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

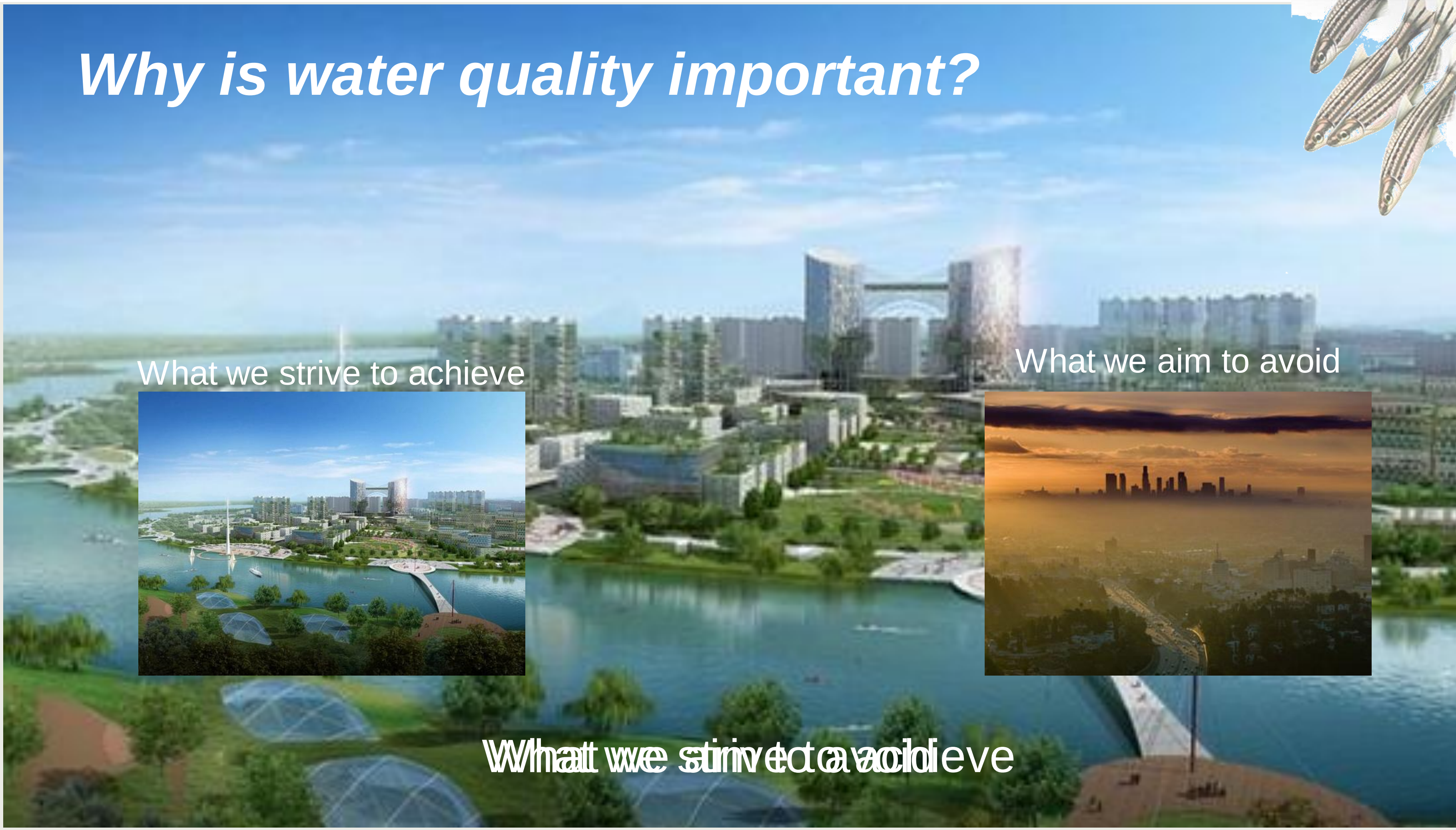
RAS Aquaria

- *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?

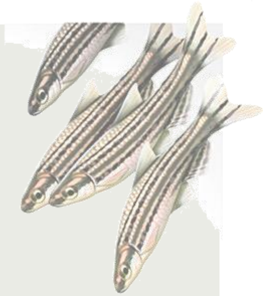


What we strive to achieve
What we aim to avoid

Water Quality is Key Stone

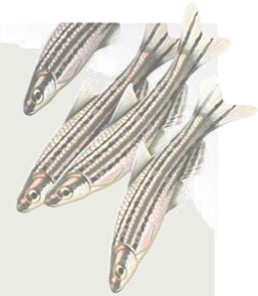
- *Aquariums are complex dynamic biological systems affected by multiple variable interactions*
- *Water quality is critical to the successful operation of any aquaria holding system*
- *Changes can range from seconds to minutes, minutes to hours, and days to months*
- *Consistent condition and routine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

Water Quality

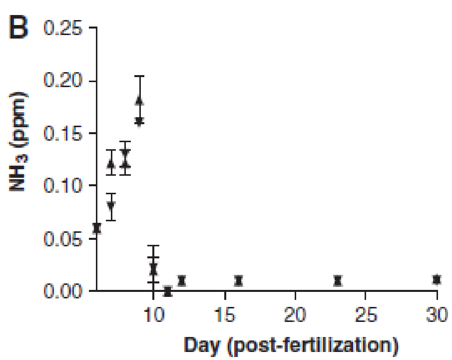


- *Minimal scientific consensus throughout industry*
- *Most current standards are based on*
  - *USEPA Red Book (1976)*
  - *What has been done traditionally*
  - *What appears successful in the laboratory setting*
- *Minimal numbers of controlled studies have been done to evaluate what parameters are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



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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 Lawrence[1]

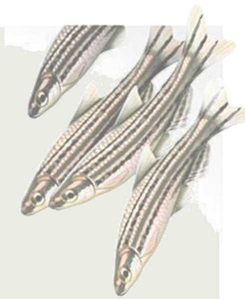
Take home message

- *Selection of water quality parameters should be*
  - *Species specific*
  - *Life stage specific*

