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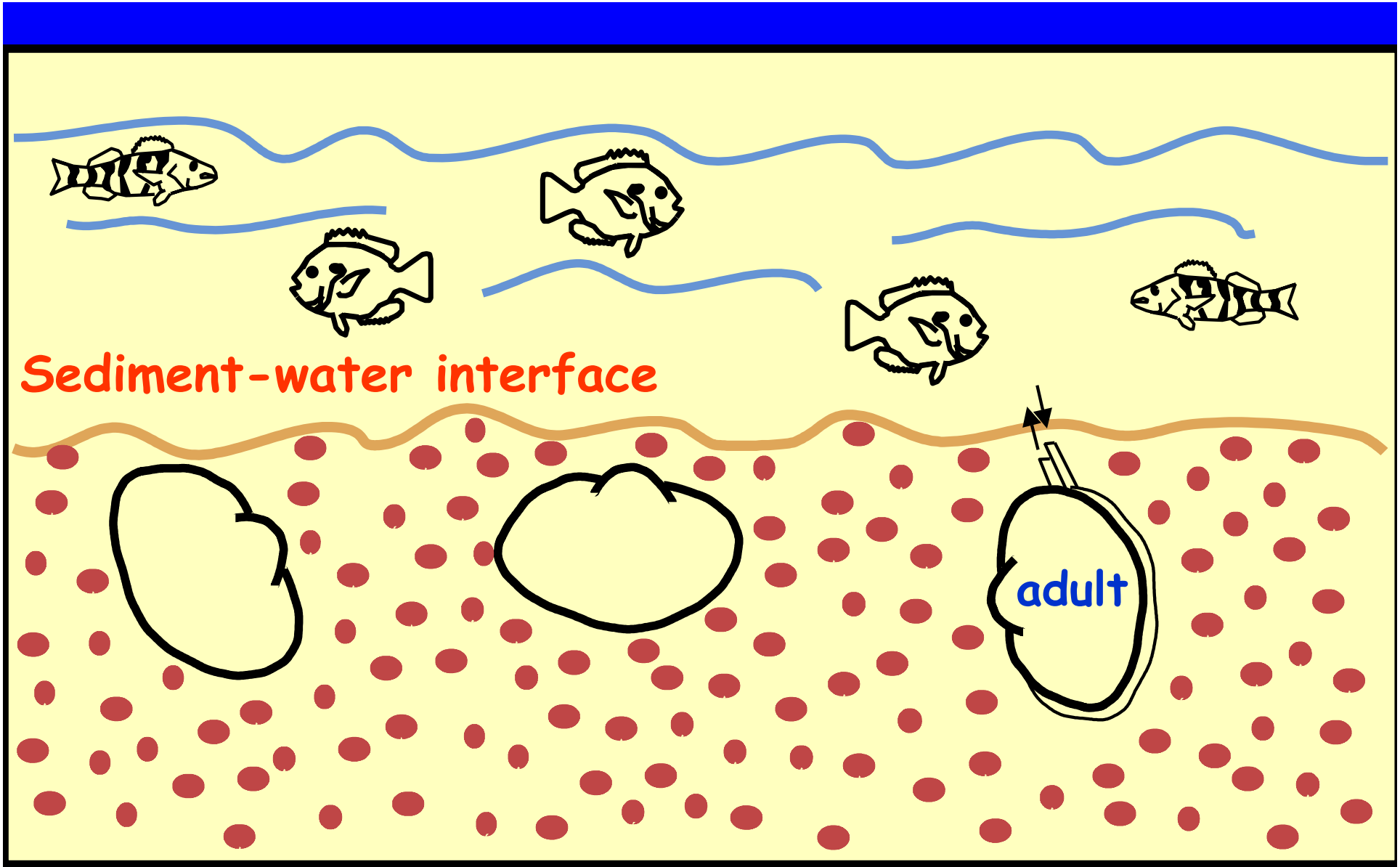
Sensitivity of Juvenile Unionids to Ammonia in Sediment and Water-Only Toxicity Tests

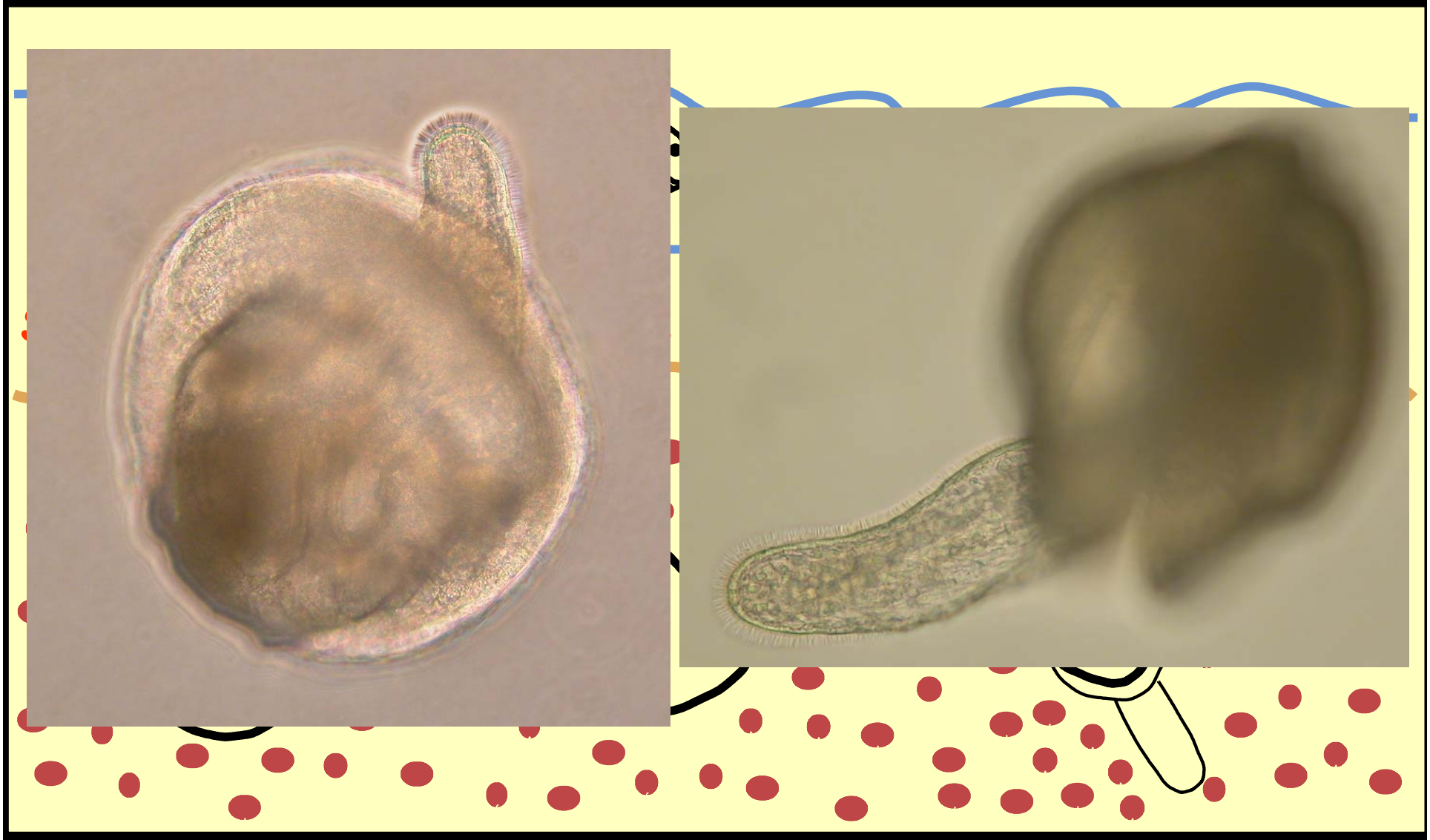
U.S. Department of the Interior
U.S. Geological Survey

Contaminant Exposure Routes

- Overlying water (adults only ?)
- Sediments (juveniles and adults)
- Pore water (juveniles and adults ?)
- Fish hosts (glochidia ?)
- Food (juveniles and adults)



Adults - suspension and deposit feeding ??



Juveniles - pedal feeding ??

Presentation Outline

- Studies conducted during 1999-2001
  - Lab—4 and 10 day sediment exposures
  - Field—4, 10 and 28-d *in situ* exposures
- Studies conducted during 2004-2005
  - Lab—4 day sediment and water-only exposures

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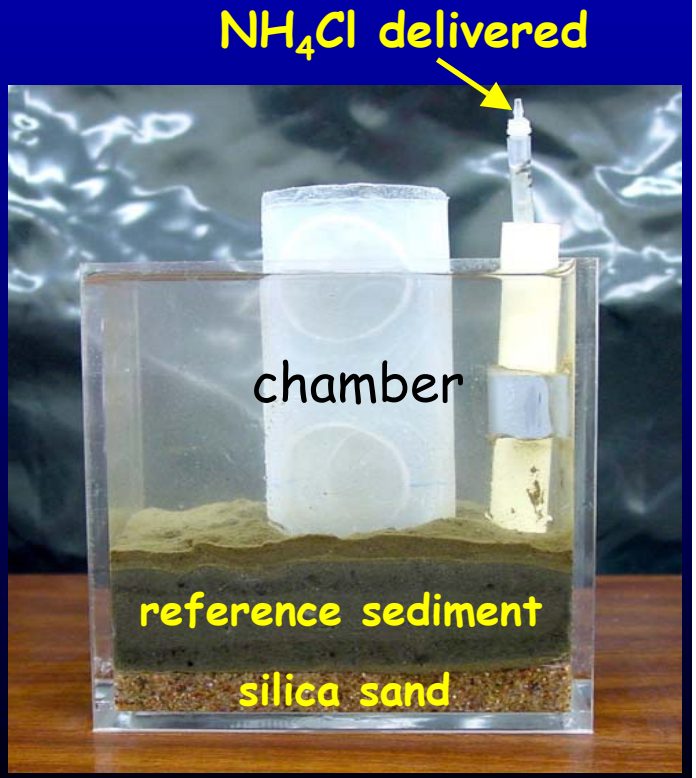
Design for Sediment Tests

Species: *Lampsilis cardium* (3-5 day old)
Number of mussels per chamber: 20
Concentration: 6 reps of 6 concns
Duration: 96 hr and 10 d
Endpoints: survival & growth
TAN measured: at end of each test in pore water



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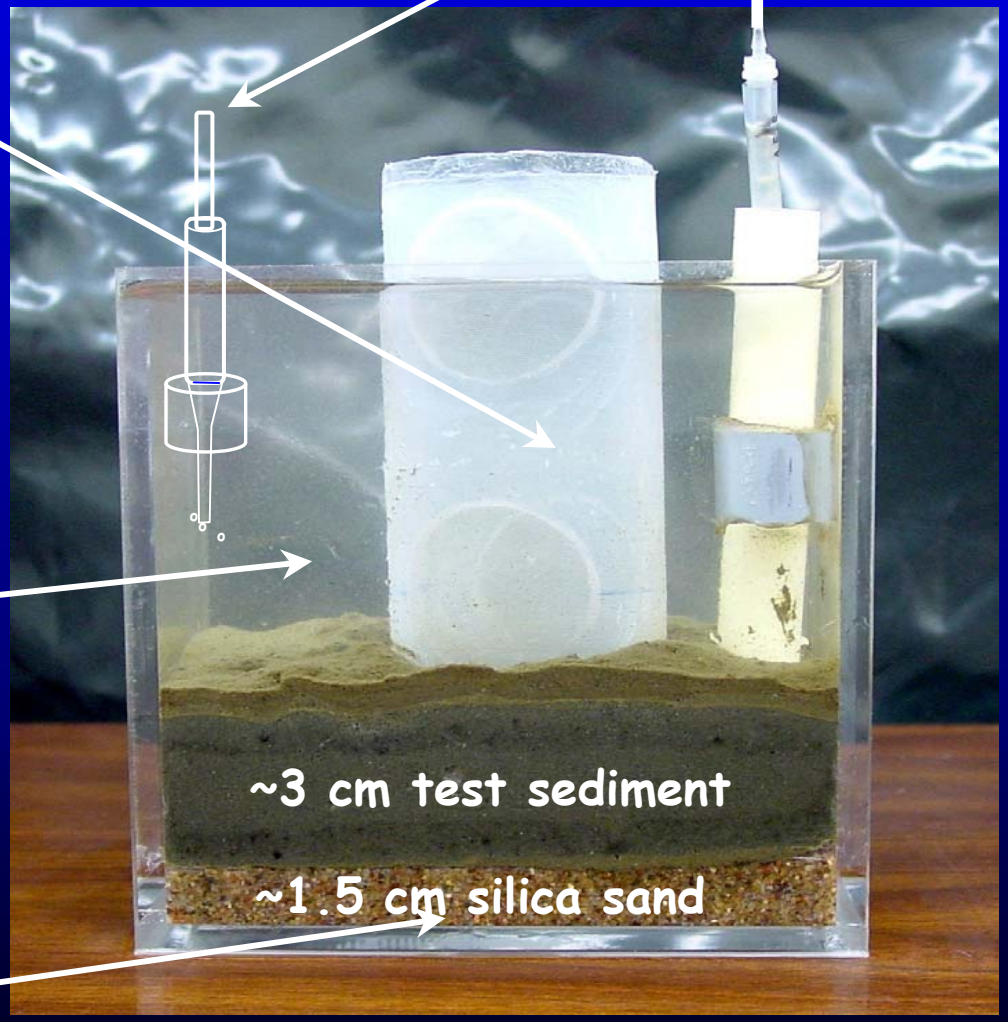
19-L Ammonia stock reservoir

