Aquaculture Water Quality Fundamentals

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Typical Zebrafish Facilities use what are known as: Recirculating Aquaculture Systems (RAS)

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

RAS Aquaria

- *Limitations of RAS*
  - *Capital outlay can be high*
  - *Disease containment can be challenging*
  - *Mechanical failure, oxygen depletion, toxin levels can be disasterous*
  - *Skilled labour is required to maintain, and monitoring is often intensified*
  - *Water Quality control can be a challenge until stabilized*

Why is water quality important?

What we strive to achieve

What we aim to

As nature intended

Water Quality Is A Keystone Component Of RAS

- *Aquariums are complex dynamic biological systems affected by multiple variable interactions*
- *Stable, adequate water quality is critical to the successful operation of any aquaria holding system*
- *Intentional changes to water quality parameters may need to occur in a range from seconds to minutes, minutes to hours, and days to months depending on the variable you aim to change*
- *Consistent conditions and routine monitoring is required*

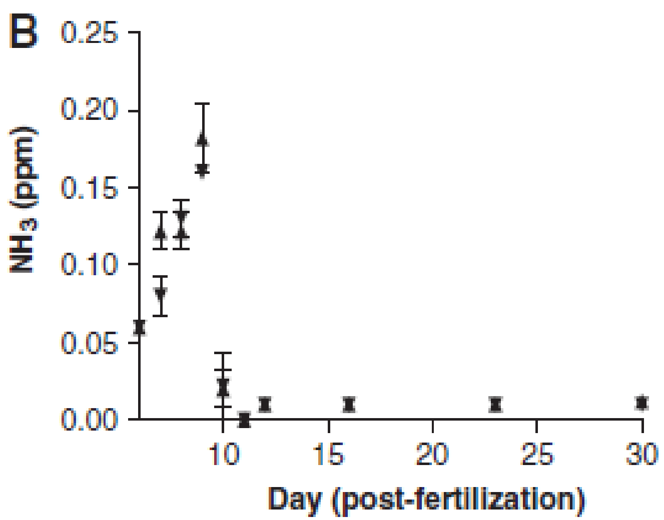
Water Quality

- Minimal scientific consensus throughout zebrafish field
- 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

Colt, 2006

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 larvae*



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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

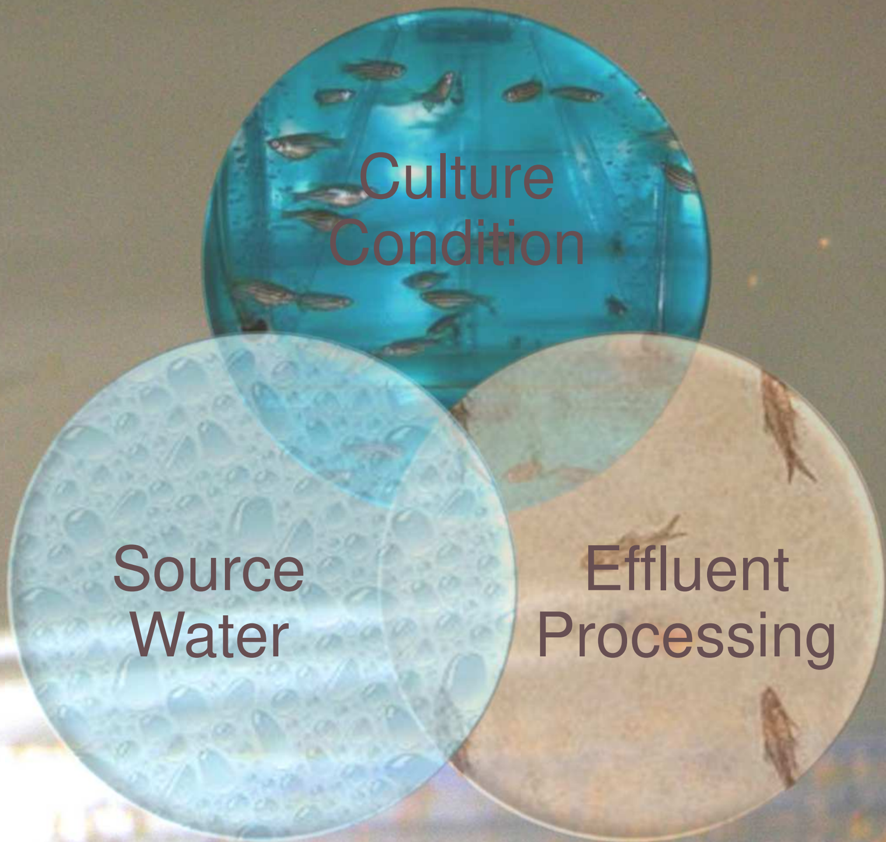
- *Your choice of water quality parameter values should be:*
  - *Species specific*
  - *Life stage specific*
    - *Larval zebrafish are more tolerant of ammonia, and may benefit from water with higher conductivity 7650uS (up to 4g/l salinity)*