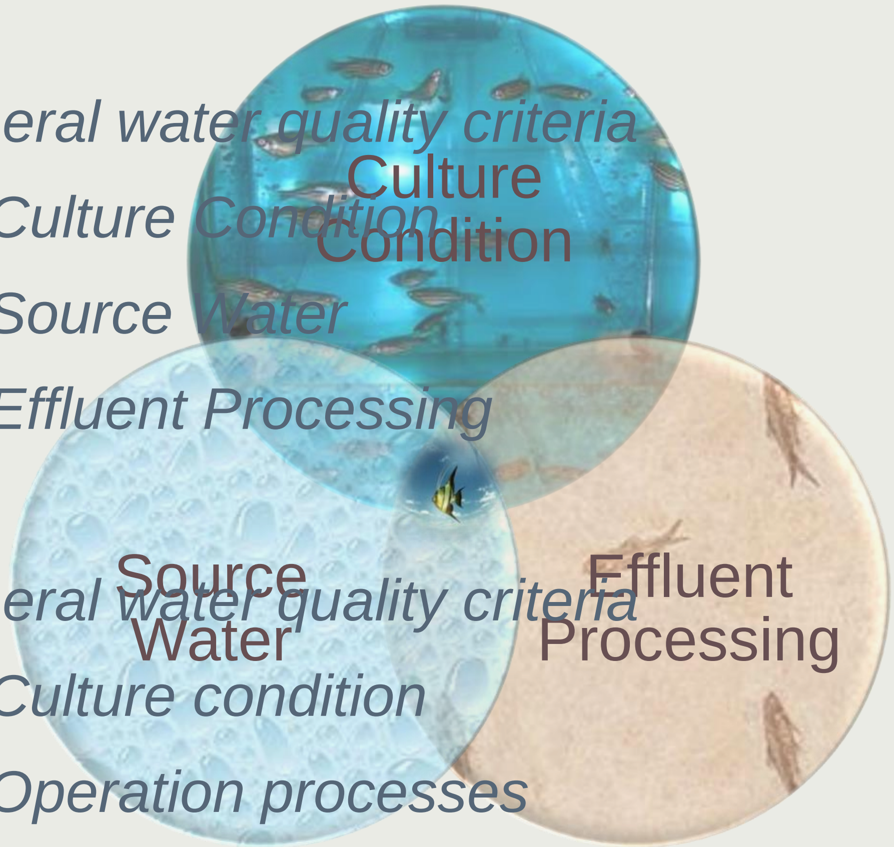
- *More controlled experimentation is required to identify optimal rearing conditions*

Understanding Water Quality

5th Annual International Zebrafish Husbandry Course



- General water quality criteria
  - Culture Condition
  - Source Water
  - Effluent Processing



- General water quality criteria
  - Culture condition
  - Operation processes

Colt, Aqua. Eng. 2006

Understanding Water Quality

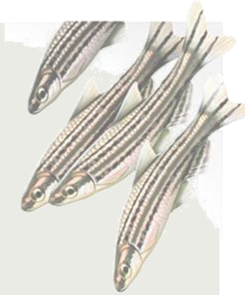
Temperature

- *Temperature tolerance*
  - *6.7-41.7^{o}C*
  - *Target = 28.5^{o}C*

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

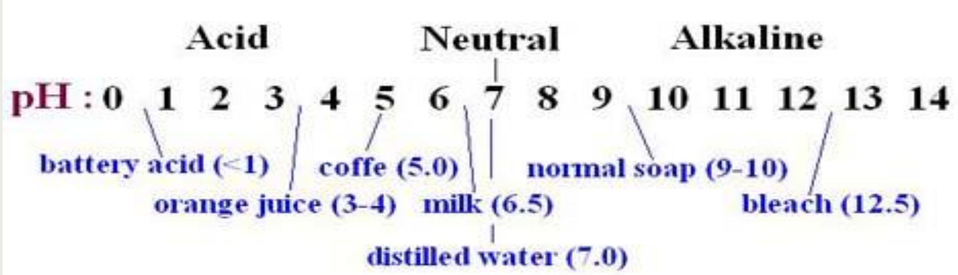
Lawrence, Aquaculture 2007

Power of Hydrogen (pH)



- *pH tolerance*
  - *6.0-9.5*
  - *Target = 7.5-8*

- Measurement of basic, acidic, or neutral qualities of a solution
- Will fluctuate in recirculating systems
  - Respiration
  - Nitrification



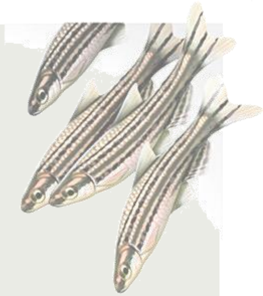
http://www.all-about-ph.com/ph-scale.html

Power of Hydrogen (pH)

- pH changes must be done gradually
- A shift from 7 to 8 represents the water becoming 10 times more alkaline

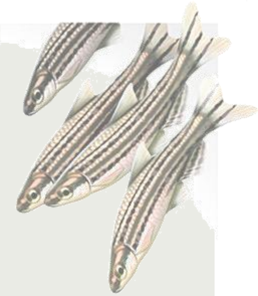
- High pH causes:
  - increases in concentration of NH_3 (most toxic form)
- Low pH causes:
  - decreases in activity of nitrifying bacteria
  - increases toxicity of heavy metals

Power of Hydrogen (pH)



- Respiration effect
  - Increased CO_2 will decrease pH
  - Depending on alkalinity, can ↓NH_3: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

Hardness



- *Hardness Tolerance*
  - *75-300ppm* $CaCO_3$
  - *Target >100ppm*

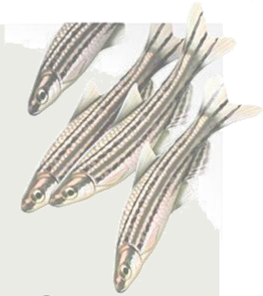
- *General Hardness (GH)*
  - *cations -* $Ca^{++} + Mg^{++}$
- *Alkalintiy / Carbonate Hardness (KH)*
  - *anions -* $HC0_3^{-} + CO_3^{--}$

General Hardness (GH)

- *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

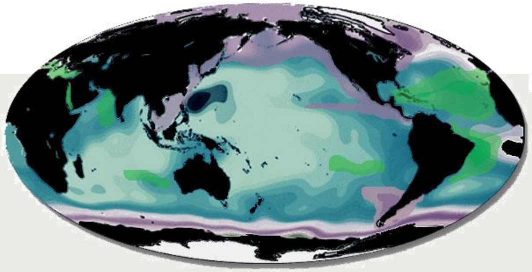
- *Increasing hardness:*
  - *Decreases osmoregulatory stress*
  - *Decreases the toxicity of dissolved metals like copper and zinc*

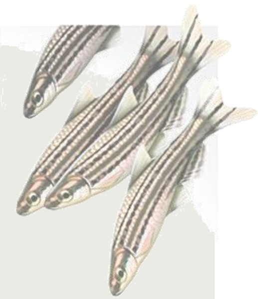
Carbonate Hardness (KH)