Peristaltic pump

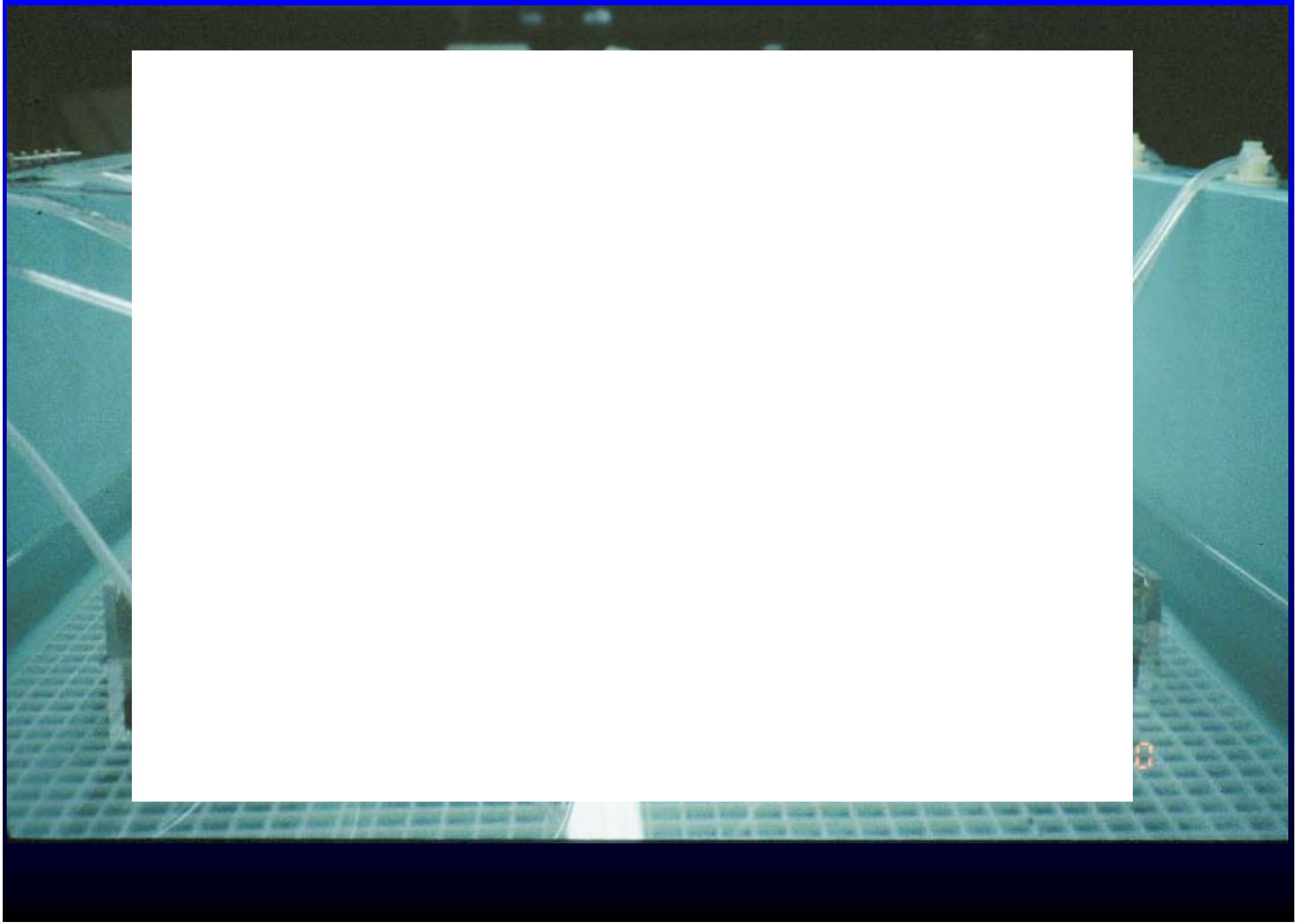
Compressed air delivered though a Pasteur pipette

Polycarbonate tube (4.5 cm ID x 11 cm H), with a 153 µm mesh bottom and 4-3.7 cm holes covered with 153 µm mesh



Acrylic unit, 12 L x 8 W x 12.7 cm H

Airstone within sand layer



Toxicity of Sedimentary Ammonia to Juveniles

Total ammonia nitrogen (mg/L) normalized to pH 8

Toxicity test	LC_{50}	EC_{50} (growth)	EPA Criteria
96 hr, rep 1	6.7	2.0	
96 hr, rep 2	6.0	3.2	8.4
10 d, rep 1	3.8	3.4	
10 d, rep 2	3.4	2.8	1.7

Design for Field Studies

Species: *Lampsilis cardium* (3-5 day old)

No. mussels per chamber: 20

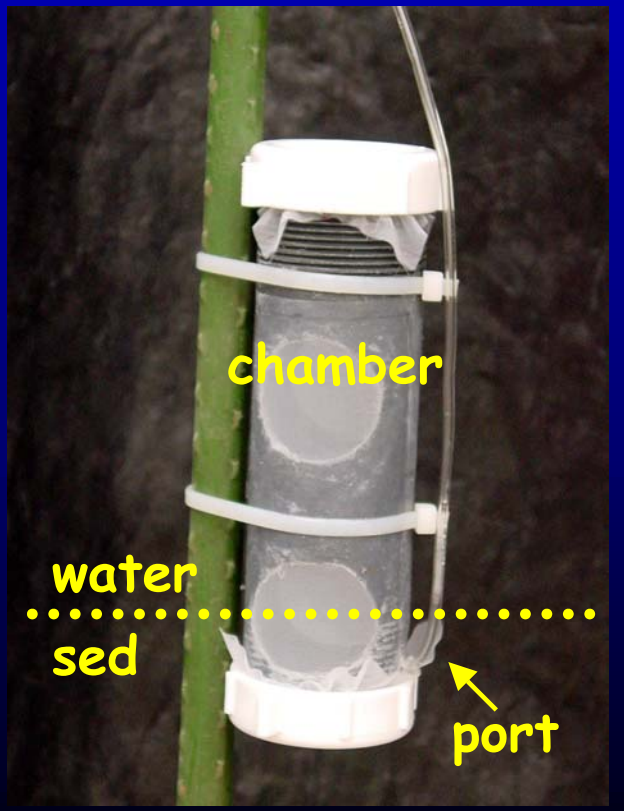
Sites: 8 sites covering 151 river km

Chambers per site: 6

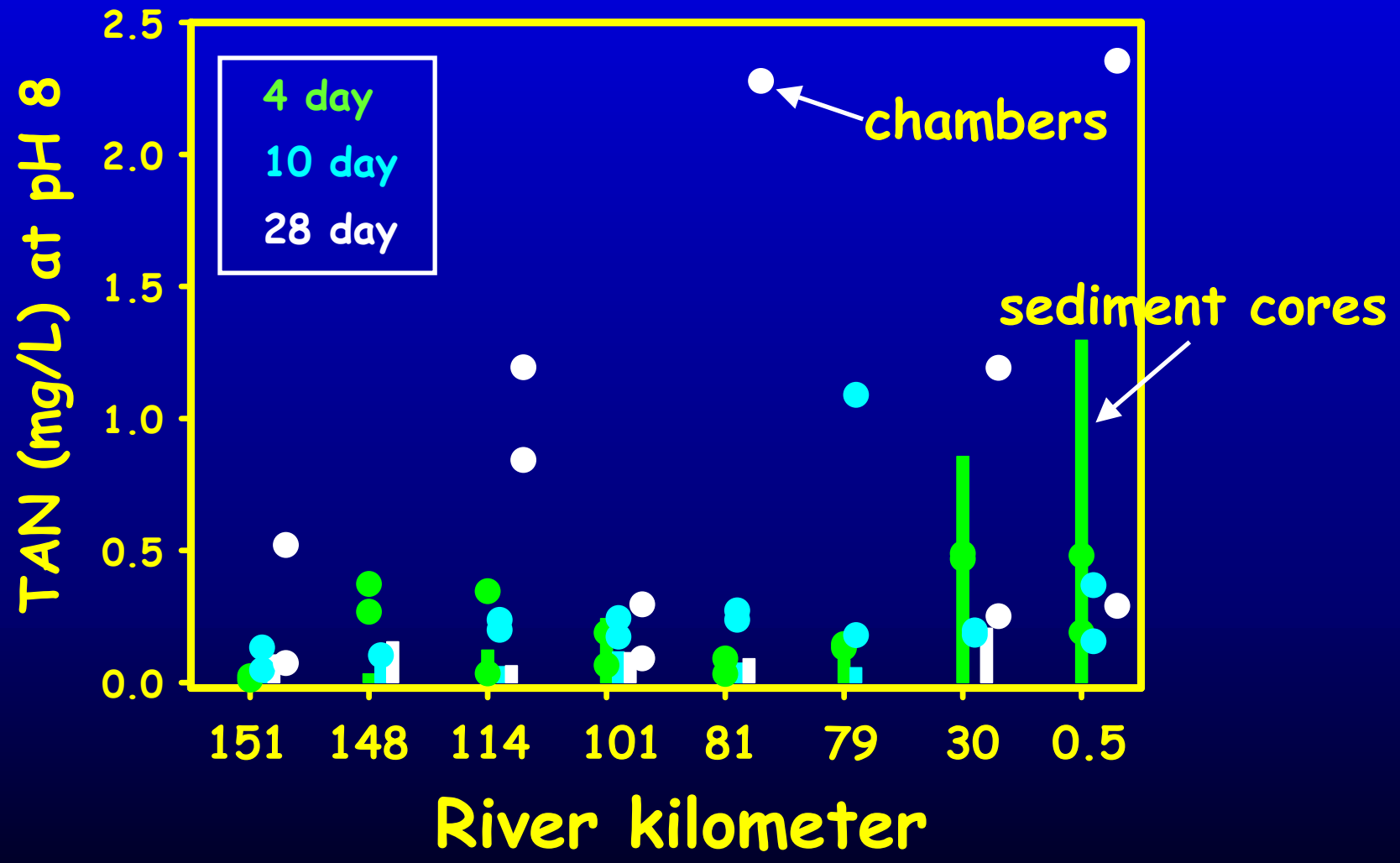
Duration: 4, 10, and 28 d

Endpoints: survival & growth

TAN measured: from within the chamber and surrounding sediments on days 4, 10, & 28

