided plastic dedicated footwear (PPE)	–
Handle on door to clean equipment cabinet	+
Hose attached to embryo medium carboy: water outlet end	–
Hose attached to RO unit: water outlet end	–
Hose for filling breeding cages: biofilm from cut cross section	+
Hose for filling breeding cages: water outlet end	+
Rotifers from vendor	–
Rotifers from laboratory culture	+
Rotifer culture bucket biofilm	+
Rotifer strainer	–
Microscope	–
Water inlet valve for housing tank	+

Summary data from sites sampled over a 6-month period from June 2013 to January 2014. *Mycobacterium* species presence was tested using PCR assays by a University of Oregon laboratory specializing in bacteriology. + indicates that this area was positive for mycobacteria at least once.

– indicates that all samples from this area were negative for mycobacteria.





PART III

In Room Implementation

DEFINE PPE REQUIREMENTS

PPE should serve two purposes!

Protection for your staff from your animals, and protection of your animals from your staff.

Which PPE to use may be determined by the health status of your colony.

Are zoonotic diseases present in your facility?

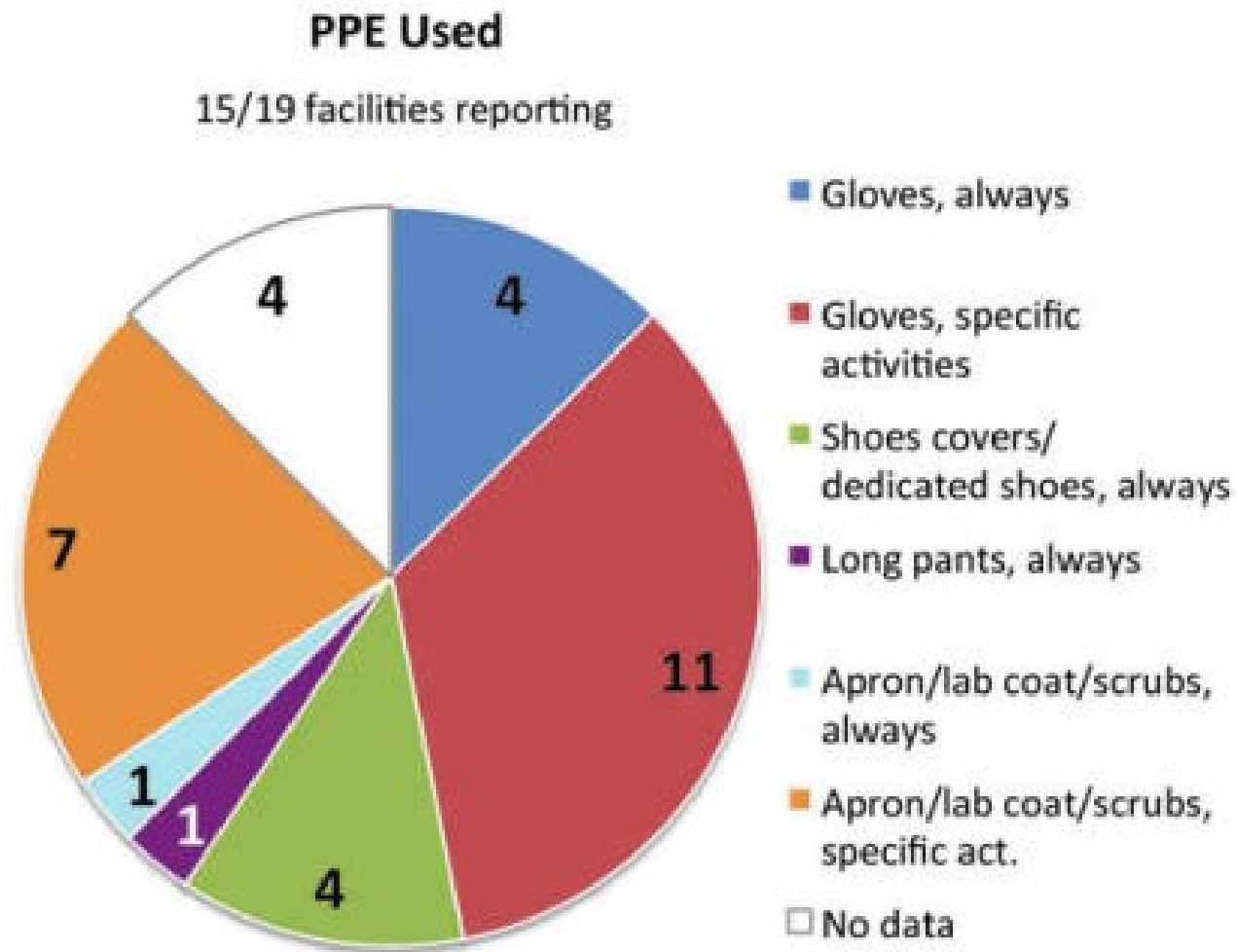
What are you trying to keep out of your colony?

