The Role of Soil-Bound Nitrogen in the Atlantic Slope Fish Kills:

Nitrite and Un-ionized Ammonia Toxicity

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Hypothesis:

Freshwater fishes in the Potomac drainage are exposed to toxic levels of nitrite and/or un-ionized ammonia as part of natural spawning behaviors. Bacteria residing in the streambed sediment "fix" abundant nitrogen, exhibiting a seasonal phenology and life history conducive to accumulation of toxicants.

Overview

- N cycle / background / sources of additional input
- Ammonia / ammonium relation to water quality
- Phenology of AOB and NOB
- Exposure of fishes /spawning behaviors
- Effects of exposure…more than just brown blood disease…un-ionized ammonia extremely toxic

Nitrogen and Soil

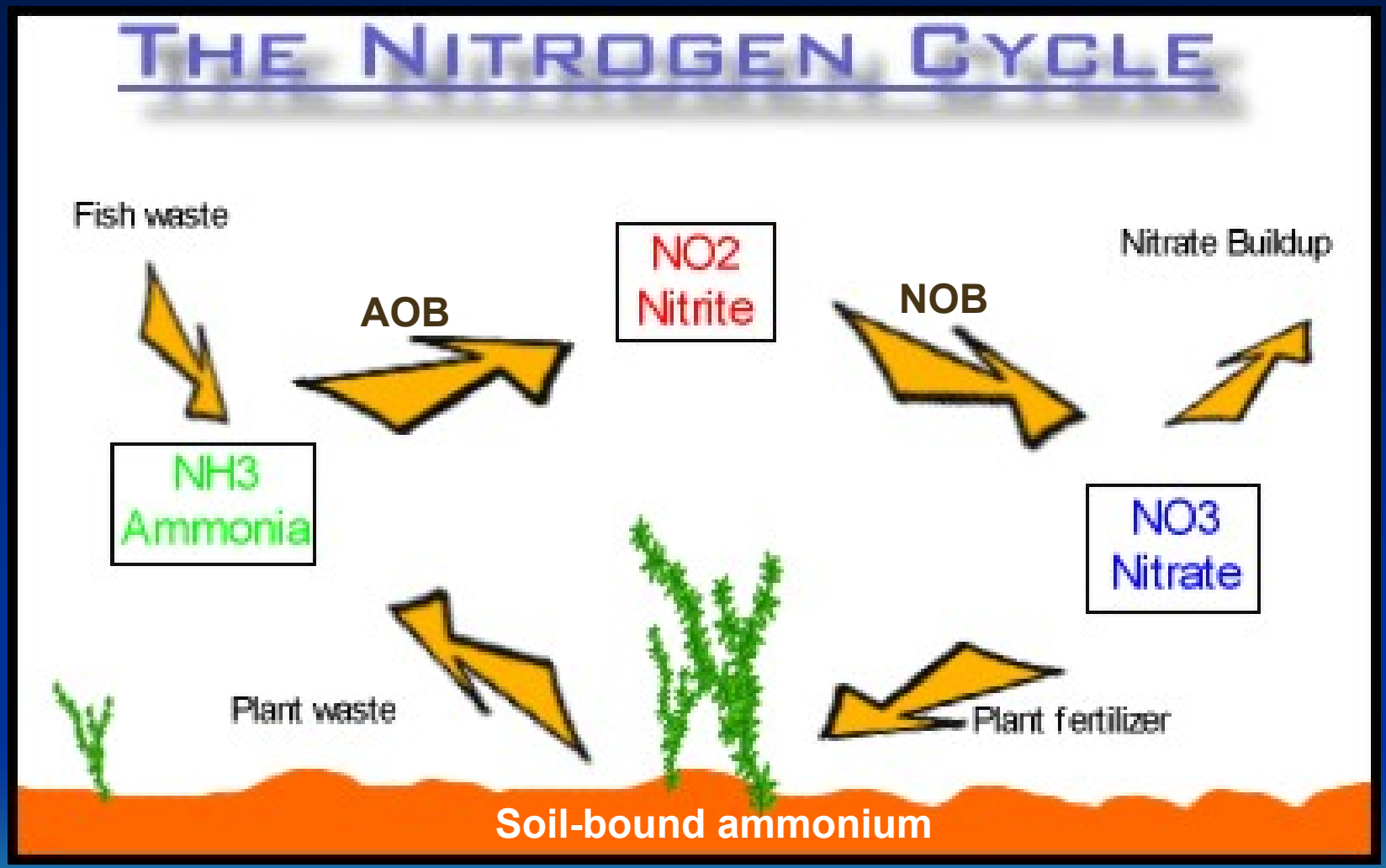
- N cycle = nutrient and food availability
- Occurs at all levels, but most prevalent in soil
- Most N from excrement is already in organic form; easy fixation to NO_3
- Some N in more concentrated forms like uric acid (birds, reptiles) and urea
- Concentrated products mobilize by acidic rainfall
- Ultimate fate of N may be as soil-bound ammonium