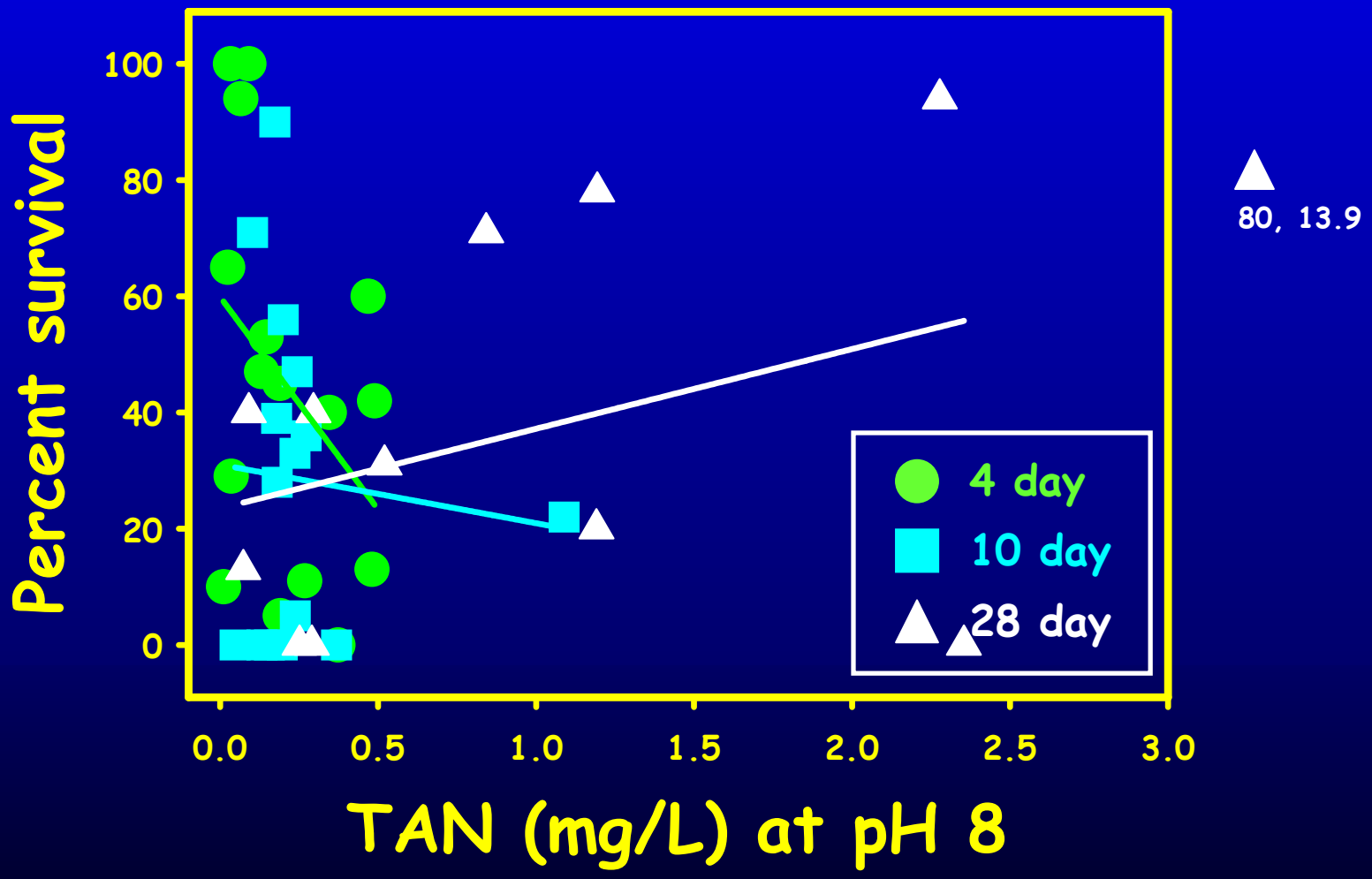


Spatial Variability in TAN



2001

Survival Data - Field



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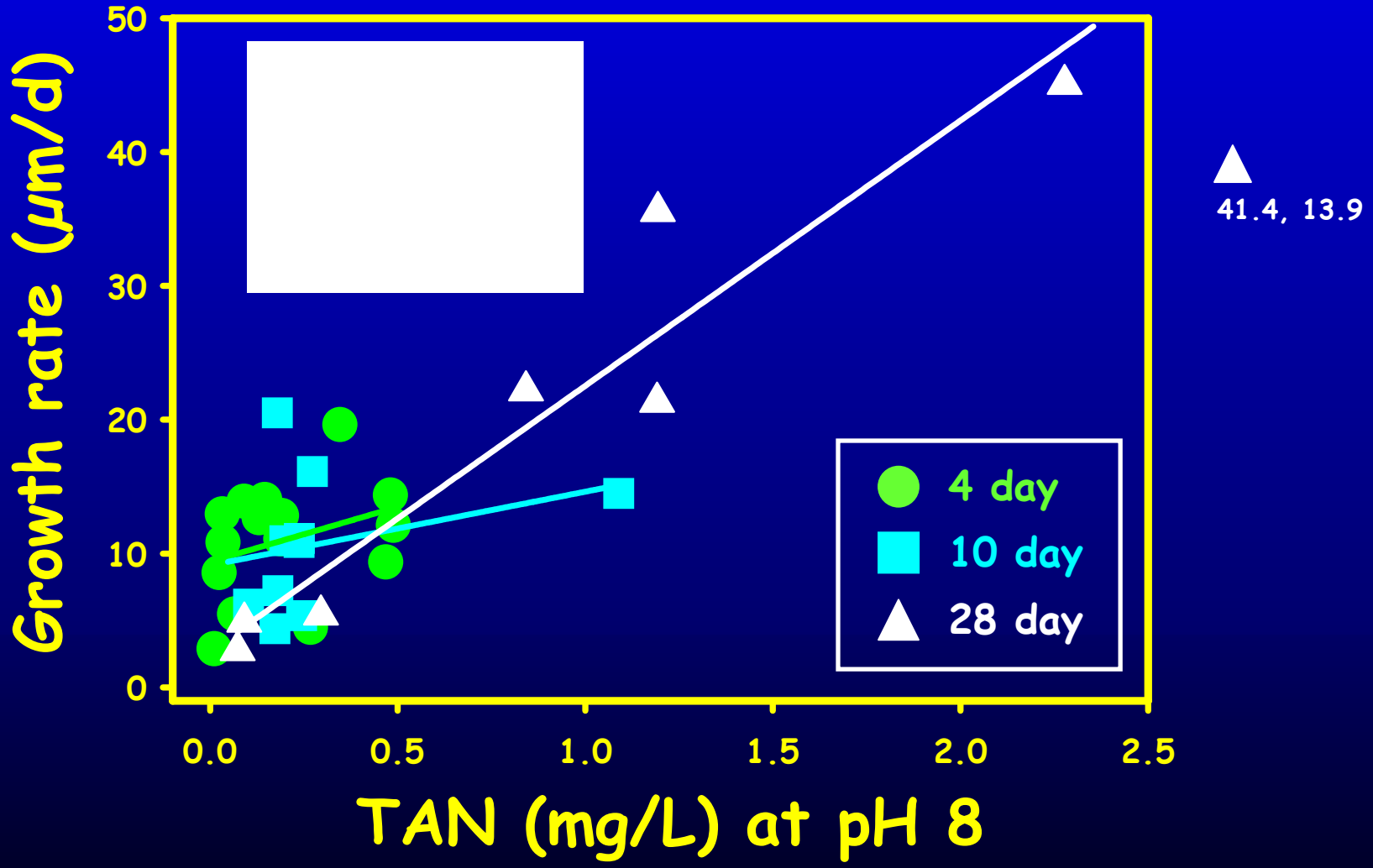
Growth Data - Field



Sedimentary TAN Data in Rivers

River	TAN (mg/L)	Reference
St. Croix 2000 2001	 0.4 - 12.7 0.1 - 2.2	Bartsch et al. 2003
Upper Mississippi	0.1 - 10	Frazier et al. 1996
Pembina	0.1 - 7.2	Chambers et al. 1992
Fox	16 - 53	Ankley et al. 1990
Illinois	24 - 60	Sparks and Ross 1992

LC50s 12-24, EC50s 5-8

Conclusions

- In the laboratory, juveniles were sensitive to ammonia; growth was affected at ~2 mg/L and survival at ~3 mg/L (TAN at pH 8)
- The current EPA criteria may not be protective of juvenile mussels
- In the field, there was no consistent pattern between survival and TAN, but growth was positively related with sedimentary TAN

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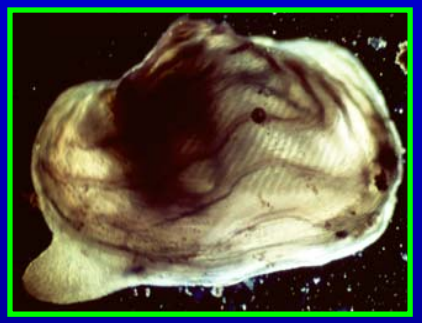
Lab vs. Field Discrepancy

- Frequency of exposure to ammonia differed between lab and field
  - lab: constant
  - field: pulsed



- Spatial and temporal variability in ammonia in the field likely masked any possible effects on survival or growth
- Not a large enough range in ammonia in the St. Croix River to see an effect on these endpoints

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Earlier Studies Published

Newton, T.J. 2003. The effects of ammonia on freshwater unionid mussels. *Environ Toxicol Chem* 22:2543-2544.

Newton, T.J. et al. 2003. Effects of ammonia on juvenile unionid mussels (*Lampsilis cardium*) in laboratory sediment toxicity tests. *Environ Toxicol Chem* 22:2554-2560.

Bartsch, M.R. et al. 2003. Effects of pore water ammonia on in situ survival and growth of juvenile mussels (*Lampsilis cardium*) in the St. Croix Riverway. *Environ Toxicol Chem* 22:2561-2568.

Design for Laboratory Tests

Species: 1-2 d old *L. cardium and L. higginsii*

Exposure: sediment and water-only (2 tests each)

No. mussels per chamber: 20

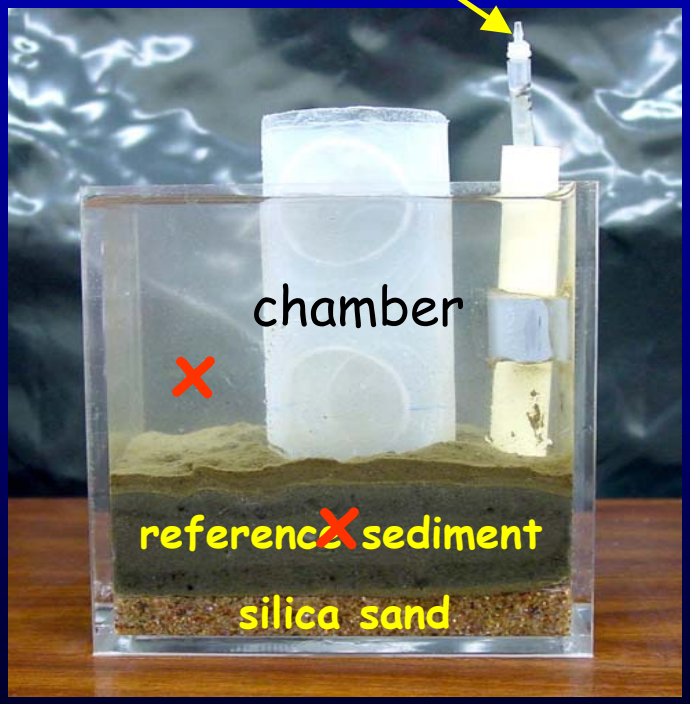
Concentration: 3 reps of 6 concns

Duration: 96 hr

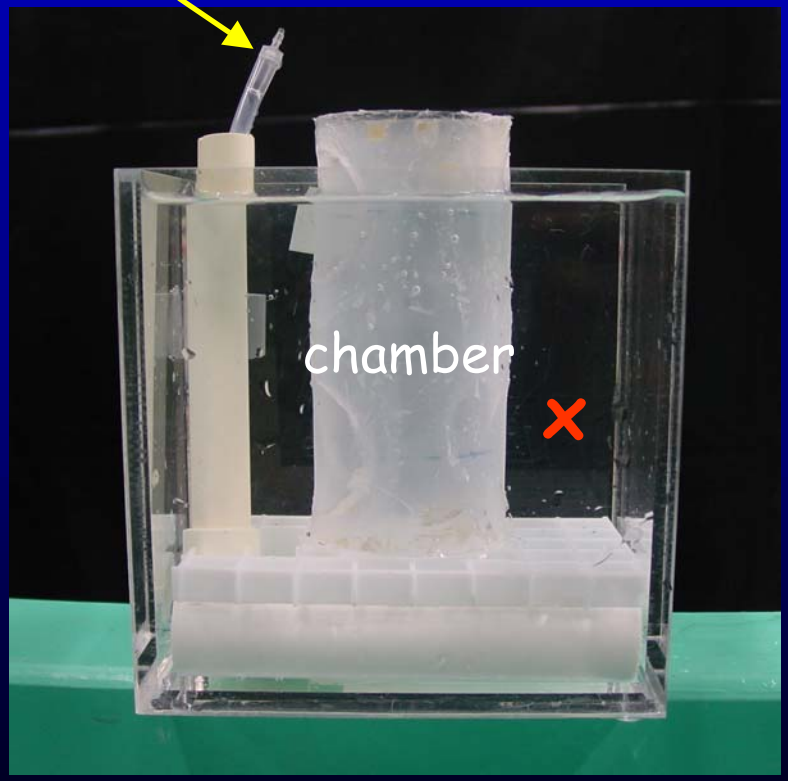
Endpoints: survival & growth

TAN measured: daily in overlying water (sediment and water-only tests) and on day 4 in pore water (sediment tests)

