








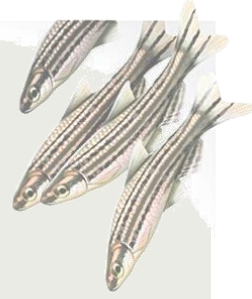
Water Quality Fundamentals

*Jason Cockington
Aquatics Manager
UQ Biological Resources*



Intensive Aquaculture

-  *Cage culture*
-  *Pond culture*
-  *Long lines*
-  *Flow through systems*
-  *Recirculating Aquaculture Systems (RAS)*



Deakin University - Aquaculture Research Centre



Cage culture in dams

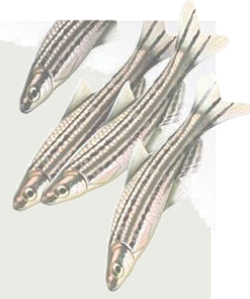


Mussel longline culture








Victorian fish hatchery raceways

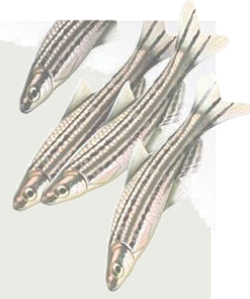
RAS Aquaria



Benefits

-  *High density stocking*
-  *Self-contained production systems*
-  *Greater control over the culture environment*
-  *Increased bio-security*
-  *Minimal water exchange ~10% system volume/24hrs*

RAS Aquaria




Limitations

 *Capital outlay*

 *Disease containment*

 *Mechanical failure, oxygen depletion, toxin levels*

 *Skilled labour required to maintain, monitoring often intensified*

 *Water Quality*

Why is water quality important?



What we strive to achieve

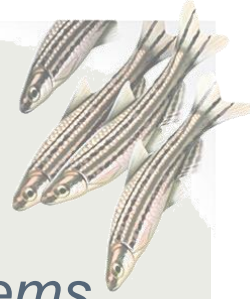






What we aim to avoid



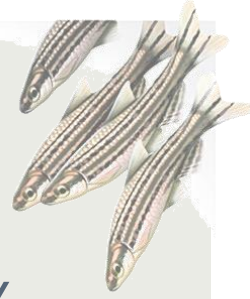
~~What we strive to achieve~~

Water Quality is Key Stone



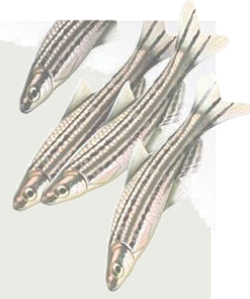
-  *Aquariums are complex dynamic biological systems affected by multiple variable interactions*
-  *Water quality is critical to the successful operation of any aquaria holding system*
-  *Changes can range from seconds to minutes, minutes to hours, and days to months*
-  *Consistent condition and routine monitoring is required*

Water Quality



- *Minimal scientific consensus throughout industry*
- *Most current standards are based on
 - *USEPA Red Book (1976)*
 - *What has been done traditionally*
 - *What appears successful in the laboratory setting**
- *Minimal numbers of controlled studies have been done to evaluate what parameters are best for captive zebrafish*

Ammonia Example

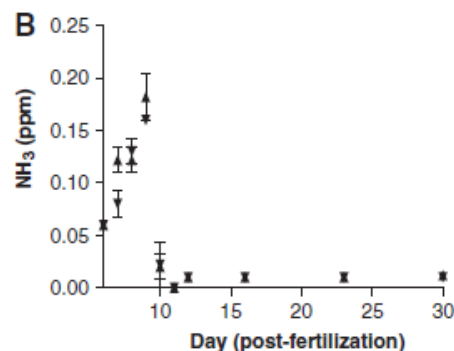


🐟 *USEPA Red Book (1976) recommends*

🐟 *<0.02mg/L NH_3 for freshwater life*

🐟 *Best et al. (2010) reported*

🐟 *0.18mg/L NH_3 with no obvious detrimental effects to 9dpf zebrafish alevin*

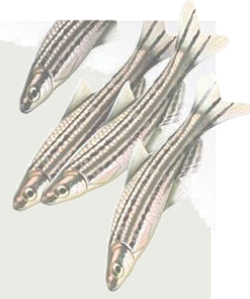






ZEBRAFISH
Volume 7, Number 3, 2010
© Mary Ann Liebert, Inc.
DOI: 10.1089/zeb.2010.0667

A Novel Method for Rearing First-Feeding
Larval Zebrafish: Polyculture with Type L Saltwater
Rotifers (*Brachionus plicatilis*)

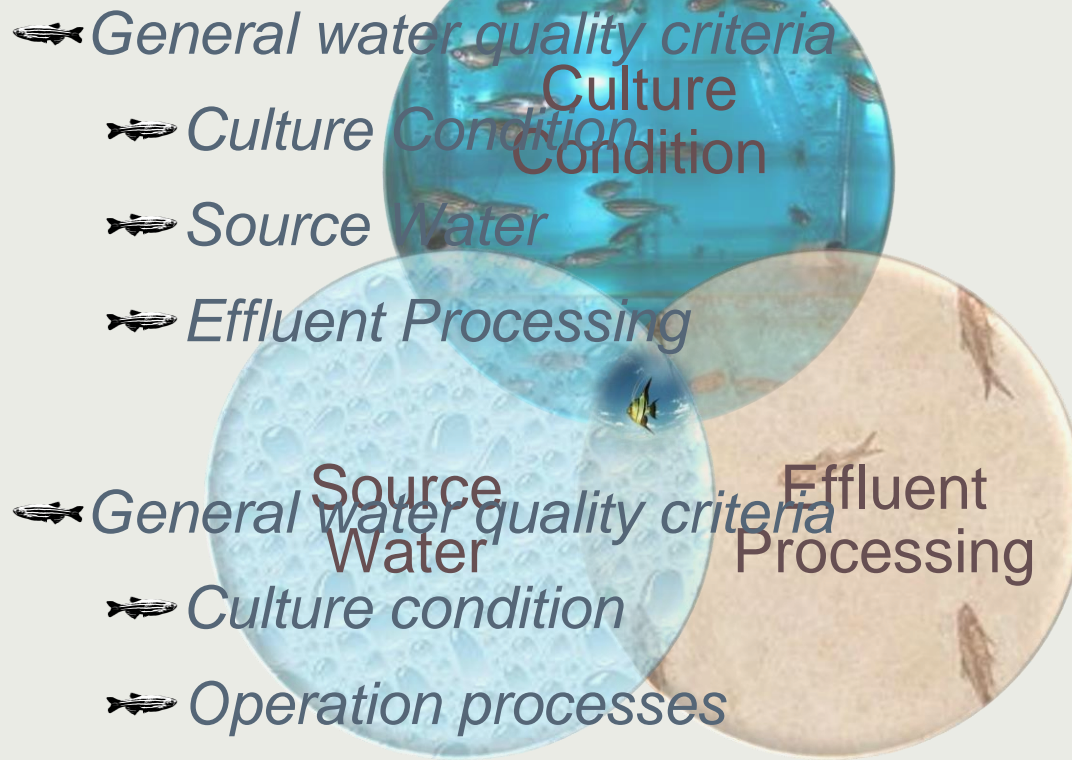
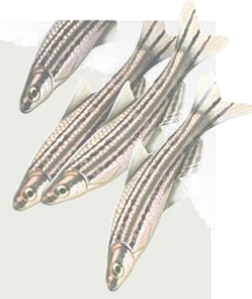
Jason Best,¹ Isaac Adatto,¹ Jason Cockington,² Althea James,¹ and Christian Lawrence¹

