Facility Management

Carrie Barton

Sinnhuber Aquatic Research Laboratory

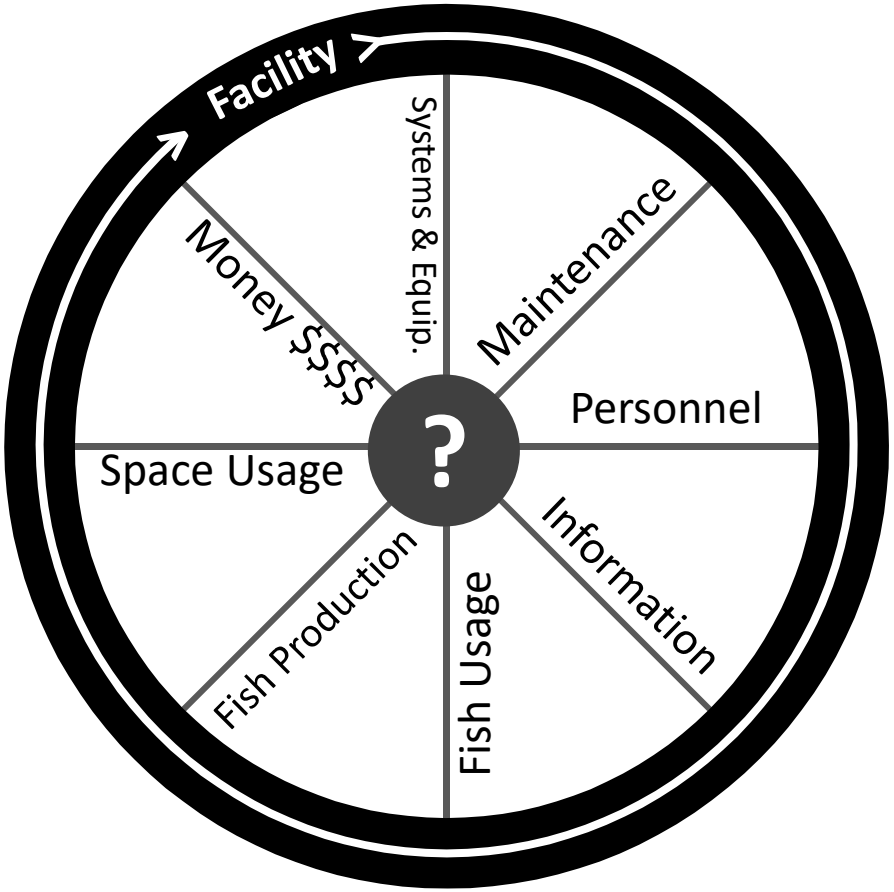
Oregon State University

7th Annual International Zebrafish Husbandry Course
Buguggiate, Italy 2018

Facility Management Scope

Facility Management

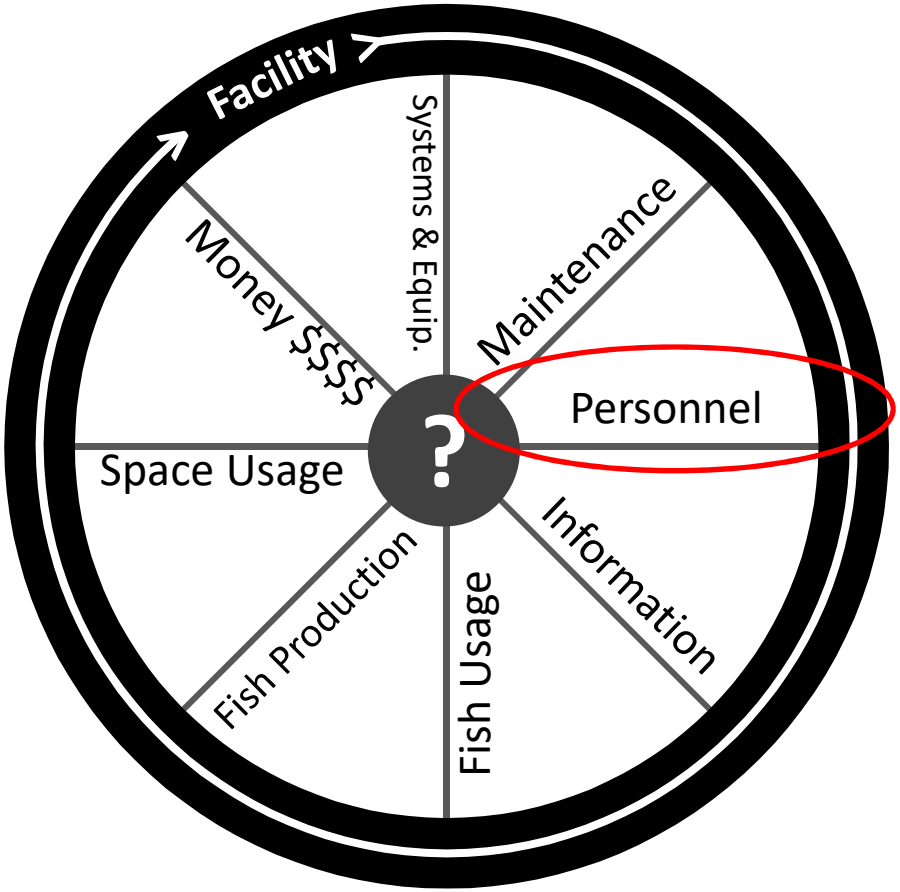
The integration of processes within an organization to maintain and develop agreed services which support and improve the effectiveness of its primary activities.



Facility Management Scope

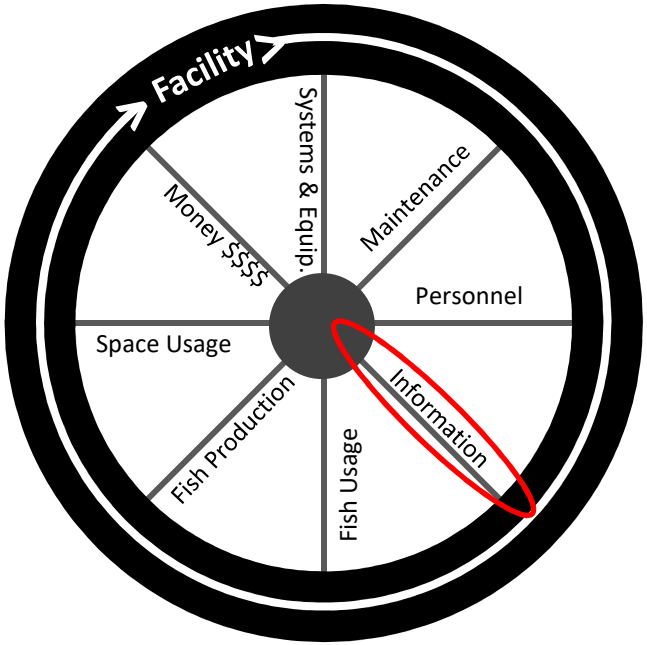
Facility Management

The integration of processes within an organization to maintain and develop agreed services which support and improve the effectiveness of its primary activities.



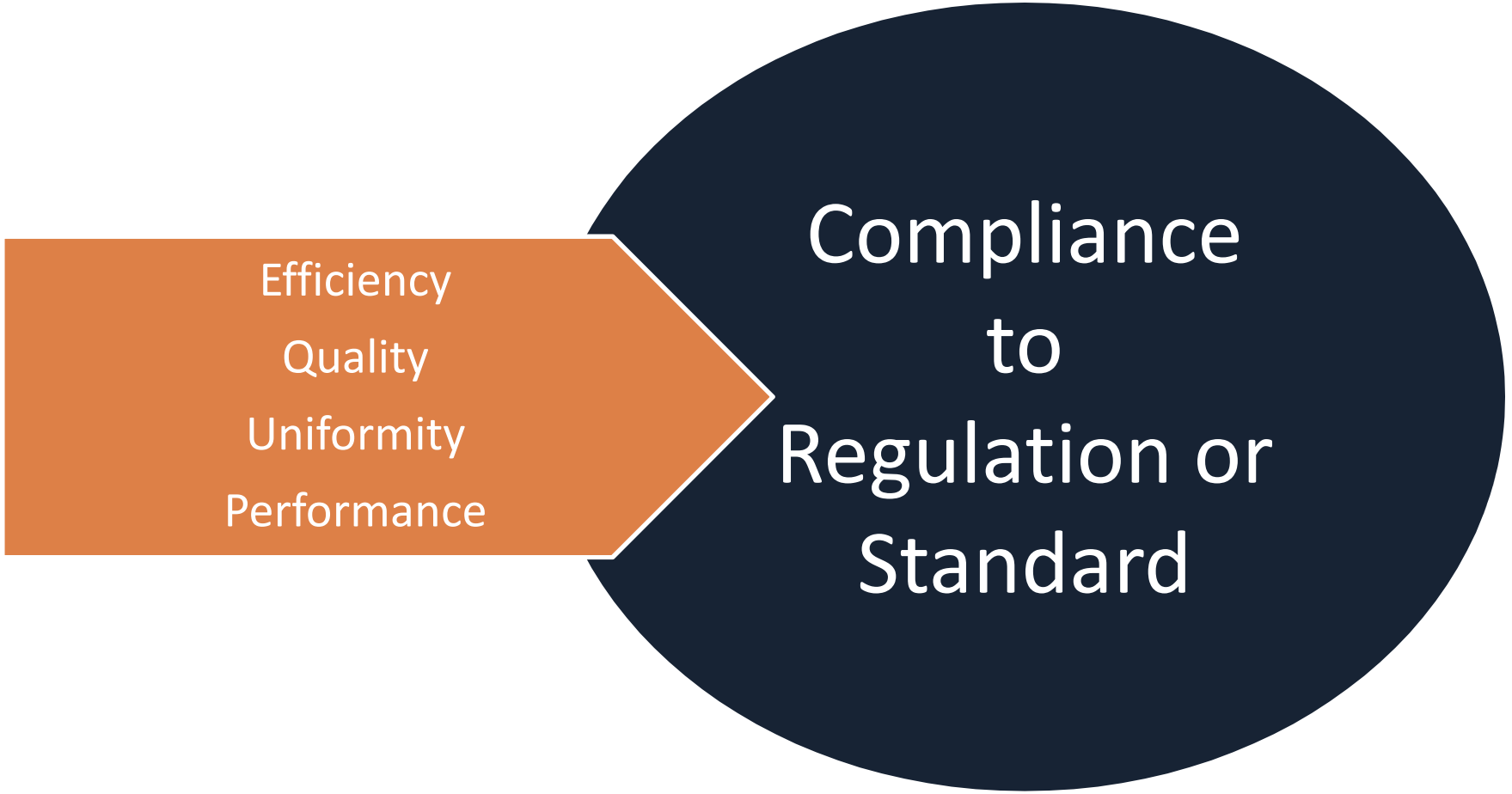
Distribution of Information

- Standard Operating Procedures
  - *Overview sheets*
- Signs and postings
- Maps
- Manuals and user guides
- Videos



Standard Operating Procedure - SOP

A **standard operating procedure**, or **SOP**, is a set of step-by-step instructions compiled by an organization to help workers carry out routine operations. SOPs aim to achieve efficiency, quality output and uniformity of performance, while reducing miscommunication and failure to comply to industry regulations. *-wikipedia*



Standard Operating Procedure - SOP

A **standard operating procedure**, or **SOP**, is a set of step-by-step instructions compiled by an organization to help workers carry out routine operations. SOPs aim to achieve efficiency, quality output and uniformity of performance, while reducing miscommunication and failure to comply to industry regulations. *-wikipedia*

Title

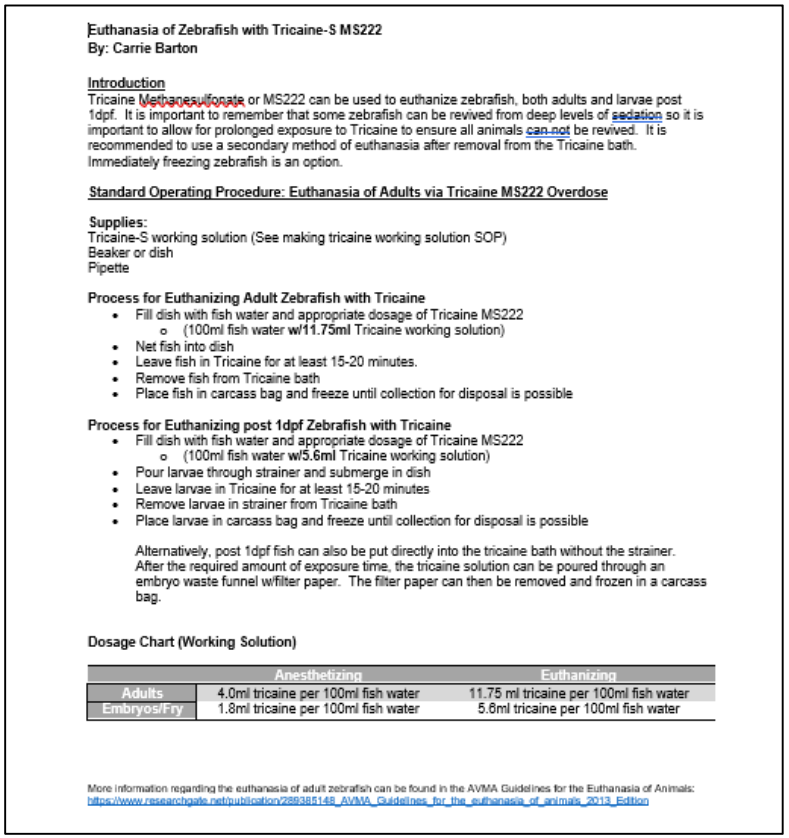
Purpose for the SOP

Materials needed to complete task

Methods: step by step instructions

Any pertinent archival information

References



Euthanasia of Zebrafish with Tricaine-S MS222
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used to euthanize zebrafish, both adults and larvae post 1dpf. It is important to remember that some zebrafish can be revived from deep levels of sedation so it is important to allow for prolonged exposure to Tricaine to ensure all animals can not be revived. It is recommended to use a secondary method of euthanasia after removal from the Tricaine bath. Immediately freezing zebrafish is an option.

Standard Operating Procedure: Euthanasia of Adults via Tricaine MS222 Overdose

Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
Beaker or dish
Pipette

Process for Euthanizing Adult Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water **w/11.75ml** Tricaine working solution)
- Net fish into dish
- Leave fish in Tricaine for at least 15-20 minutes.
- Remove fish from Tricaine bath
- Place fish in carcass bag and freeze until collection for disposal is possible

Process for Euthanizing post 1dpf Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water **w/5.6ml** Tricaine working solution)
- Pour larvae through strainer and submerge in dish
- Leave larvae in Tricaine for at least 15-20 minutes
- Remove larvae in strainer from Tricaine bath
- Place larvae in carcass bag and freeze until collection for disposal is possible

Alternatively, post 1dpf fish can also be put directly into the tricaine bath without the strainer. After the required amount of exposure time, the tricaine solution can be poured through an embryo waste funnel w/filter paper. The filter paper can then be removed and frozen in a carcass bag.