Sediment Tests



Water-only Tests



Minimal Species Variation

range in 95% confidence limits around TAN (mg/L at pH 8) LC50s

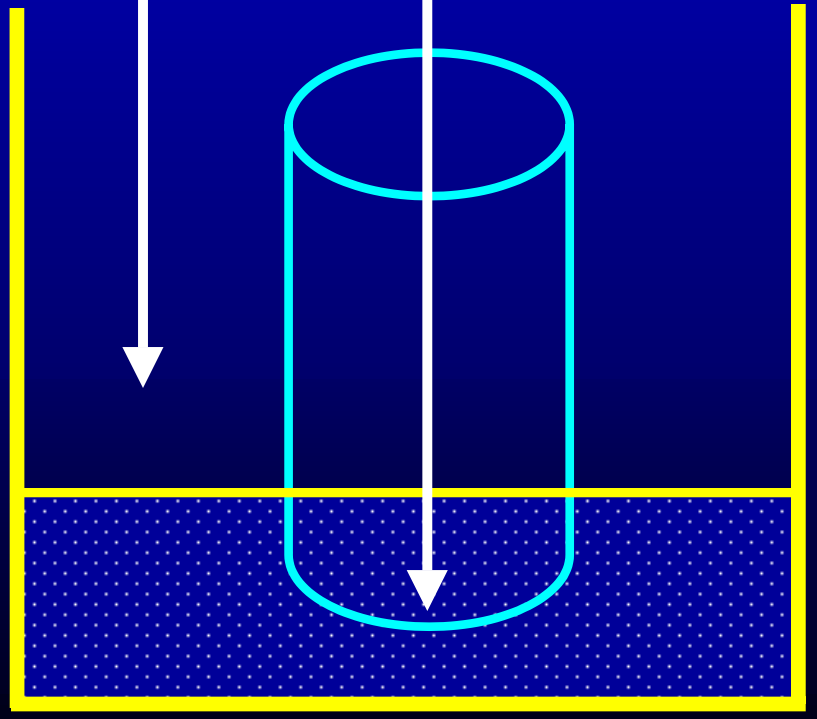
	L. cardium	*L. higginsii*
sediment tests	4.7-6.0	4.1-6.5
water-only tests	8.2-12.5	5.7-10.5

Possible Chamber Artifacts

sediment test 2
3 EU/conc
day 4

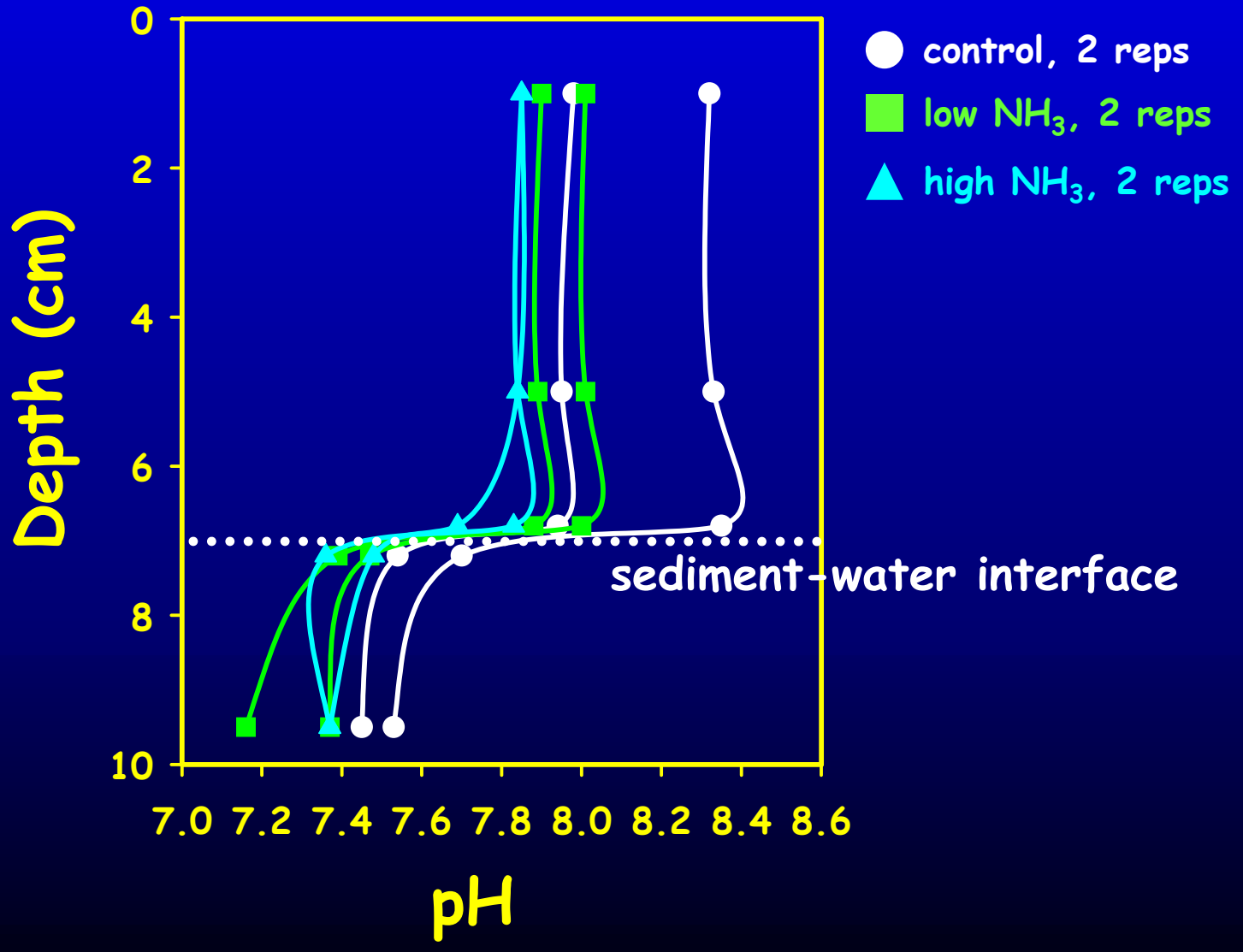
inside chamber
temp: 21.0
pH: 7.85
TAN: 1-48
NH_3: 5-1570