Nitrogen Sources

- Atmospheric
- WWTP's
- Land application of:
  - Human wastes
  - Agricultural wastes
- Fertilizer runoff
- De-icing / Blasting Agents

How Bacteria Factor In

- N cycle in freshwater and soil is initiated by bacteria (e.g. ammonia oxidizing bacteria)
- N cycle fulfilled for plants when nitrite oxidizing bacteria produce nitrate
- Nitrate is only form of N usable by plants
- Some plants, including stargrass, have rhizospheres to house N-fixing bacteria


THE NITROGEN CYCLE
Fish waste
NH3
Ammonia
AOB
NO2
Nitrite
NOB
Nitrate Buildup
NO3
Nitrate
Plant waste
Plant fertilizer
Soil-bound ammonium

Proof in the Plants

Fate of Ammonium in Streams

- Ammonium is quickly attracted to soil particles (+/-)
- Soil-bound ammonium accumulates in depositional areas
- Steambed sediments become rich for bacterial metabolism
- Inherently held by clay soils

WQ and Ammonium

- Alkaline conditions conducive to ammonia formation
- High pH drives the reaction
- Un-ionized ammonia has irreparable impact on fish gills (only few tolerate)
- Especially toxic to freshwater fishes adapted to low salinity

Nitrogen Fixing Bacteria

- Ammonia Oxidizing Bacteria (AOB)
  - Prefers higher pH
  - Lower temperature for metabolic activity
- Nitrogen Oxidizing Bacteria (NOB)
  - Metabolizes nitrite into nitrate
  - Most active later in season as NO_2 becomes available

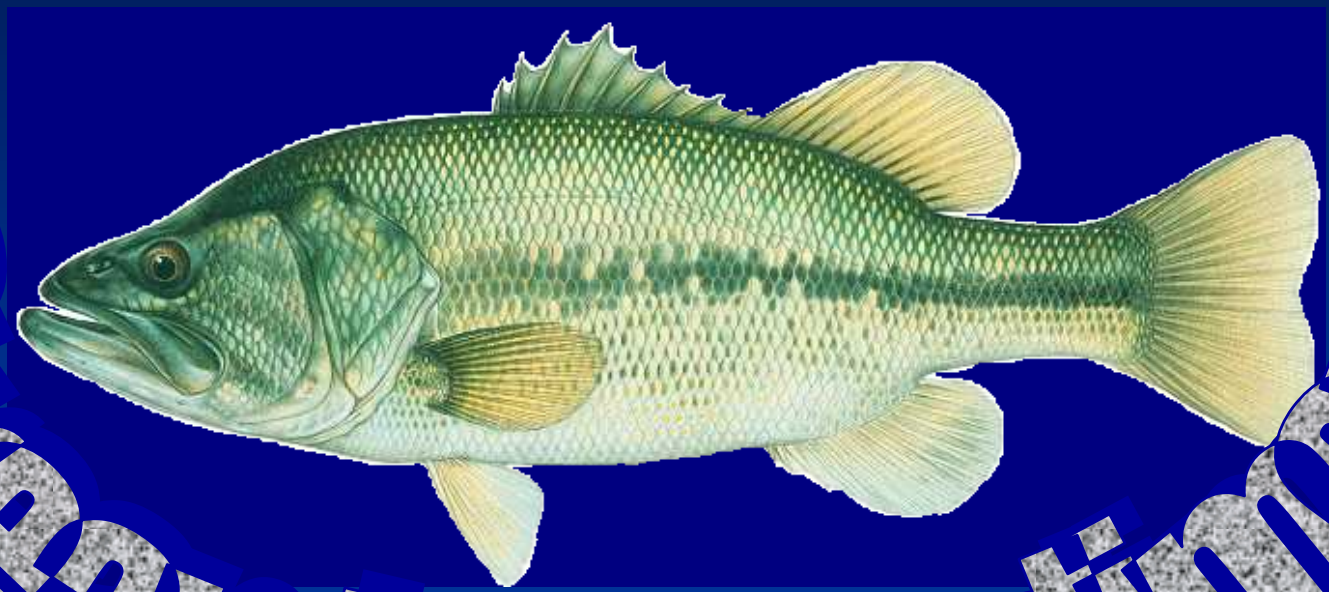
Phenology of AOB and NOB

- AOB becomes active in early spring, prior to plant production
- NOB becomes most active as nitrite is available for food
- Both bacteria have approximately same temperature of max. metabolism
- Exhibit feedback mechanism and seasonal response