Dosage Chart (Working Solution)

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Fry	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of Animals: https://www.researchgate.net/publication/288385148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition

Standard Operating Procedure - Formatting

Title: Euthanasia and Anesthesia of Zebrafish

Avoid the bulk approach to building SOPs

Euthanasia of Zebrafish with Tricaine-S MS222
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used to euthanize zebrafish, both adults and larvae post 1dpf. It is important to remember that some zebrafish can be revived from deep levels of sedation so it is important to allow for prolonged exposure to Tricaine to ensure all animals can not be revived. It is recommended to use a secondary method of euthanasia after removal from the Tricaine bath. Immediately freezing zebrafish is an option.

Standard Operating Procedure: Euthanasia of Adults via Tricaine MS222 Overdose

Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
Beaker or dish
Pipette

Process for Euthanizing Adult Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water **w/1.75ml** Tricaine working solution)
- Net fish into dish
- Leave fish in Tricaine for at least 15-20 minutes.
- Remove fish from Tricaine bath
- Place fish in carcass bag and freeze until collection for disposal is possible

Process for Euthanizing post 1dpf Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water **w/5.6ml** Tricaine working solution)
- Pour larvae through strainer and submerge in dish
- Leave larvae in Tricaine for at least 15-20 minutes
- Remove larvae in strainer from Tricaine bath
- Place larvae in carcass bag and freeze until collection for disposal is possible

  Alternatively, post 1dpf fish can also be put directly into the tricaine bath without the strainer. After the required amount of exposure time, the tricaine solution can be poured through an embryo waste funnel w/filter paper. The filter paper can then be removed and frozen in a carcass bag.

Dosage Chart (Working Solution)

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Fry	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of Animals: https://www.researchgate.net/publication/289385148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition

Making Tricaine for Anesthesia and Euthanasia
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 is a popular anesthetic for zebrafish. It can also be prepared in a lethal dose for euthanasia. Tricaine can degrade if exposed to light and pH can shift. Tricaine stock solution can be frozen in aliquots to prolong life. Do not thaw and refreeze Tricaine stock solution. It is important to make Tricaine regularly and implement a use by date and appropriate storage system within your facility.

Standard Operating Procedure: Making Tricaine Stock Solution

Purpose: This document describes the processes of making tricaine stock solution.

Supplies:
Tricaine-S Powder (Western Chemical)
RO or DD water
Tris – pH9
Scale and weigh boats
Beaker
Stir plate or stir stick
pH meter
Plastic, non-transparent bottles or tubes for storage
Labels
Chemical hood (tricaine is classified as a respiratory irritant)

Process for Making the Stock Solution:
- Place scale inside hood with the blower turned off
- Measure out the appropriate volume of RO or DD water per chart below
- Weigh Tricaine-S powder in a hood
- Carefully add powder to water and stir to dissolve
- Turn hood on as soon as the powder has been added to the water
- Insert pH probe
- Slowly add Tris- pH 9 until pH of tricaine solution has reached 7.00-7.2
- Aliquot into amber containers and tubes and store back stock in freezer
- Bottles and tubes can be removed and thawed as needed

Tricaine Stock Solution					
Desired Amount	300mL	500mL	600mL	800mL	1L
3x Tricaine Powder	1200 mg	2000mg	2400mg	3200mg	4000mg
RO water	293.7mL	489.5mL	587.4mL	783.2mL	979mL
Tris- pH 9	~6.3mL	~10.5mL	~12.6mL	~16.8mL	~21mL

Anesthesia of Adult Zebrafish with Tricaine
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used as a submersible anesthetic. It is important to know the levels of anesthesia of fish prior to doing the procedure.

Levels of Anesthesia (McFarland and Klontz 1969)
Level 1: Light Sedation
Reaction to some stimuli (both visual and tactile) is slightly reduced

Level 2: Deep Sedation
Opercular movement rates are slightly reduced, and fish do not react to most external stimuli except pressure.

Level 3: Narcosis
Muscle tone is decreased, swimming is erratic and the opercular rate is increased. Fish continue to react to strong tactile and vibration stimuli. This is appropriate for external sampling and fin/gill biopsies.

Level 4: Light Anesthesia
Equilibrium and muscle tone are lost, opercular rates decreases, fish respond only to strong pressure. This is appropriate for minor surgical procedures.

Level 5: Surgical plane of Anesthesia
Opercular movements are shallow and the heart rate is decreased. There is no reaction to external stimuli and no reflex activity.

Level 6: Medullary Collapse
Opercular movement ceases and the heartbeat is shallow or absent.

Standard Operating Procedure: Anesthesia via Tricaine MS222 for Zebrafish

Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
2 dishes (finger bowls/deli cups/or other shallow container that will hold ~200mls)
Plastic spoon with holes
Paper Towels
Metal spatula
Breeding tank with fresh fish water for recovery
Net

Process for Anesthetizing Fish:
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - For Adults: 100ml fish water w/4ml tricaine working solution
  - For Larvae: 100ml fish water w/1.8ml tricaine working solution
- Fill second dish with just fresh fish water.
- Net fish and drop into the anesthetic bath
- Observe fish for signs of distress (bleeding from the gills or reddening around the base of the fins)
- When fish has reached the appropriate level of anesthesia, remove from bath with spoon.
- Dip the fish in the dish containing just fish water to rinse anesthetic from the fish.
- Move the fish to the paper towel, rolling once to remove excess water.
- Use spatula to transfer the fish to the procedure surface and do the procedure.
- Once procedure is done, move fish to the recovery tank and observe for signs of recovery.
- Allow fish to completely recover before returning to the system.
- Signs of full recovery include normal opercular movement and normal swimming behavior.

Standard Operating Procedure: Making Tricaine Working Solution

Purpose: This document describes the processes of making tricaine working solution.

Supplies:
3x Tricaine-S Stock Solution (See Making Tricaine Stock Solution SOP)
Beaker
Fish water
Stir stick

Process for Making the Working Solution:
- Thaw stock solution
- Combine with fish water in a ratio for the life stage and desired outcome (anesthesia or euthanasia)
- See chart below for ratios
- Mix thoroughly
- Use working solution that day. Do not reuse!

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Fry	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

Process of Euthanizing Larval (1dpf-7dpf) Zebrafish

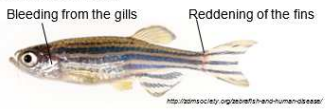
- Fill bottom of 2L breeding tank 2/3 full of crushed ice
- Add fish water until full
- Nest breeding tank insert in the tank bottom, pushing it down into the ice
  - This will create a cold water bath, free of ice
- Insert thermometer to ensure temperature of 36-39F have been achieved
- Pour larvae through strainer and nest into the water
- Wait until fish have full loss of orientation/equilibrium and opercular movements have cea
- Start timer for minimum of **20 minutes**
- After **20 minutes** have passed, remove strainer
- Transfer larvae into carcass bag and freeze until time of collection and disposal

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of A
https://www.researchgate.net/publication/289385148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition

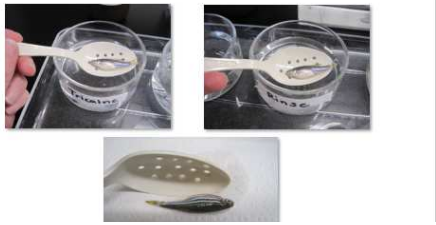
Dosage Chart (Working Solution)

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Fry	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

Signs of Anesthetic Stress in Zebrafish



Moving fish from Tricaine bath, to rinse bath, to the towel:



Euthanasia of Zebrafish via Hypothermic Shock
By: Carrie Barton

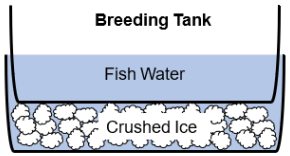
Introduction
Prolonged immersion in 36-39F water (hypothermic shock) can be used to euthanize both adult and larval zebrafish. It is important to remember that some zebrafish can survive hypothermic shock if slowly rewarmed. It is recommended to use a secondary method of euthanasia after removal from the ice bath. Immediately freezing all animals post hypothermic shock exposure is an appropriate method.

Standard Operating Procedure:
Euthanasia of Zebrafish via Hypothermic Shock (Ice Bath Immersion)

Supplies:
Crushed ice
2 L breeding cage w/insert
Fish water
Thermometer
Embryo strainer (for larval euthanasia)

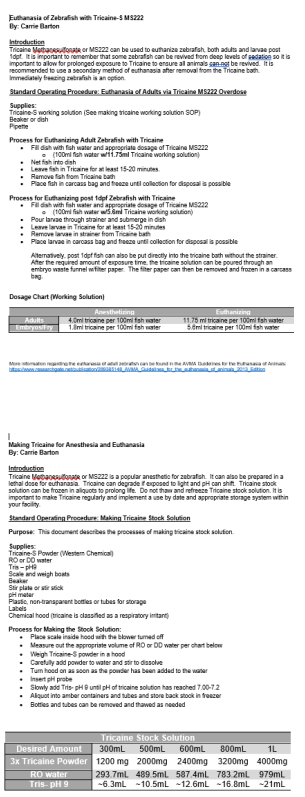
Process for Euthanizing Adult Zebrafish via Hypothermic Shock

- Fill bottom of 2L breeding tank 2/3 full of crushed ice
- Add fish water until full
- Nest breeding tank insert in the tank bottom, pushing it down into the ice
  - This will create a cold water bath, free of ice
- Insert thermometer to ensure temperature of 36-39F have been achieved
- Net fish into cold water bath
- Wait until fish have full loss of orientation/equilibrium and opercular movements have ceased
- Start timer for minimum of **10 minutes**
- After **10 minutes** have passed, remove fish from ice bath
- Transfer fish into carcass bag and freeze until collection and disposal



Standard Operating Procedure - Formatting

Title: Euthanasia and Anesthesia of Zebrafish



Euthanasia of Zebrafish with Tricaine-S MS222
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used to euthanize zebrafish, both adults and larvae post 1dpf. It is important to remember that some zebrafish can be revived from deep levels of sedation so it is important to allow for prolonged exposure to Tricaine to ensure all animals can not be revived. It is recommended to use a secondary method of euthanasia after removal from the Tricaine bath. Immediately freezing zebrafish is an option.

Standard Operating Procedure: Euthanasia of Adults via Tricaine MS222 Overdose

Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
Beaker or dish
Pipette

Process for Euthanizing Adult Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water w/1.75ml Tricaine working solution)
- Net fish into dish
- Leave fish in Tricaine for at least 15-20 minutes.
- Remove fish from Tricaine bath
- Place fish in carcass bag and freeze until collection for disposal is possible

Process for Euthanizing post 1dpf Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water w/5.6ml Tricaine working solution)
- Pour larvae through strainer and submerge in dish
- Leave larvae in Tricaine for at least 15-20 minutes
- Remove larvae in strainer from Tricaine bath
- Place larvae in carcass bag and freeze until collection for disposal is possible

Alternatively, post 1dpf fish can also be put directly into the tricaine bath without the strainer. After the required amount of exposure time, the tricaine solution can be poured through an embryo waste funnel w/filter paper. The filter paper can then be removed and frozen in a carcass bag

Dosage Chart (Working Solution)

	Anesthesia	Euthanasia
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Larvae	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of Animals: https://www.researchgate.net/publication/280380148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition

Making Tricaine for Anesthesia and Euthanasia
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 is a popular anesthetic for zebrafish. It can also be prepared in a lethal dose for euthanasia. Tricaine can degrade if exposed to light and pH can shift. Tricaine stock solution can be frozen in aliquots to prolong life. Do not thaw and refreeze Tricaine stock solution. It is important to make Tricaine regularly and implement a use by date and appropriate storage system within your facility.

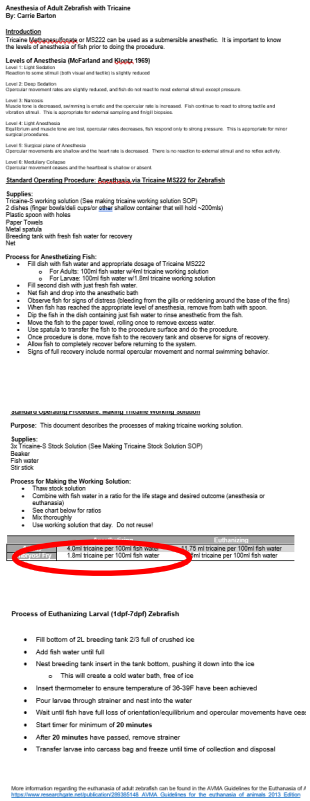
Standard Operating Procedure: Making Tricaine Stock Solution

Purpose: This document describes the processes of making tricaine stock solution.

Supplies:
Tricaine-S Powder (Western Chemical)
RO or DD water
Tris – pHB
Scale and weigh boats
Beaker
Stir plate or stir stick
pH meter
Plastic, non-transparent bottles or tubes for storage
Labels
Chemical hood (tricaine is classified as a respiratory irritant)

Process for Making the Stock Solution:
- Place scale inside hood with the blower turned off
- Measure out the appropriate volume of RO or DD water per chart below
- Weigh Tricaine-S powder in a hood
- Carefully add powder to water and stir to dissolve
- Turn hood on as soon as the powder has been added to the water
- Insert pH probe
- Slowly add Tris- pH 9 until pH of tricaine solution has reached 7.00-7.2
- Aliquot into amber containers and tubes and store back stock in freezer
- Bottles and tubes can be removed and thawed as needed

Tricaine Stock Solution					
Desired Amount	300mL	500mL	600mL	800mL	1L
3x Tricaine Powder	1200 mg	2000mg	2400mg	3200mg	4000mg
RO water	293.7mL	489.5mL	587.4mL	783.2mL	979mL
Tris- pH 9	~6.3mL	~10.5mL	~12.6mL	~16.8mL	~21mL



Anesthesia of Adult Zebrafish with Tricaine
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used as a submersible anesthetic. It is important to know the levels of anesthesia of fish prior to doing the procedure.

Levels of Anesthesia (McFarland and Klontz, 1969)
Level 1: Light Sedation
Reaction to some stimuli (both visual and tactile) is slightly reduced

Level 2: Deep Sedation
Opercular movement rates are slightly reduced, and fish do not react to most external stimuli except pressure.

Level 3: Narcosis
Muscle tone is decreased, swimming is erratic and the opercular rate is increased. Fish continue to react to strong tactile and vibration stimuli. This is appropriate for external sampling and freight biopsies.

Level 4: Light Anesthesia
Equilibrium and muscle tone are lost, opercular rates decreases, fish respond only to strong pressure. This is appropriate for minor surgical procedures.

Level 5: Surgical plane of Anesthesia
Opercular movements are shallow and the heart rate is decreased. There is no reaction to external stimuli and no reflex activity.

Level 6: Medullary Collapse
Opercular movement ceases and the heartbeat is shallow or absent.