Gloves
Dedicated Shoes
Scrubs
Safety glasses

Gowns
Shoe covers
Lab coats
Ear protection



PPE USAGE SURVEY



<http://online.liebertpub.com/doi/pdfplus/10.1089/zeb.2016.1309>

PPE TRAINING

What items to use in which room

How to put each item on and take it off

How to dispose of each item



What's wrong with this picture??



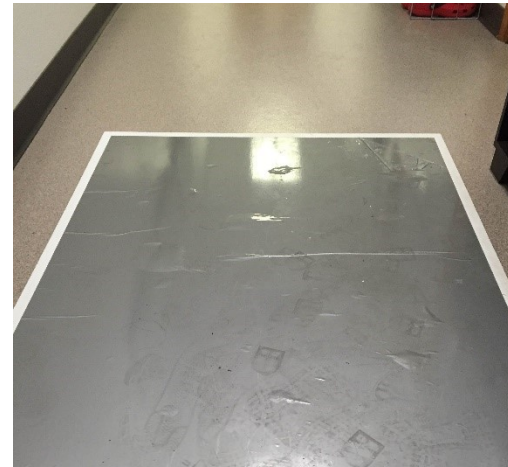
STICKY MATS, DEDICATED SHOES, AND SHOE COVERS

Efficacy of shoe sanitation, covers and mats is widely debated

Little to no published data relating to aquatic facilities

Published data from rodent facilities is contradictory and highly variable

Published data from hospitals suggest Efficacy is depended on type of organism



ENTRY POLICY CONSIDERATIONS

- Wait times for people

- 24 hours between contact – no exceptions
- Declaring contact with other aquatic colonies



- Are animals allowed to leave the fish rooms and return?

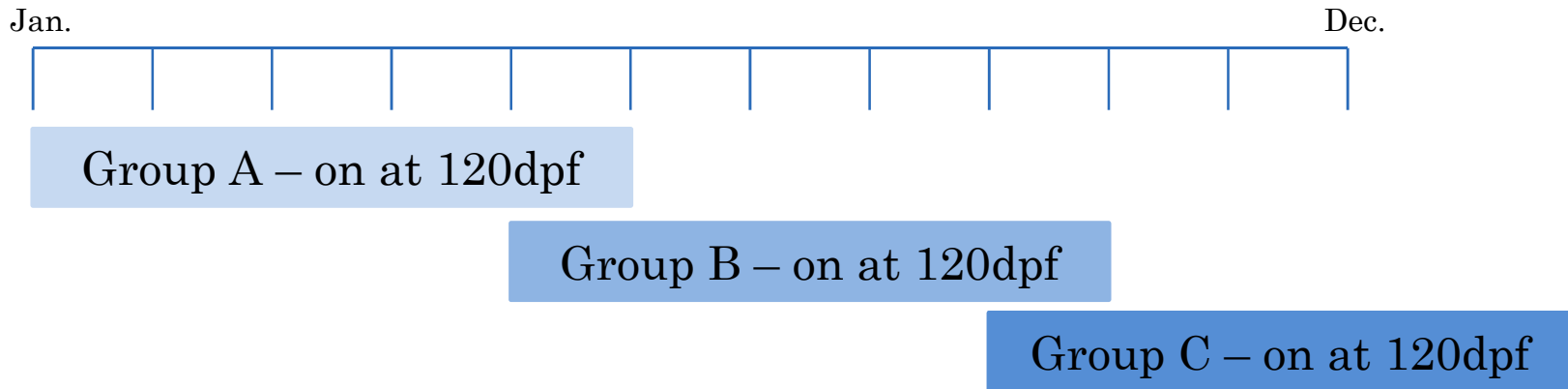
- Why type of experiments are being done?
- Are procedure rooms within the facility?
- How long can animals be off of the system?



