



STONEHILL COLLEGE



Evaluating Sediment Denitrification Under Three Oyster Aquaculture Systems in Waquoit Bay

Webinar, March 23, 2021

Daniel Rogers, Ginny Edgcomb, Paraskevi (Vivian) Mara, Chuck Martinsen, Christina Lovely, Tonna-Marie Rogers



**National Estuarine
Research Reserves**
Science Collaborative



Imagine our coastal community.
What do you think of?

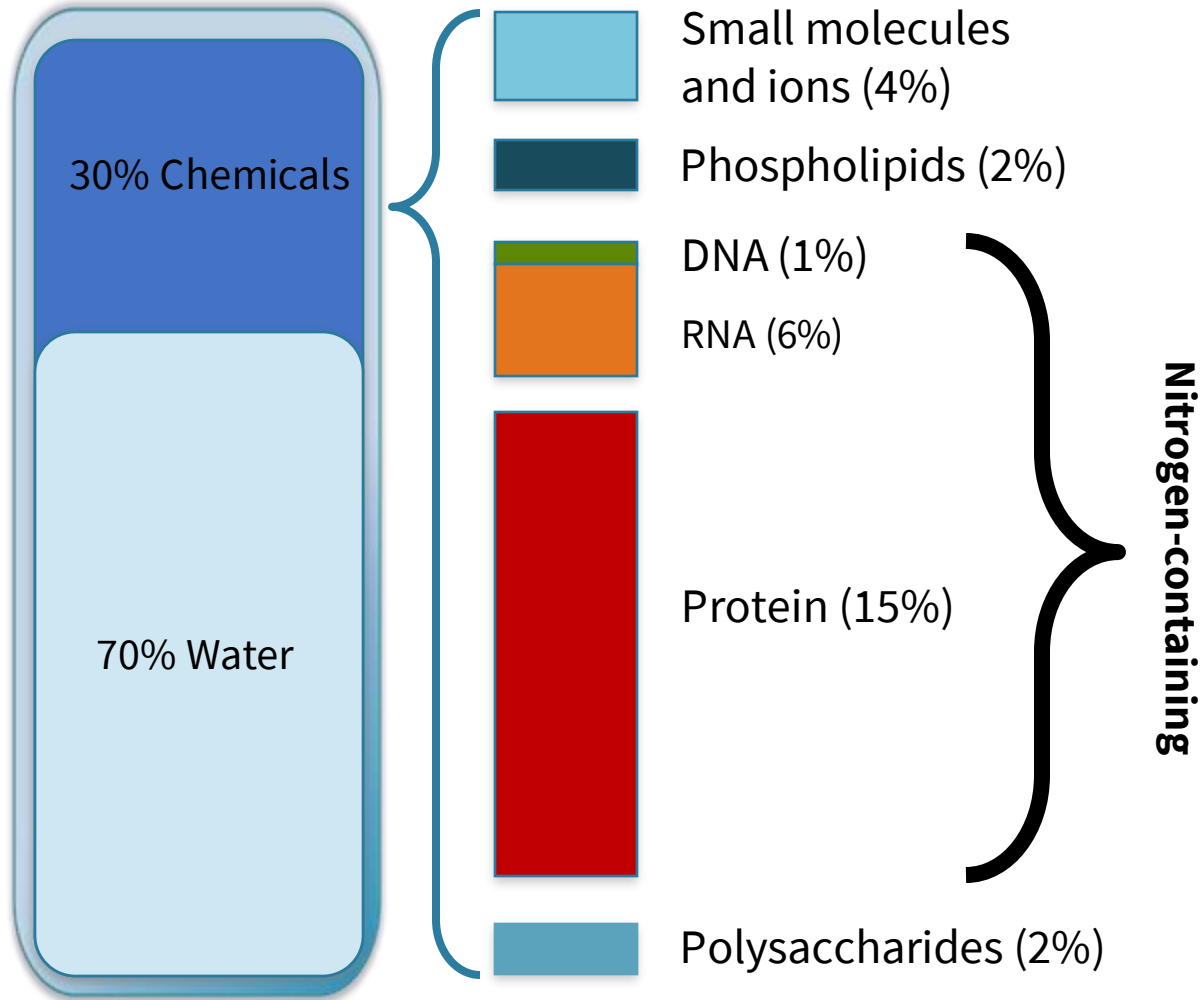


Human activity impacts our coastal waters.

- Major cities located on waters or coasts
- Large population on the coasts
- Activities have altered the coastal ecosystem.
 - Overfishing of many species.
 - Loss of habitat
 - **Nitrogen pollution**



Why do we care about nitrogen?