Zebrafish 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 -1g/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

- *General water quality criteria*
  - *Source Water*
    - *Ex: Reverse Osmosis*
  - *Culture Condition*
    - *Conditions in your fish tanks*
  - *Effluent Processing*
    - *How your waste products are managed and mitigated*



Colt, Aqua. Eng. 2006

Controlling Water Quality

Source Water examples

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

Example: 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+}

Example: Municipal Supply Water

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*

Example: Municipal Supply Water

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

Example: Municipal Supply Water

- *2017 St. Louis, MO Water Conditions*
  - *Alkaline* *8.65-10.29pH*
  - *Hard* 56-194 mg/l $CaCO_3$
  - *Conductivity* *283-711µS/cm*
  - *Copper* *0.0202 mg/l* Cu^{++}
  - *Lead* *0999* µg/l
  - *Chloramine* *2.03-3.36mg/l*

City of St. Louis Water Division 2017

Example: 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

Example: RO Supply Water

Class3 RO Supply Water

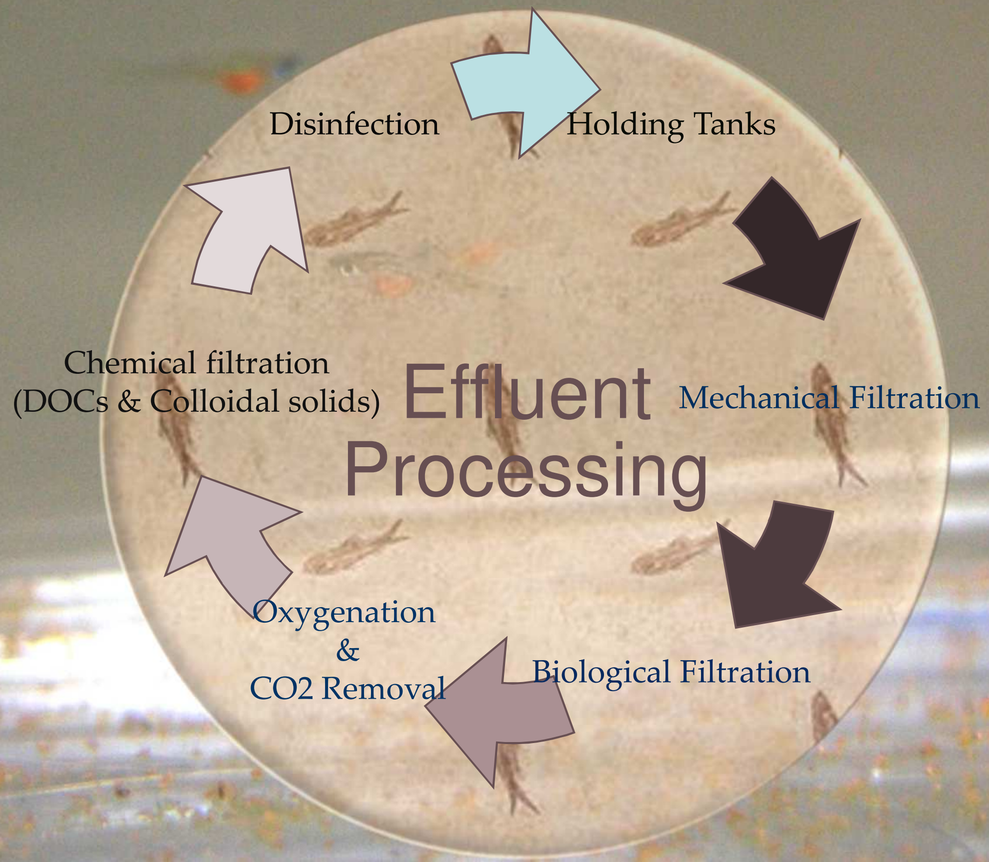
- *Low alkalinity* *Acid pH ~6.3*
- *Soft* *0mg/L* $CaCO_3$
- *Conductivity* ~20 μS/cm^2 *conductivity*