Standard Operating Procedure: Anesthesia via Tricaine MS222 for Zebrafish

Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
2 dishes (Pyrex bowls/deli cups/or other shallow container that will hold ~200mls)
Plastic spoon with holes
Paper Towels
Metal spatula
Breeding tank with fresh fish water for recovery
Net

Process for Anesthetizing Fish:
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - For Adults: 100ml fish water w/4ml tricaine working solution
  - For Larvae: 100ml fish water w/1.8ml tricaine working solution
- Fill second dish with just fresh fish water.
- Net fish and drop into the anesthetic bath
- Observe fish for signs of distress (bleeding from the gills or reddening around the base of the fins)
- When fish has reached the appropriate level of anesthesia, remove from bath with spoon.
- Dip the fish in the dish containing just fish water to rinse anesthetic from the fish.
- Move the fish to the paper towel, rolling once to remove excess water.
- Use spatula to transfer the fish to the procedure surface and do the procedure.
- Once procedure is done, move fish to the recovery tank and observe for signs of recovery.
- Allow fish to completely recover before returning to the system.
- Signs of full recovery include normal opercular movement and normal swimming behavior.

Purpose: This document describes the processes of making tricaine working solution.

Supplies:
3x Tricaine-S Stock Solution (See Making Tricaine Stock Solution SOP)
Beakar
Fish water
Stir stick

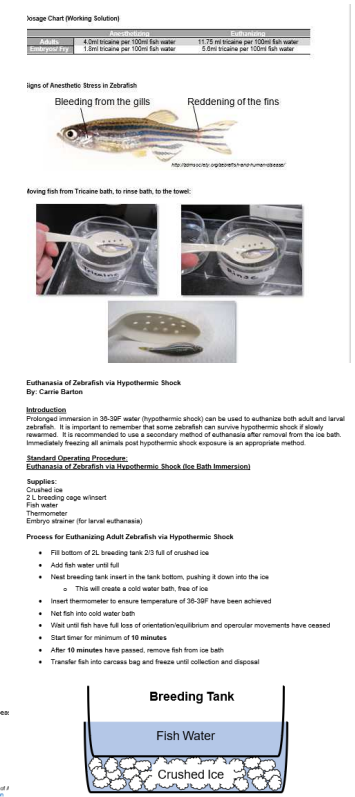
Process for Making the Working Solution:
- Thaw stock solution
- Combine with fish water in a ratio for the life stage and desired outcome (anesthesia or euthanasia)
- See chart below for ratios
- Mix thoroughly
- Use working solution that day. Do not reuse!

	Anesthesia	Euthanasia
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Larvae	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

Process of Euthanizing Larval (1dpf-7dpf) Zebrafish

- Fill bottom of 2L breeding tank 2/3 full of crushed ice
- Add fish water until full
- Nest breeding tank insert in the tank bottom, pushing it down into the ice
  - This will create a cold water bath, free of ice
- Insert thermometer to ensure temperature of 36-39F have been achieved
- Pour larvae through strainer and nest into the water
- Wait until fish have full loss of orientation/equilibrium and opercular movements have ceas
- Start timer for minimum of **20 minutes**
- After **20 minutes** have passed, remove strainer
- Transfer larvae into carcass bag and freeze until time of collection and disposal

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of A
https://www.researchgate.net/publication/280380148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition



losage Chart (Working Solution)

	Anesthesia	Euthanasia
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryos/Larvae	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

ligns of Anesthetic Stress in Zebrafish

http://domesticsociety.org/zebrafish-and-human-disease/

loving fish from Tricaine bath, to rinse bath, to the towel:

Euthanasia of Zebrafish via Hypothermic Shock
By: Carrie Barton

Introduction
Prolonged immersion in 36-39F water (hypothermic shock) can be used to euthanize both adult and larval zebrafish. It is important to remember that some zebrafish can survive hypothermic shock if slowly rewarmed. It is recommended to use a secondary method of euthanasia after removal from the ice bath. Immediately freezing all animals post hypothermic shock exposure is an appropriate method.

Standard Operating Procedure:
Euthanasia of Zebrafish via Hypothermic Shock (Ice Bath Immersion)

Supplies:
Crushed ice
2 L breeding cage w/insert
Fish water
Thermometer
Embryo strainer (for larval euthanasia)

Process for Euthanizing Adult Zebrafish via Hypothermic Shock

- Fill bottom of 2L breeding tank 2/3 full of crushed ice
- Add fish water until full
- Nest breeding tank insert in the tank bottom, pushing it down into the ice
  - This will create a cold water bath, free of ice
- Insert thermometer to ensure temperature of 36-39F have been achieved
- Net fish into cold water bath
- Wait until fish have full loss of orientation/equilibrium and opercular movements have ceased
- Start timer for minimum of **10 minutes**
- After **10 minutes** have passed, remove fish from ice bath
- Transfer fish into carcass bag and freeze until collection and disposal

6 page SOP covering:

- Making tricaine stock solution
- Making tricaine working solution
- Euthanizing zebrafish with Tricaine
- Anesthesia of zebrafish with Tricaine
- Alternate methods of euthanasia

Standard Operating Procedure - Formatting

4 Specific SOPs

Information is: Easier and faster to find = More likely to be used

Making tricaine solutions

I

Making Tricaine for Anesthesia and Euthanasia
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 is a popular anesthetic for zebrafish. It can also be prepared in a lethal dose for euthanasia. Tricaine can degrade if exposed to light and pH can shift. Tricaine stock solution can be frozen in aliquots to prolong life. Do not thaw and refreeze Tricaine stock solution. It is important to make Tricaine regularly and implement a use by date and appropriate storage system within your facility.

Standard Operating Procedure: Making Tricaine Stock Solution

Purpose: This document describes the processes of making tricaine stock solution.

Supplies:
Tricaine-S Powder (Western Chemical)
RO or DD water
Tris – pH9
Scale and weigh boats
Beaker
Stir plate or stir stick
pH meter
Plastic, non-transparent bottles or tubes for storage
Labels
Chemical hood (tricaine is classified as a respiratory irritant)

Process for Making the Stock Solution:
- Place scale inside hood with the blower turned off
- Measure out the appropriate volume of RO or DD water per chart below
- Weigh Tricaine-S powder in a hood
- Carefully add powder to water and stir to dissolve
- Turn hood on as soon as the powder has been added to the water
- Insert pH probe
- Slowly add Tris- pH 9 until pH of tricaine solution has reached 7.00-7.2
- Aliquot into amber containers and tubes and store back stock in freezer
- Bottles and tubes can be removed and thawed as needed

Tricaine Stock Solution					
Desired Amount	300mL	500mL	600mL	800mL	1L
3x Tricaine Powder	1200 mg	2000mg	2400mg	3200mg	4000mg
RO water	293.7mL	489.5mL	587.4mL	783.2mL	979mL
Tris- pH 9	~6.3mL	~10.5mL	~12.6mL	~16.8mL	~21mL

Supplies:
3x Tricaine-S Stock Solution (See Making Tricaine Stock Solution SOP)
Beaker
Fish water
Stir stick

Process for Making the Working Solution:
- Thaw stock solution
- Combine with fish water in a ratio for the life stage and desired outcome (anesthesia or euthanasia)
- See chart below for ratios
- Mix thoroughly
- Use working solution that day. Do not reuse!

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryo/Early larvae	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

Euthanizing zebrafish with Tricaine

Euthanasia of Zebrafish with Tricaine-S MS222
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used to euthanize zebrafish, both adults and larvae post 1dpf. It is important to remember that some zebrafish can be revived from deep levels of sedation so it is important to allow for prolonged exposure to Tricaine to ensure all animals can not be revived. It is recommended to use a secondary method of euthanasia after removal from the Tricaine bath. Immediately freezing zebrafish is an option.

Standard Operating Procedure: Euthanasia of Adults via Tricaine MS222 Overdose

Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
Beaker or dish
Pipette

Process for Euthanizing Adult Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water **w/**11.75ml Tricaine working solution)
- Net fish into dish
- Leave fish in Tricaine for at least 15-20 minutes.
- Remove fish from Tricaine bath
- Place fish in carcass bag and freeze until collection for disposal is possible

Process for Euthanizing post 1dpf Zebrafish with Tricaine
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - (100ml fish water **w/**5.6ml Tricaine working solution)
- Pour larvae through strainer and submerge in dish
- Leave larvae in Tricaine for at least 15-20 minutes
- Remove larvae in strainer from Tricaine bath
- Place larvae in carcass bag and freeze until collection for disposal is possible

Alternatively, post 1dpf fish can also be put directly into the tricaine bath without the strainer. After the required amount of exposure time, the tricaine solution can be poured through an embryo waste funnel w/filter paper. The filter paper can then be removed and frozen in a carcass bag.

Dosage Chart (Working Solution)

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryo/Early larvae	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of Animals: https://www.researchgate.net/publication/280360148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition

Anesthesia of zebrafish with Tricaine

Anesthesia of Adult Zebrafish with Tricaine
By: Carrie Barton

Introduction
Tricaine Methanesulfonate or MS222 can be used as a submersible anesthetic. It is important to know the levels of anesthesia of fish prior to doing the procedure.

Levels of Anesthesia (McFarland and Klontz 1969)
Level 1: Light Sedation
Reaction to some stimuli (both visual and tactile) is slightly reduced

Level 2: Deep Sedation
Opercular movement rates are slightly reduced, and fish do not react to weak external stimuli except pressure.

Level 3: Narcosis
Opercular rates are decreased, swimming is erratic and the opercular rate is increased. Fish continue to react to strong tactile and vibration stimuli. This is appropriate for external sampling and fin/gill biopsies.

Level 4: Light Anesthesia
Equilibrium and muscle tone are lost, opercular rates decreases, fish respond only to strong pressure. This is appropriate for minor surgical procedures.

Level 5: Surgical plane of Anesthesia
Opercular movements are shallow and the heart rate is decreased. There is no reaction to external stimuli and no reflex activity.

Level 6: Medullary Collapse
Opercular movement ceases and the heartbeat is shallow or absent.

Standard Operating Procedure: Anesthesia via Tricaine MS222 for Zebrafish

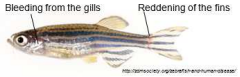
Supplies:
Tricaine-S working solution (See making tricaine working solution SOP)
2 dishes (finger bowls/deli cups)or other shallow container that will hold ~200mls)
Plastic spoon with holes
Paper Towels
Metal spatula
Breading tank with fresh fish water for recovery
Net

Process for Anesthetizing Fish:
- Fill dish with fish water and appropriate dosage of Tricaine MS222
  - For Adults: 100ml fish water w/4ml tricaine working solution
  - For Larvae: 100ml fish water w/1.8ml tricaine working solution
- Fill second dish with just fresh fish water.
- Net fish and drop into the anesthetic bath
- Observe fish for signs of distress (bleeding from the gills or reddening around the base of the fins)
- When fish has reached the appropriate level of anesthesia, remove from bath with spoon.
- Dip the fish in the dish containing just fish water to rinse anesthetic from the fish.
- Move the fish to the paper towel, rolling once to remove excess water.
- Use spatula to transfer the fish to the procedure surface and do the procedure.
- Once procedure is done, move fish to the recovery tank and observe for signs of recovery.
- Allow fish to completely recover before returning to the system.
- Signs of full recovery include normal opercular movement and normal swimming behavior.

Dosage Chart (Working Solution)

	Anesthetizing	Euthanizing
Adults	4.0ml tricaine per 100ml fish water	11.75 ml tricaine per 100ml fish water
Embryo/Early larvae	1.8ml tricaine per 100ml fish water	5.6ml tricaine per 100ml fish water

Signs of Anesthetic Stress in Zebrafish



http://zebrafish.org/zirc/health/commonDiseases.php

Moving fish from Tricaine bath, to rinse bath, to the towel:

Alternate methods of euthanasia

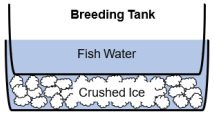
Euthanasia of Zebrafish via Hypothermic Shock
By: Carrie Barton

Introduction
Prolonged immersion in 36-39F water (hypothermic shock) can be used to euthanize both adult and larval zebrafish. It is important to remember that some zebrafish can survive hypothermic shock if slowly rewarmed. It is recommended to use a secondary method of euthanasia after removal from the ice bath. Immediately freezing all animals post hypothermic shock exposure is an appropriate method.

Standard Operating Procedure:
Euthanasia of Zebrafish via Hypothermic Shock (Ice Bath Immersion)

Supplies:
Crushed ice
2 L breeding cage w/insert
Fish water
Thermometer
Embryo strainer (for larval euthanasia)

Process for Euthanizing Adult Zebrafish via Hypothermic Shock
- Fill bottom of 2L breeding tank 2/3 full of crushed ice
- Add fish water until full
- Nest breeding tank insert in the tank bottom, pushing it down into the ice
  - This will create a cold water bath, free of ice
- Insert thermometer to ensure temperature of 36-39F have been achieved
- Net fish into cold water bath
- Wait until fish have full loss of orientation/equilibrium and opercular movements have ceased
- Start timer for minimum of **10 minutes**
- After **10 minutes** have passed, remove fish from ice bath
- Transfer fish into carcass bag and freeze until collection and disposal



Process of Euthanizing Larval (1dpf-7dpf) Zebrafish

- Fill bottom of 2L breeding tank 2/3 full of crushed ice
- Add fish water until full
- Nest breeding tank insert in the tank bottom, pushing it down into the ice
  - This will create a cold water bath, free of ice
- Insert thermometer to ensure temperature of 36-39F have been achieved
- Pour larvae through strainer and nest into the water
- Wait until fish have full loss of orientation/equilibrium and opercular movements have ceased
- Start timer for minimum of **20 minutes**
- After **20 minutes** have passed, remove strainer
- Transfer larvae into carcass bag and freeze until time of collection and disposal

More information regarding the euthanasia of adult zebrafish can be found in the AVMA Guidelines for the Euthanasia of Animals: https://www.researchgate.net/publication/280360148_AVMA_Guidelines_for_the_euthanasia_of_animals_2013_Edition

Standard Operating Procedure – Reference Sheets

Quick Reference Sheets

A **reference card** or **reference sheet** (or quick reference card) is a concise bundling of condensed notes about a specific topic. It serves as an ad hoc **memory** aid for an experienced user.