SENTINEL PROGRAM

- Determine relevant sample #'s and schedule

- Groups should overlap



- Determine line used for sentinel program

- Most commonly used line? Most susceptible line? Multiple lines?

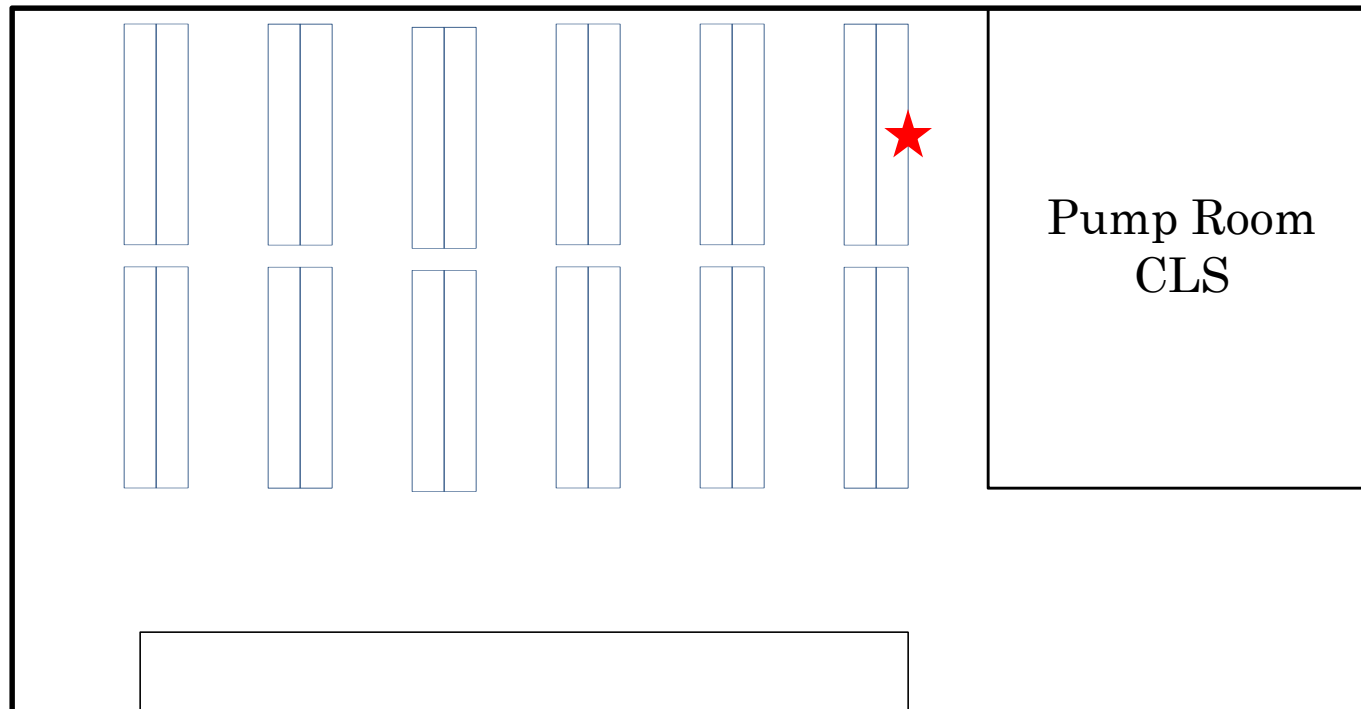
- Choose approach

- Pre filtration, post filtration or both?