Take home message



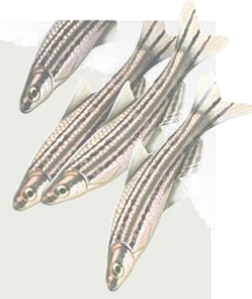
-  *Selection of water quality parameters should be*
 -  *Species specific*
 -  *Life stage specific*
-  *More controlled experimentation is required to identify optimal rearing conditions*

Controlling Water Quality



Colt, Aqua. Eng. 2006

Temperature Water Quality



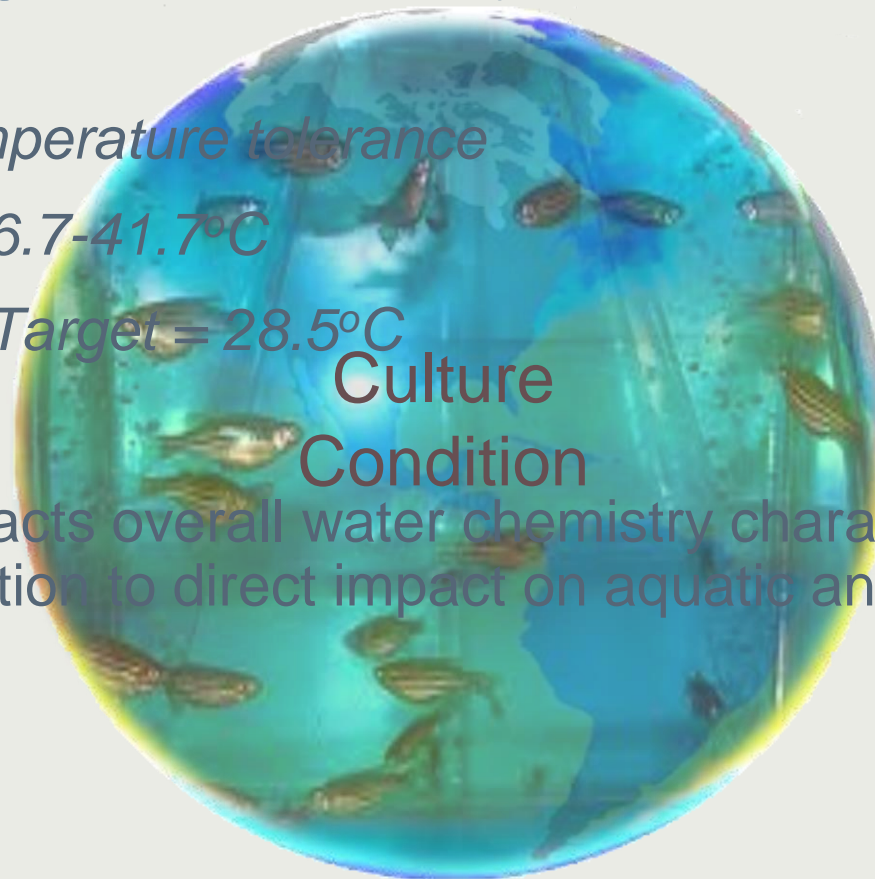
➤ *Temperature tolerance*

➤ 6.7-41.7°C


➤ *Target = 28.5°C*

**Culture
Condition**

➤ Impacts overall water chemistry characteristics in addition to direct impact on aquatic animals



Lawrence, Aquaculture 2007

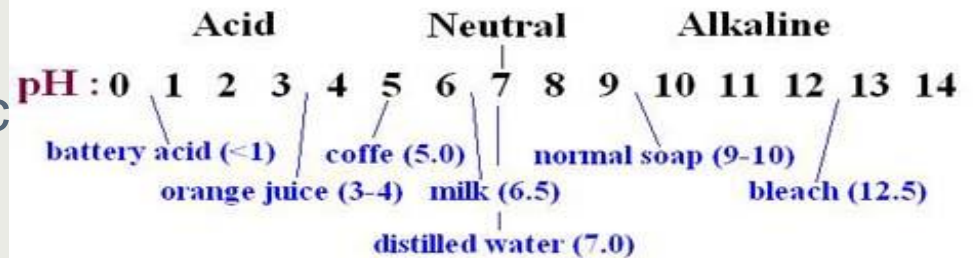
 6.0-9.5

🐟 Measurement of basic, acidic, or neutral qualities of a solution

Respiration

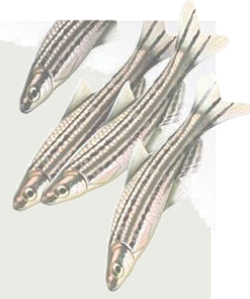


Nitrification



<http://www.all-about-ph.com/ph-scale.html>

Power of Hydrogen (pH)



- 🐟 pH changes must be done gradually
- 🐟 A drop from 7 to 6 represents the water becoming 10 times more acidic
- 🐟 High pH causes:
 - 🐟 increases in concentration of NH_3 (most toxic form)
- 🐟 Low pH causes:
 - 🐟 decreases in activity of nitrifying bacteria
 - 🐟 increases toxicity of heavy metals



Power of Hydrogen (pH)

Respiration effect


 Increased CO_2 will decrease pH

 Depending on alkalinity, can $\downarrow \text{NH}_3:\text{NH}_4^+$ in TAN

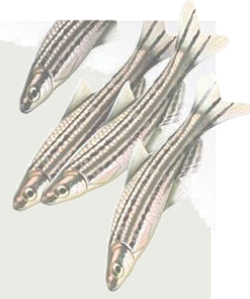
Nitrification effect

 Nitrification consumes alkalinity, decreasing pH

 NH_3 toxicity decreases with decreasing pH

 Nitrifying bacteria have reduced growth and activity at pH levels below 6.4

Power of Hydrogen (pH)