- *Requires conditioning for culture use*
  - *Hardness Generators (↑pH + Ca^+ cations)*
  - Marine Sea Salt (↑μS + essential minerals)
- *Typically 7.5pH, >100mg/L* $CaCO_3$, *~400* μS/cm^2


SIEMENS

Controlling Water Quality

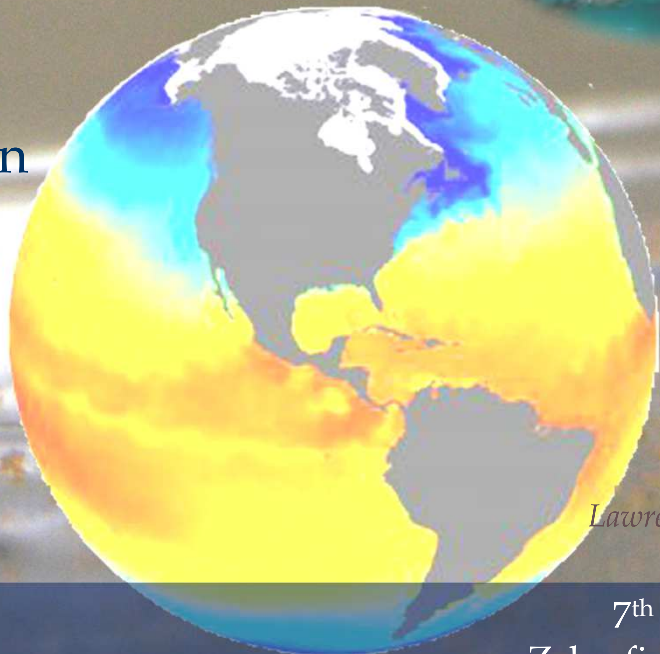
RAS Central Life Support Systems (CLS)



Controlling Water Quality

Temperature

- *Temperature tolerance of zebrafish*
  - *6.7-41.7°C*
  - *Target = 28.5°C*
    - *This is the standard temperature for developmental purposes*
- Temperature impacts overall water chemistry characteristics in addition to direct impact on aquatic animals

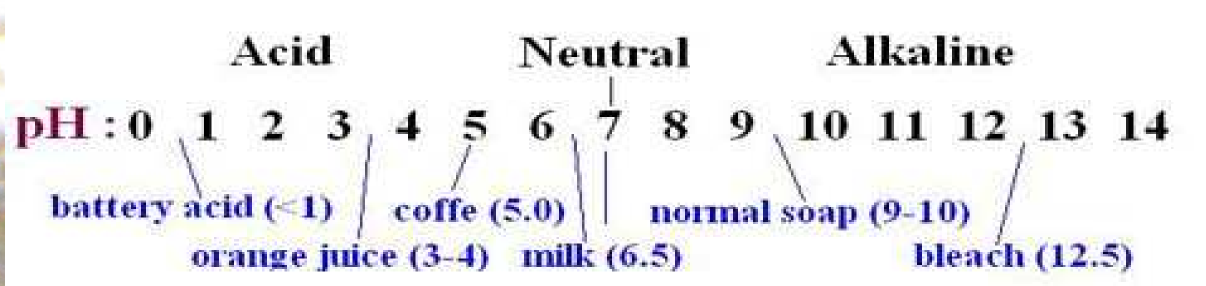


Culture Condition

Lawrence, Aquaculture 2007

Power of Hydrogen (pH)

- *pH tolerance*
  - *6.0-9.5+*
  - *Target = 7.2-8.0*
- Measurement of basic, acidic, or neutral qualities of a solution
- Will fluctuate in recirculating systems due to:
  - Respiration
  - Nitrification