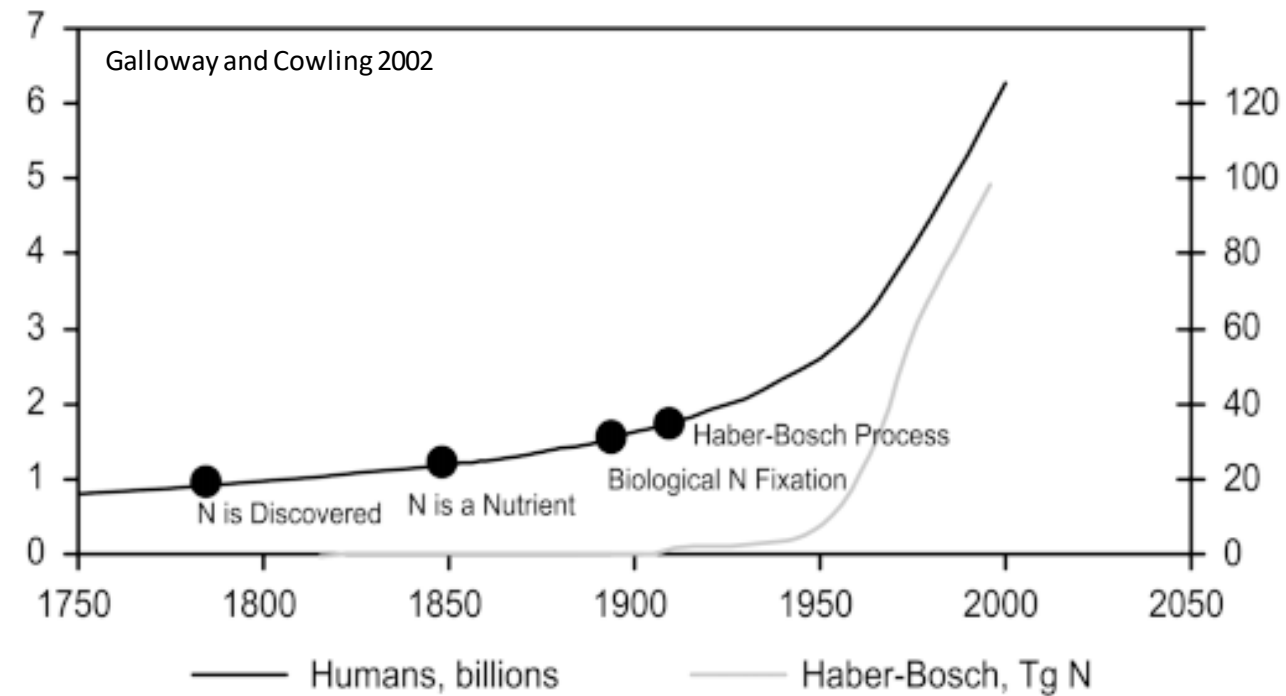
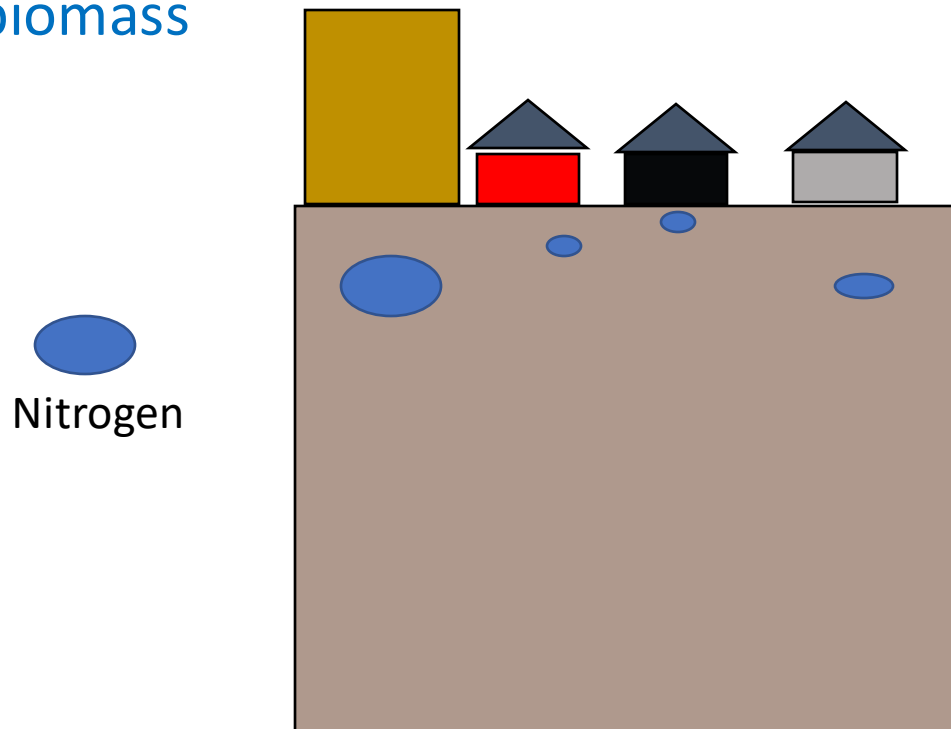


More than 20% of the cell weight is built with nitrogen.

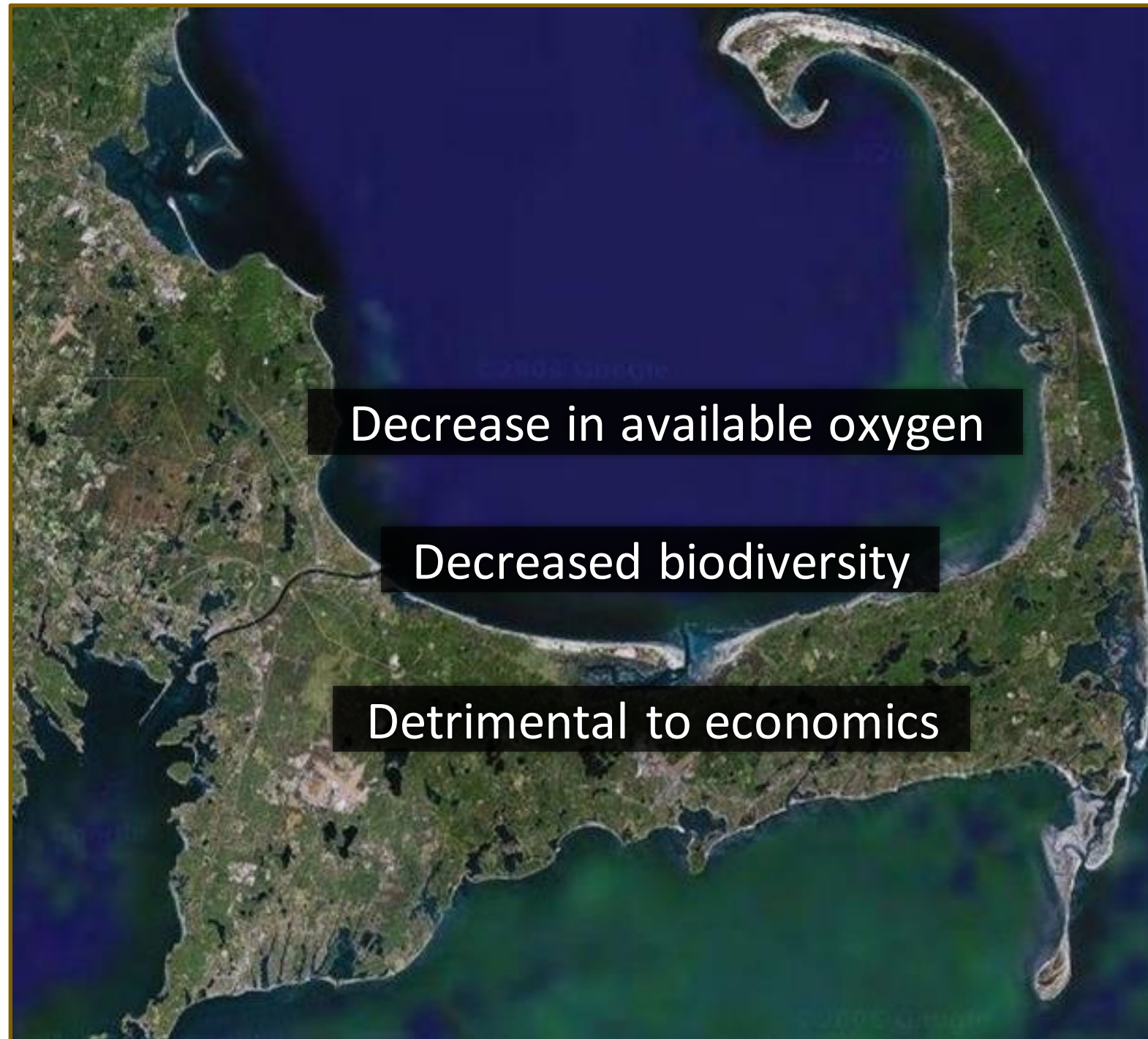
- One of six elements required for all life (CHONPS).
- It's abundant. Nitrogen is everywhere!
- However, the most abundant form, N_2 gas is largely unavailable to life.
- Humans have gone to great lengths to produce more (anthropogenic) biologically available nitrogen.