- Buffering pH often necessary
 - Daily – Sodium Bicarbonate (NaHCO_3)
 - Periodic – Coral or Oyster shells (CaCO_3)
 - Must be done slowly to avoid rapid and excessive pH level changes

- Important to understand water hardness and alkalinity before adjusting pH

Hardness



🐟 *Hardness Tolerance*

🐟 *75-200ppm CaCO_3*

🐟 *Target >100ppm*

🐟 *General Hardness (GH)*

🐟 *cations - $\text{Ca}^{++} + \text{Mg}^{++}$*

🐟 *Alkalintiy / Carbonate Hardness (KH)*

🐟 *anions - $\text{HCO}_3^- + \text{CO}_3^{--}$*

General Hardness



- *The sum concentrations of calcium, magnesium, and other divalent cations*
- *Effected by the geology of the watershed of the source*
- *Freshwater fish blood ions are higher than the water*

- *Increasing hardness:*
 - *Decreases osmoregulatory stress*
 - *Decreases the toxicity of dissolved metals like copper and zinc*

Carbonate Hardness



- *Sum of bicarbonate (HCO_3^-) and carbonate (CO_3^{--}) anions in the water*
- *Reflects the buffering capacity of the water or the stability of pH*
- *Dissolved metals (copper, zinc, and aluminium) are more toxic to fish in water of low alkalinity*

Salinity and Conductivity



➤ *Salinity Tolerance*

➤ *0.2-2.0ppt*

➤ *Target 0.25-0.75ppt*

➤ *Salinity measures salts of the alkali metals or magnesium*

➤ *Conductivity Tolerance*

➤ *Can both be modified by addition of balanced salt formulations*

➤ *Evaporation of water will increase both*

➤ *300-4000 μ S/cm²*

➤ *Target 300-1200 μ S*

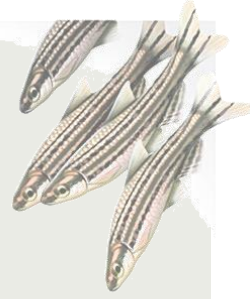
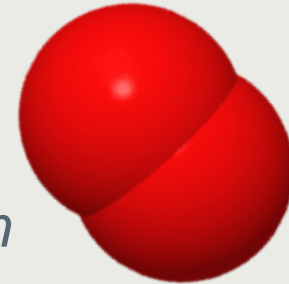
➤ *Capacity of water to conduct an electrical current*

Salinity and Conductivity



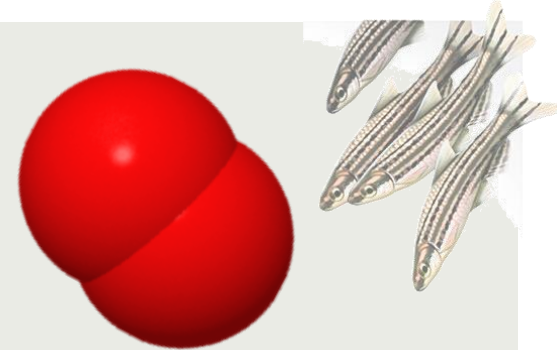
- Na^+ are necessary for ammonium (NH_4^+) excretion and ion regulatory function
- High salt
 - Fresh water animals cannot excrete enough ions
- Low salt
 - Fresh water animals will fight to retain ions
- These processes have a high metabolic cost to the animals

Dissolved Oxygen



- *Target > 6 mg/L or > 80 % saturation*
- *100% saturation @28°C = 7.8mg/L*
- *As water temperature increases the maximum DO saturation level decreases*
- *Can be modified by the use of mechanical aeration devices, degassing towers, trickle filters, or by the introduction of oxygen gas*
 - *>100% saturation can be dangerous*
 - *Different than total gas pressure (TGP)*

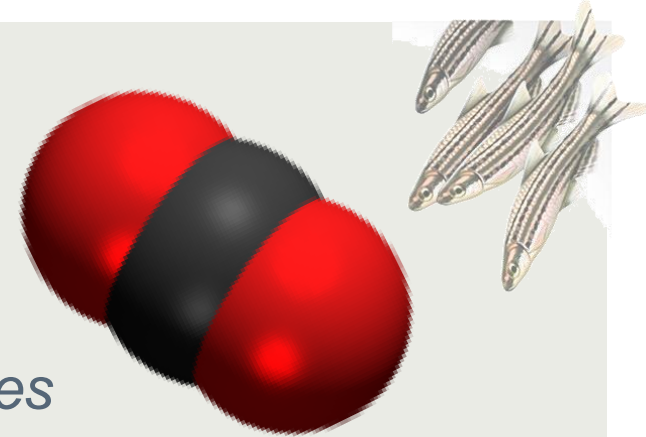
Dissolved Oxygen



- 🐟 Saturation >100%
- 🐟 Hyperoxia (delicate to manage)
- 🐟 Indicator of Gas Bubble Disease (GBD)
- 🐟 Hyperoxia
 - 🐟 Used to manage densely populated, docile species
 - 🐟 Respiration decreases (CO_2 is retained)
 - 🐟 Kidneys retain HCO_3^- to balance blood pH

Carbon Dioxide

- *Target < 5mg/L*
- *Elevated CO₂ reduces growth rates*
- *High CO₂ causes nephrocalcinosis*
- *Increasing CO₂ reduces pH*
 - *↓ NH₃ toxicity, ↑ heavy metal toxicity*
- *Tested and recorded infrequently*
- *Can be reduced by use of degassing towers, packed columns, trickle filters, etc*