- *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



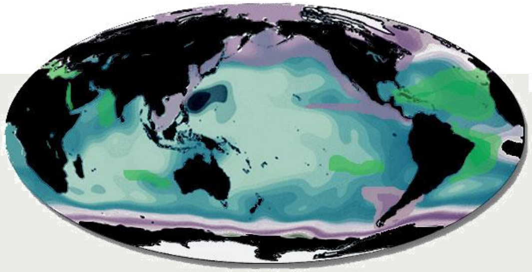


- *Salinity Tolerance*
  - *0.2-2.0ppt*
  - *Target 0.25-0.75ppt*
- *Salinity measures salts of the alkali metals or magnesium*

- *Conductivity Tolerance*
  - *300-4000µS/cm^2*
  - *Target 300-1200µS*
- *Capacity of water to conduct an electrical current*

- *Both modified by addition of balanced salt formulations*
- *Evaporation of water will increase both*

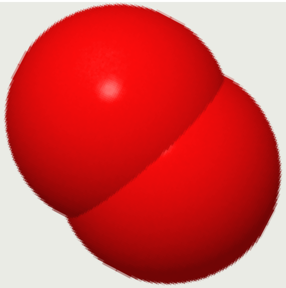
Salinity and Conductivity

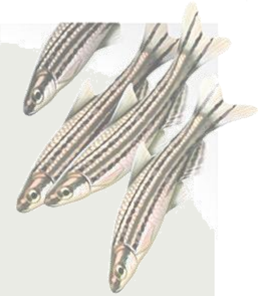


- Na^+ are necessary for ammonium (NH_4^+) excretion and ion regulatory function
- High salt
  - Fresh water animals cannot excrete enough ions
- Low salt
  - Fresh water animals will fight to retain ions

- These processes have a high metabolic cost to the animals

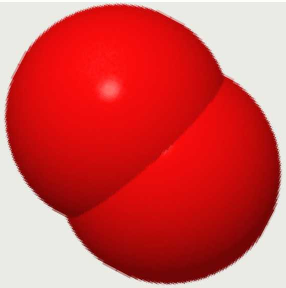
Dissolved Oxygen

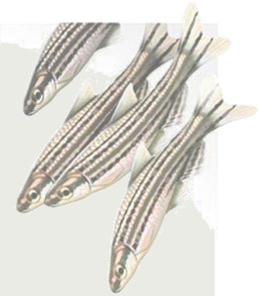




- Target > 6 mg/L or > 80 % saturation
- 100% saturation @28^{o}C = 7.8mg/L
- As water temperature increases the maximum DO saturation level decreases
- Can be modified by the use of mechanical aeration devises, degassing towers, trickle filters, or by the introduction of oxygen gas
  - >100% saturation 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 CO_2 reduces growth rates
- High CO_2 causes nephrocalcinosis
- Increasing CO_2 reduces pH
  - ↓ NH_3 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

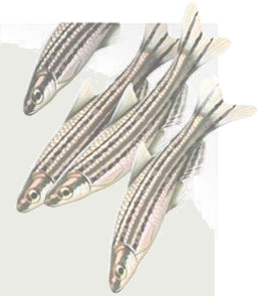
Total Gas Pressure

- 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

Culture Condition Guidelines

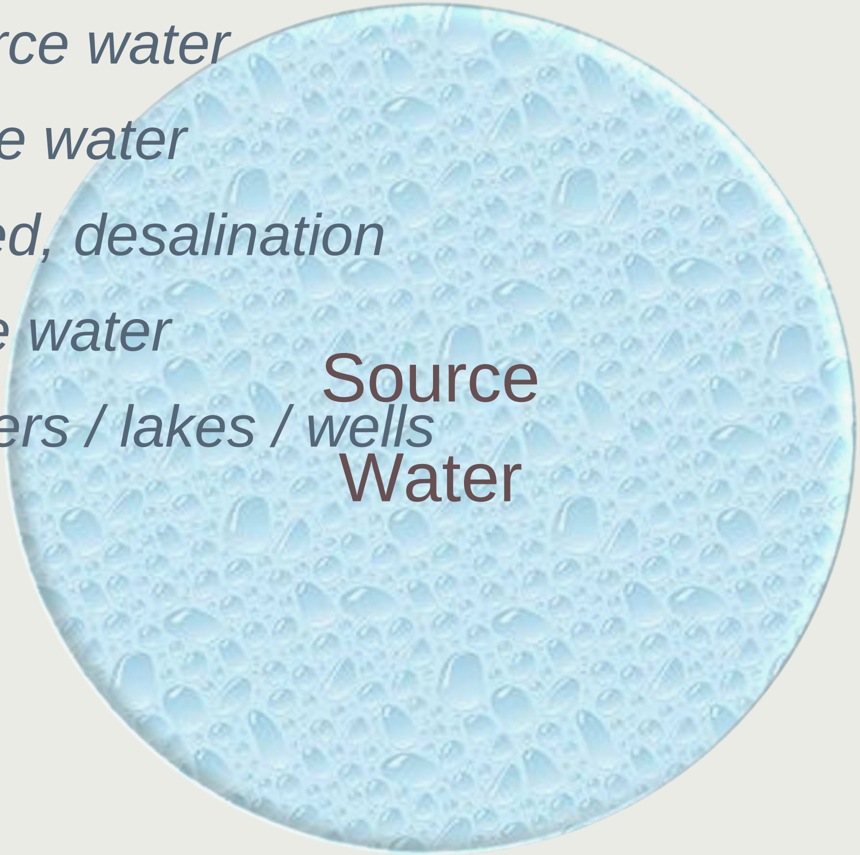


- Temperature: 24-28^{o}C
- Alkalinity: 50-150 mg/L
- Hardness: 80-300+ mg/L
- pH: 6.0-8.0
- Salinity: 0.5 -2g/L(ppt)
- Conductivity: 300-1500μS
- Un-ionized ammonia: (NH_3): < 0.02mg/L
- Nitrite: (NO_2^-): < 1mg/L
- Nitrate: (NO_3^-): < 50mg/L
- Chlorine: 0mg/L
- DO_2 : > 6 mg/L or > 80 % saturation
- CO_2: < 5mg/L

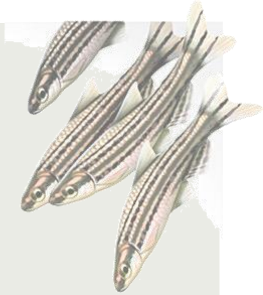
Controlling Water Quality

Source Water

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



Municipal Supply Water



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

Chlorine and Chloramine

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

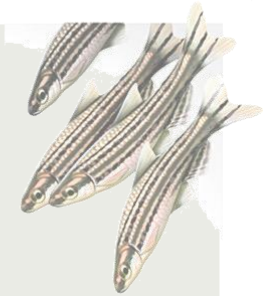
Chlorine and Chloramine

- 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

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)*

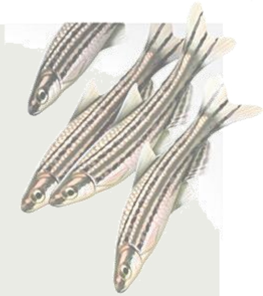
Metal	Freshwater				Seawater
	500[a]	100[a]	10[a]	1[a]	
Copper	35	9	1.3	0.18	3.1
Zinc	460	120	17	2.4	81
Cadmium	0.75	0.25	0.049	0.01	8.8

[a] Hardness (mg/L as $CaCO_3$).