Exposure Vectors

- Soil-bound ammonium not typically encountered in water column
- Spawning behaviors initiate disturbance of interstitial soil bacteria and toxic N products
- Most fishes occurring in stony habitats are lithophilic to some degree
- Nest guarding and delayed egg laying exaggerate exposure

Probable Exposure Scenario



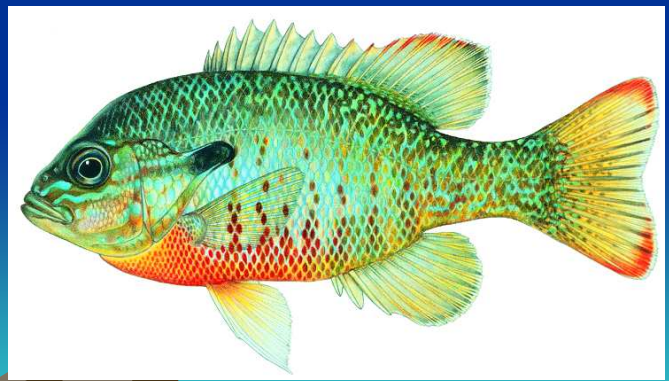
Streambed Sediment

Spawning Phenology

- Lithophilic spawners begin nest building as AOB metabolizes ammonia into nitrite
- Depression makers are particularly vulnerable
- Both sexes susceptible at certain times / peak NO_2 production / high pH and NH_3
- Side-flopping and nest fanning may intensify exposure
- Drive to complete life history stage is intense

Observations on Impacted Spawners

- Mound builders vs depression makers
- Nest guarding
- Mature / nuptial male stonerollers
- Common and striped shiner males
- Golden redhorses, both sexes
- Some white and hogsuckers