Colt, Aqua. Eng. 2006

Total Gas Pressure

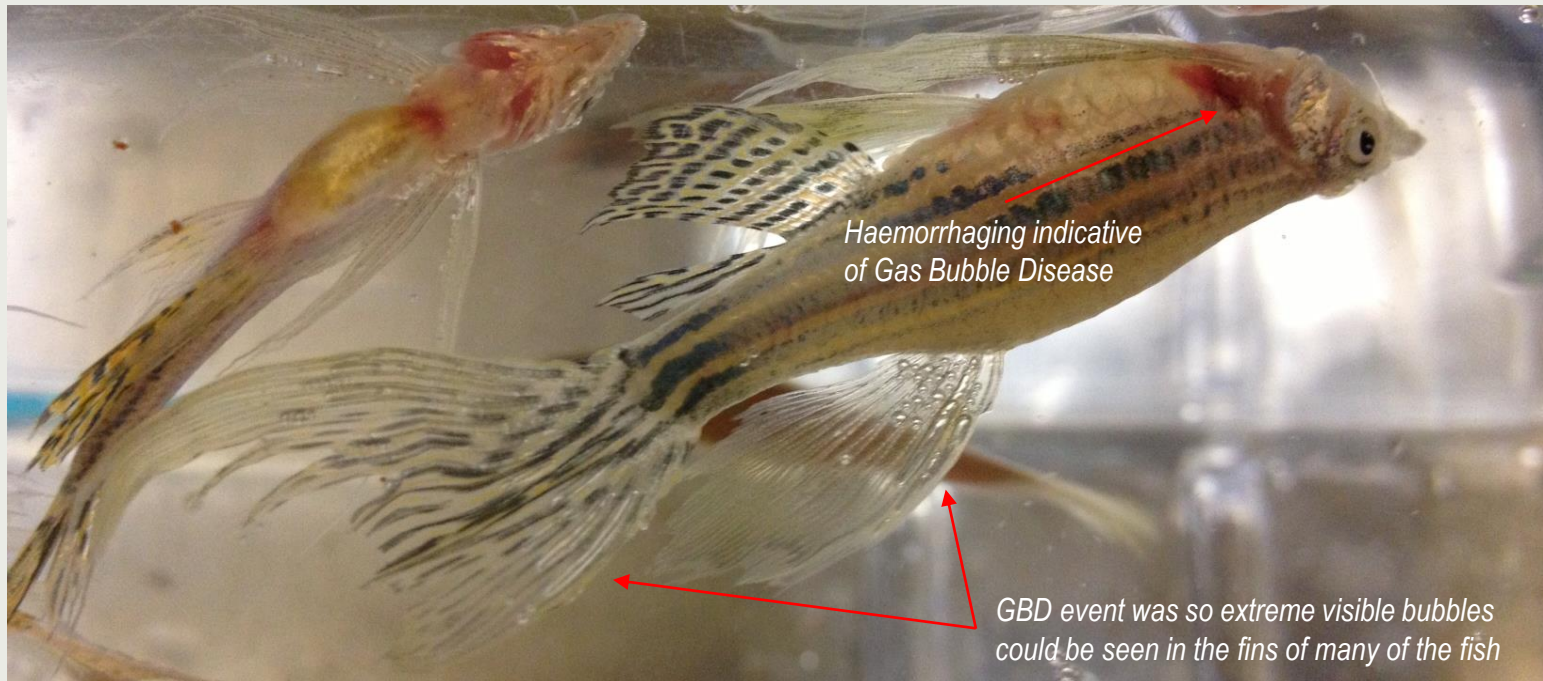


- When the total pressure of all the gases in the water exceeds the ambient atmospheric pressure at the water surface, supersaturation exists
- The effect of excessive supersaturation on fish has been well documented, and if supersaturation exceeds the established safe levels, massive fish kills can occur

➤ Gas Bubble Disease

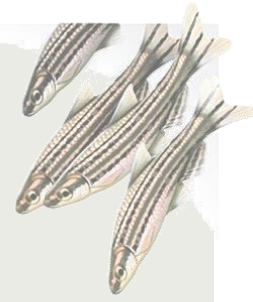
Colt, Aqua. Eng. 2006

Gas Bubble Disease



*Haemorrhaging indicative
of Gas Bubble Disease*

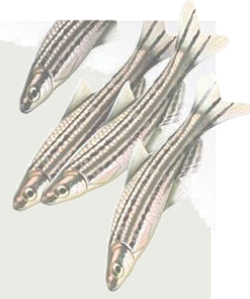
*GBD event was so extreme visible bubbles
could be seen in the fins of many of the fish*









Chlorine and Chloramine

- 🐟 Chloramine
 - 🐟 Target = 0 mg/L (ppm)
 - 🐟 Chloramine = Chlorine + Ammonia
 - 🐟 0.01 ppm is acutely toxic to fish
- 🐟 Neutralise - commercial products (Nov-Aqua[®], AmQuel[®] or Safe[®]), or Sodium Thiosulfate
- 🐟 Remove - filtration using activated carbon

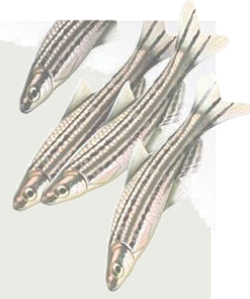
Chlorine and Chloramine



Chlorine

-  Target = 0 mg/L (ppm)
-  Zebrafish can tolerate low Cl_2 (0.5 -1ppm)
-  Human smelling threshold is ~ 0.2 – 0.4 ppm
-  Neutralise or remove – same as chloramine, or aeration over time (only for chlorine)
-  Chronic exposure can damage skin, eyes, and gills
-  Municipal water systems typically have 0.5 to 1.0 mg/L residual concentrations of chlorine present

Culture Condition Guidelines

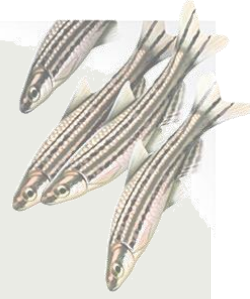


- Temperature: 18 – 24 (24-28°C)
 - Alkalinity: 50-150 mg/L
 - Hardness: 80-300+ mg/L
 - pH: 6.0-8.0
 - Salinity: 0.5 -1g/L(ppt)
 - Conductivity: 300-1500 μ S
 - Un-ionized ammonia:
- (NH₃): < 0.02mg/L
 - Nitrite: (NO₂⁻): < 1mg/L
 - Nitrate: (NO₃⁻): < 50mg/L
 - Chlorine: 0mg/L
 - DO₂ : > 6 mg/L or > 80 % saturation
 - CO₂: < 5mg/L

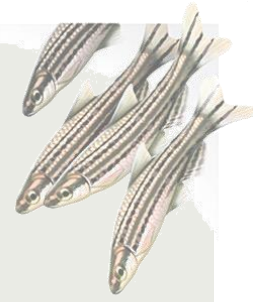
Control Water Quality

- *Municipal source water*
- *Artificial source water*
- *RO, distilled, desalination*
- *Natural source water*
- *Bores / rivers / lakes / wells*

Source
Water

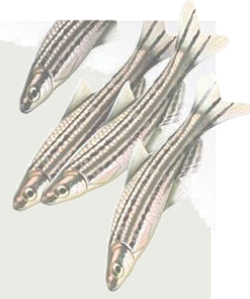


Municipal Supply Water



<i>Benefits</i>	<i>Limitations</i>
<i>Availability</i>	<i>Government regulated quality</i>
<i>Cheap</i>	<i>Limited pathogen control</i>
<i>Conditioning optional for culture use</i>	<i>Limited control over culture condition</i>
<i>Minimal waste volumes</i>	<i>Requires pre-treatment Activated Carbon – Chlorine/Chloramine/Cu^{2+}</i>

Heavy Metals




 *Most important*

 *Cadmium, copper, zinc, aluminium*

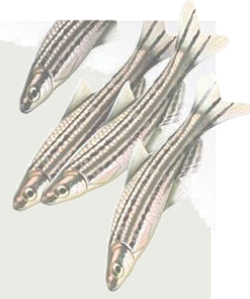
 *Source*

 *Corrosion of pipes and fittings*

 *Poor quality feed stuffs*

 *Dissolved metals are more toxic to fish in water of low alkalinity*

Heavy Metals



 *Action level for heavy metal presence (µg/L)*

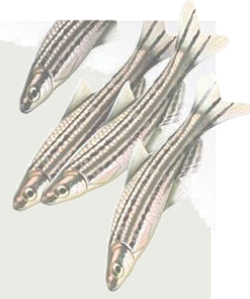
Metal	Freshwater				Seawater
	500 ^a	100 ^a	10 ^a	1 ^a	
Copper	35	9	1.3	0.18	3.1
Zinc	460	120	17	2.4	81
Cadmium	0.75	0.25	0.049	0.01	8.8

^a Hardness (mg/L as CaCO₃).