- *Zebrafish hardness target >100mg/L*

Colt, Aqua. Eng. 2006

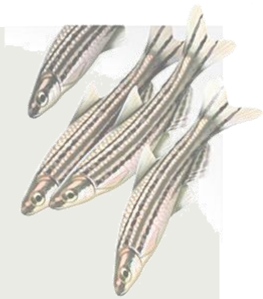
Municipal Supply Water



- *Typical Brisbane Water Conditions*
  - *Alkaline* 6.7-8.3pH
  - *Hard* 60-220 mg/L $CaCO_3$
  - *Conductivity* 210-950µS/cm^2
  - *Heavy metals* 5-110 µg/L Cu^{++}
  - *Free Chlorine* <0.1-1.6mg/L

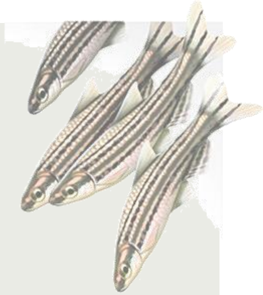
Urban Utilities, 2012

RO Supply Water



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

Class3 RO Supply Water

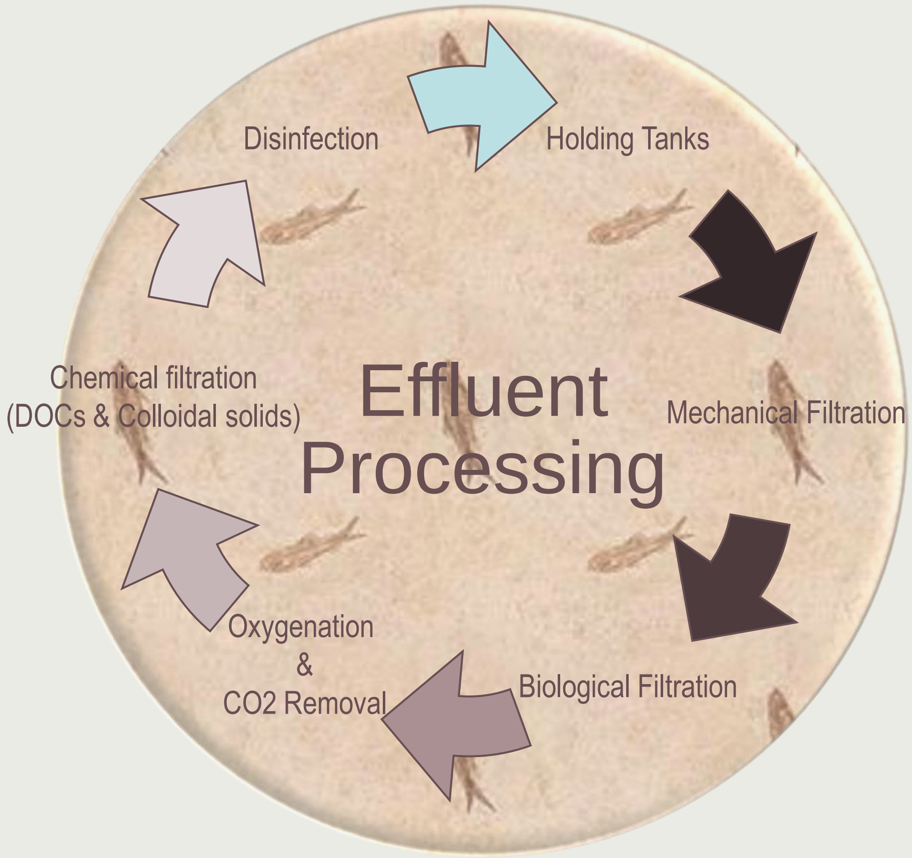


- *Low alkalinity* *Acid pH ~6.3*
- *Soft* *0 mg/L* $CaCO_3$
- *Salinity* *~20* µS/cm^2 *conductivity*

- *Requires conditioning for culture use*
  - *Hardness Generators (↑pH +* Ca^+ *cations)*
  - Marine Sea Salt (↑µS + essential minerals)
- *Typically 7.5pH, >100mg/L* $CaCO_3$*, ~1,000* µS/cm^2

RAS Central Water Supply Systems

Restoring Water Quality

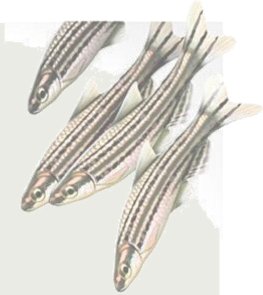


Mechanical Filtration

Mechanical Filtration

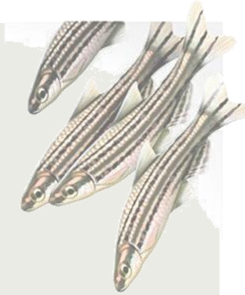
- *Purpose*
  - *Remove large suspended debris*
    - *Range : 10-100µm*
  - *Allows for healthy biofilter growth*
  - *Enhances UV efficacy*

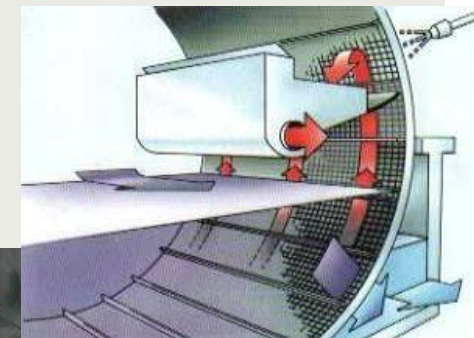
Mechanical Filtration



- Considerations
  - Removes or Isolates waste?
    - Welfare impact
  - Consumables?
  - Technical skill level for operation / maintenance?
  - Automation?
    - Operating cost impact