outside chamber
temp: 21.1
pH: 7.96
TAN: 1-49
NH_3: 6-1874



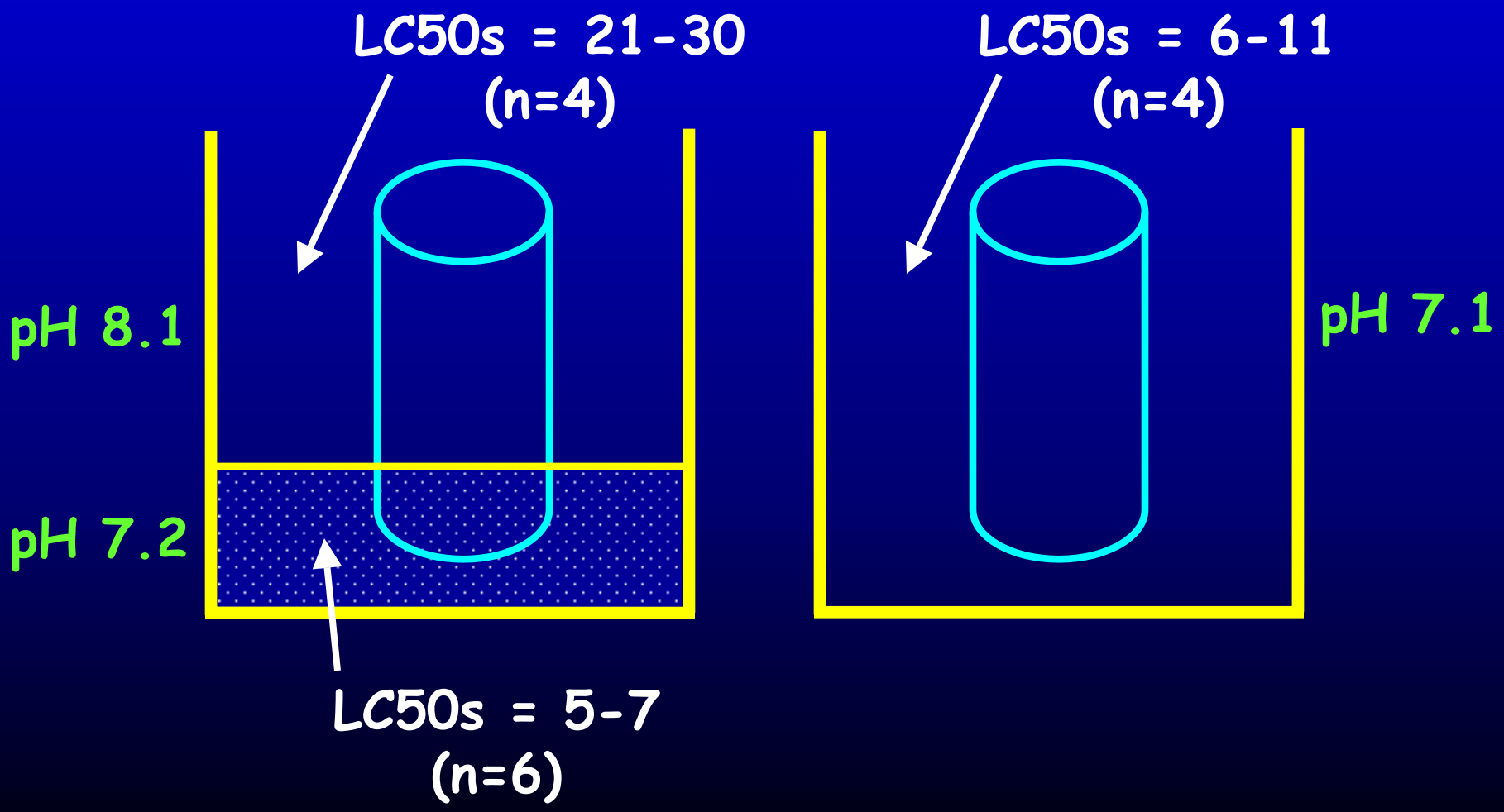
Temp: °C
TAN: mg/L
NH_3: µg/L

Spatial Variability in pH

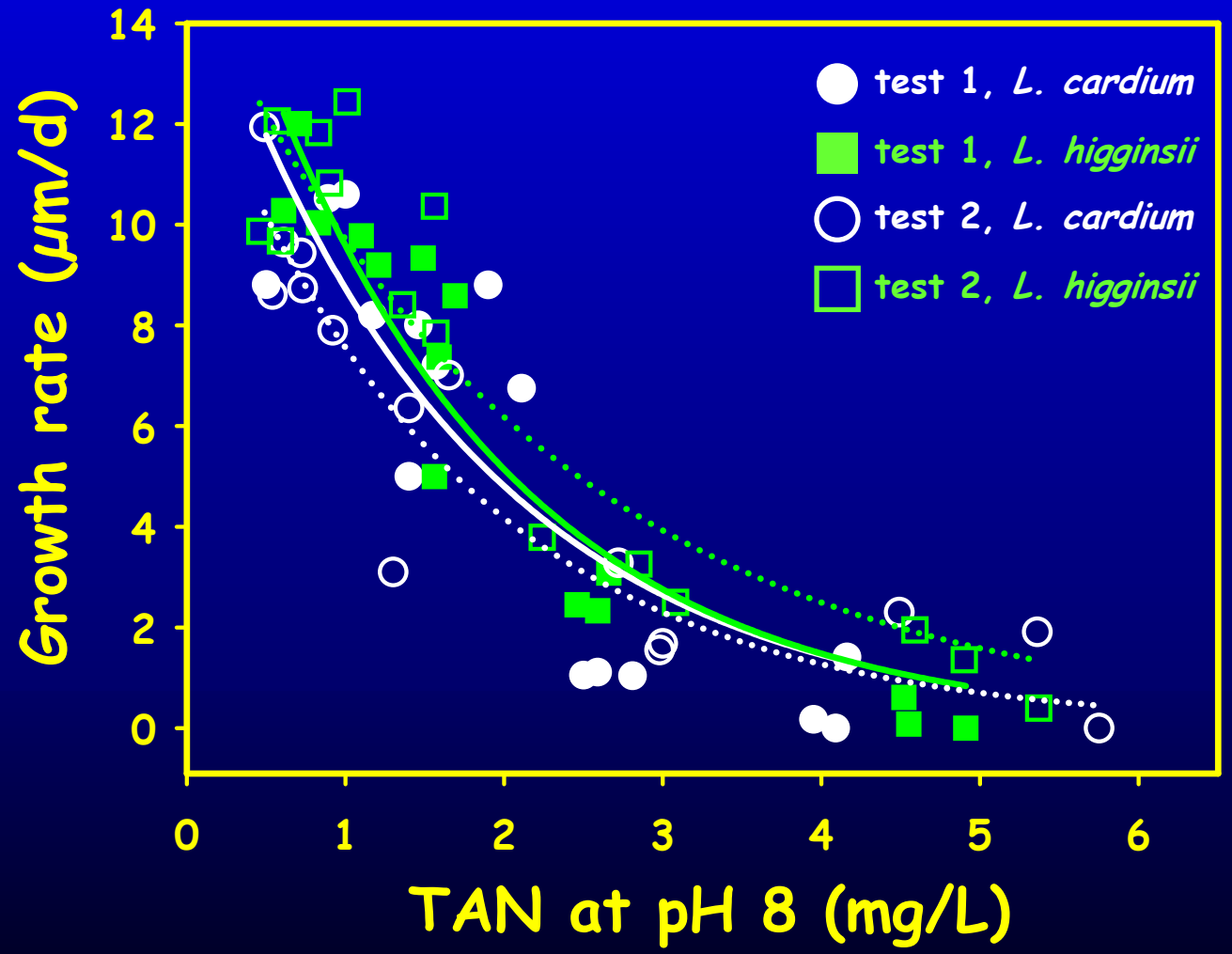


Lethality: Total Ammonia Nitrogen

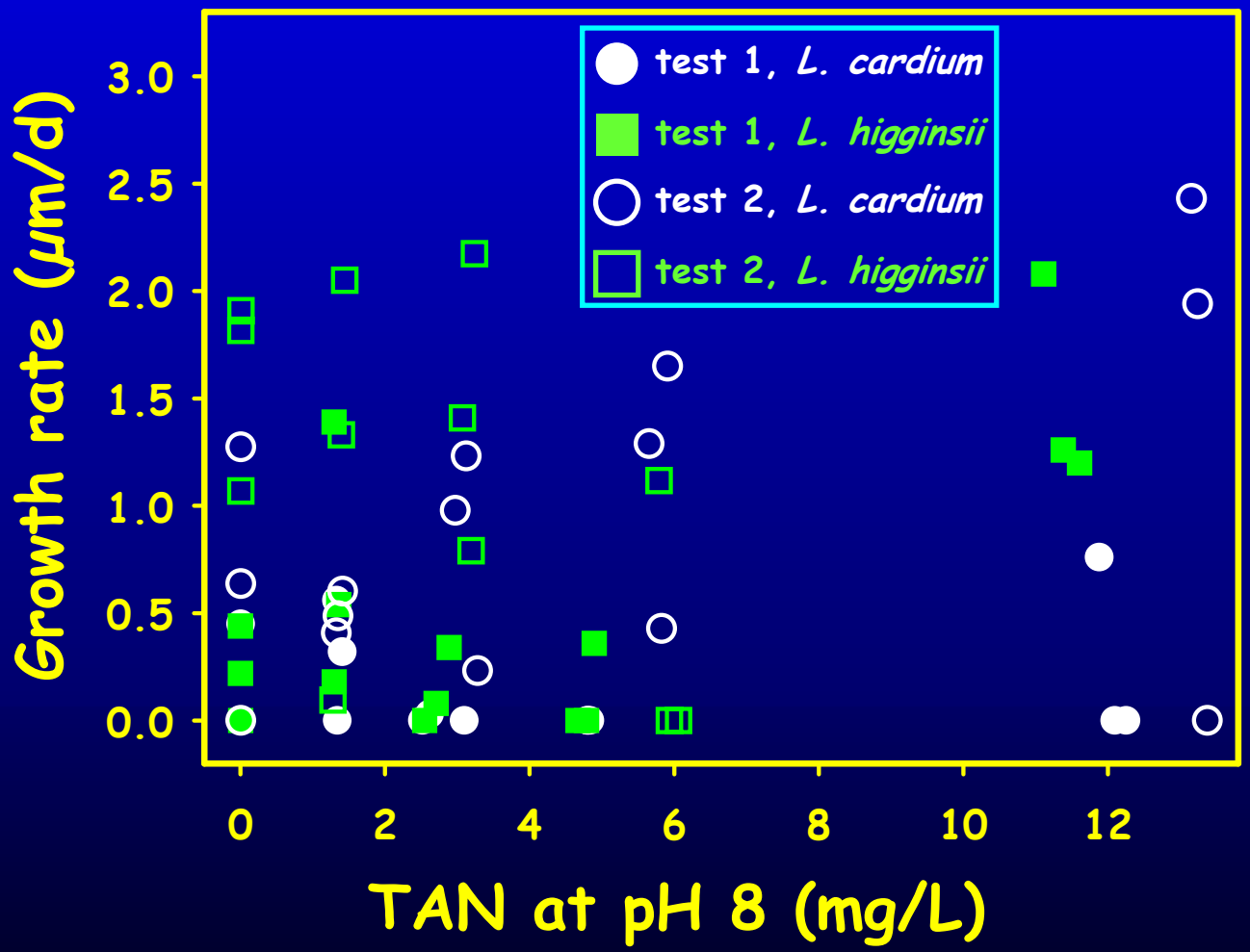
normalized to pH 8 in mg/L



Growth Rate: Sediment Tests

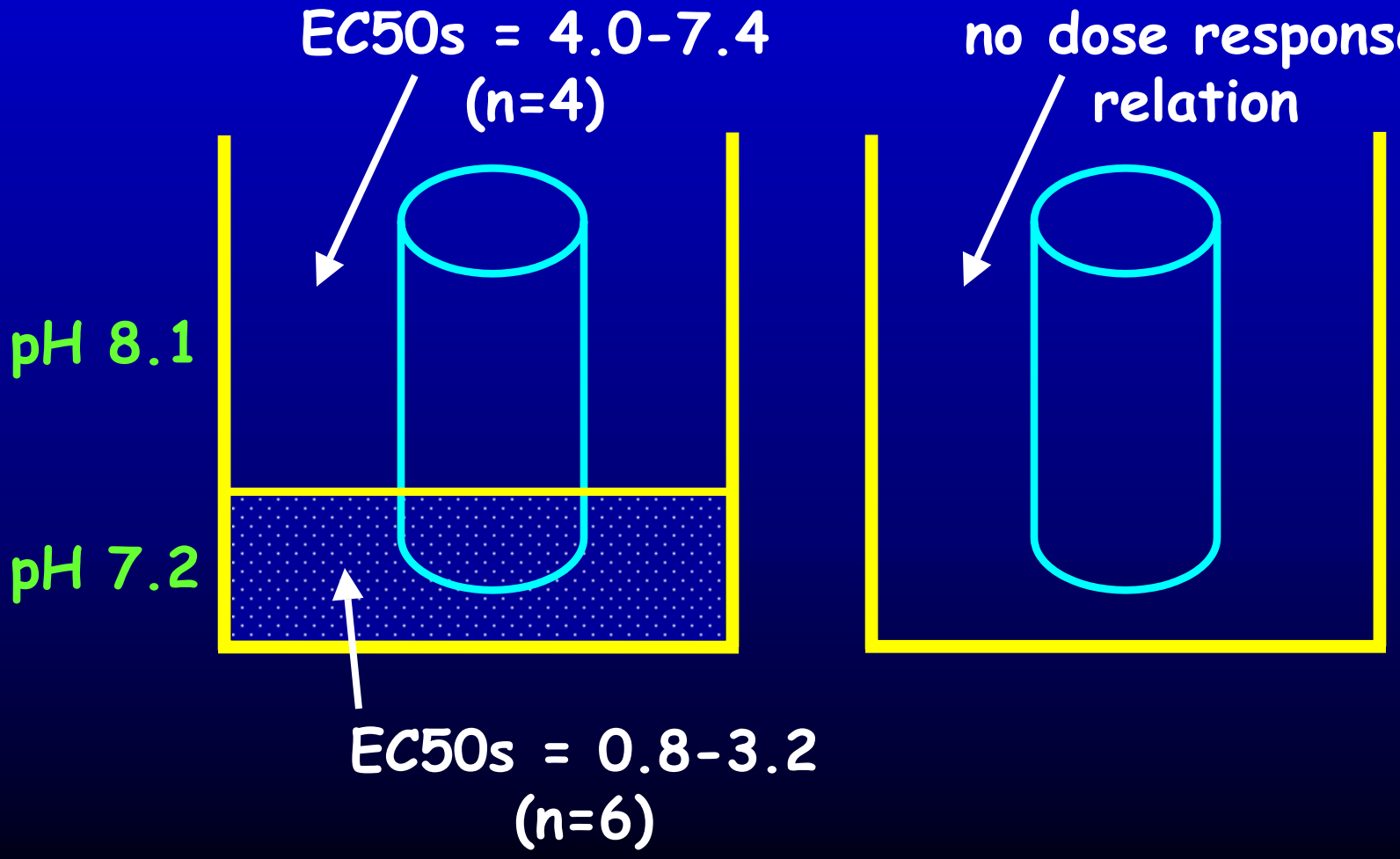


Growth Rate: Water-Only Tests



Growth: Total Ammonia Nitrogen

normalized to pH 8 in mg/L



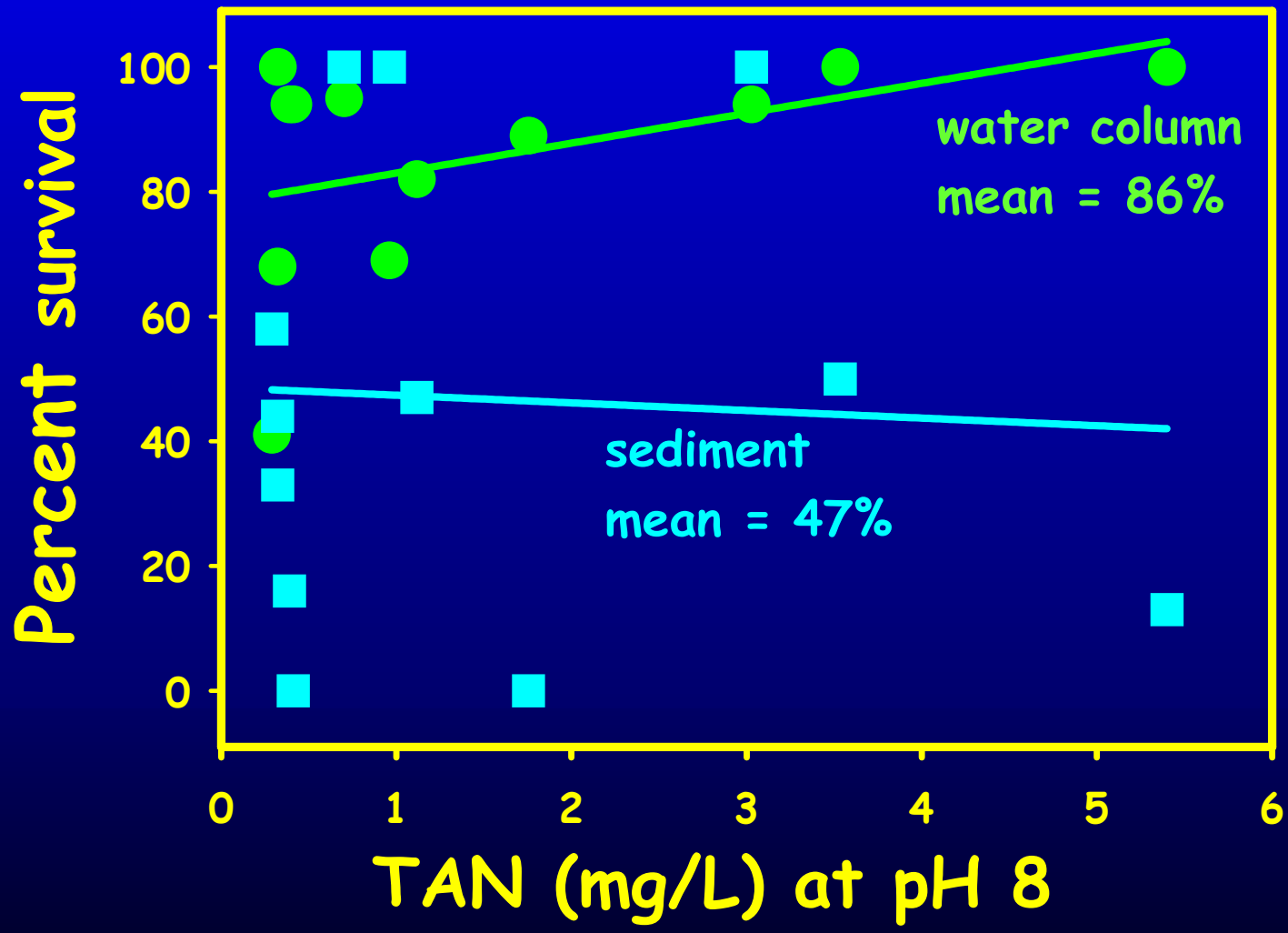
Preliminary Interpretations

- There were no differences in the lethal or sublethal responses of *L. higginsii* and *L. cardium* to ammonia
- The LC50s in the water-only tests were similar to those in the sediment tests when TAN was based on pore water concn, but were ~4X greater when TAN was based on concn in water overlying sediments
- Growth of juveniles was an order of magnitude greater in the controls in the sediment tests relative to the water-only tests

Preliminary Interpretations, cont..