Nitrogen Ecology

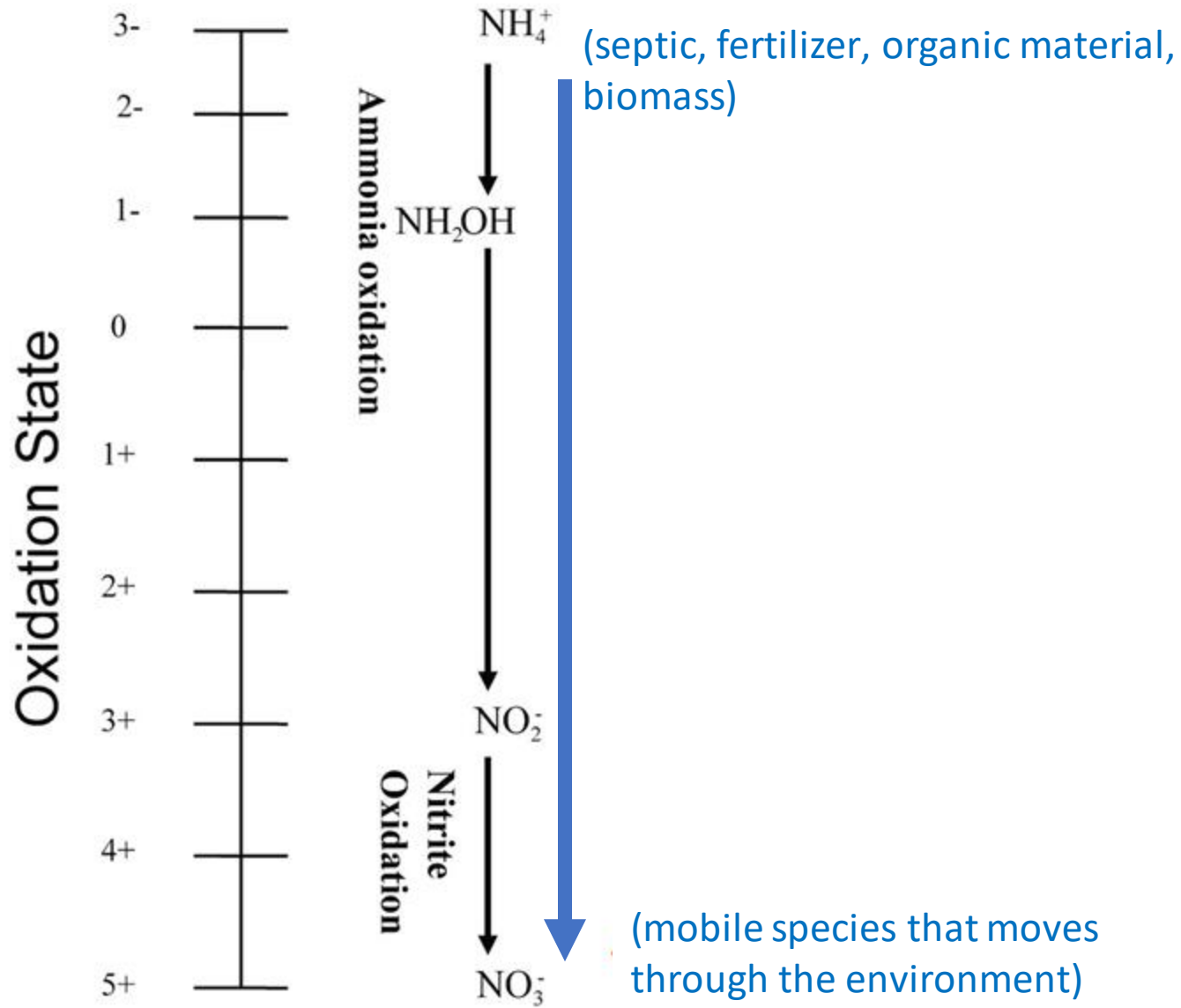
- Nitrogen is produced through human activity
- Nitrogen acts as a fertilizer to drive primary production – more overall biomass