-wikipedia

Facility Feeding Quick Reference

AM Feeding: 8:00am-10:00am

Color	Size of Tank	Volume of Feed
Red	2.8 L	¼ scoop
Yellow	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops
Orange	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops
Purple	Bulk Tanks	Instruction on each tank
Special Diets	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops

Mid-day Feeding: 12:00pm-1:30pm

Color	Size of Tank	Volume of Feed
Red	2.8 L	¼ scoop
Yellow	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops

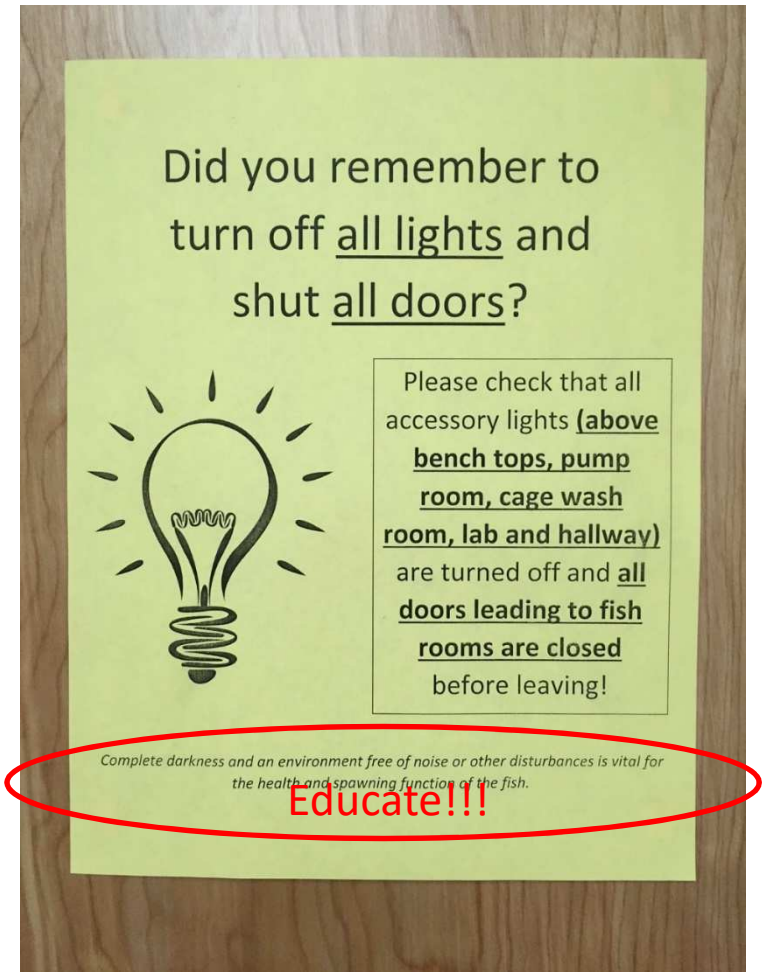
PM Feeding: 3:00pm-6:00pm

Color	Size of Tank	Volume of Feed
Red	2.8 L	¼ scoop
Yellow	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops
Orange	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops
Purple	Bulk Tanks	Instruction on each tank
Special Diets	2.8 L 6 L 9 L	½ scoop 1 scoop 2 scoops

- Not a substitute for full length SOP or proper training
- Meant as a supplement to accessible procedure book
- Make these water proof and available within facility were the specific task is done

Visual Aid – Signage

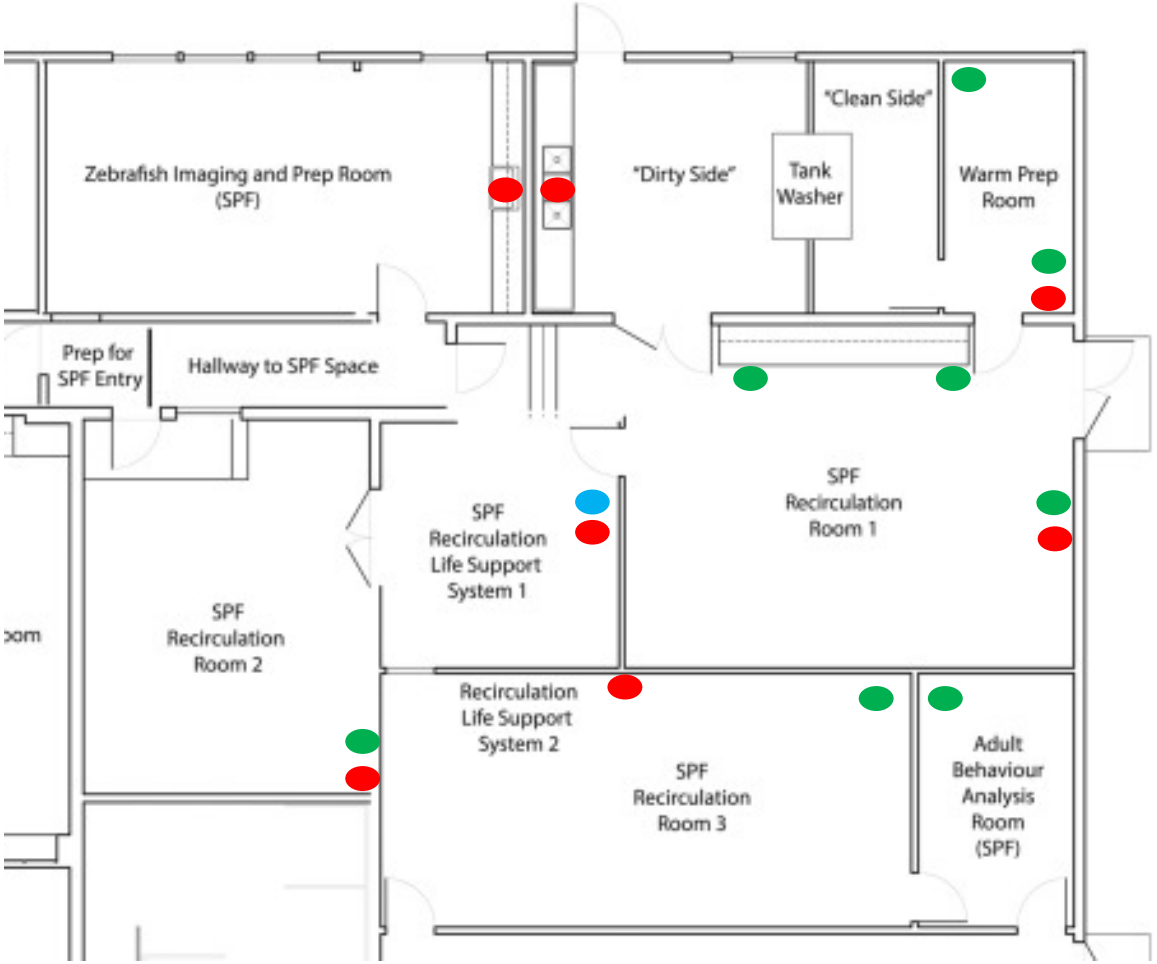
Signs are a useful tool for conveying a brief and concise message or providing basic reminders



Visual Aid - Map

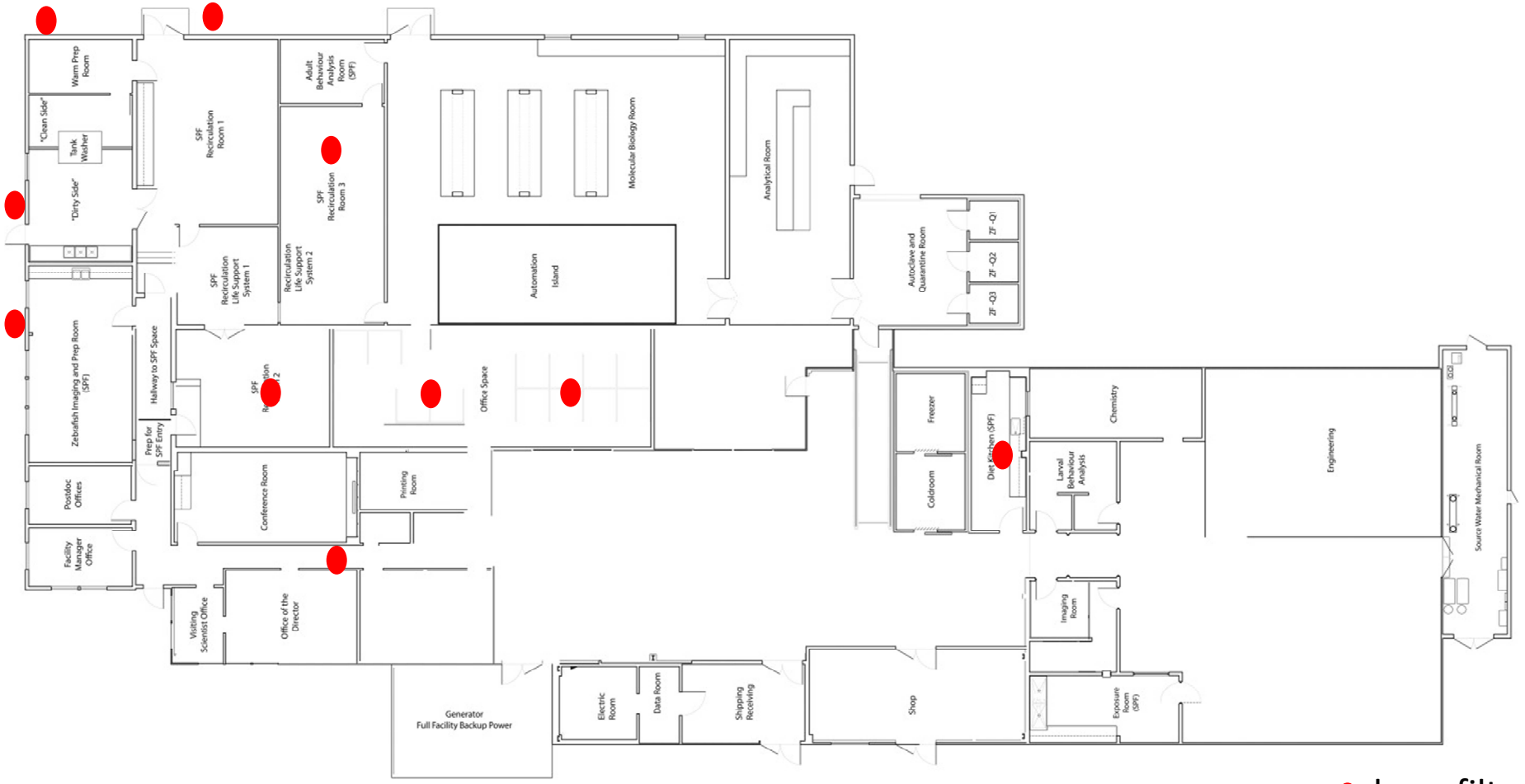
Location of all water spigots in the facility

- Line water tap
- Fish water tap
- RO water tap



Visual Aid – Map

Equipment locations – for maintenance purposes



hvac filter

Availability of Information

SOP binders should be available at facility entry point

-Check in sheets can help track entry in the event of a problem

Husbandry Staff

Research Staff

- Facility entry guidelines
- All animal care SOP's
- Emergency contacts
- ACUP's

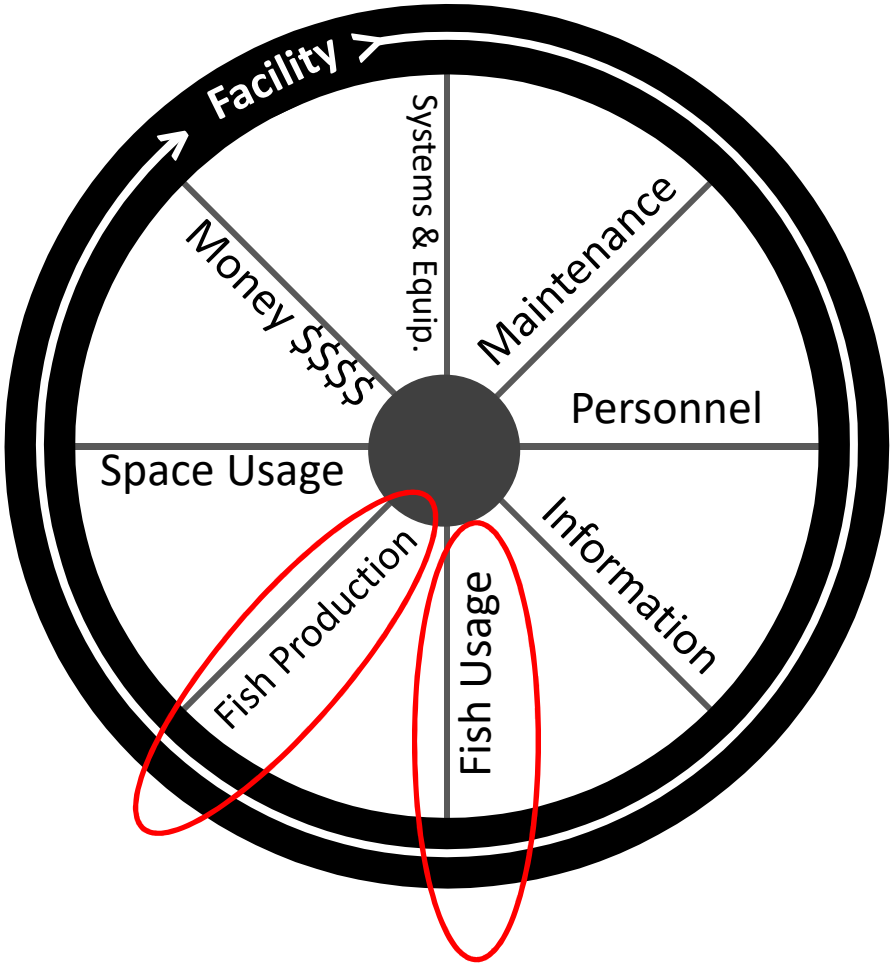
Facility Services

Outside Contractors

- Facility entry guidelines

  -what tools can come in?
- Facility maps
- Environmental conditions
- Emergency contacts

Fish Usage and Production



Fish Usage and Production

A systematic approach to the use and production of fish is vital for:

- Animal welfare
- Reduce production of unneeded animals
- Support research – minimize disruptions

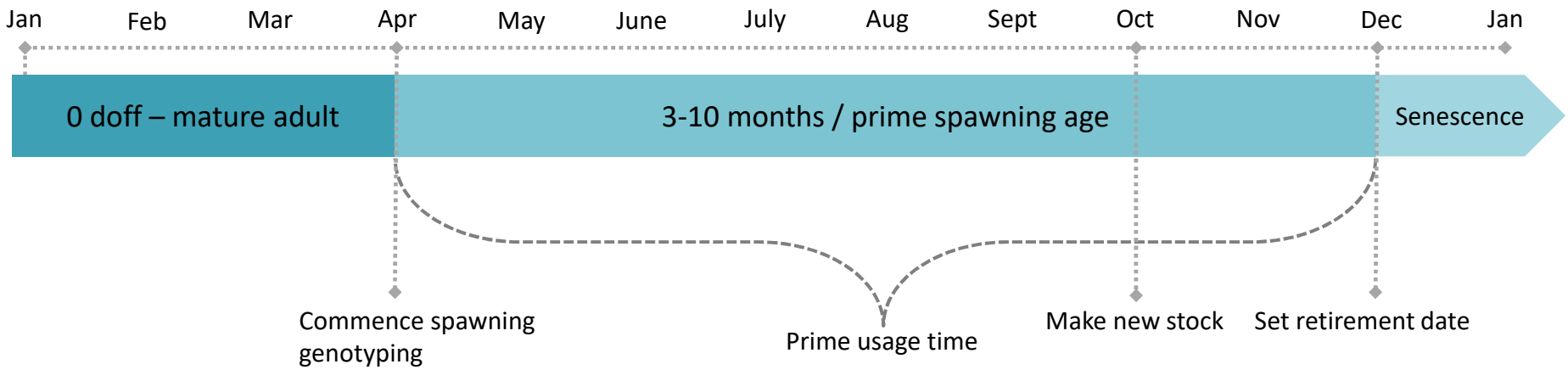
Production and usage schedules

Fish Production

Production schedule should take into account milestone events such as:

- Birth date
- Maturation time
- Time to ID if needed
- Respawn to make new generation of fish

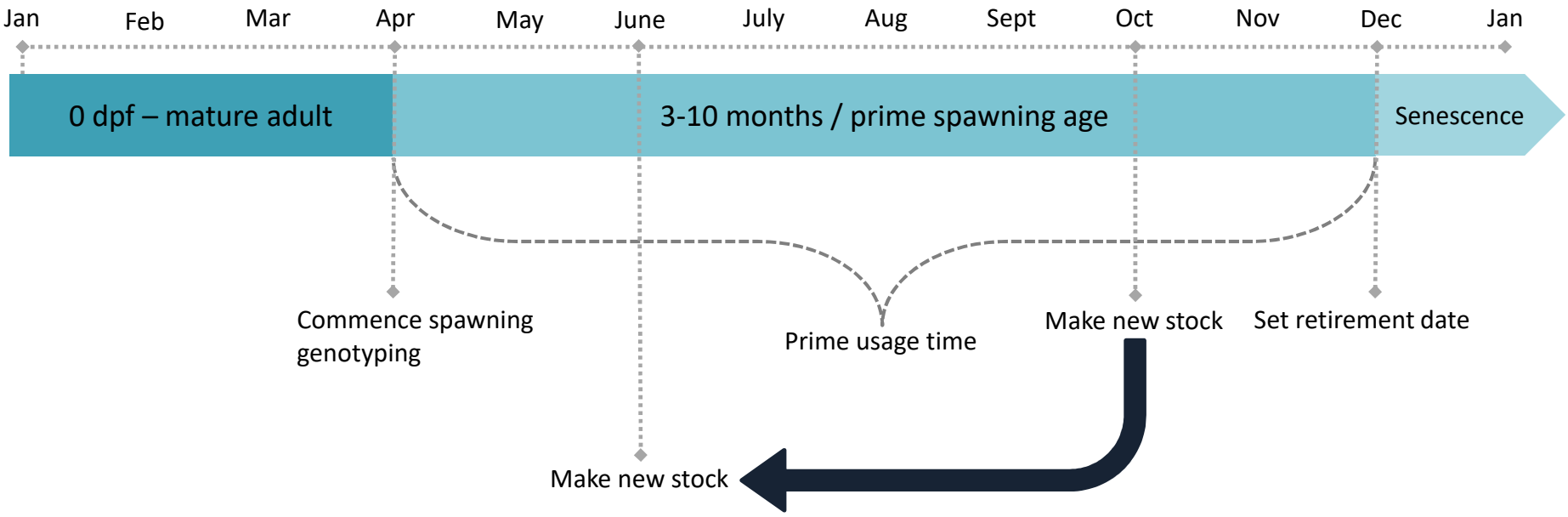
Establish generic timeline – life and usage cycle



Fish Production

- Does this line require extra time for genotyping?
- Is this a heavily used line?
- Is lag time between generations acceptable?

Establish customizable timelines for special or heavily used lines

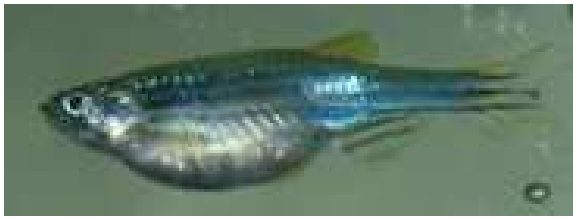


Fish Usage – Spawning Rotation

Rotation system benefits heavily and minimally used animals

- Color code on tank label is a visual que of animal status
- Helps prevent over and under spawning
- Reduction of egg associated inflammation (egg bound)
- Works well for situations with multiple users
- Improved spawn success

www.zebrafish.org



Joerg Mayer, DVM, 2002

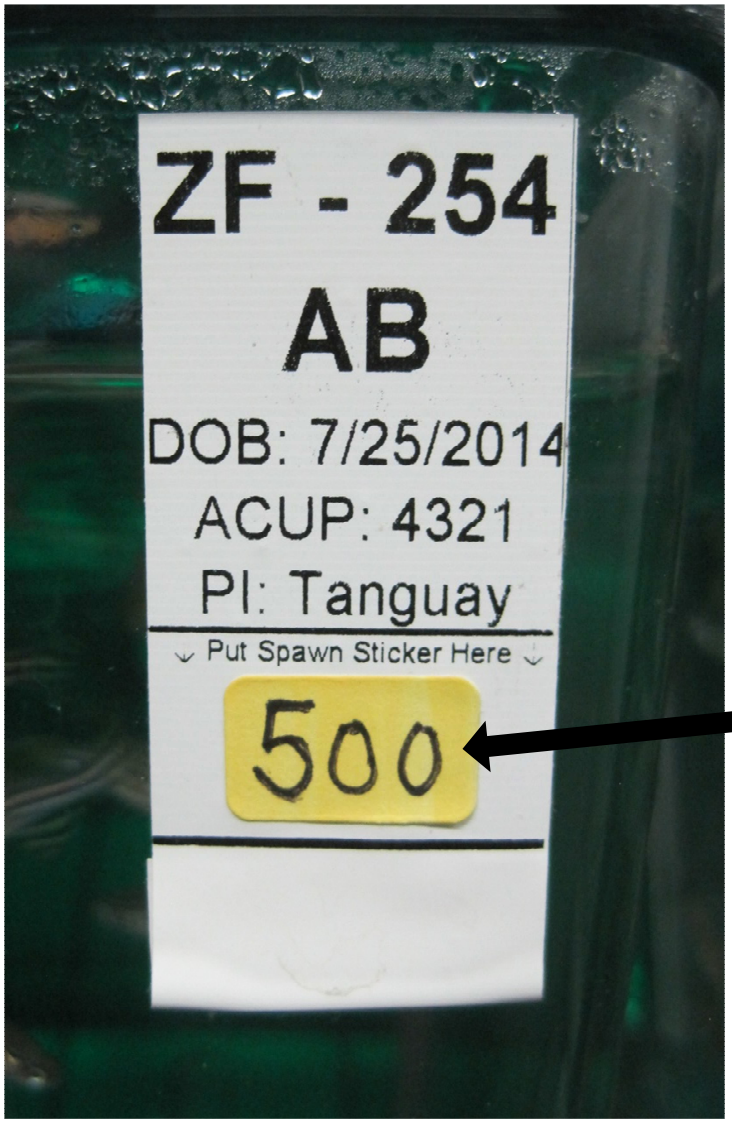
Fish Usage – Spawning Rotation

How does it work?

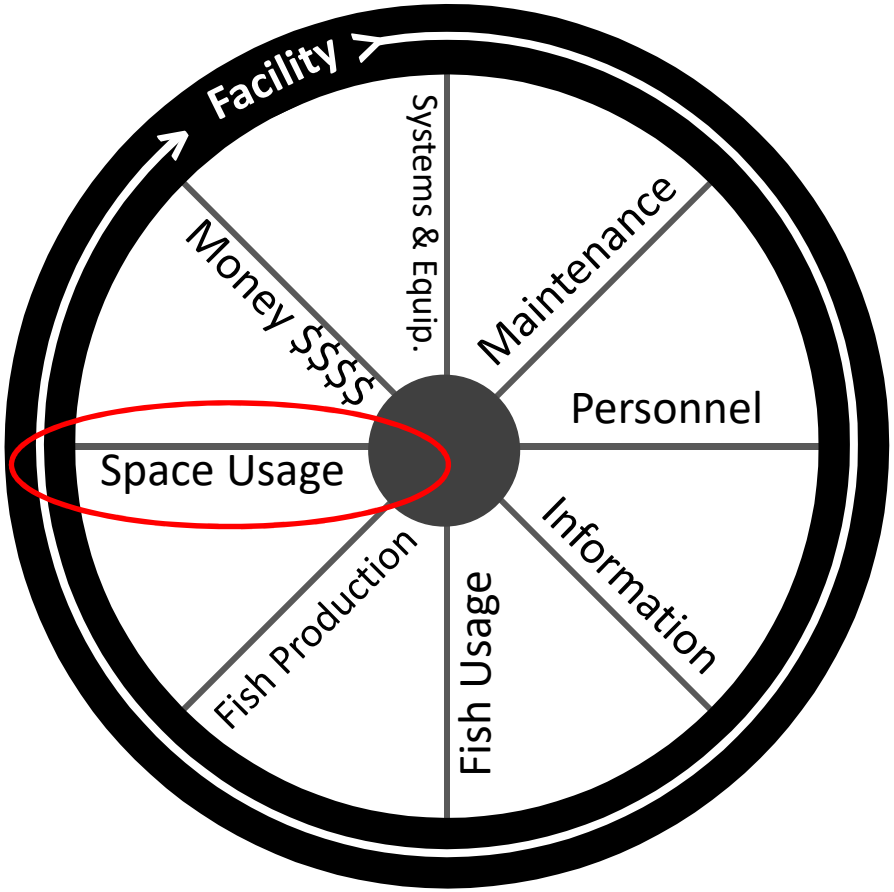
- All tanks spawned within the current week are tagged with a specific color by affixing a small flag to their tank label.
- This color is used as an indicator of how long ago the tank was spawned.
- Researchers and staff can easily see when the tank was used last by matching the label color to the rotation chart.

Minimum spawning interval 7-10 days
Maximum spawning interval 6 weeks

Fish Usage – Spawning Rotation



Space and Equipment Usage

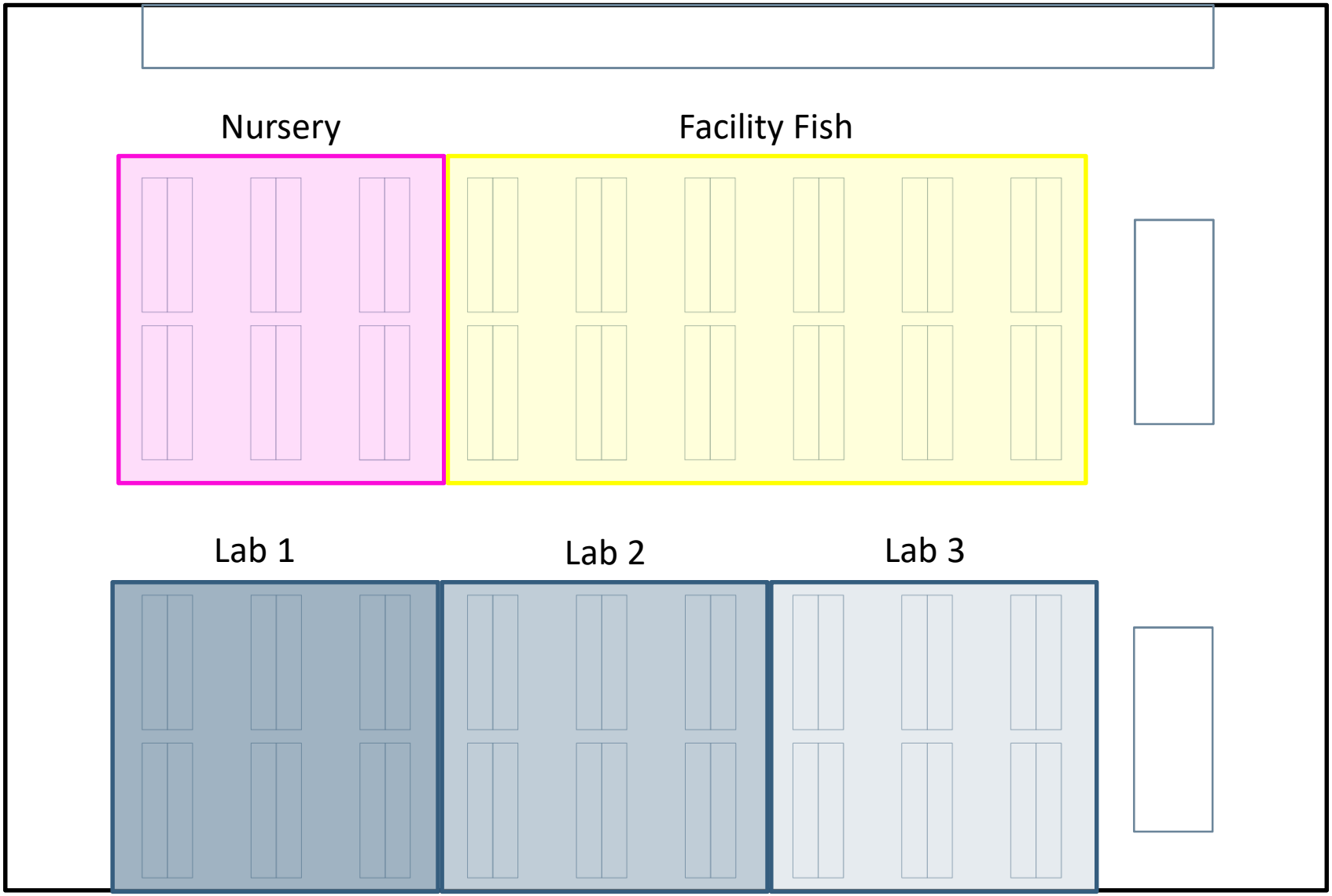


Space and Equipment Usage

A systematic approach to managing space usage is vital for:

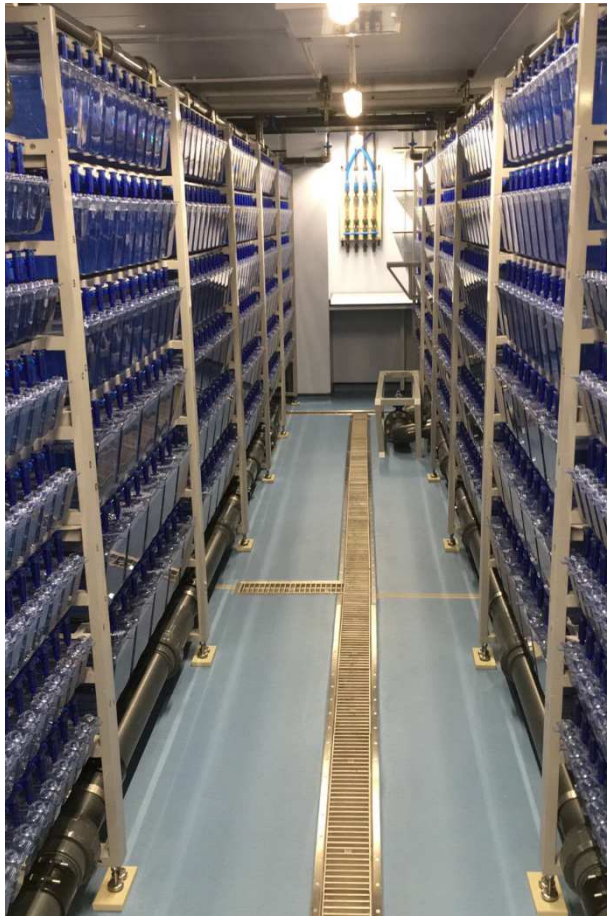
- Animal welfare
  - *Reduction of animals produced and used*
- Eliminating (okay, maybe minimizing) user conflict
- Time management and efficiency
- Productivity = more research outcomes