 *Zebrafish hardness target >100mg/L*

Colt, Aqua. Eng. 2006

Municipal Supply Water

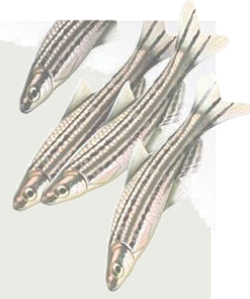


2012 Brisbane Water Conditions

 <i>Alkaline</i>	<i>6.7-8.3pH</i>
 <i>Hard</i>	<i>60-220 mg/L CaCO₃</i>
 <i>Conductivity</i>	<i>210-950µS/cm²</i>
 <i>Heavy metals</i>	<i>5-110 µg/L Cu⁺⁺</i>
 <i>FreeChlorine</i>	<i><0.1-1.6mg/L</i>

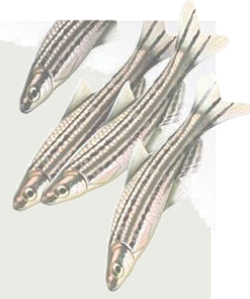
Urban Utilities, 2012

RO Supply Water



<i>Benefits</i>	<i>Limitations</i>
<i>User specified product quality</i>	<i>Dedicated equipment</i>
<i>Good pathogen control (clean water)</i>	<i>Higher operating cost (membrane replacement)</i>
<i>Specific control over culture condition</i>	<i>Requires conditioning for culture use</i>
	<i>↑ product quality = ↑ waste volumes</i>

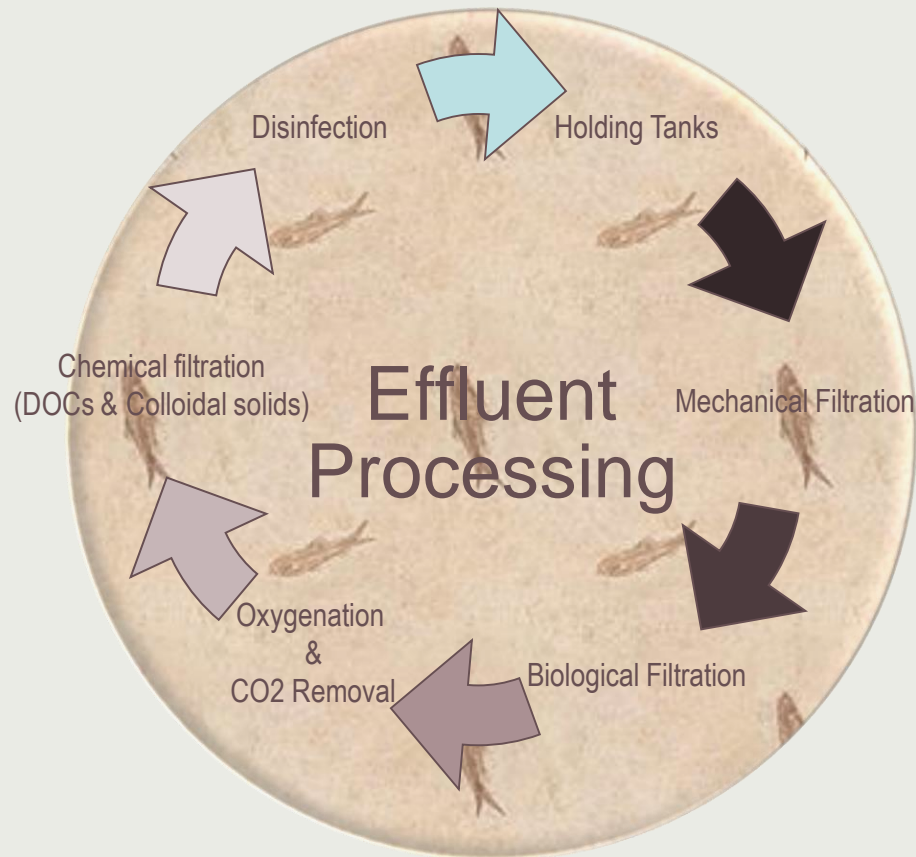
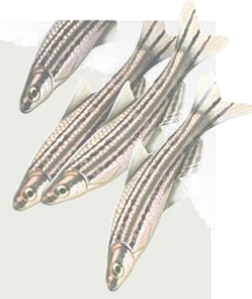
Class3 RO Supply Water



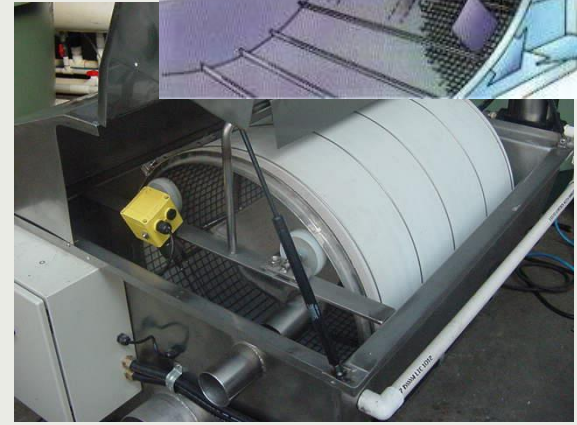
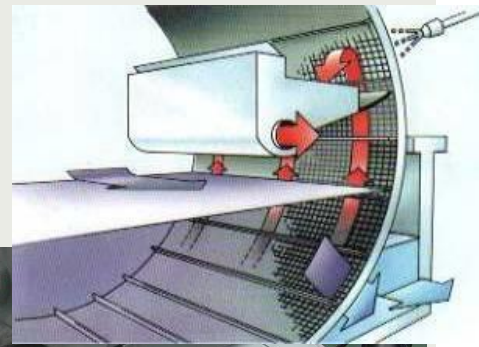
- *Low alkalinity* *Acid pH ~6.3*
- *Soft* *0mg/L CaCO₃*
- *Conductivity* *~20 µS/cm² conductivity*