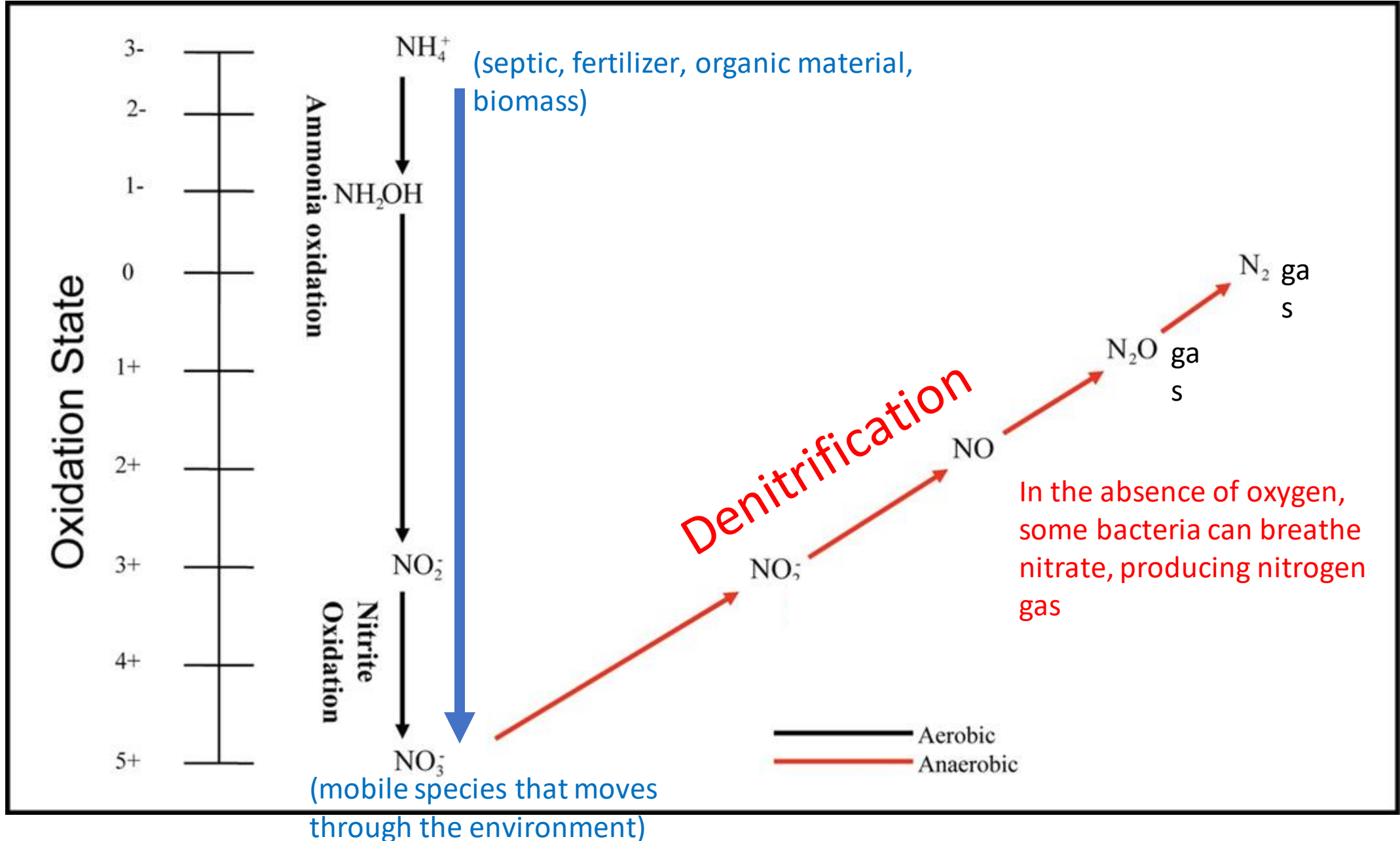
Too much nitrogen
is bad for the
health of
the coastal
ecosystem



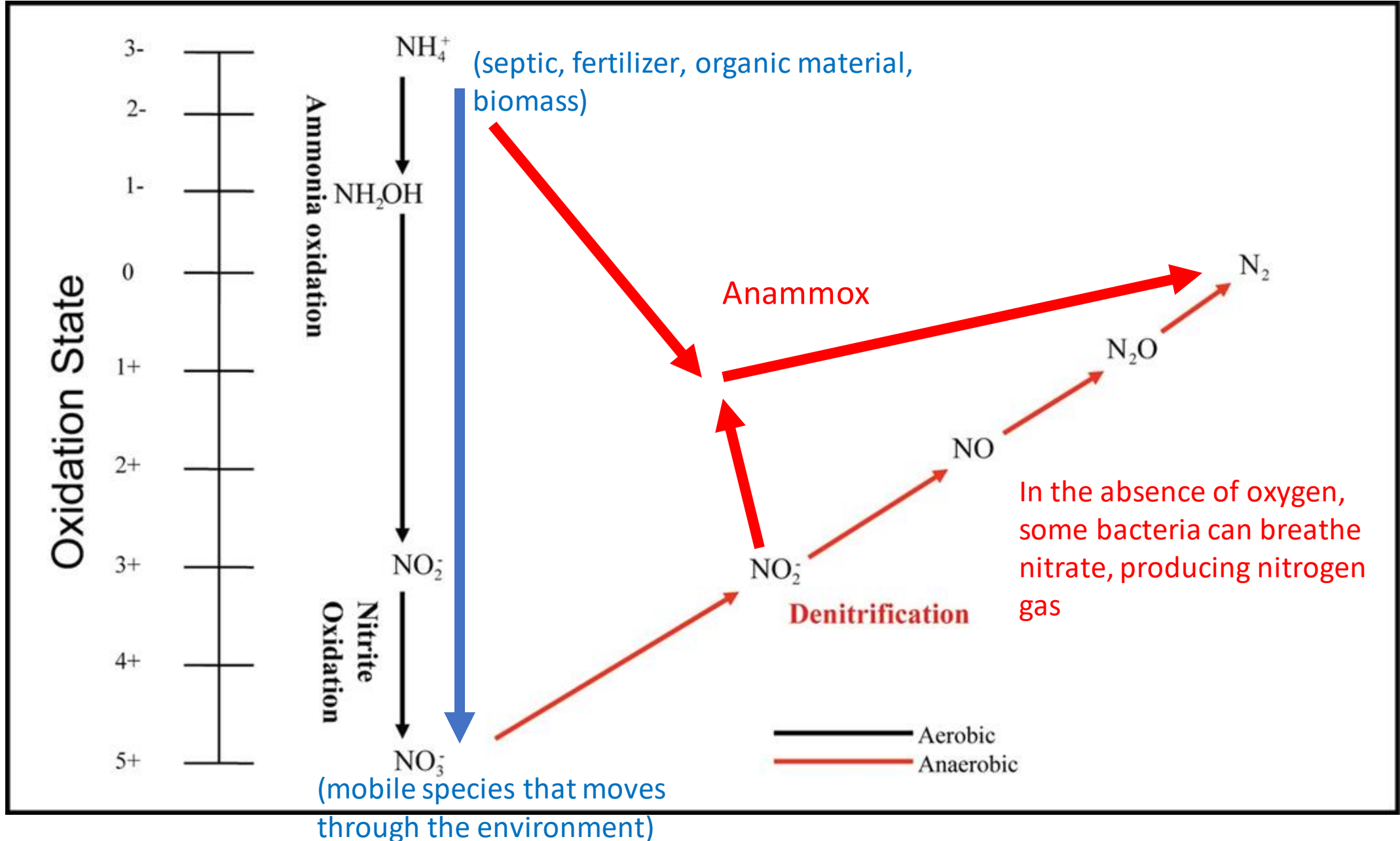
Nitrogen transformations in the environment