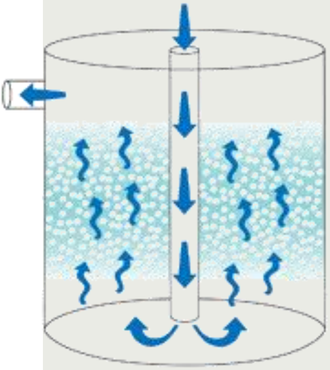
Mechanical Filtration



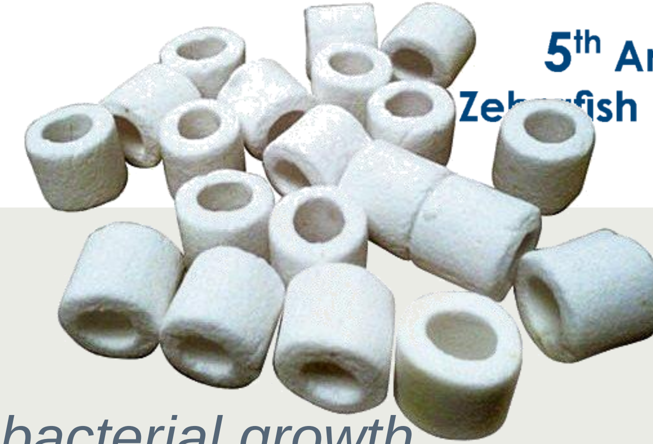


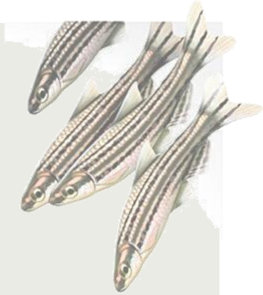
Biological Filtration

- Includes
  - Under-gravel filters
  - Fluidized beds
  - Trickle filters (wet/dry filters)
  - Bead filters
  - Media varies by type, shape, size

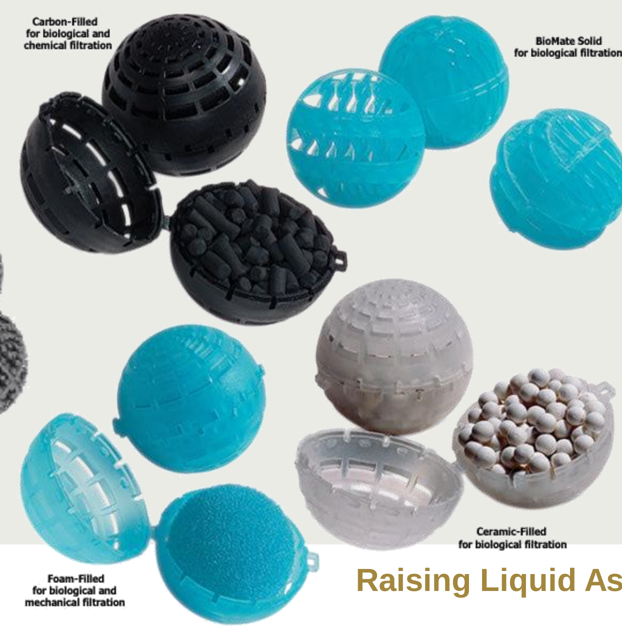


Biological Filtration

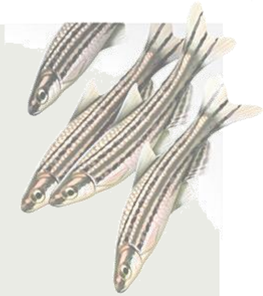




- *Dedicated media*
  - *Provides substrate for bacterial growth*
  - *Bacteria break down metabolic waste products*

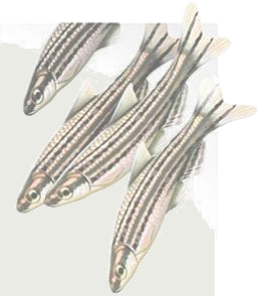


Ammonia



- 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 – <0.02 ppm

pH effect on Ammonia



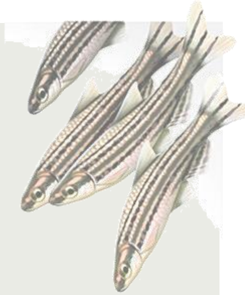
- *Total Ammonia Nitrogen (TAN) =* $NH_4^+ + NH_3$
- *TAN species ratio influenced by pH*
  - *pH 7.5, 28.5°C, 1000µS*
    - *TAN = 1.0ppm,* NH_3 *= 0.0234ppm*
  - *pH 6.5, 28.5°C, 1000µS*
    - *TAN = 10ppm,* NH_3 *= 0.0239ppm*
  - *http://www.hamzasreef.com/Contents/Calculators/FreeAmmonia.php*

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.*
  - $2\ NH_3 + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 2\ H^+$