- Growth was a sensitive endpoint in the sediment tests, but not in the water-only tests
- Ammonia fractionation is pH dependent; understanding where juveniles reside in sediments is critical because of the sharp drop in pH in the top few cm of sediment
- Stay tuned…..

Sediment vs Water Column



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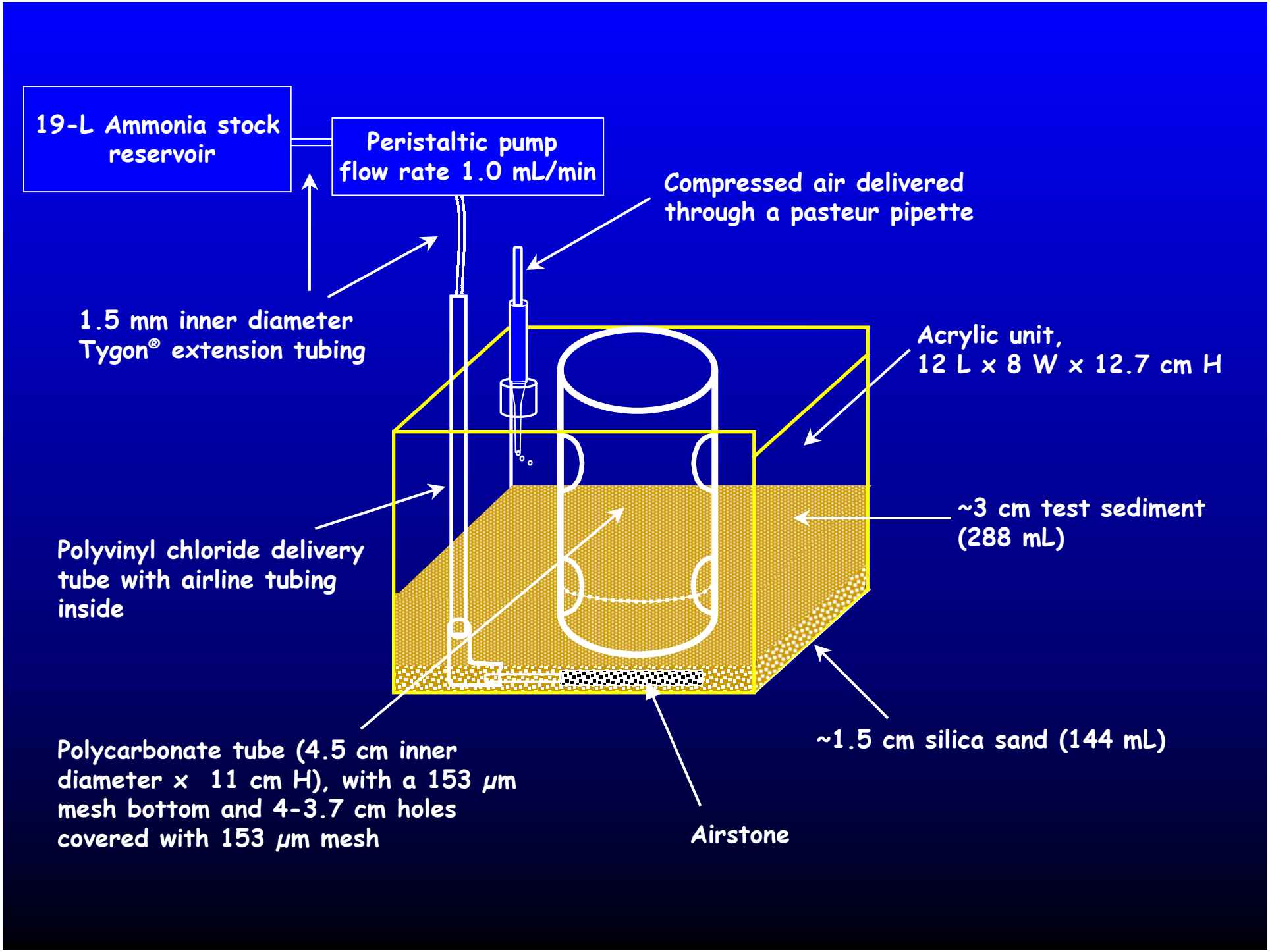
2000

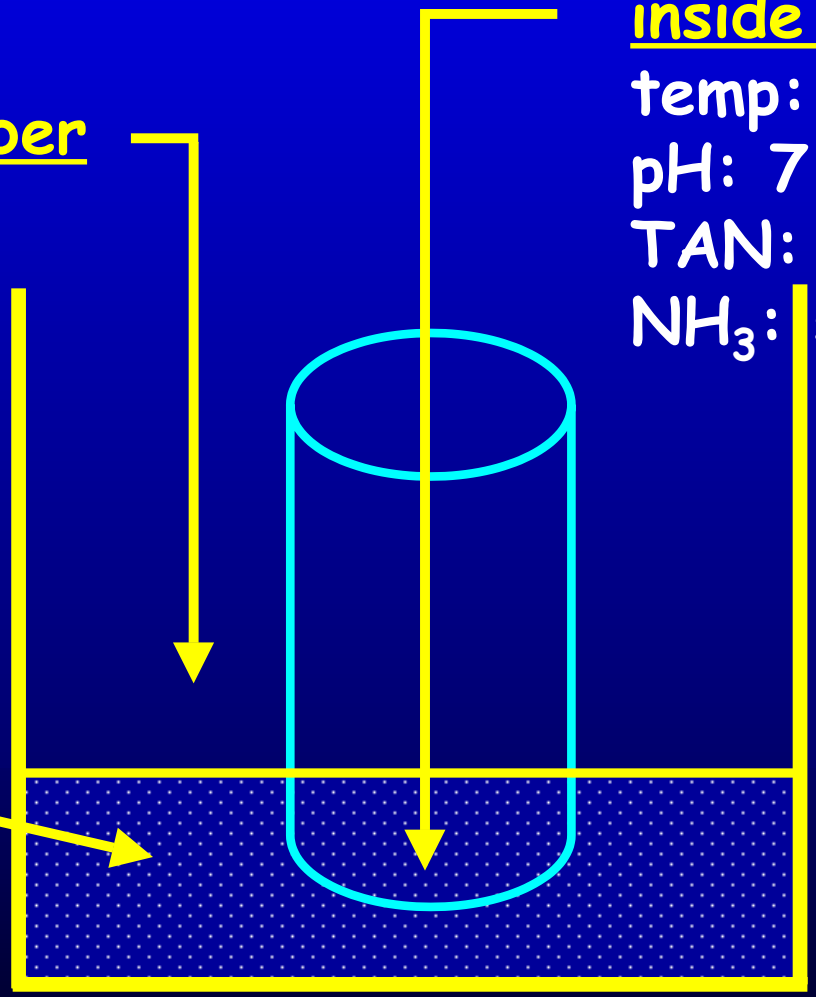

19-L Ammonia stock reservoir
Peristaltic pump flow rate 1.0 mL/min
Compressed air delivered through a pasteur pipette
1.5 mm inner diameter Tygon® extension tubing
Acrylic unit, 12 L x 8 W x 12.7 cm H
~3 cm test sediment (288 mL)
Polyvinyl chloride delivery tube with airline tubing inside
Polycarbonate tube (4.5 cm inner diameter x 11 cm H), with a 153 µm mesh bottom and 4-3.7 cm holes covered with 153 µm mesh
Airstone
~1.5 cm silica sand (144 mL)