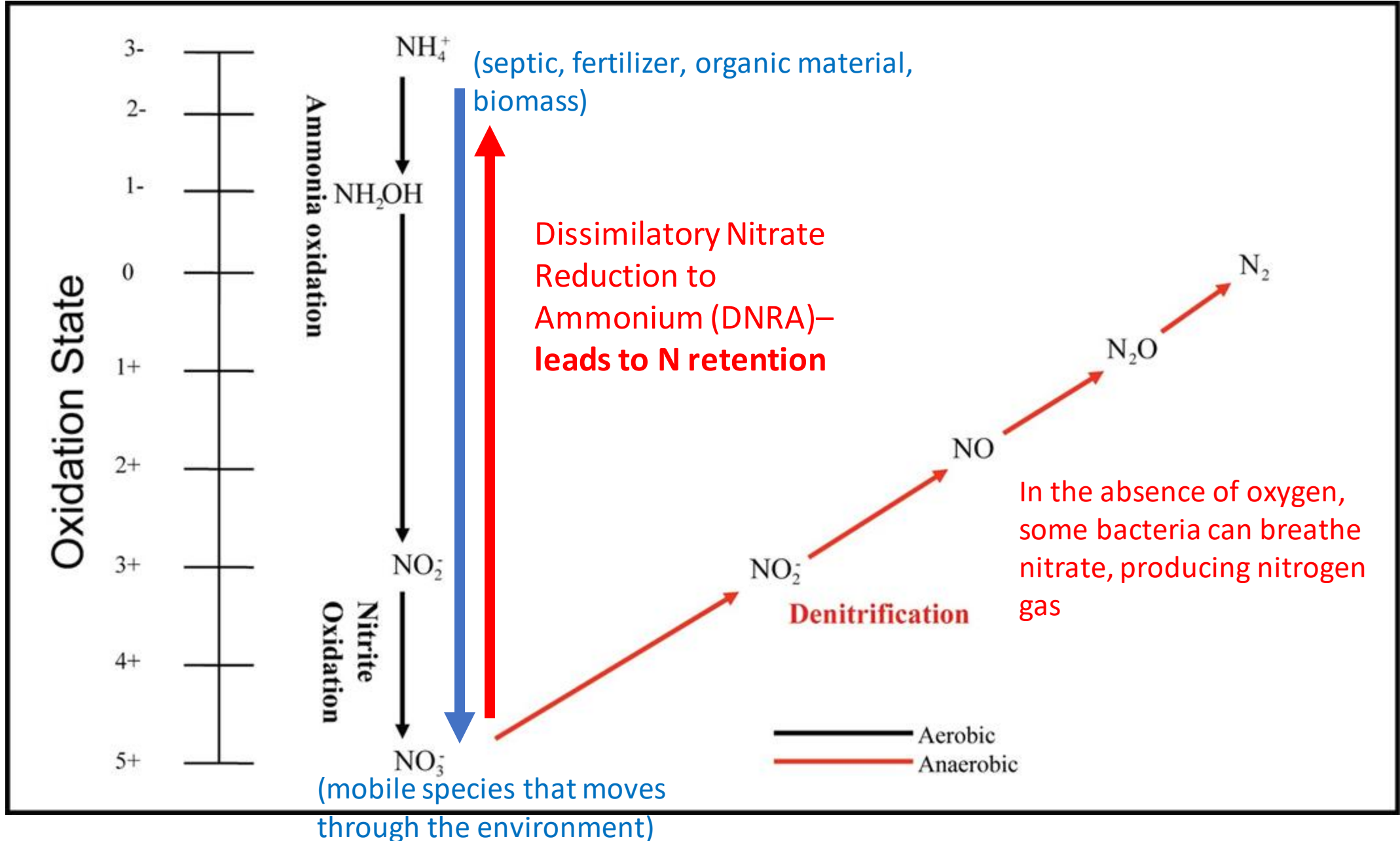
Nitrogen transformations in the environment