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

Power of Hydrogen (pH)

- pH changes should be performed gradually in RAS where possible
- 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)

- Effect of Respiration by fish
  - Increased CO_2 will decrease pH
  - Depending on alkalinity, can ↓NH_3:NH_4^+ in TAN

- Effect of increased Nitrification due to increased fish waste
  - 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 of pH is often necessary in RAS
  - 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

- *Total Hardness Tolerance of zebrafish*
  - *75-200ppm* $CaCO_3$
  - *Target >100ppm*
- *Total Hardness is made of two components:*
  - *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*

General Hardness (GH)

- Soft water = low in ions,
  - create difficulties in maintaining homeostasis through osmoregulation
- Higher hardness levels often necessary for facilitating normal osmoregulation and healthy oocyte production

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

- *Conductivity, or Specific Conductance, describes the capacity of water to conduct an electrical current*
- *Conductivity Tolerance of zebrafish*
  - *300-4000$\mu S/cm^2$*
  - *Target 300-1200μS*
- *Salinity Tolerance of zebrafish*
  - *0.2-2.0ppt*
  - *Target 0.25-0.75ppt*
- *Salinity measures salts of the alkali metals or magnesium*
- *Can both be modified by addition of balanced salt formulations*
- *Evaporation of water will increase both*

Salinity and Conductivity

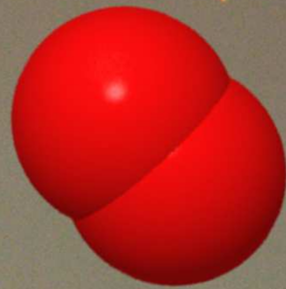
- Na^{+} are necessary for ammonium (NH_4^{+}) excretion and ion regulatory function
- High salt
  - Fresh water animals cannot excrete enough ions
- Low salt
  - Fresh water animals will fight to retain ions

- These processes have a high metabolic cost to the animals

Sources of Conductivity/Salinity

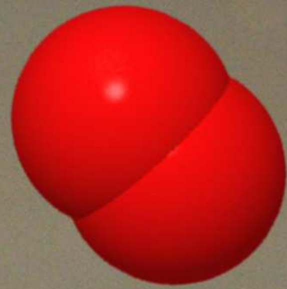
- Synthetic Sea Salt/Reef Salt
  - Amongst most commonly used in zebrafish RAS
  - Poorly defined recipe, proprietary in nature
  - When used at concentrations desired for zebrafish, trace minerals become irrelevant
  - High cost
  - Saturation ~80g/l
- High Purity NaCl
  - Widely available as food ingredient
  - 99.999% purity available (ex. Culinox 999)
  - Can be used to create stock solutions ~350g/l

Dissolved Oxygen



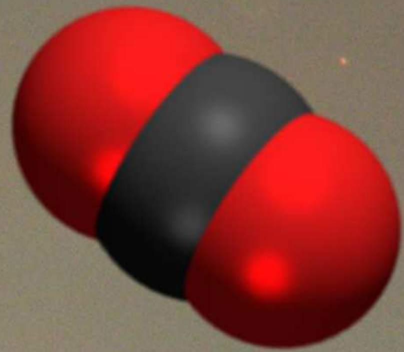
- *Target > 6 mg/L or > 80 % saturation*
- *100% saturation @28^{o}C = 7.8mg/L*
- *As water temperature increases the maximum DO saturation level decreases*
- *Can be modified by the use of mechanical aeration devises, degassing towers, trickle filters, or by the introduction of oxygen gas*
  - *>100% 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