- *Requires conditioning for culture use*
 - *Hardness Generators (↑pH + Ca⁺ cations)*
 - *Marine Sea Salt (↑µS + essential minerals)*
 - *Typically 7.5pH, >100mg/L CaCO₃, ~400 µS/cm²*

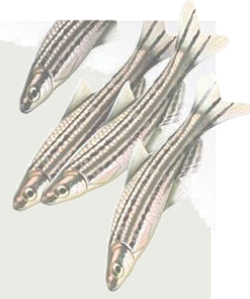
RAS Ceiling Water Supply Systems (CLS)



Mechanical Filtration



Mechanical Filtration



Purpose

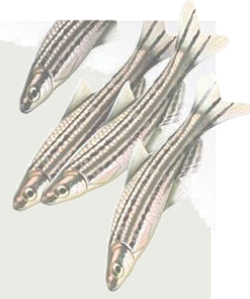
 *Remove large suspended debris*

 *Range : 10-100µm*

 *Allows for healthy biofilter growth*

 *Enhances UV efficacy*

Mechanical Filtration



 *Considerations*

 *Removes or Isolates waste?*

 *Welfare impact*

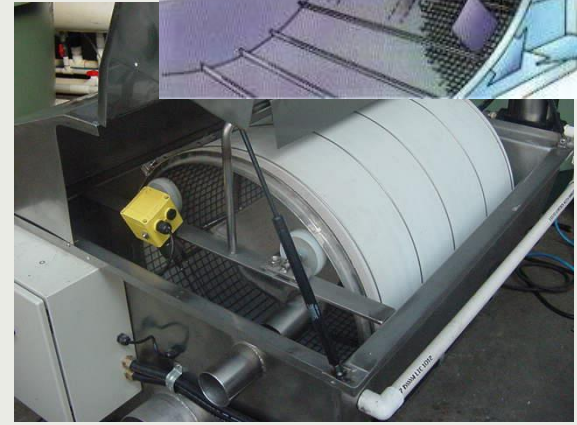
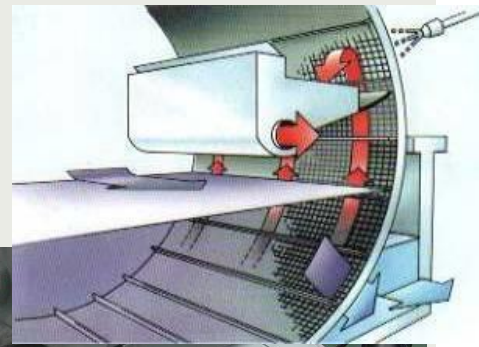
 *Consumables?*

 *Technical skill level for operation / maintenance?*

 *Automation?*

 *Operating cost impact*

Mechanical Filtration



Biological Filtration

 *Includes*

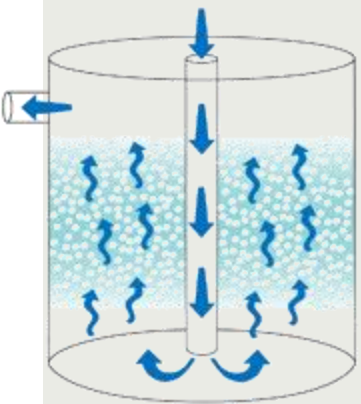
 *Under-gravel filters*

 *Fluidized beds*

 *Trickle filters (wet/dry filters)*

 *Bead filters*

 *Media varies by type, shape, size*



Biological Filtration



🐟 *Dedicated media*

🐟 *Provides substrate for bacterial growth*

🐟 *Bacteria break down metabolic waste products*

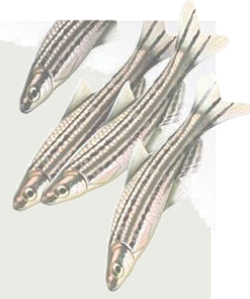




Ammonia

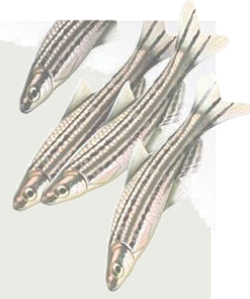
- *Total Ammonia Nitrogen (TAN) = NH_4^+ + NH_3*
- *TAN species ratio influenced by pH*
- *Majority of waste nitrogen in fish is excreted as NH_3 through gills not as urea*
- *Requires positive gradient between fish and ambient water*
- *As ambient water concentrations increase the outward flow of NH_3 decreases or may stop altogether*
- *Should be kept as low as possible – 0 ppm*

Biological Filter



- 2 types of aerobic microorganisms that colonize aquatic biofilters
 - Heterotrophic bacteria utilize dissolved organic compounds (DOCs)
 - Chemosynthetic bacteria utilize ammonia and nitrite as a food source
- Heterotrophic bacteria grow 5X faster than Chemosynthetic bacteria