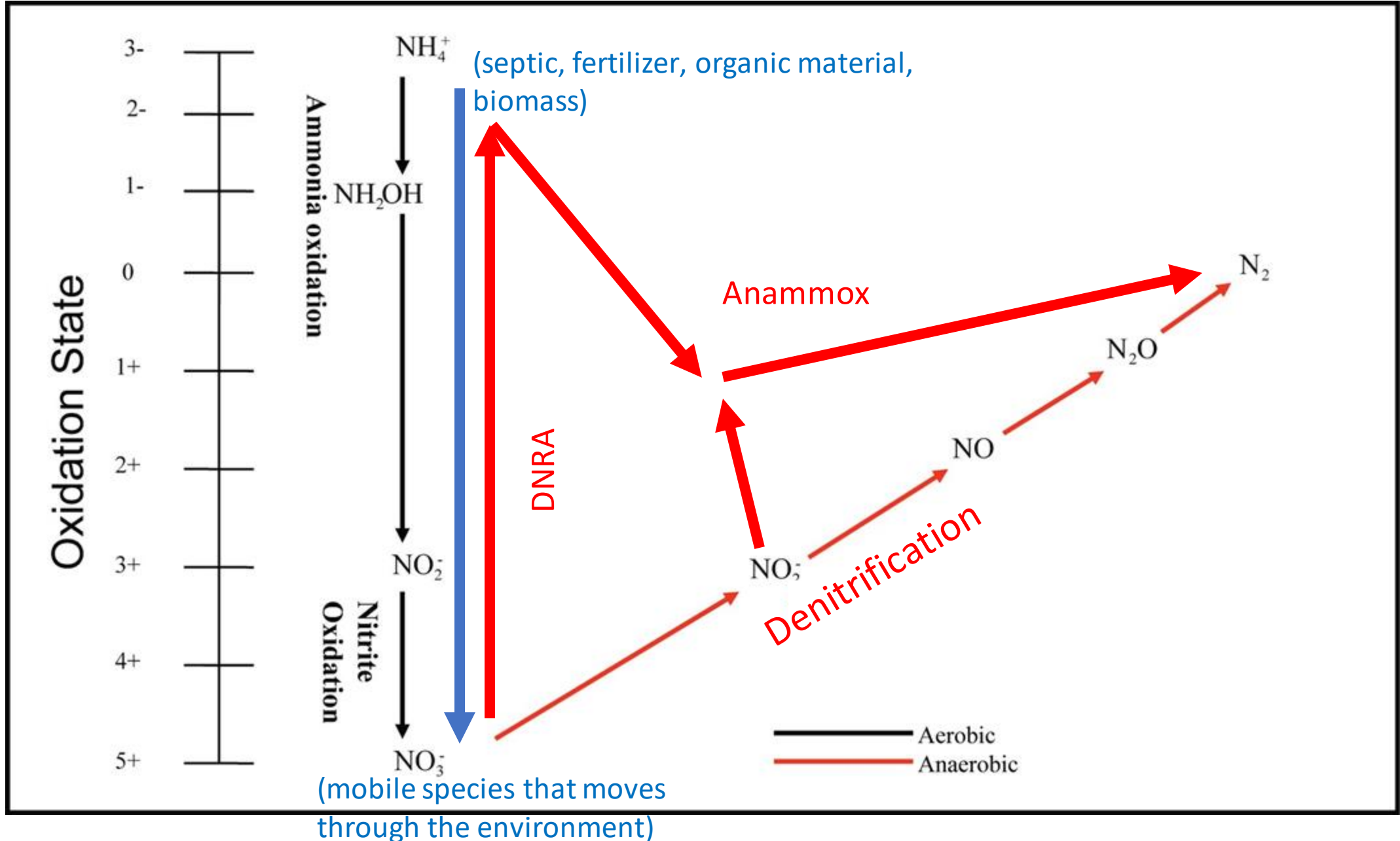
Nitrogen transformations in the environment



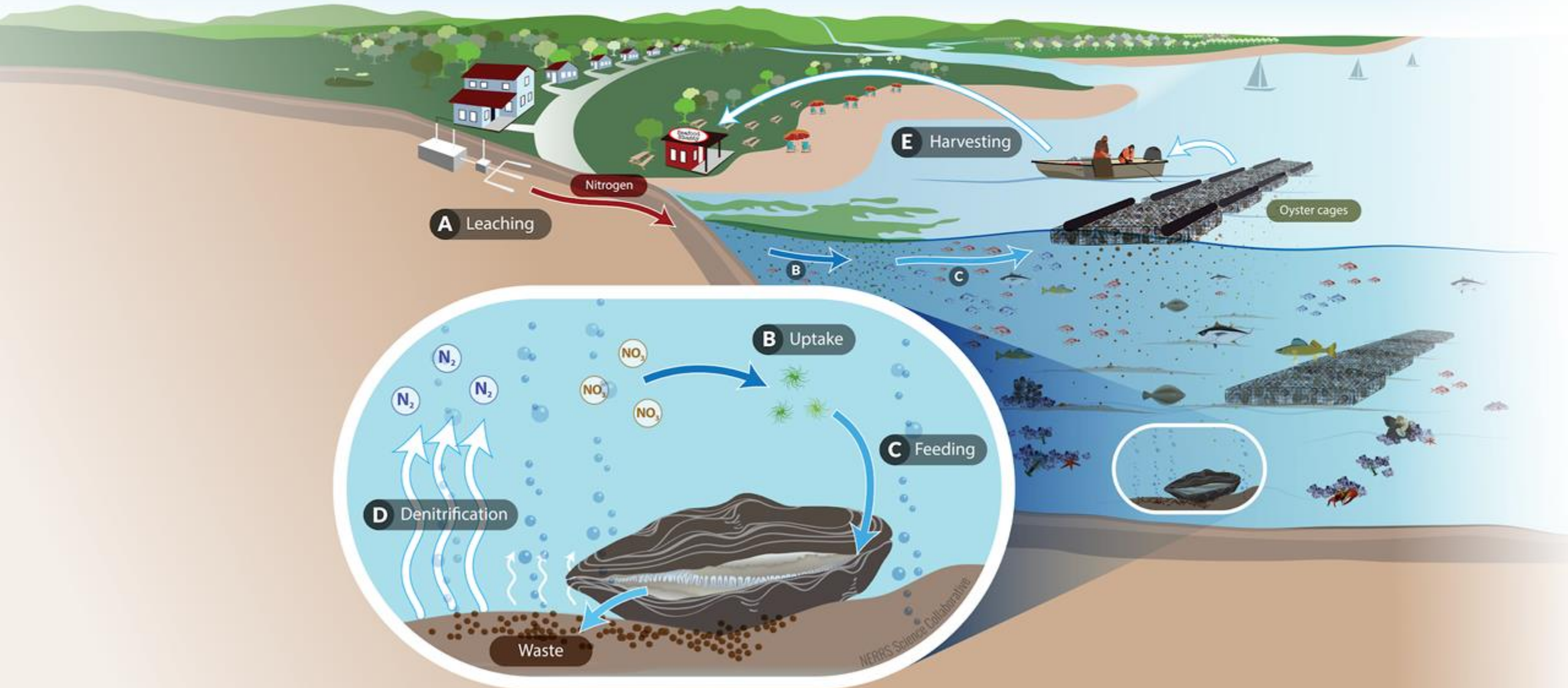
Nitrogen transformations in the environment