Possible Chamber Artifacts

sediment test 2
3 EU/conc
day 4

outside chamber
temp: 21.1
pH: 7.96
TAN: 1-49
NH_3: 6-1874

inside chamber
temp: 21.0
pH: 7.85
TAN: 1-48
NH_3: 5-1570

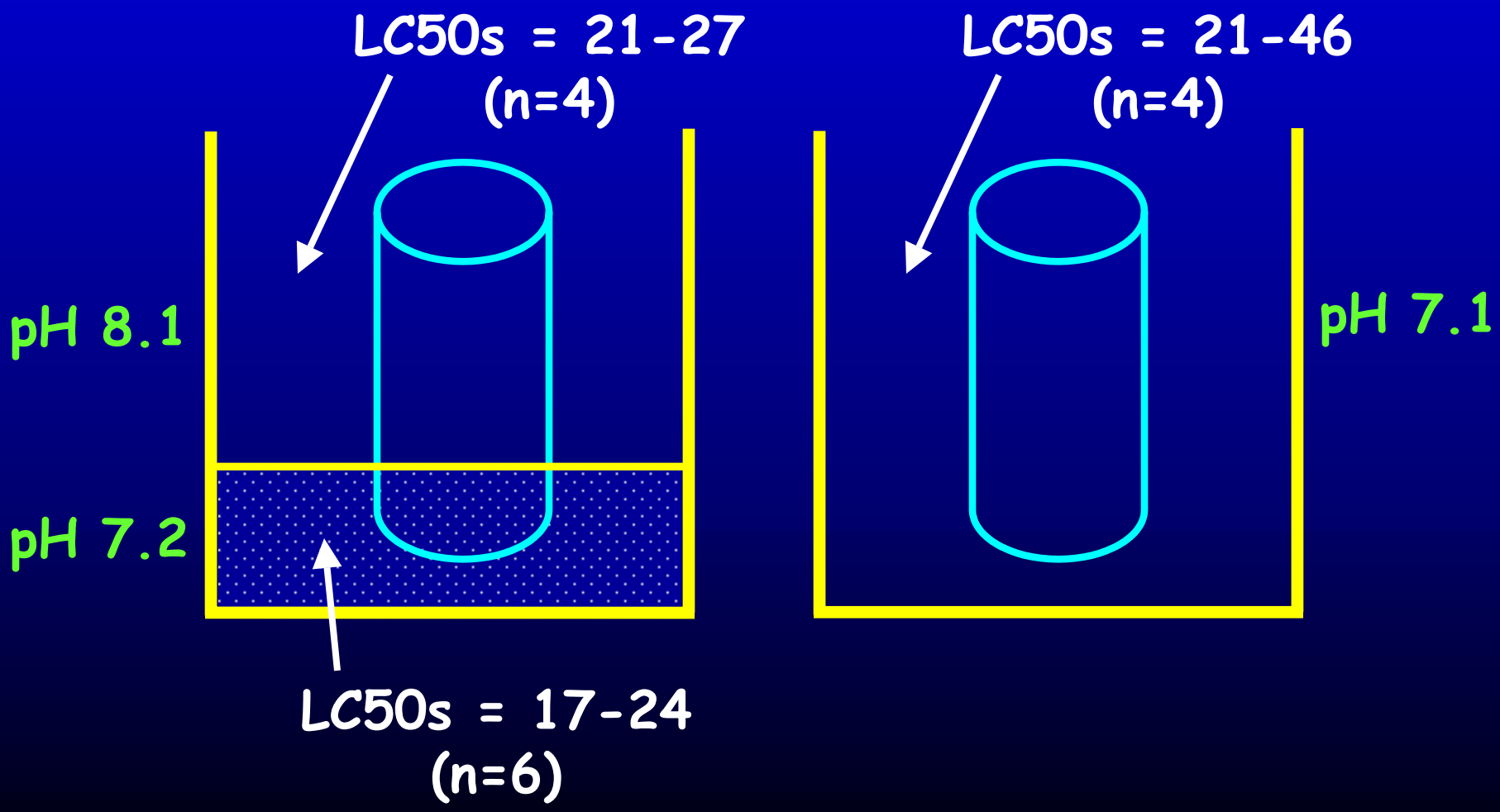


pore water
temp: 21.4
pH: 7.2
TAN: 2-38
NH_3: 14-245

Temp: °C
TAN: mg/L
NH_3: µg/L

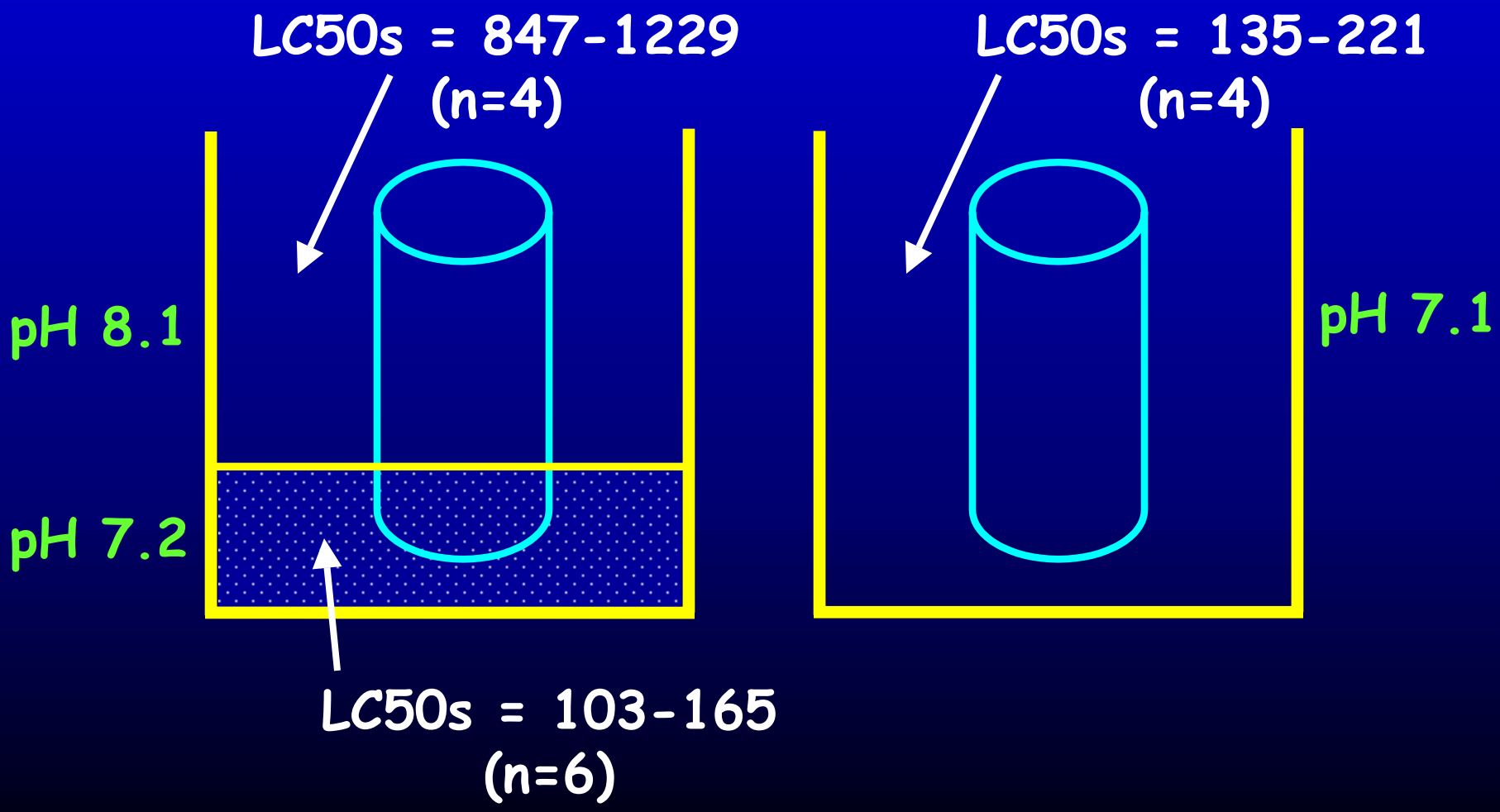
Lethality: Total Ammonia Nitrogen

units mg/L



Lethality: Un-ionized Ammonia

units µg/L



Growth Rate: Controls

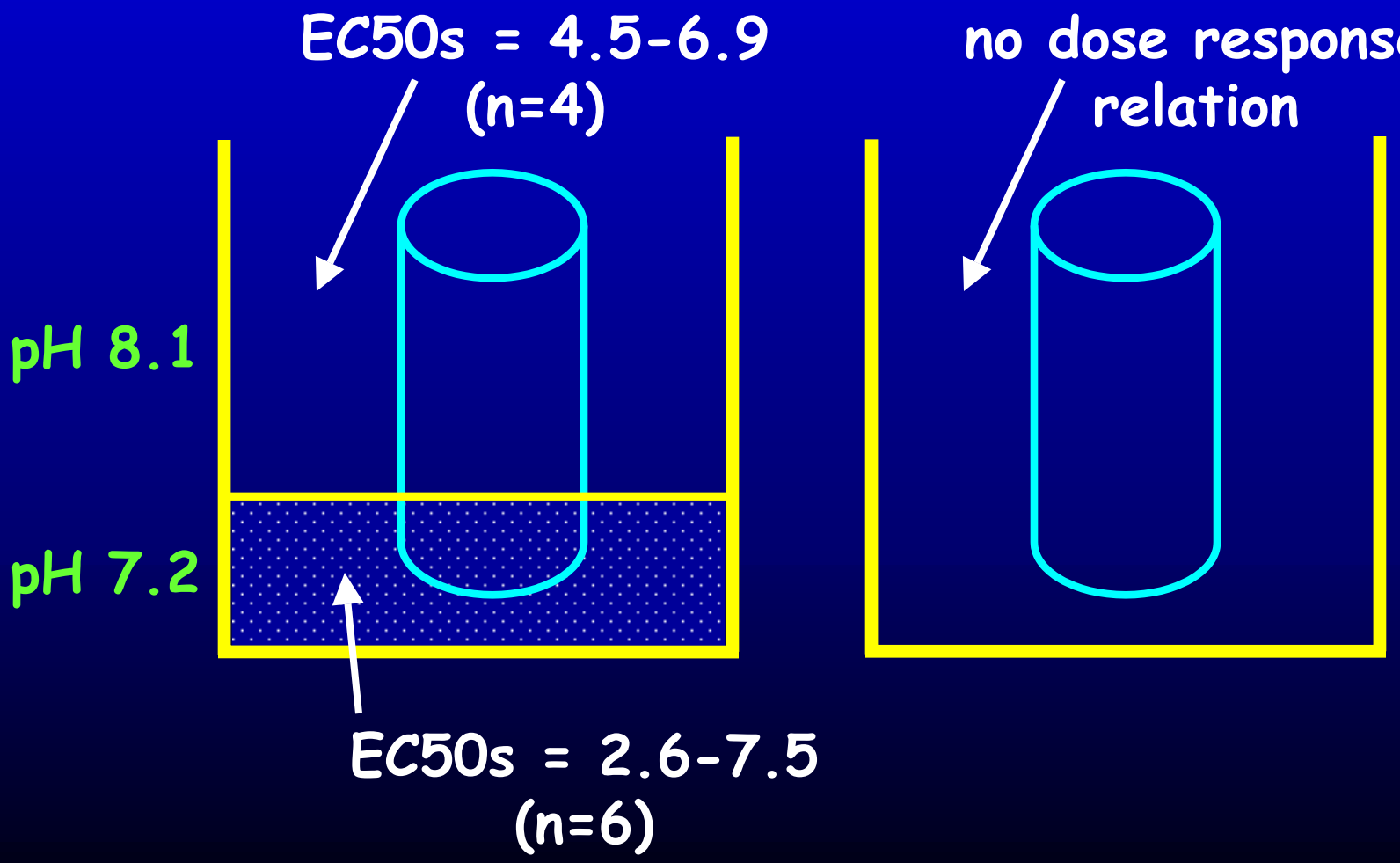
units µm/d

Test	*L. cardium*	*L. higginsii*
sediment 1	10.0	10.8
sediment 2	10.1	10.5
water-only 1	0.2	0.2
water-only 2	0.6	1.6