Interstitial AOB, NO_2, and NH_3

Gill Exposure

Soil-bound N Source

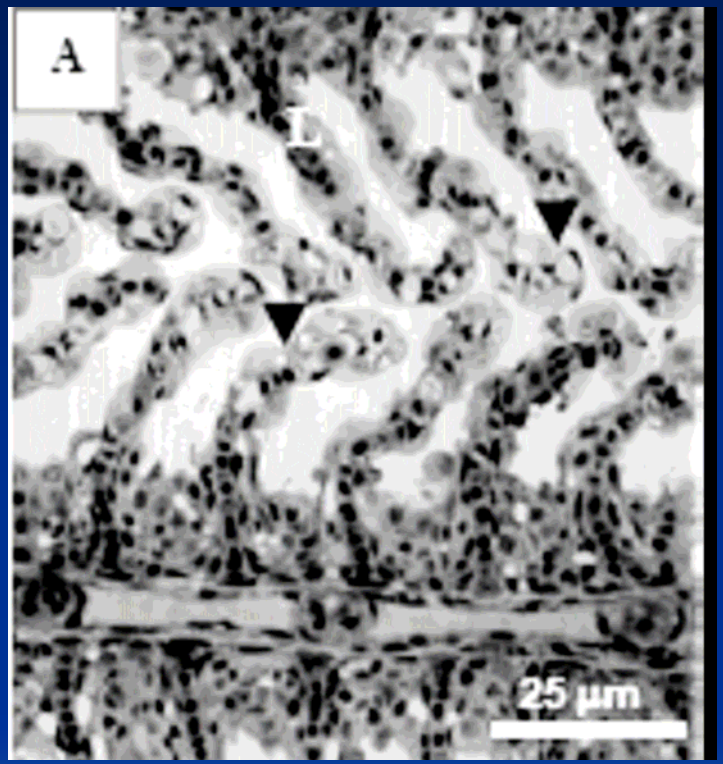
Nest Depressions

NO_2 and Fishes' Gills

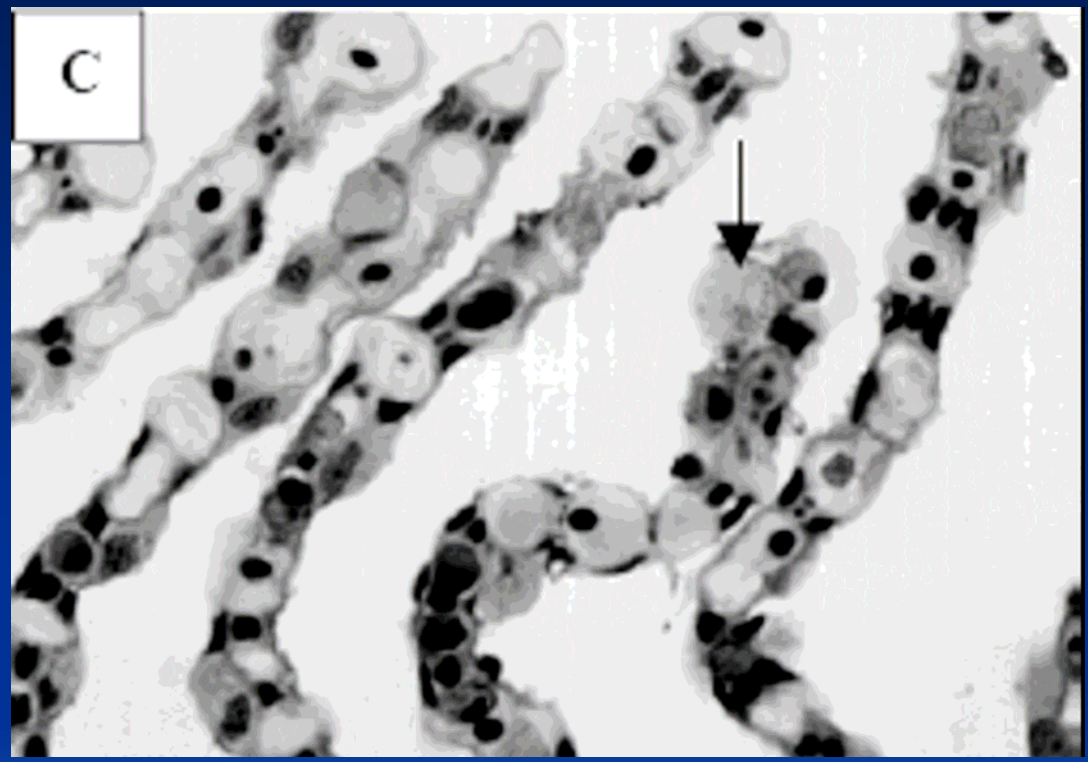
- NO_2 incurred by chloride cells on gill surfaces
- NO_2 changes structural confirmation of chloride cells and impedes functionality
- NO_2 does not always reach blood stream
- Survival depends on degree of damage
- Younger fish more tolerant via alternative respiratory mechanisms

Gill Filament and Lamellae

Control Group



Experimental Group



▼Arrowheads show chloride cells

Electron Microscopy Results

- Reduction in chloride cells in lamellar epithelium
- Irreparable damage to mitochondria of chloride cells
- Cessation of ATP production
- Leads to energy starvation and death

Causes of Death

- Suffocation in most individuals
- Severe liver damage due to anaerobic respiration
- Common bacterial infections:
  - Aeromonas
  - Columnaris
  - Pseudomonas

Supporting Evidence

- Rooted vegetation demonstrates N fixation occurring in soils
- Seasonal phenology has some biological signature
- Exposure among fishes likely mirrors spawning behaviors
- Behavior of moribund fishes indicates respiratory / osmoregulatory / fatigue

Preliminary Sample Results

- Sediment ammonia samples – six locales
  - 3 within South Branch watershed
    - Old Fields (215.4 mg/kg)
    - Upper Tract (61.4 mg/kg)
    - South Fork (25.7 mg/kg)
  - 3 Charleston samples
    - Large river – Elk River (<MDL)
    - WWTP – Campbells Creek (45.6 mg/kg)
    - Headwater – Davis Creek (~MDL)

Questions

Poultry Excrement

- Waste products stem from evolutionary past / water conservation
- Most kidney wastes (like reptiles) are highly concentrated uric acid
- Combined liquid and solid wastes before excretion from cloaca
- 3X more N per volume than beef / 2X more N than swine

Poultry Manure on the Landscape

- Combined excrement and other materials (e.g. alum, organic materials) to make litter
- Plowed into soil to bind N to soil
- Rainfall moves uric acid as:
  - Whole insoluble particles
  - Ammonia from H_2SO_4 rxn (as mostly ammonium) in free form
  - Soils with bound ammonium

Litter Statistics

- 1000 chickens create 1 ton of manure
- 83 million birds per year in watershed (2002 Ag. census)
- 83,000 tons of excrement annually
- Insert WV Ag. Stat. here for N content