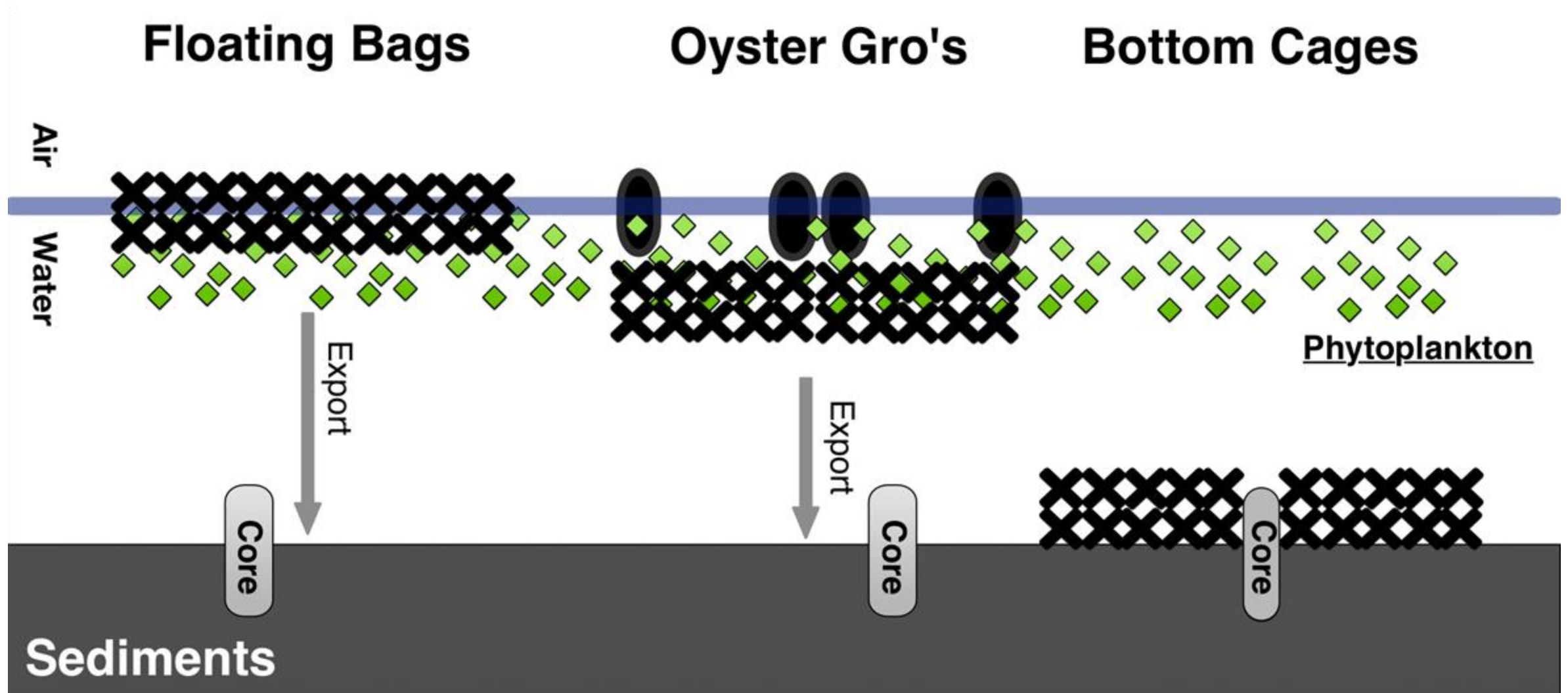
Nitrogen transformations in the environment



How oyster aquaculture may impact the N cycle



Project Overview:



The Big Questions

- Are there differences in oyster growth between gear types?
- Does aquaculture activity change N_2 release (flux) from the sediments?
 - Is the amount of N_2 released enough to be included in management planning?
- Are the microbial communities in the underlying sediments changed?
- Are the activities of these communities changed in the presence of aquaculture?

Set-up, Growth and Management of the Aquaculture Systems

Chuck Martinsen and Christina Lovely,

Town of Falmouth, Department of Marine and Environmental Services.