initial sizes varied <5% among all tests and both species

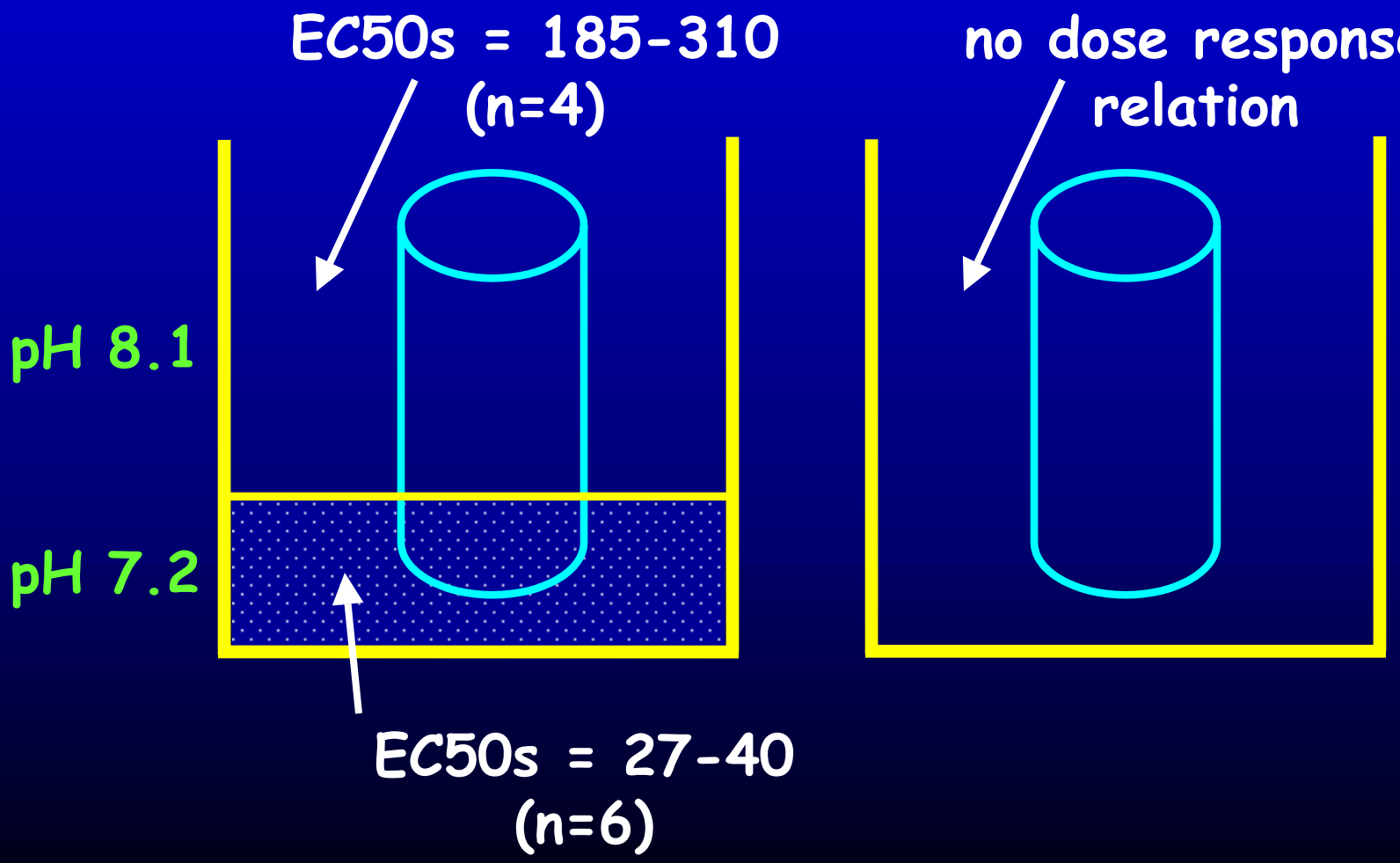
Growth: Total Ammonia Nitrogen

units mg/L

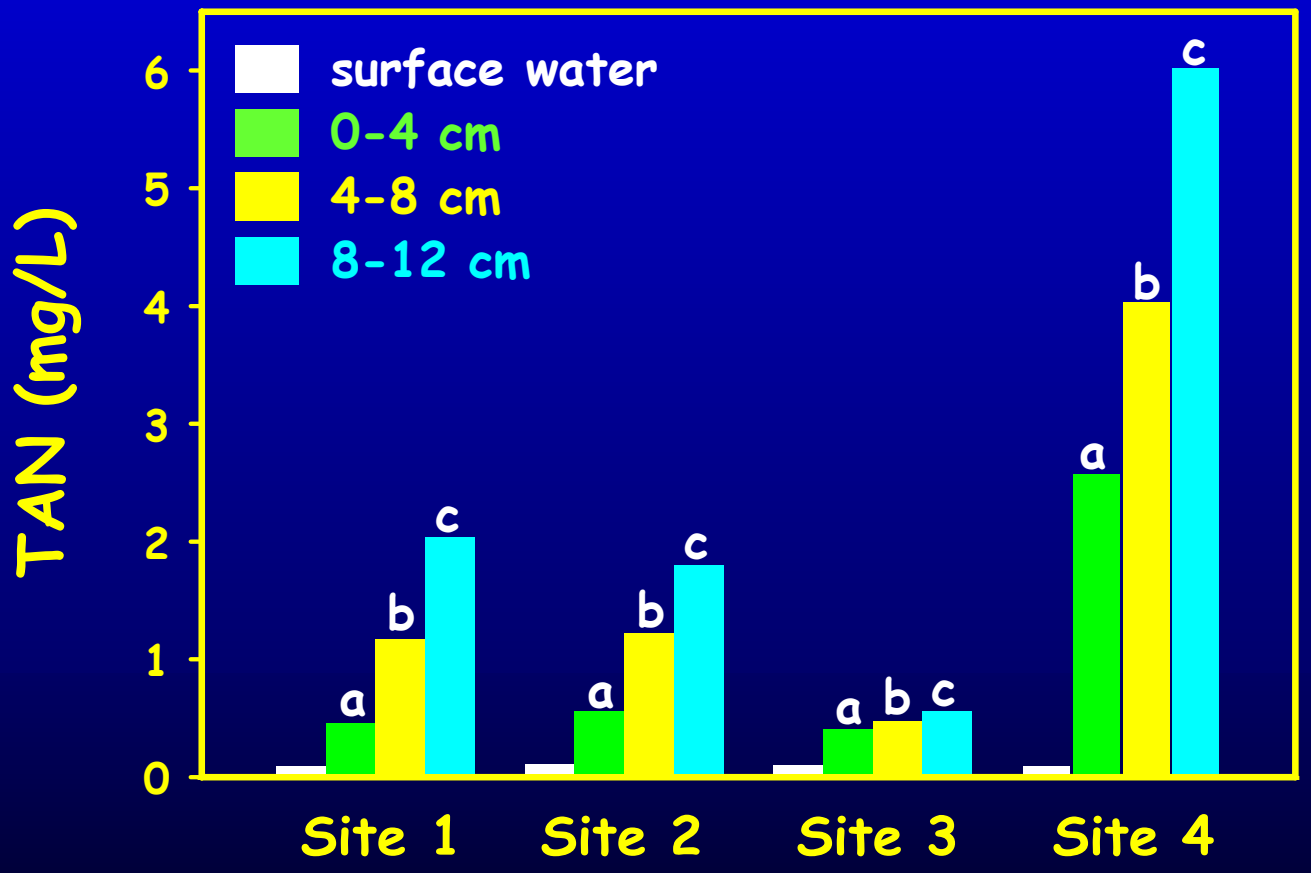


Growth: Un-ionized Ammonia

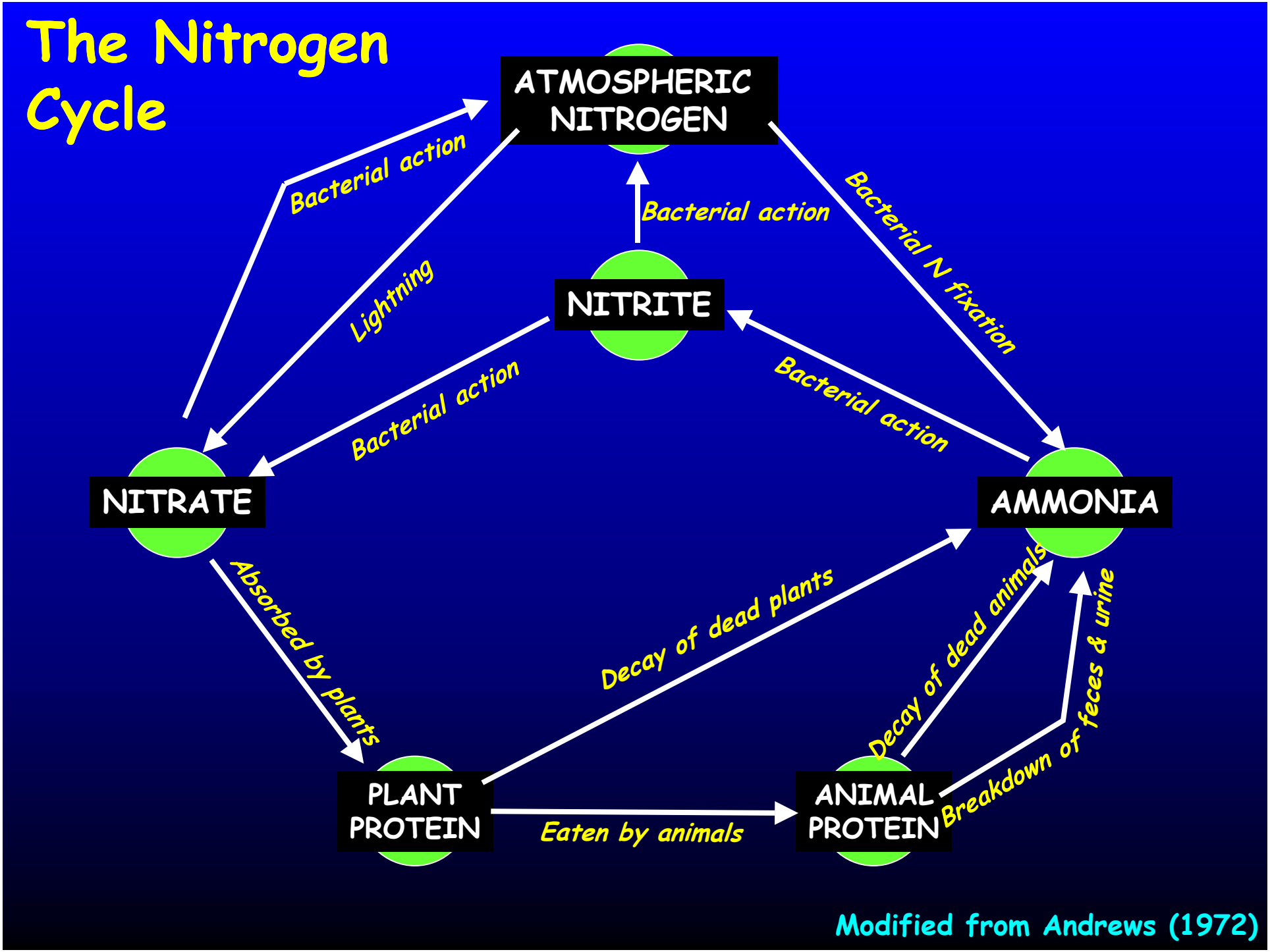
units µg/L

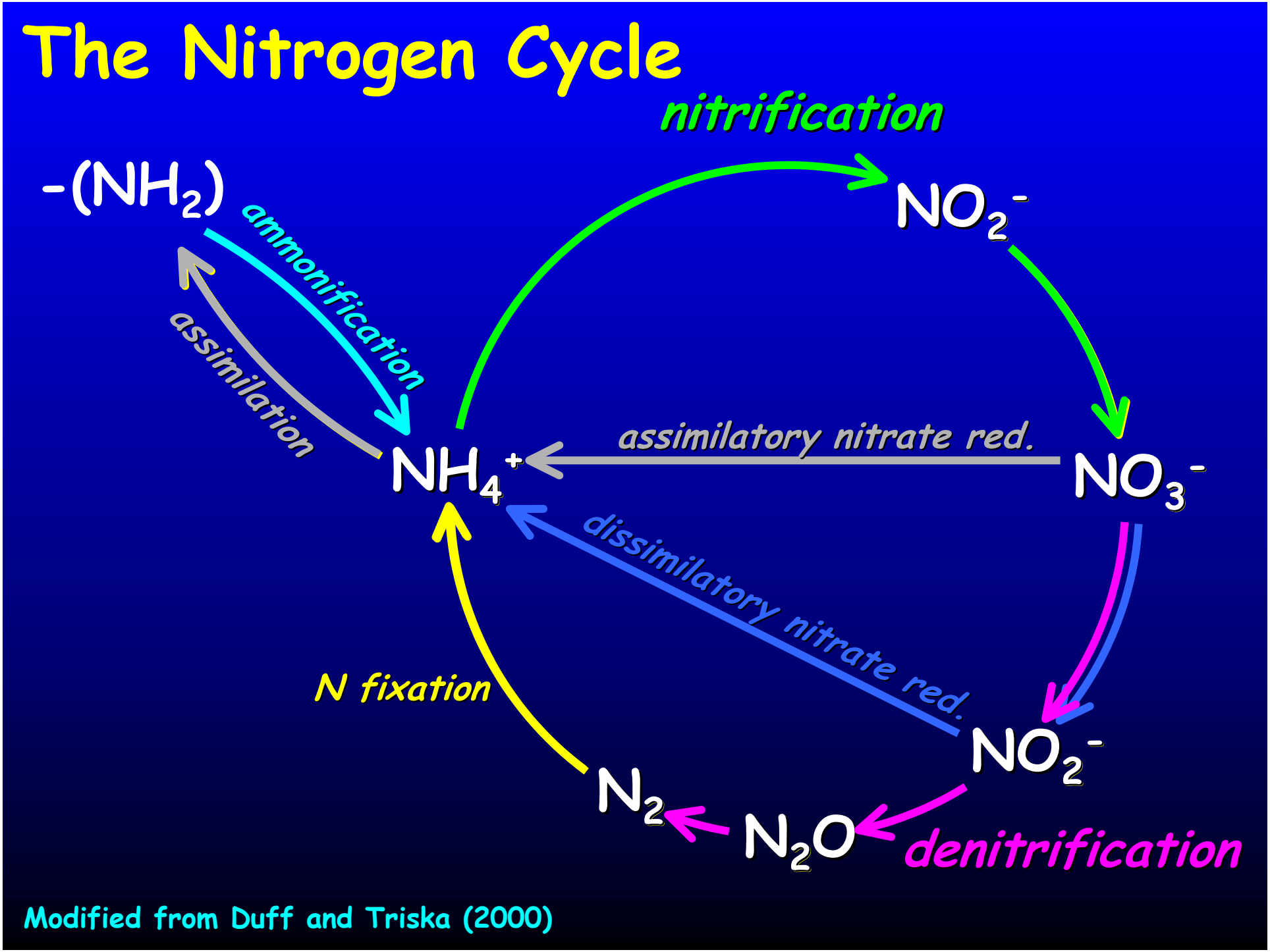


Vertical Distribution of TAN in Sediments



Upper Mississippi River, Frazier et al. 1996


The Nitrogen Cycle
ATMOSPHERIC NITROGEN
Bacterial action
Lightning
Bacterial action
Bacterial N fixation
NITRITE
Bacterial action
Bacterial action
NITRATE
AMMONIA
Absorbed by plants
Decay of dead plants
Decay of dead animals
Breakdown of feces & urine
PLANT PROTEIN
Eaten by animals
ANIMAL PROTEIN
Modified from Andrews (1972)


The Nitrogen Cycle
nitrification
-(NH2)
NO2-
ammonification
assimilation
assimilatory nitrate red.
NH4+
NO3-
dissimilatory nitrate red.
N fixation
NO2-
N2
N2O
denitrification
Modified from Duff and Triska (2000)