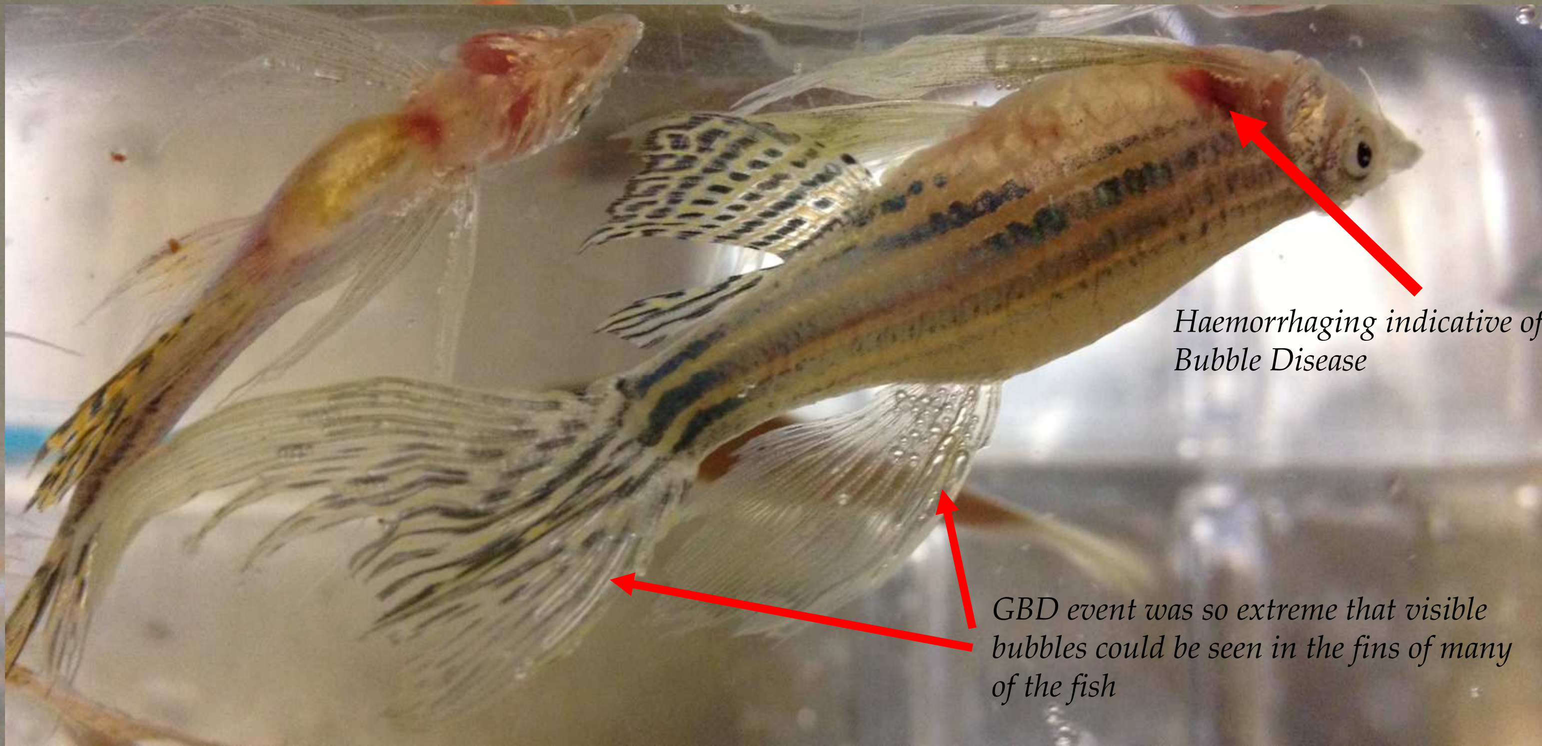
Colt, Aqua. Eng. 2006

Total Dissolved Gas Pressure

- When the total pressure of all the gases in the water exceeds the ambient atmospheric pressure at the water surface, supersaturation exists
- May be measured in mg/l or % saturation or mmHg
- The effect of prolonged and excessive supersaturation on fish has been well documented, and if supersaturation exceeds the established safe levels, massive fish kills can occur very quickly, this has been termed: Gas Bubble Disease

Colt, Aqua. Eng. 2006

Gas Bubble Disease



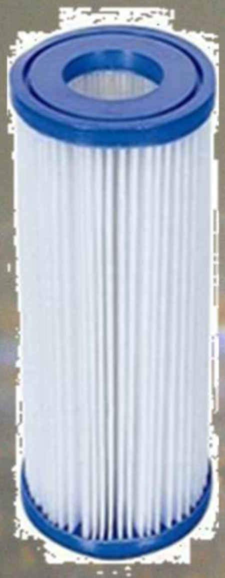
Chlorine and Chloramine

- Chloramine
  - Target = 0 mg/L (ppm)
  - Chloramine = Chlorine + Ammonia
    - Very stable molecule
  - 0.01 ppm is acutely toxic to fish
- To neutralize chlorine and chloramine - commercial products (Nov-Aqua® or AmQuel ®)
- To remove chlorine - filtration using activated carbon, or aeration over time