- *Nitrobacter, Nitrospina sp.*
  - $2\ NO_2^- + 1\ O_2 \rightarrow 2\ NO_3^-$

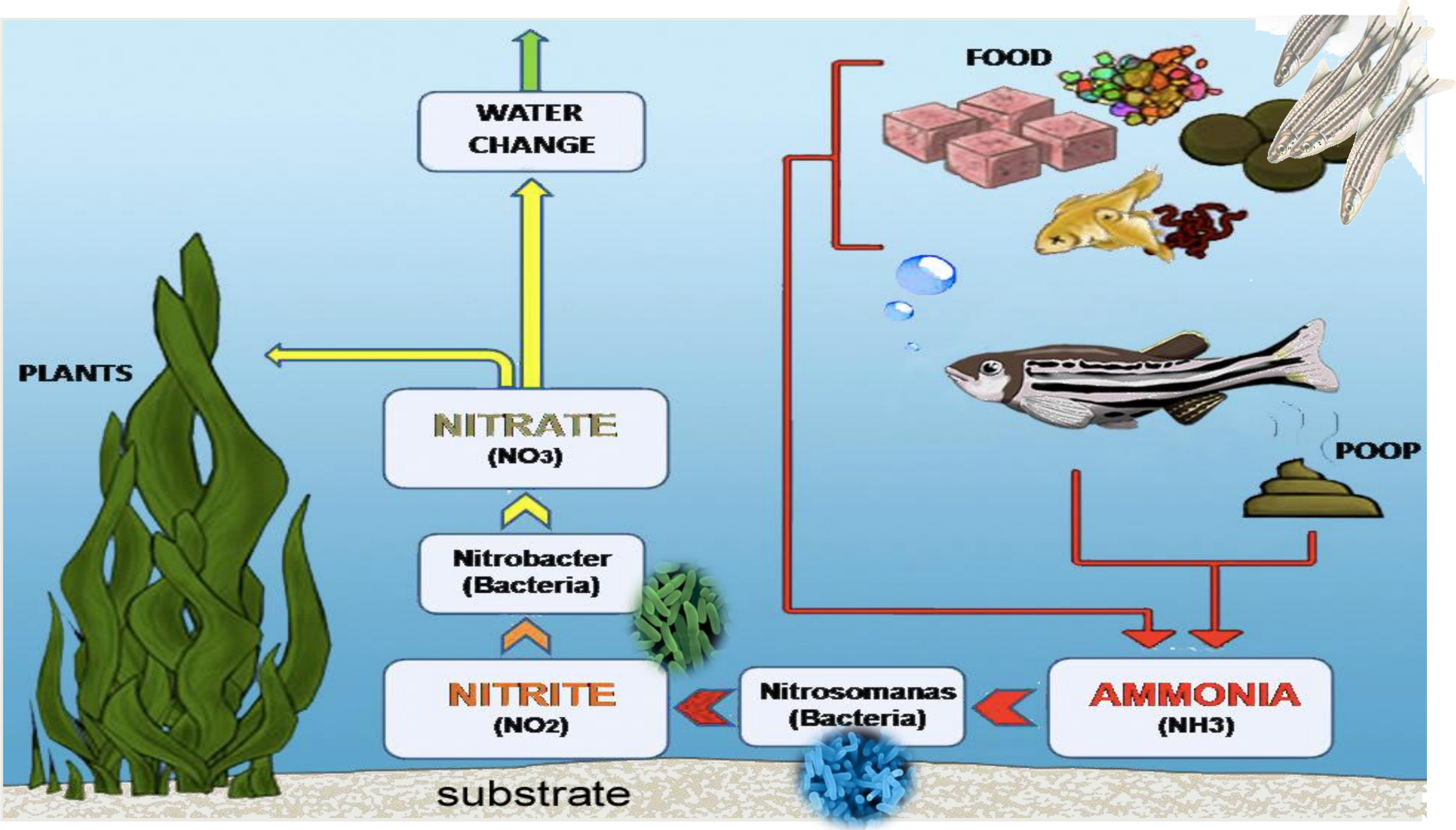
Chen et al, Aqua. Eng. 2006

Nitrification

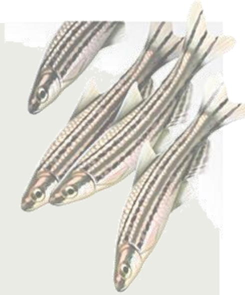
- Requires oxygen and alkalinty (↓pH)
- 1g TAN oxidised to nitrate nitrogen requires
  - 4.18g of O_2
  - 7.07g of CO_3^-
  - 0.17g of bacteria biomass generated

Chen et al, Aqua. Eng. 2006

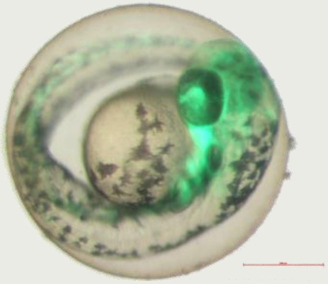
Aquatic Nitrogen Cycle



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
- Emerging Technology
  - Quantum Disinfection

Ultraviolet irradiation

- Bacteriostatic agent
- Effectiveness depends on flow rates, plumbing diameter and unit size/power and Water Quality
    - Presence of particulates decreases efficacy
- Irradiation dose expressed as µJs/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

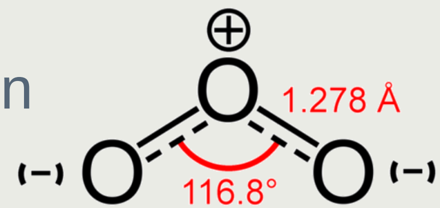
Ozone

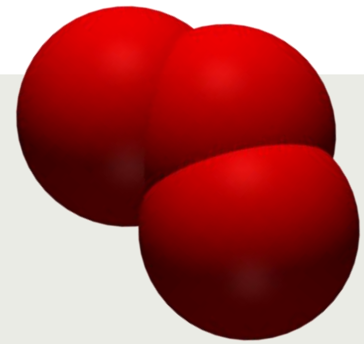
- *Biocidal agent*
- *Superior disinfecting power to UV*

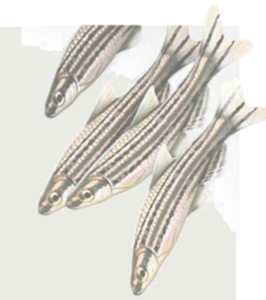
- *Why not commonly implemented?*
  - Higher risk to animals
  - Limited by contact time

Ozone

- Triatomic oxygen





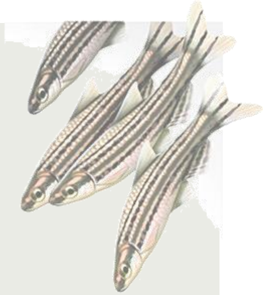


- Pale blue coloured gas
- Forms naturally from lightning/electrical sparks, with pungent odour at concentrations above 0.01mg/l
- Highly reactive oxidizer
- Extremely toxic
  - 10cm rainbow trout 96-h LC50 = 0.0093mg/l
  - Monitor both gaseous and aqueous presence

Wedemeyer et al, J. Fish. Res. Bd. Can. 1979

Ozone	UV
Constant disinfection dose	*Dose ↓ over time*
High capital	*Low capital*
Low cost consumable (electricity + silica)	*High cost consumables (annual lamp replacement)*
Safety monitors required (residues and leaks)	*No residues or leaks*
Moderate penetration (impurities consume O_3)	*Weak penetration (impurities block UV)*
Biocidal + additional effects	*Bacteriostatic action only*

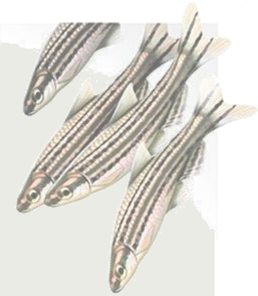
Ozone in aquaculture



- Removal of fine and colloidal solids
  - 1-30 µm and 0.001-1 µm respectively
    - Microflocculation = clumping of the solids

- Removal of dissolved organic compounds (DOCs)
  - non-biodegradable and accumulate
  - High levels stress fish and reduce nitrification efficiencies of the biofilter
    - Oxidises DOCs

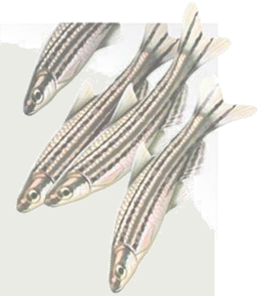
Ozone in aquaculture



- Biofilter supplementation (Removal of Nitrite)
  - Direct oxidation to nitrate
  - Reduces organic loading

- Disinfection (pathogen control)
  - Concentration and exposure time dependant

Ozone in aquaculture



- Rapid reaction rate (15sec half-life)
  - Few harmful reaction bi-products
  - Produces additional O_2 as a reaction end product

- Drawbacks
  - Harmful to humans and aquatic animals
    - Must be applied appropriately
  - High initial capital outlay

Bullock et al, Aquaculture 1997

Ozone application essentials

- Gas generation
  - Corona discharge
  - UV generator
- Gas-to-liquid adsorption
  - Ozone cone
  - Protein skimmer