Nitrification



 *Toxic ammonia is converted to non-toxic nitrate*



 *Nitrosomonas sp.*

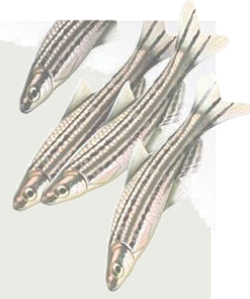


 *Nitrobacter, Nitrospina sp.*



Chen et al, Aqua. Eng. 2006

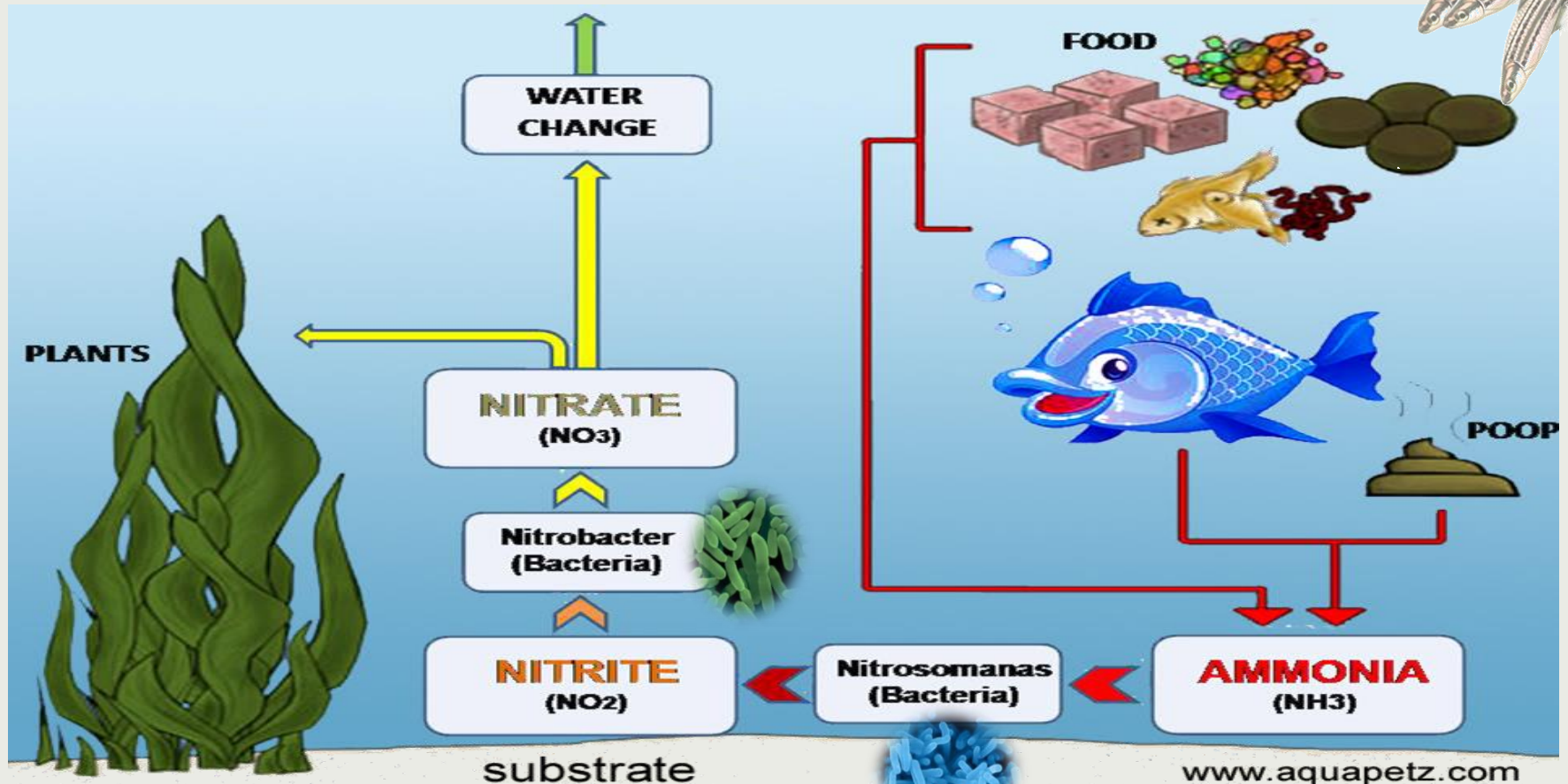
Nitrification



- *Requires oxygen and alkalinity (\downarrow pH)*
- *1g TAN oxidised to nitrate nitrogen requires*
 - *4.18g of O_2*
 - *7.07g of CO_3^-*
 - *0.17g of bacteria biomass generated*

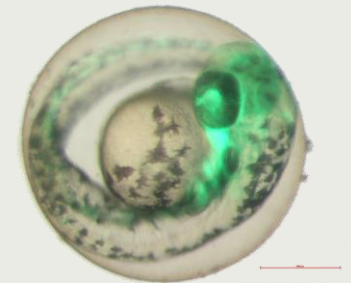
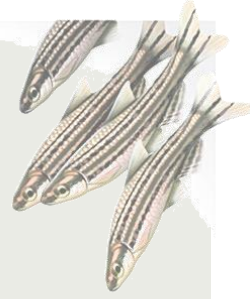
Chen et al, Aqua. Eng. 2006

Aquatic Nitrogen Cycle

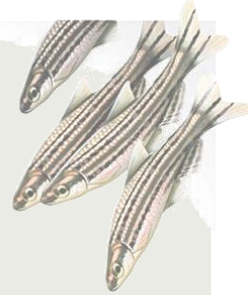


Chemical Filtration

- Dissolved wastes are more difficult to remove
- 2 main options for chemical filtration
 - Activated Carbon
 - Foam fractionation (protein skimming)



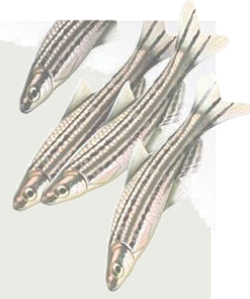
Activated Carbon




- 🐟 Works by adsorption
 - 🐟 pollutant molecules in the water are trapped inside the pore structure of the carbon substrate
- 🐟 Removes
 - 🐟 Chlorine and Copper ions
 - 🐟 Dissolved Organic Compounds (DOC's)
 - 🐟 Colloidal solids




Protein Skimming



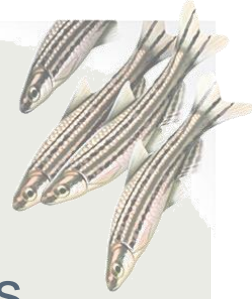
 Works by adsorption

 Hydrophobic pollutant molecules in the water bind to micro bubbles (of air or ozone) rising through a column

 At the surface the bubbles form a foam and the waste is discharged to the foamate stream



Disinfection



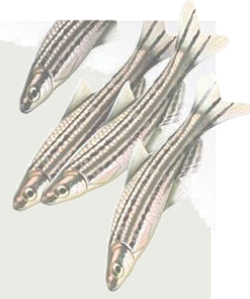
- Focussed at reducing microorganism populations
- 2 main options to consider
 - Ultraviolet irradiation
 - Ozone



Ultraviolet irradiation

- Effectiveness depends on flow rates, plumbing diameter and unit size/power and Water Quality
 - Presence of particulates decreases efficacy
- Irradiation dose expressed as $\mu\text{Js}/\text{cm}^2$
- Effective bulb life is short (~12 months)
- Critical: bulb must be replaced regularly and quartz sleeve cleaned and replaced when it becomes cloudy