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

Mechanical Filtration examples



Mechanical Filtration

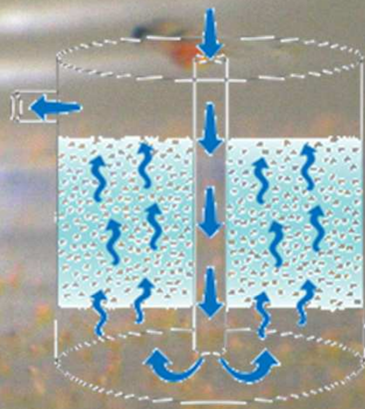
- *Purpose*
  - *Remove large suspended debris*
    - *Range : 10-100µm*
  - *Allows for healthy biofilter growth*
  - *Enhances UV efficacy*

Mechanical Filtration

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

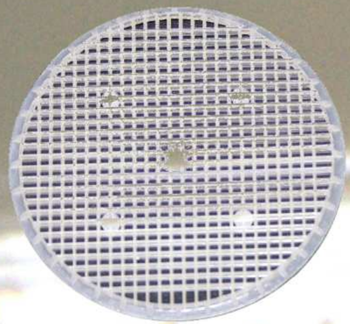
Biological Filtration examples

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



Biological Filtration Examples

- *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 for fish culture** – 0 .0ppm*

Nitrification

- Toxic ammonia is converted to non-toxic nitrate

- Nitrosomonas-like sp.
  - $2\ NH_3 + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 2\ H^+$

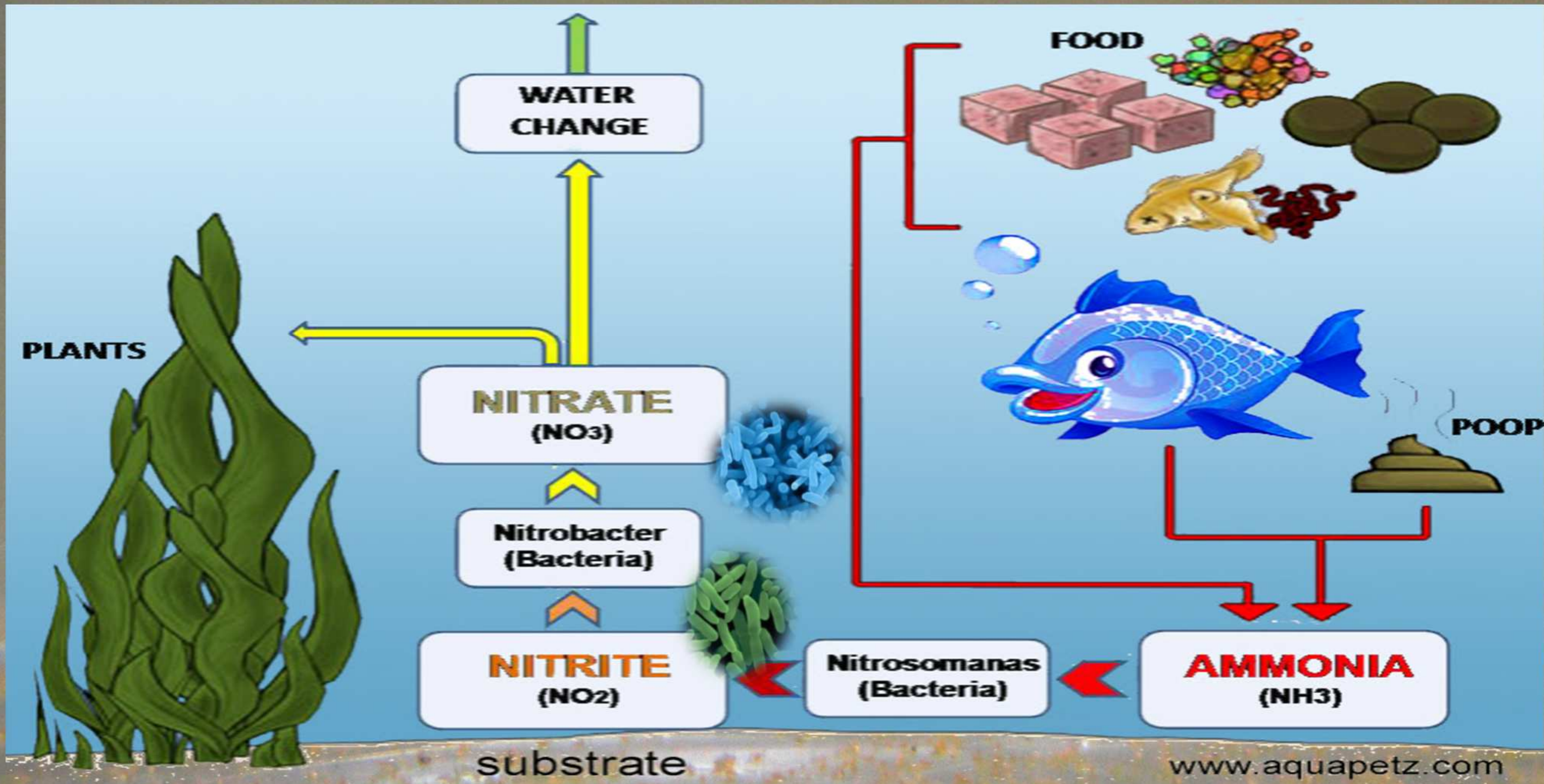
- Nitrobacter-like, Nitrospira-like sp.
  - $2\ NO_2^- + 1\ O_2 \rightarrow 2\ NO_3^-$