Space Usage – Facility Organization



Space Usage – At the lab level

Rack 1	*Rack 2*	*Rack 3*	*Rack 4*
Shared Lines	Grad Student 1	Grad Student 3	Post Doc 2
Nursery Space	Grad Student 2	Post Doc 1	Post Doc 3



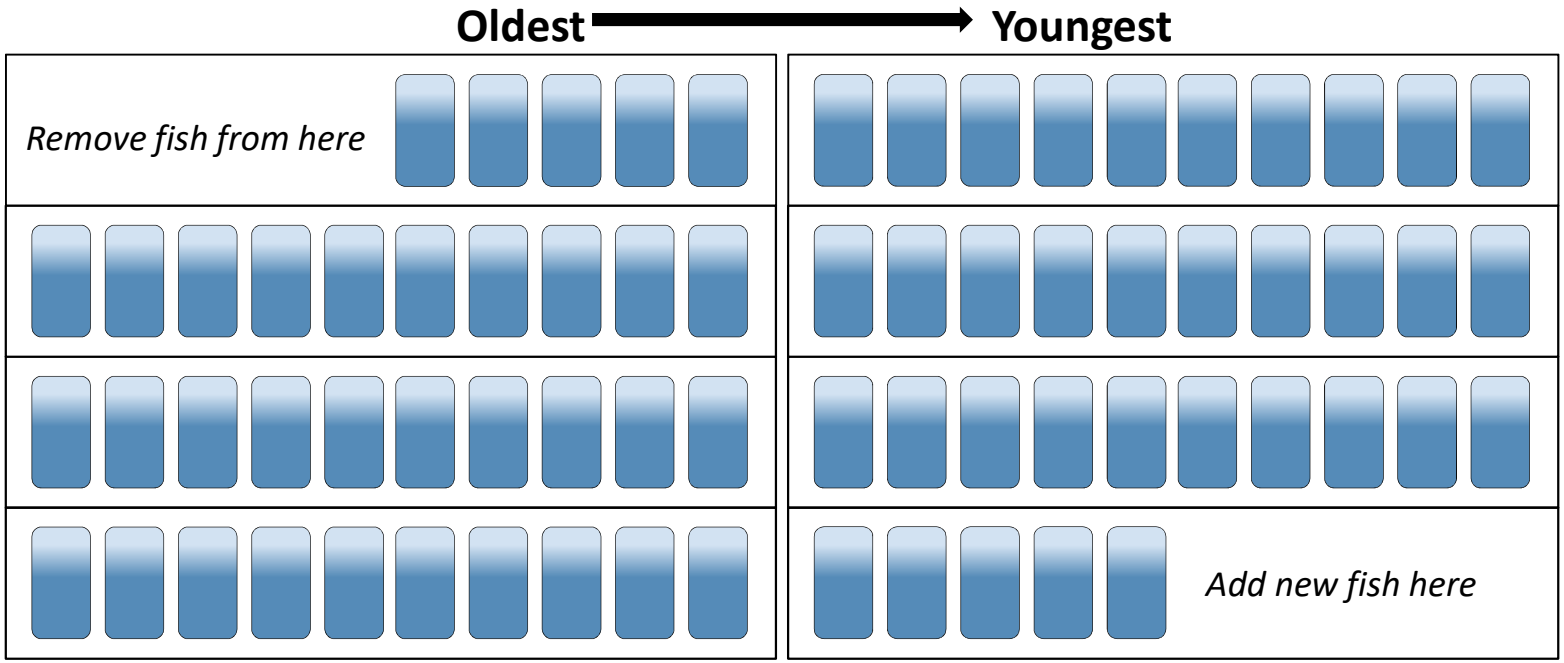
Space Usage – Nursery

Regardless of type of facility, consolidate all juvenile fish into one nursery space!

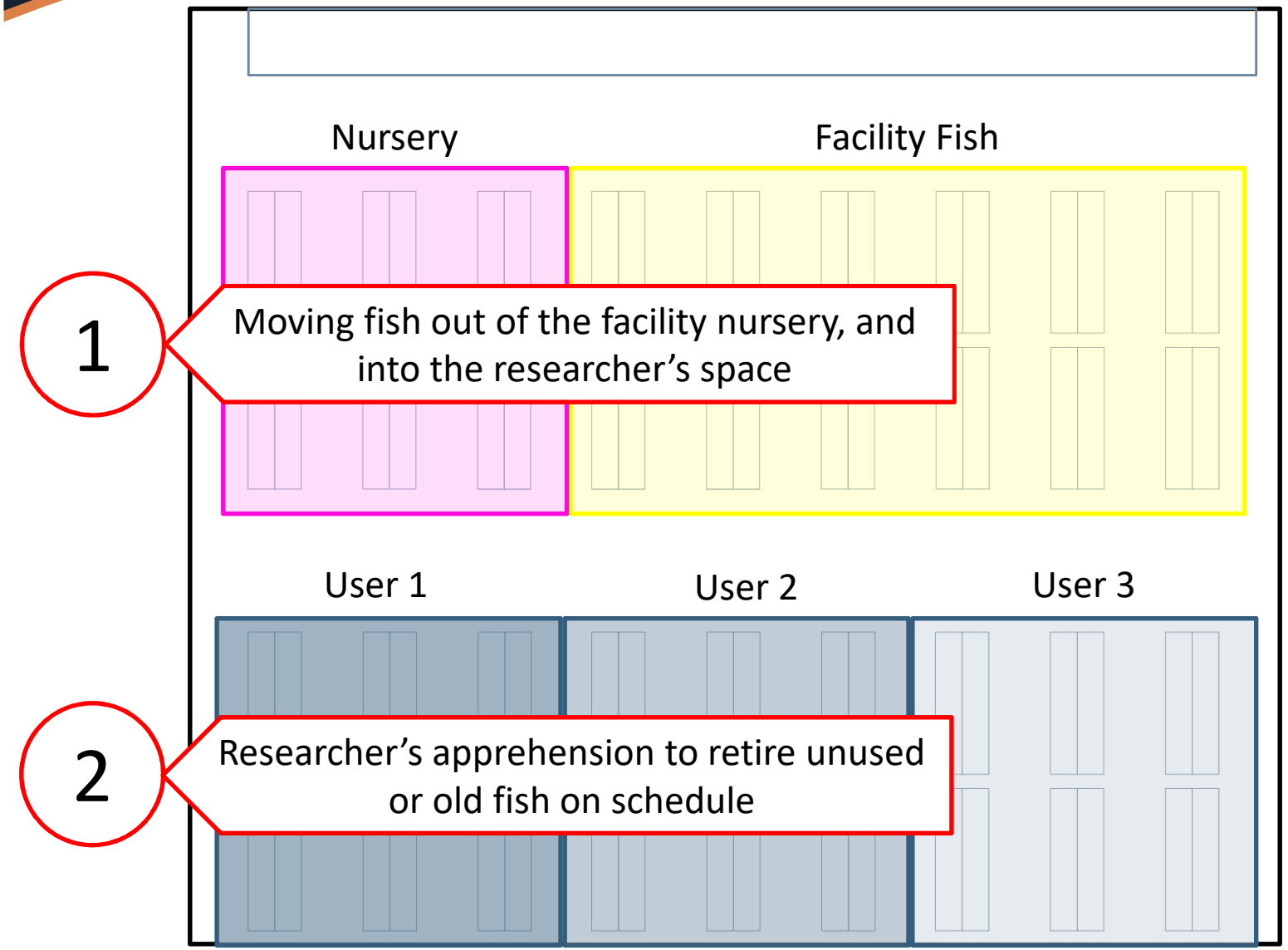
Create a chronological rotation system that:

1) allows researchers to know where to put fish

2) organizes the fish by age and ultimately feed size



Space Usage – Bottlenecks

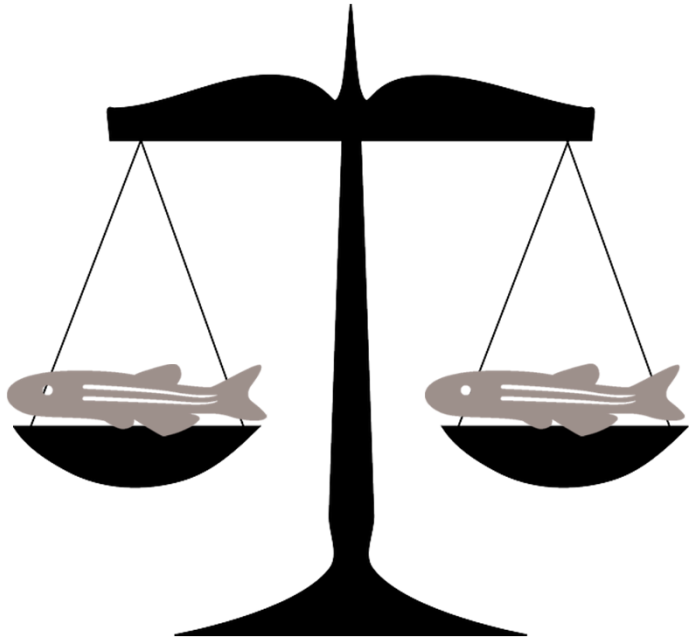


Space Usage – Bottlenecks

Fish Math

1000 fish stock enters nursery
-10% mortality
=900 fish leave nursery ~3 months later

At 95-100% occupancy, ~900 fish need to be retired within 3 months of stock creation



number of fish in must = number of fish out

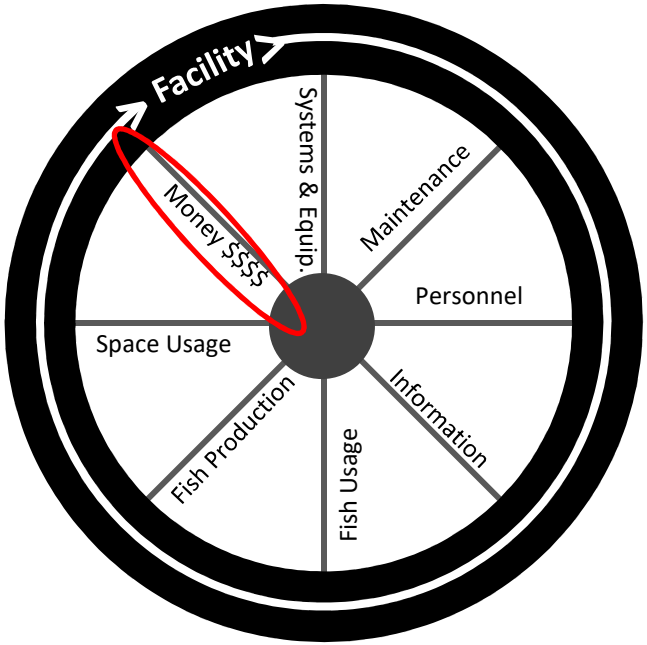
Space Usage – Bottlenecks

An effective system to prevent bottlenecks related to tank space in the main facility is to:

1) Create a sign up and occupancy system that accounts for maximum capacity in both researcher space and nursery that has notifications for impending fish transfers

2) Education users about the implications of elderly fish and the impact on whole colony health

3) Impose increased per diem rates or penalties for non-compliance



Facility Per Diem

Determine total operating expense per tank or per animal, at all stages within the facility.

- Nursery
- Grow out
- Adult tanks
- Quarantine
- Special services

Example:

Facility	Cost per tank (3.5L) Per day (Adult)	Cost per tank (3.5L) Per day (nursery)
WASHU-MCDS	$0.20	$0.40

Typically within a per diem system, all other associated expenses are captured.

Fee Book Model

<u>Tank charges similar to per diem system:</u>

Fish housing/husbandry – tank rate or fish/embryo procurement

Operating expenses

Supplies

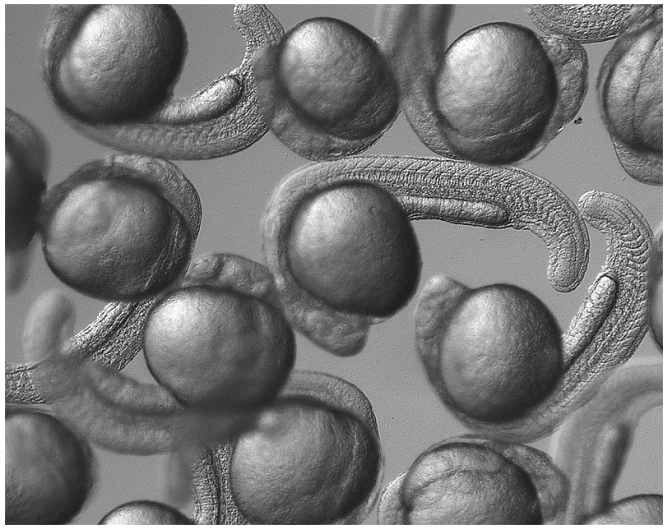
PPE

<u>Independent fees for specific services:</u>

Special procedure support (microinjections, mutant line maintenance, dissection

Equipment usage (microscopes, PCR equipment, etc.)