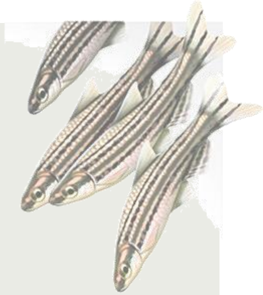
Ozone application essentials

- Contact time for reaction
    - k = C(mg/l) . T (min)
    - IPNV – k = 2.25
        - 45sec exposure at 3mg/l

- Residual removal
    - Activated carbon or UV

Summerfelt, Aqua. Eng. 2003

Ozone with zebrafish



- Enhance water quality processing
  - Reduce DOCs
  - Remove colloidal solids
  - Enhance UV efficacy
- Best applied in large scale facilities
  - High effluent production

SILECTE™
QUANTUM DISINFECTION

5th Annual International
Zebrafish Husbandry Course

A BREAKTHROUGH TECHNOLOGY
SO POWERFUL
IT ~~WILL CHANGE~~ THE WORLD!
is changing

What is it?

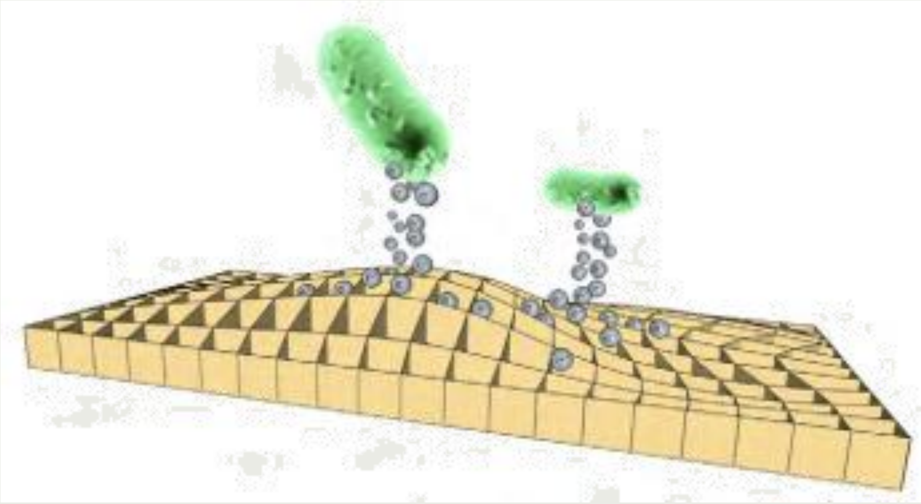
Silecte Quantum Disinfection

- Is a cationic electron magnet
  - Utilizes elemental P-type semiconductor technology and solid-state theory

- Discovered 2012 by Cristian Chris
  - Silica gel, TiO2(Cl), Ag
  - No chemical leaching
  - Biocidal activity on contact
    - Log 6 kill in <0.01sec

Silecte Quantum Disinfection

- How it works
  - Absence of valance electrons on outermost surface
  - On contact, electrons are “zapped” from the microorganism



EPA Test Results

Silecte™ Disinfection Media

Microorganism performance data sheet

Micro Organism (MO)	MO Type	MO Reduction
Pseudomonas aeruginosa *Escherichia coli* *Staphylococcus aureus* *Enterococcus hirae* *Legionella adelaidensis*	*Bacteria*	*Log 6*
Candida albicans	*Yeast*	*Log 6*
Anabaena constricta	*Algae*	*Log 5*
MS2 Phage (ATCC 15597)	*Virus*	*Log 4*
Cryptosporidium	*Protozoa*	*Log 2*

Wastewater Disinfection

- Island community (1,000 residents)
- Existing plant
  - Micron filter, Carbon clarifier, Chlorine dosing
  - Silecte added post carbon filter

Wastewater Disinfection

MICROBAC®
Fayetteville Division

Certificate of Analysis

[redacted]

Project: **Water Samples -** [redacted]

Date Reported: 09/11/14
Date Received: 09/10/14
Date Sampled: 09/10/14
Sampled By: [redacted]

Pre (Raw Water)

K4I0342-01

Analyte	Result	Units	Analyzed	Analyzed By	Method	Qualifier
Analyzed by: Microbac Laboratories, Inc. - Fayetteville						
Coliform, Fecal	TNTC	per 100 ml	09/10/14 15:55	JAO	SM 9222 D-1997	

Micron Screens

K4I0342-02

Analyte	Result	Units	Analyzed	Analyzed By	Method	Qualifier
Analyzed by: Microbac Laboratories, Inc. - Fayetteville						
Coliform, Fecal	<10	per 100 ml	09/10/14 15:55	JAO	SM 9222 D-1997	

One Carbon

K4I0342-03

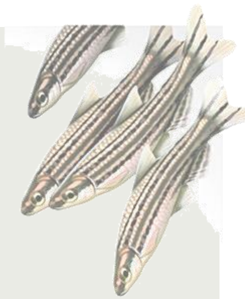
Analyte	Result	Units	Analyzed	Analyzed By	Method	Qualifier
Analyzed by: Microbac Laboratories, Inc. - Fayetteville						
Coliform, Fecal	<1.0	per 100 ml	09/10/14 15:55	JAO	SM 9222 D-1997	

Wastewater Disinfection

- Effluent process
  - 30um disc filter, UV disinfection
- Silecte – 2x side stream application pre-UV