SENTINEL PROGRAM - IMPLEMENTATION

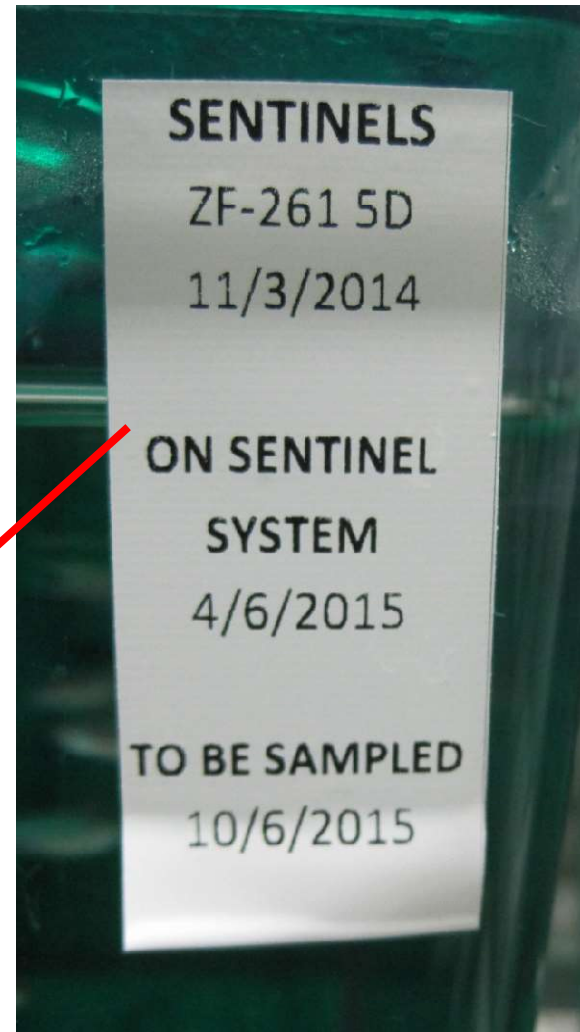
Determine the best location for your sentinels and work with your CLS/Rack manufacturer to build this into your system



SENTINEL PROGRAM - IMPLEMENTATION

Make informative labels that have the following information:

- Date on sentinel row
- Date to be sampled
- All other stock info



NON-SENTINEL TESTING

- How does husbandry and research staff know what to test and when to report?
 - Create training program to help staff and researchers ID problems
 - Picture books available in fish rooms
 - Log books to track morts/moribund



Permanent Sample Station

camera
ruler
tubes of fixative
dissection tools
histology marker

Information obtained from sampling station can also be integrated into reporting system



WORKING WITH YOUR VETERINARY STAFF

- Create effective methods of communicating and reporting
- Establish guidelines for who can determine if diagnostics are needed
- Utilize vet to train staff and users about fish health and disease management
- Don't wait for an inspection to ask for advice!

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Animal Health

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Animal Health Report - Sinnhuber Aquatic Research Lab (SARL) - 09/12/2015

Facility Name	Sinnhuber Aquatic Research Lab (SARL)
Report date	09/12/2015
Status	Complete
Submitter	Barton, Carrie L
Submitted at	09/12/2015 at 02:30pm
To the best of my knowledge, all animals at the facility have been checked	Yes

Comments

There are no comments for this Animal Health Report

QUESTIONS?