Good alternative for facilities supporting a large volume of labs exclusively using embryos or offering more technical support or full service closed core facilities



Procedure Spaces

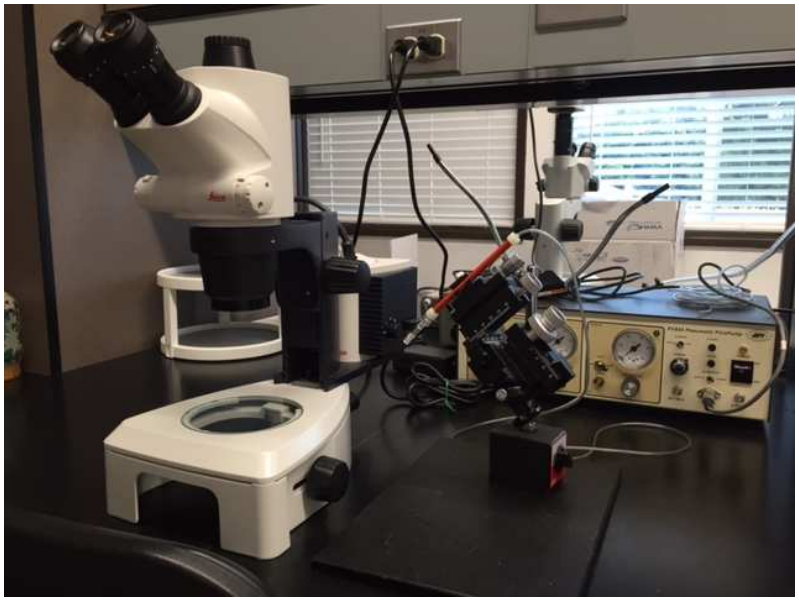
Create sign up system and log book

- *help mitigate overlaps*
- *track equipment usage*
- *identify problems*

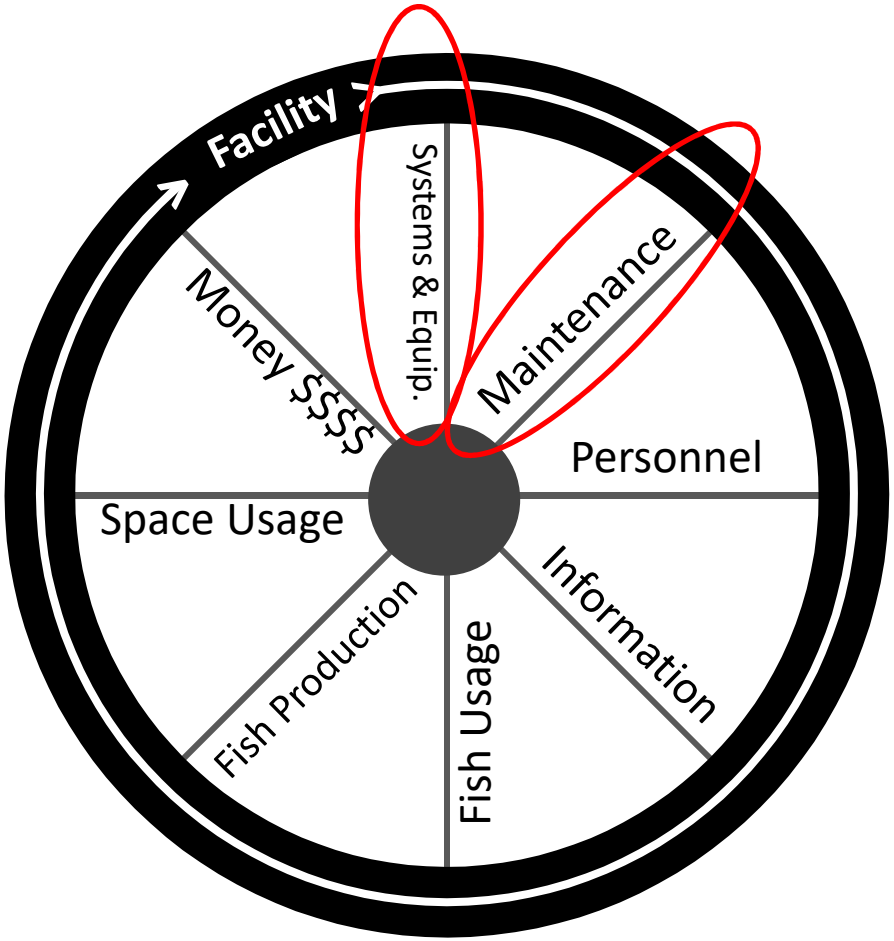
Determine who is responsible for equipment repair, consumables, and service contracts!

- *fee book system?*
- *rolled into per diem?*
- *university core support?*

Ensure equitable system for all users



Systems


Facility
Systems & Equip.
Maintenance
Personnel
Information
Fish Usage
Fish Production
Space Usage
Money $$$$

Systems

Water Systems

- central life support
- recirculating aquaculture system
- supply water systems

Environmental Conditions

- lighting
- humidity and temperature control

Emergency Systems

- Power
- Water

Sanitation Systems

- Tank washers
- Autoclaves
- Waste water processing

Systems

Water Systems

- central life support
- recirculating aquaculture system
- supply water systems

Environmental Conditions

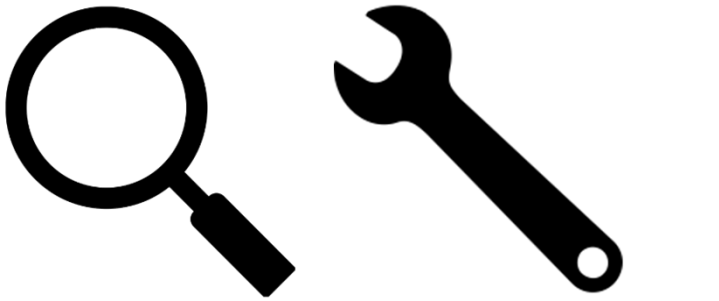
- lighting
- humidity and temperature control

Emergency Systems

- Power
- Water

Sanitation Systems

- Tank washers
- Autoclaves
- Waste water processing



Monitoring Maintenance

Water Systems (CLS, RAS, Water Plant)

The heart of every facility is the fish water system

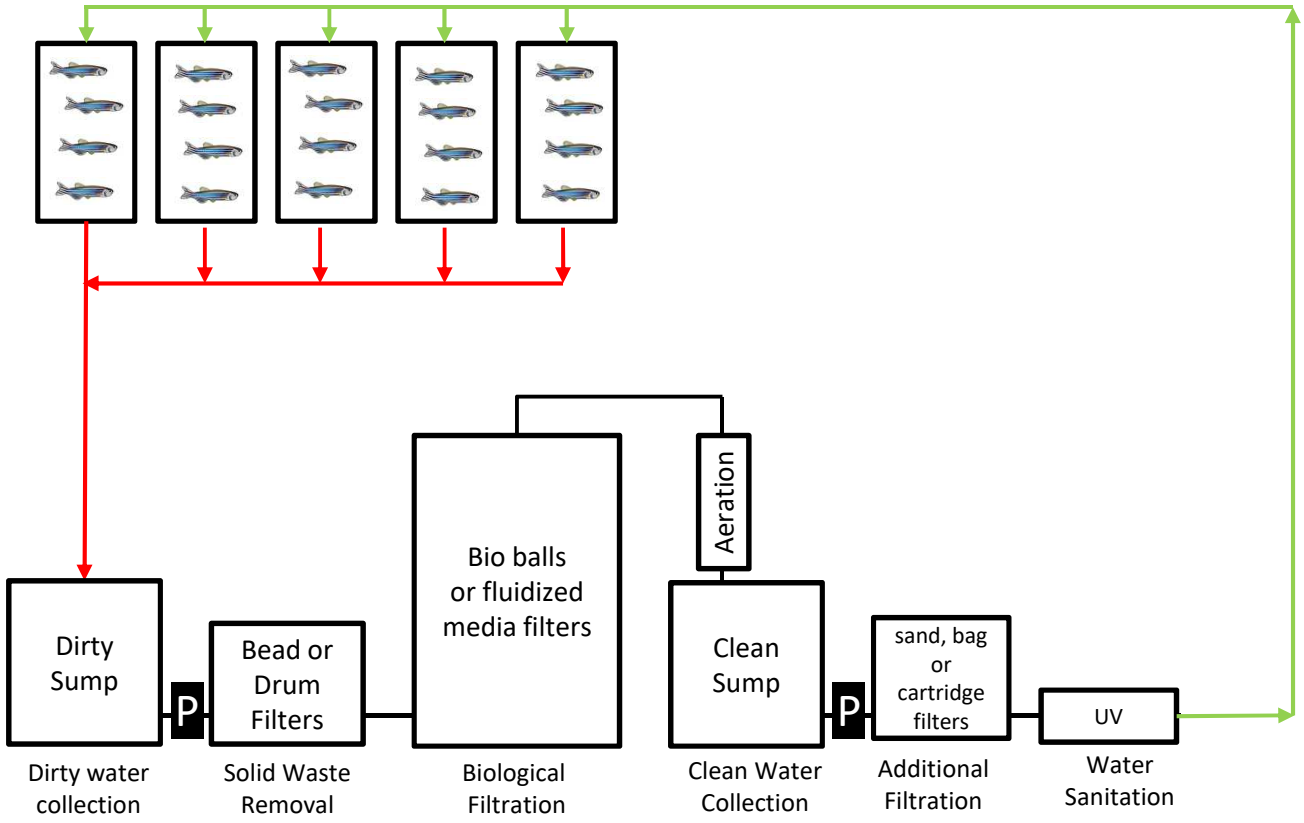
One of the few systems we have complete control over in terms of care and maintenance

Challenge – Not all systems are alike

Although the basic processes are the same, many components are different.

Differences in building services impact this as well

Basic RLS schematic



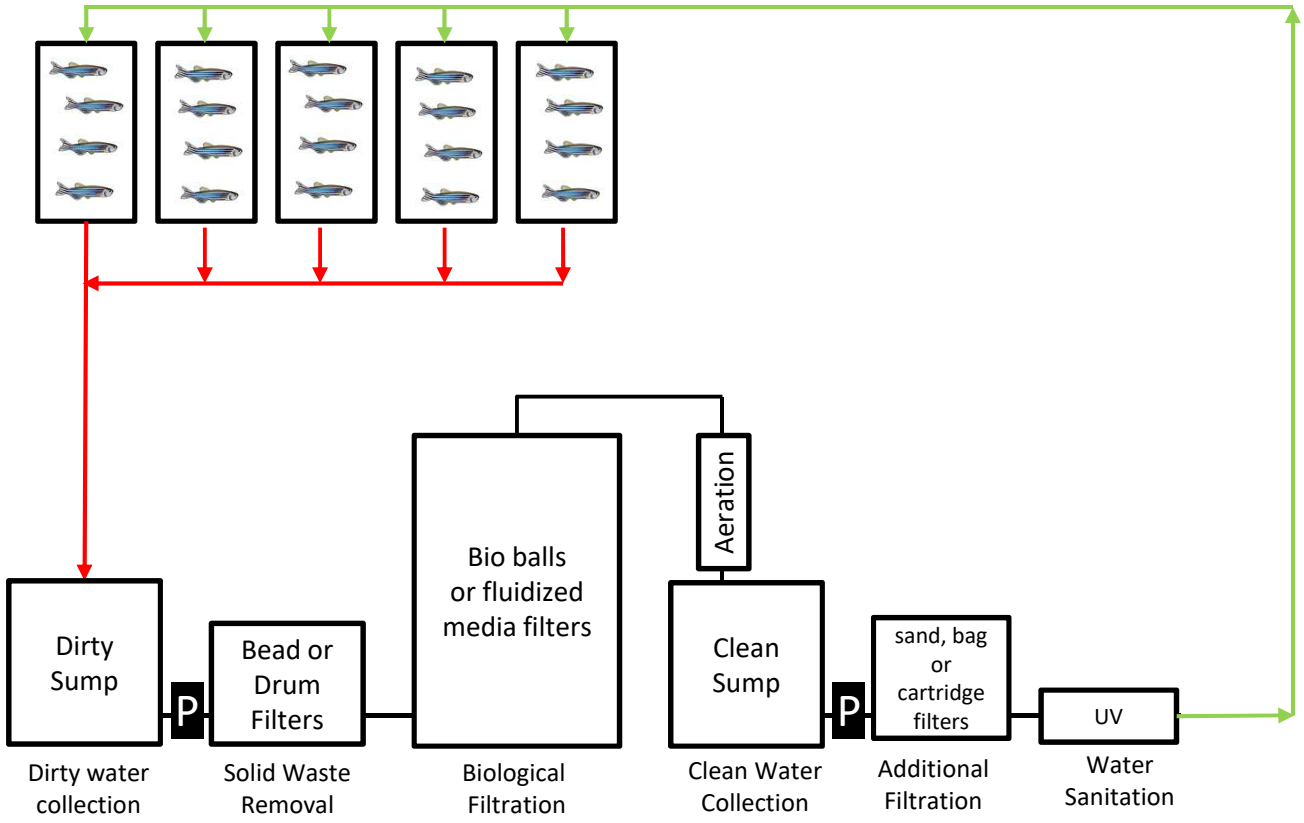
Basic RLS schematic

Multiple components working together

- Identify the maintenance needs and potential failure points
- Have back ups to things like pumps in place and tested
- Identify proactive maintenance intervals on critical equipment

  -UV lamps

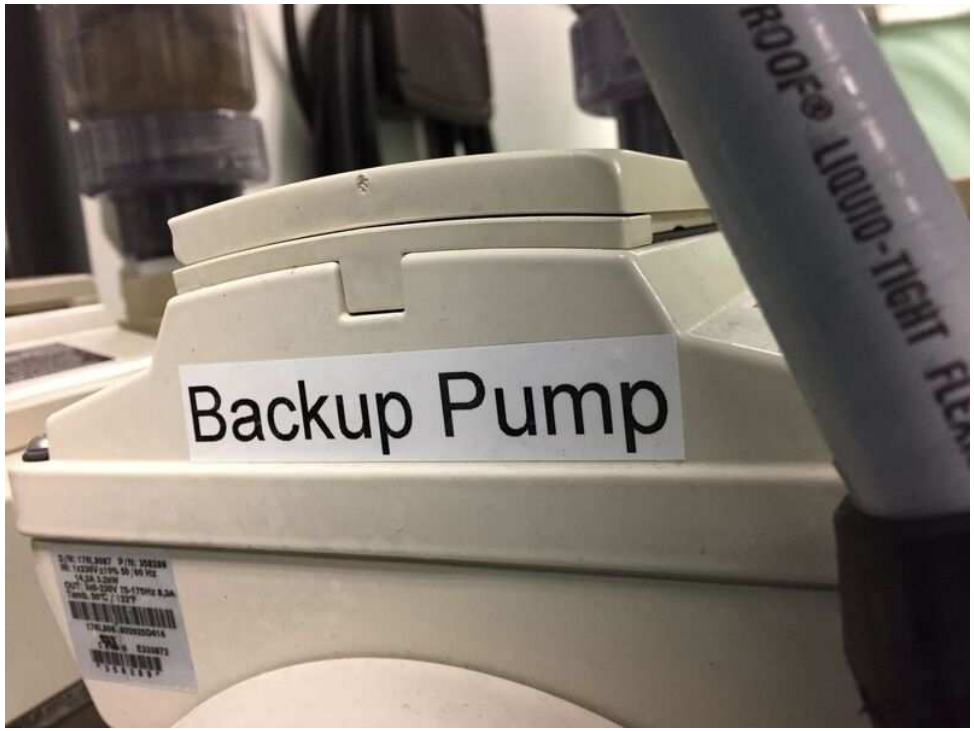
  -filters



Pumps

Having back up pumps onsite is crucial to avoiding negative outcomes in the event of an equipment failure

- if these can be integrated into the system, changing pumps is easier and reduces down time
- ensures spare pumps are actually operational
- like generators, pump need to be cycled!