Wastewater Disinfection



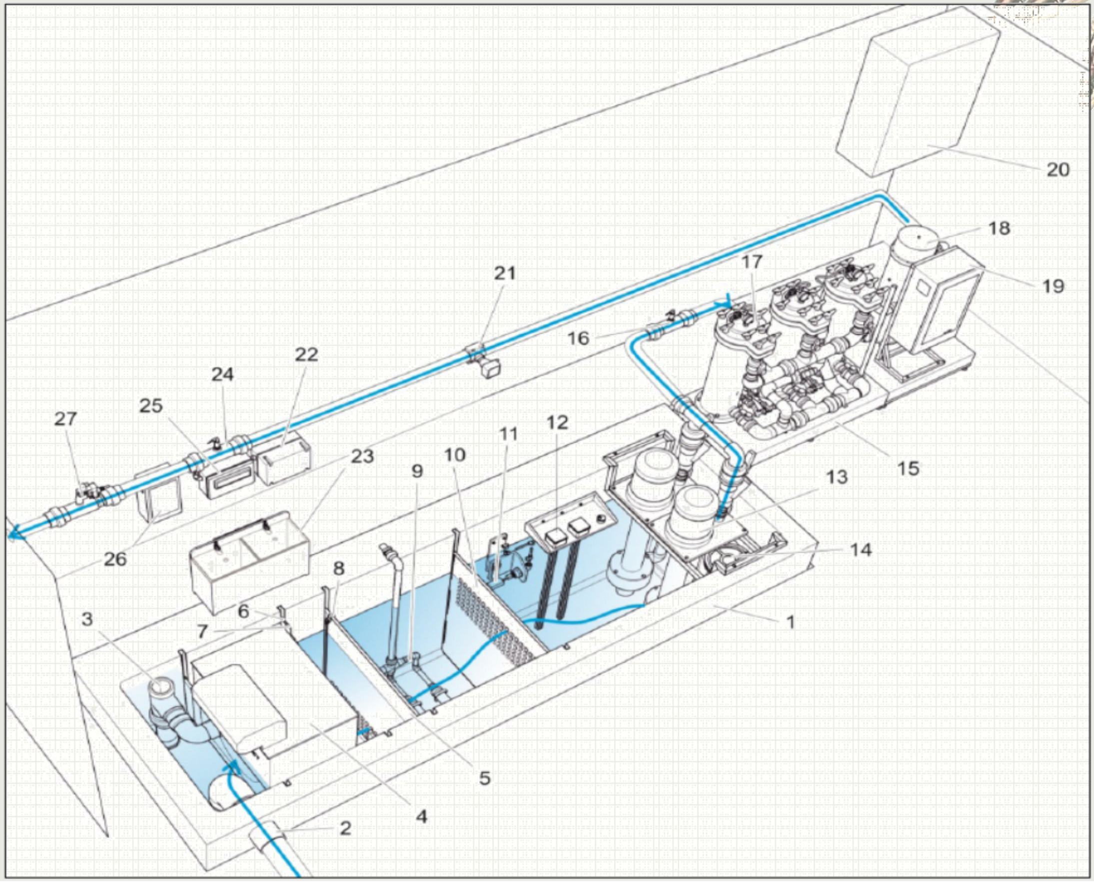
Parameter	Units	Date Analyzed	Site #1	Site #2
Fecal Coliform	col/100ml	12/4/13	2	10
Fecal Coliform	col/100ml	1/15/14	<1	<1
Fecal Coliform	col/100ml	3/5/14	<1	<1
Fecal Coliform	col/100ml	4/2/14	5	<1
Fecal Coliform	col/100ml	4/23/14	<1	<1
Fecal Coliform	col/100ml	4/29/14	<1	<1
Fecal Coliform	col/100ml	5/7/14	<1	<1
Fecal Coliform	col/100ml	10/22/14	<1	<1
Fecal Coliform	col/100ml	11/26/14	<1	<1

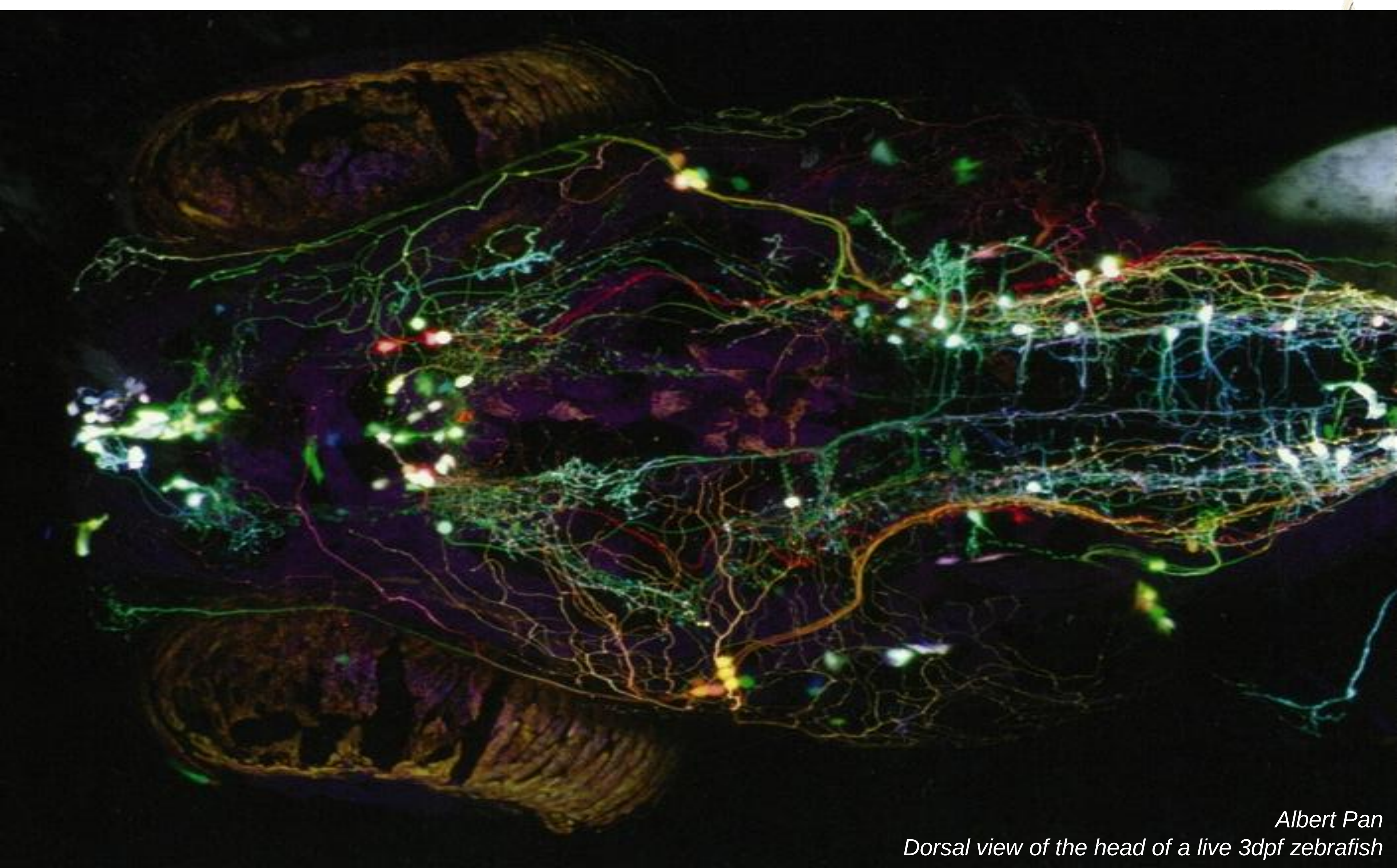
Silecte Quantum Disinfection

- Potential zebrafish application
  - Unequalled biocidal activity (on contact, <0.01sec)
  - No power required
  - No chemicals added to water (vs Ozone)
  - Cheap consumables (claiming 31% saving over UV)

- Limitations
  - Dependent on influent water quality

Effluent Processing Assembly





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