Nitrification

- Requires oxygen and alkalinty (↓pH)
- 1g TAN oxidized to nitrate nitrogen requires
  - 4.18g of O_2
  - 7.07g of $CaCO_3$
  - 0.17g of bacteria biomass generated

Chen et al, *Aqua. Eng.* 2006

Aquatic Nitrogen Cycle



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

Chemical Filtration

- Dissolved wastes are more difficult to remove
- 2 main options for chemical filtration
  - Activated Carbon- most common in zebrafish RAS
  - Foam fractionation (protein skimming), rare in zebrafish RAS

Activated Carbon, also called GAC or Granulated 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

Activated Carbon

- Can be made from a variety of source material
  - Bituminous coal, may be acid-washed, may contain phosphorous
  - Coconut shell- pore size too small for liquids

- Considerations before placing in service
  - Rinse dust as much as possible
  - Granule size must be appropriate for media vessel
  - May impact pH if not properly sourced and pre-treated

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

Protein Skimming

- Benefits
  - Removal or oxidation of Dissolved Organic Compounds (DOC's)
  - Improved performance of biofilters, conventional filters
  - Removal of fine and colloidal solids through micro-flocculation
  - Removal of nitrites, and chemical loading oxidation
  - Increase Dissolved Oxygen (DO) levels in water
  - Increased water clarity

Disinfection Technologies

- Focus on reducing microorganism populations
- 2 main options to consider
  - Ultraviolet irradiation (UV-C), typical in zebrafish RAS
  - Ozone, rare in zebrafish RAS

Ultraviolet irradiation (UV-C)

- Effectiveness depends on flow rates, plumbing diameter and unit size/power
- Irradiation dose expressed as mJs/cm^2 (millijoule per square centimeter)
- Effective bulb life is short (~12 months)
- Presence of particulates decreases efficacy
- Not all target pathogens are susceptible to UV-C
- Does not always kill organism outright, but may damage DNA to degree that reproduction is impossible
- Critical: bulb must be replaced regularly and quartz sleeve cleaned and replaced when it becomes cloudy

Ozone (O_3)

- *Higher disinfecting power than UV*
- *Why not commonly implemented?*
  - Higher risk to animals and people
  - Effectiveness is limited by contact time
  - Often requires additional processing steps, such as degassing towers, to render the treated water safe for use in the RAS
- *UV may be used to neutralize residual 0_3*

Thank You

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