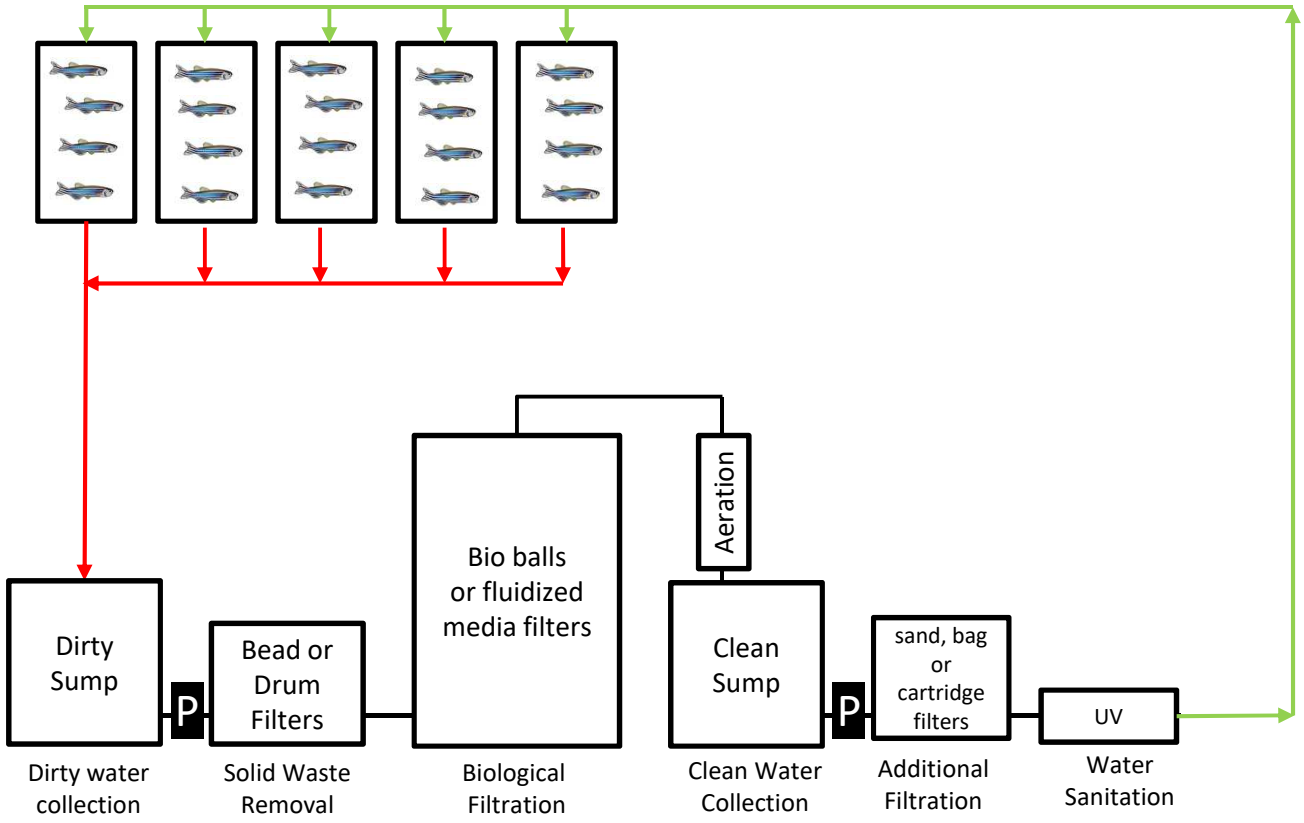
Auxiliary Systems

Supply water

- Reverse Osmosis
- Deionized
- Tap water??

  -requires treatment to remove chlorine or chloramines
- Dedicated equipment or building supplied

  -know the other users and who does the maintenance



Municipal Water Supply

Municipal water supply reports for Corvallis, Oregon

Learn about your local water!

-is it routinely treated? With what?

-will they notify you of changes?

Primary Standards

(see glossary of abbreviations and definitions on page 11)

Results from different sites/times are averaged; range may be higher than maximum reported value

Treatment Plants

Taylor Treatment Plant data are not shaded. | Rock Creek Treatment Plant data are shaded

Parameter	MCL	MCLG	Maximum Reported	Range	Likely Source	Meets Regs?
Turbidity [1]	TT = 95% of samples < 0.3 NTU	N/A	0.03 NTU	0.02 - 0.06 NTU	Soil runoff and stream sediment	Yes
			0.03 NTU	0.02 - 0.12 NTU		
Fluoride [2]	4 mg/L	4 mg/L	0.64 mg/L	0.00 - 0.88 mg/L	Added to promote dental health	Yes
			0.68 mg/L	0.72- 1.01 mg/L		
TOC, Raw Water	TT = 4 mg/L	N/A	1.53 mg/L	1.12 - 1.77 mg/L	Naturally occurring carbon, often from leaves or other organics	Yes
			1.06 mg/L	0.55 - 1.29 mg/L		
TOC, Finished Water	TT = 2 mg/L	N/A	0.71 mg/L	0.50 - 0.58 mg/L		Yes
			0.65 mg/L	0.50 - 0.70 mg/L		
Nitrate[3]	10 mg/L	10 mg/L	No detect	N/A	Fertilizer, septic tanks, sewage, or erosion.	Yes
			0.10 mg/L	N/A		
Sodium[3]	20 mg/L	N/A	17.6 mg/L	N/A	Chlorination with Sodium Hypochlorite	Yes
			6.64 mg/L	N/A		

1. Turbidity has no health effects but can interfere with disinfection and provide a medium for microbial growth. "TT" means a treatment technique is required if the limit is exceeded.

2. Fluoride is added to City drinking water and has been since 1952. Known for its cavity-fighting benefits, fluoride is of special interest to parents with young children. See article on page 6.

3. Most inorganic contaminants are measured on a nine-year reduced monitoring cycle; the last sample was in May 2011. Nitrate is sampled annually. The MCL for nitrite is 1.0 mg/L and for nitrate is 10.0 mg/L; Corvallis tests the combination of nitrate+nitrite.

Primary Standards

(see glossary of abbreviations and definitions on page 11)

Results from different sites/times are averaged; range may be higher than maximum reported value

Distribution System

Parameter	MCL	MCLG	Maximum Reported	Range	Likely Source	Meets Regs?
Total Trihalo-methanes [4]	80 µg/L	0 µg/L	21.35 µg/L	6.3 - 25.7 µg/L	By-products of disinfection process	Yes
Haloacetic Acids [4]	60 µg/L	N/A	19.53 µg/L	9.7 - 29.3 µg/L	By-products of disinfection process	Yes
Copper [5]	Action level: 90% of homes tested have less than 1.3 mg/L	1.3 mg/L	90% of homes tested had less than 0.305 mg/L	No homes tested were above 1.3 mg/L	Corrosion of household plumbing	Yes
Lead [5]	Action level: 90% of homes tested have less than 15 µg/L	0 µg/L	90% of homes tested had less than 3 µg/L	No homes tested were above 15 µg/L	Corrosion of household plumbing	Yes

4. This test is performed on a quarterly basis at four locations in the distribution system most likely to have elevated levels (places in the distribution system where water is likely to have remained in the pipes longer).

5. This test is performed every three years (most recently in 2017) in homes most likely to test positive for lead and/or copper; if levels reach the action level in 10% of homes sampled, water providers must begin extra treatment. **Lead and copper have never been detected in the City's raw water sources.** More information about lead and copper is on page 14.

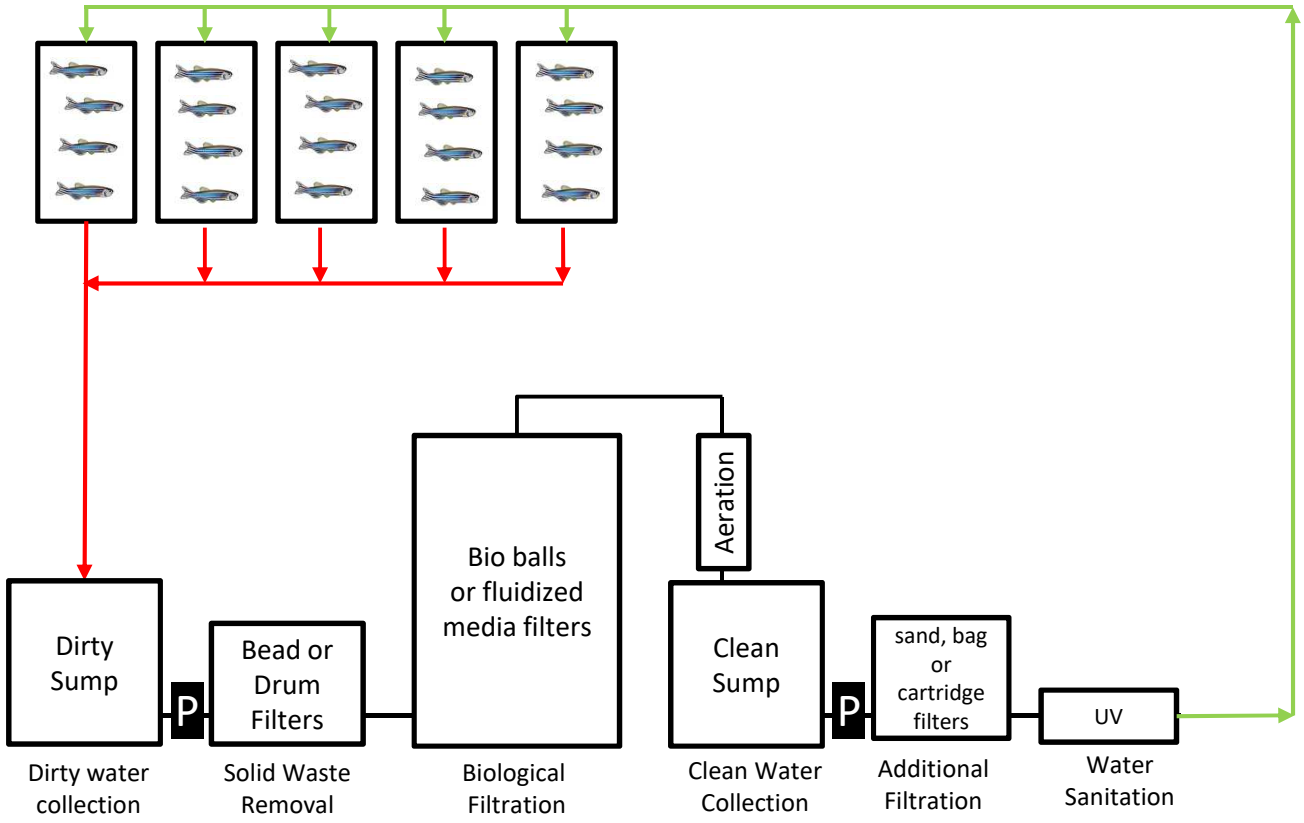
Detected Levels of Secondary Standards

Parameter	MCL (non-enforceable)	Taylor Plant Reported	Rock Creek Plant Reported
Calcium	n/a	4.75 mg/L	10.1 mg/L
Chloride	250 mg/L	2.4 mg/L	4.2 mg/L
Sulfate	250 mg/L	9.6 mg/L	8.2 mg/L
Alkalinity	n/a	24.9 mg/L	39.5 mg/L
Hardness	250 mg/L	20 mg/L	40 mg/L
pH	6.5 - 8.5 pH units	6.90 - 7.40 pH units	7.00 - 7.20 pH units
Total Dissolved Solids	500 mg/L	67.0 mg/L	58.0 mg/L

UV Disinfection

UV Failure poses a significant risk to health of the colony

- Do not use lamp failure as your maintenance interval
  -Recommendations on bulb degradation are available
- Check for lamp failure daily
- Keep lamps on site
- Keep sleeves and rebuild kits on site



Systems – Maintenance

Proactive

Preventing problems **before** they arise

- Automatic monitoring systems
- Routine checks on equipment
- Alarms are set for early detection
- Test back up equipment
- Disposables and supplies on hand
- Preventative maintenance contracts
- Replace critical elements on schedule vs. when they fail

Reactive

Reacting to problems **after** they arise

- Relying on alarms alone to drive your maintenance and system management plan
- Source what you need when it fails or needs replacement

Systems – The moving parts

Supply lines
- power and water
- shutoff points

Waste lines
- drains
- water treatment

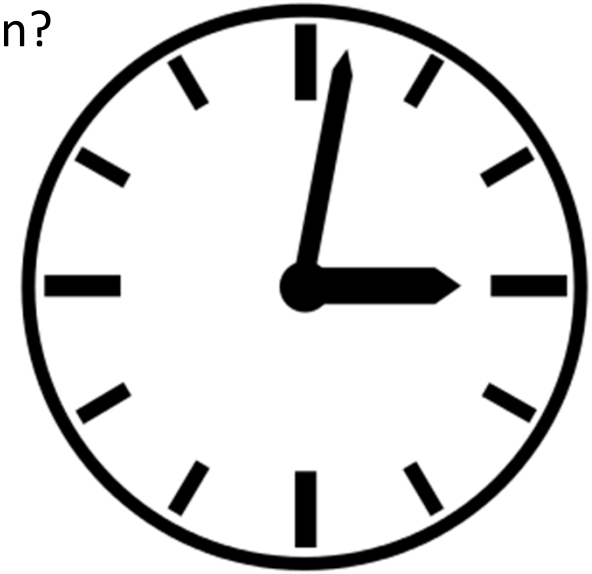
Wear items and <u>disposables</u>
- what needs replacing and when
- are there spares on site?
- if not, how fast can you get them?

Repairs
- internal (facility services or operations)
- external (service contracts)

Systems – How much time do you have?

Learn the limits of your system in the event of an emergency or major repair which requires a system shutdown.

- Biological filter: how long can it sit without circulation?
- Fish in tanks: when will they need manual care?



Systems – Monitoring

Real time availability of system parameters

- temperature (room air and water)
- pH
- conductivity

System logs are required at some institutions

- have the ability to look back at trends
- key for troubleshooting

Have back up systems

- handheld multi meters
- Test kits

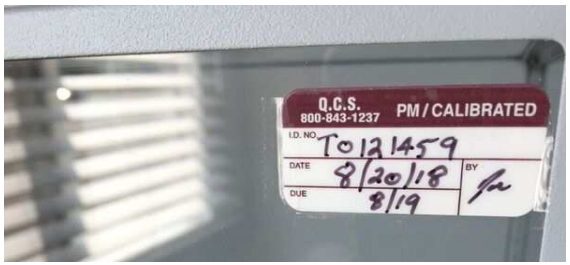
Not just for your system water but for source/supply water too!

Diagnosis and troubleshooting of a problem will be impossible without these things!!

Systems – Monitoring

Probes and instruments used for water monitoring, production of solutions or additives, and any type of life support require maintenance and calibration.

- pH and conductivity probes (on system)
- Multi meters (back up for system)
- Temperature probes (ambient and water)
- Pipettes
- Scales
- Lighting timers
- Incubators
- Flow meters



Systems – Observation

Use your senses!!

Routinely walk through your facilities and look, listen, smell and touch things!

Do things look right?
- water on floors
- levels on pH and salt solutions
- lights

Do things smell right?
- no smells
- extra smells
- food smells

Do things sound right?
- silence
- extra background noise
- pumps/motors/equipment

Do things feel right?
- vibration
- heat

Thank You!

Oregon State University

TANGUAY LAB
THINKING OUTSIDE THE TANK

EHSQ
new discoveries