Ozone



🐟 *Higher disinfecting power than UV*

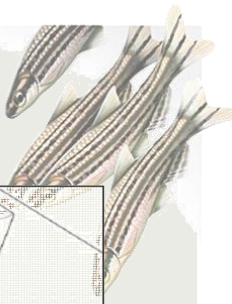
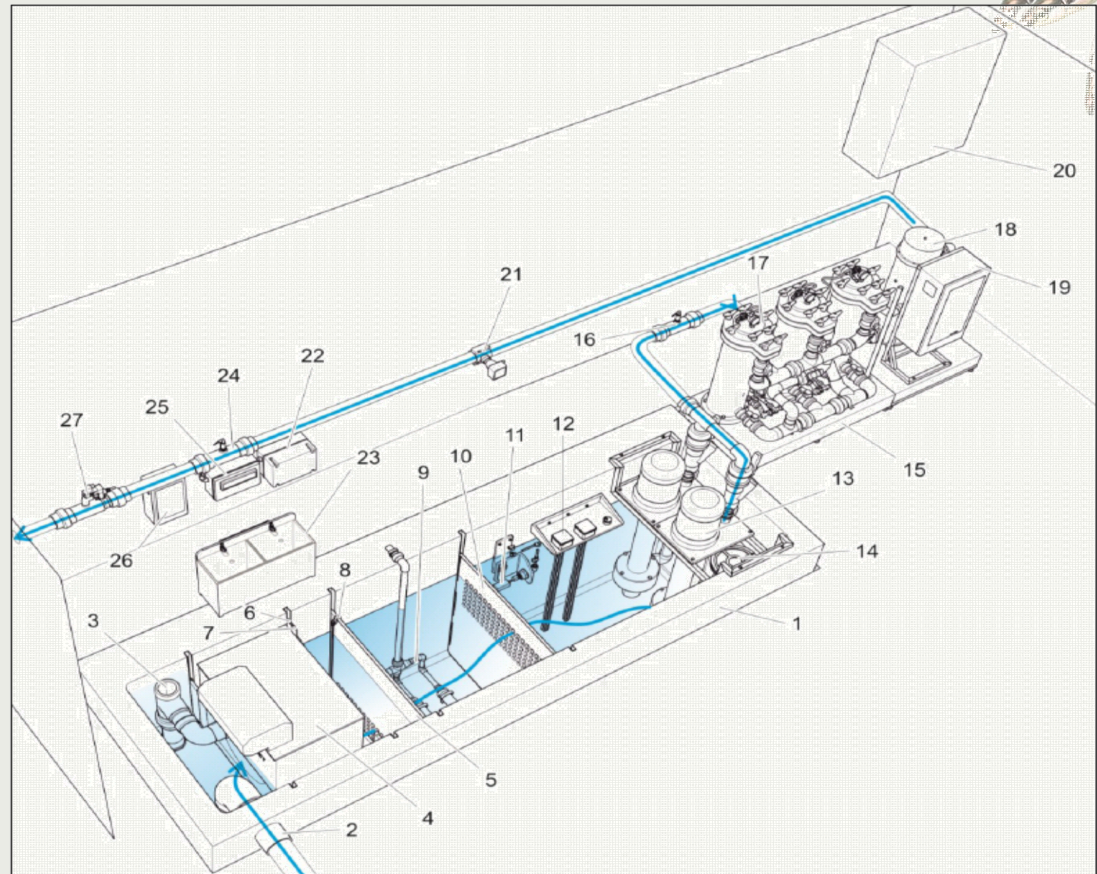
🐟 *Why not commonly implemented?*

🐟 Higher risk to animals

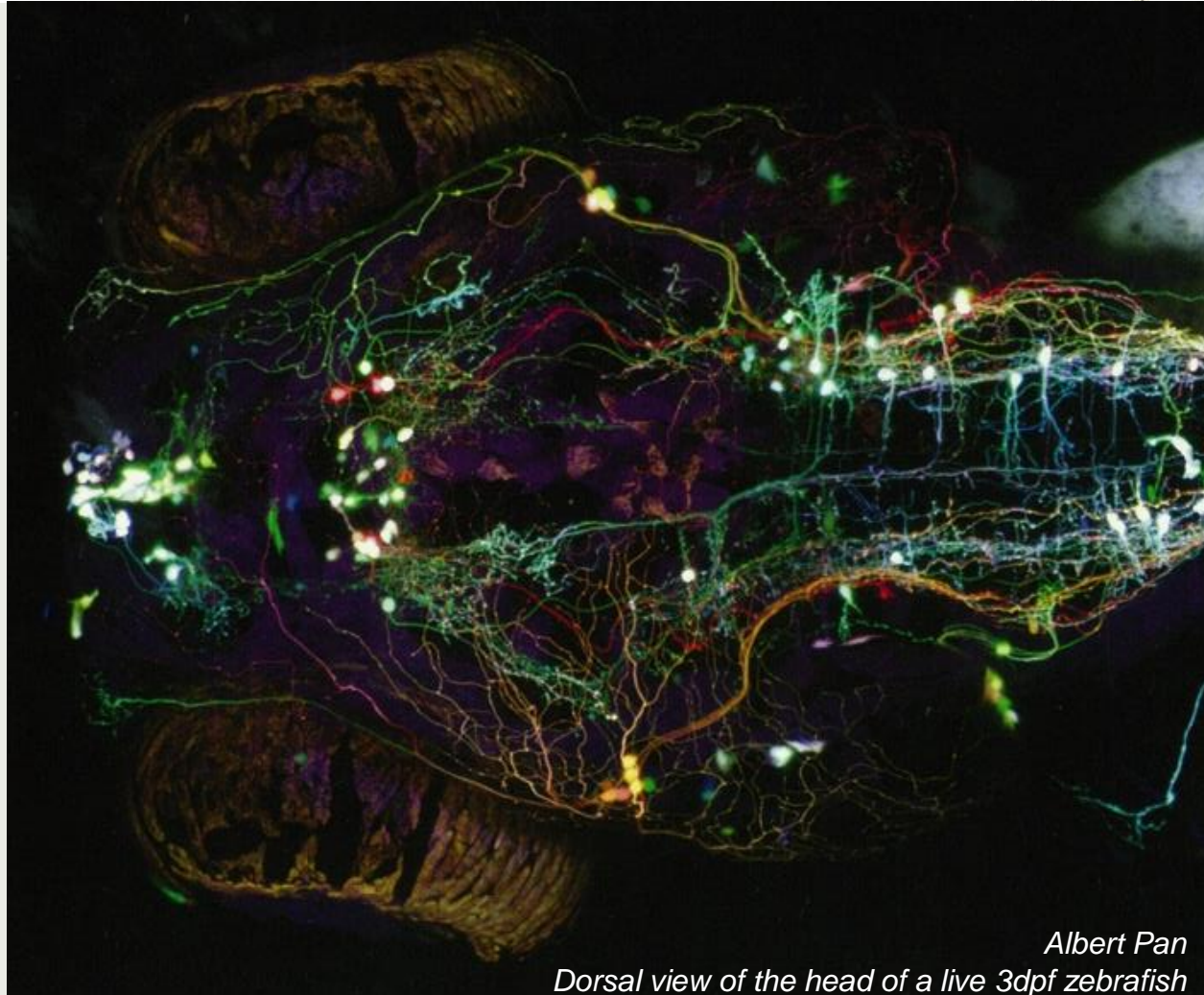
🐟 Limited by contact time

🐟 We'll discuss more in next session

Effluent Processing Assembly



Questions?



Albert Pan
Dorsal view of the head of a live 3dpf zebrafish