Town of Falmouth
Marine and Environmental Services

BEST SHELLFISH PRACTICES



METHOD CONSIDERATIONS

It is important to consider certain geographical aspects of the growing area:

- Site access
- Water Depth
- Substrate/sediment type

METHOD COMMONALITIES

1. Bags are sleeves with a closed (sealed) end and an open end
2. Oysters are contained in bags within a larger system
3. Access to oysters within bags via removal of a PVC sliding fastener
4. As oysters grow, smaller mesh bags may need to be swapped for larger mesh bags
5. Systems can be used as primary grow-out of first-year oyster seed as well as secondary grow-out of second-year oyster seed

METHOD DIFFERENCES

1. Position in the water column the oysters are located
2. Vulnerability to damage from storms
3. Costs of equipment and labor to construct systems
4. Oyster growth



FLOATING BAG SYSTEM



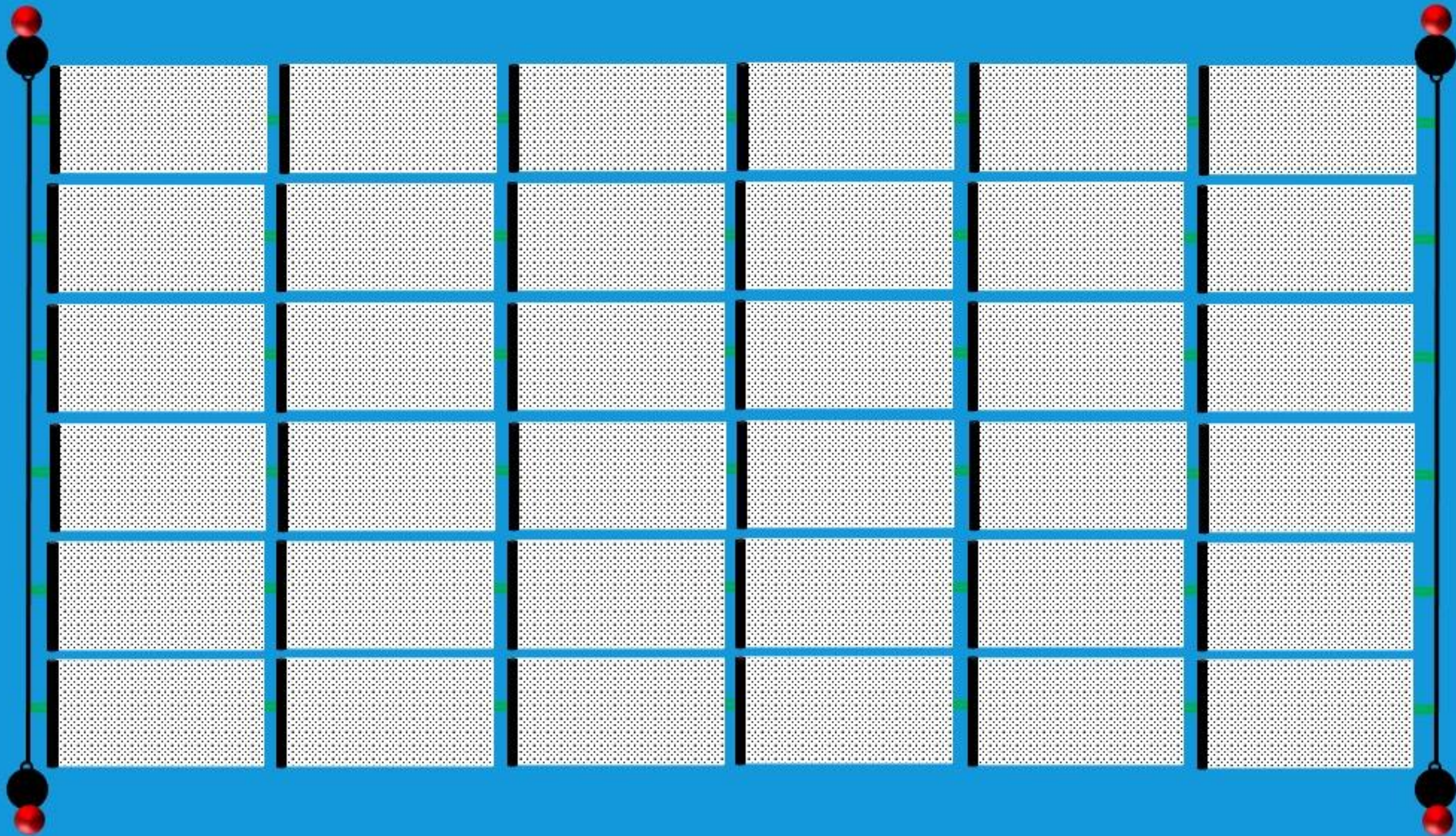
What the system looks like:

- Bags each have one end with a clip and the opposite end with a loop
- Bags are attached end to end, with the clip from one bag attaching to the loop end of the next bag to form a string of bags
- Strings of bags are attached into buoyed main-lines at each end
- Main-lines are buoyed with floatation that rises and falls with the tide

FLOATING BAG SYSTEM

Installation

- Maximum water depth is not a factor in site selection
 - System can be installed in both shallow-water and deep-water sites
- Bottom substrate is not a primary factor in site selection
 - It is a secondary factor needed for anchoring the main-line system that bags clip into
- System should be positioned with direction of prevailing winds considered to limit tension on the system
 - System is vulnerable to damage from storms (e.g. sustained high winds)



FLOATING BAG SYSTEM

Maintenance

- Depending on site water-depth, maintenance on a low tide may be helpful
- String of bags is flipped over such that each previously submerged side of each bag is out of the water and vice versa
- Bags are flipped bi-weekly at minimum
- Maintenance is less labor-intensive and less time-consuming than on other gear systems

FLOATING BAG SYSTEM



Costs

- Total gear costs for 90-bag floating array
 - \$2,078.85 (cost in 2018)
- Cost relative to other growing methods used:
 - Most inexpensive system
 - Labor is required to build the system components into desired forms
 - Relatively inexpensive system, but cannot purchase gear “ready-made”

FLOATING BAG SYSTEM

Growth Summary

- First-year oysters experienced an intermediate increase in shell-height compared to other systems (2018)
- First-year oysters experienced a similar increase in shell-height compared to other systems (2019)
- First-year oysters experienced a similar increase in mass compared to bottom system (2018 and 2019)
- Second-year oysters experienced the greatest increase in shell-height compared other systems (both 2018 and 2019)
- Second-year oysters experienced the greatest increase in mass compared to other systems (2018)
- Second-year oysters experienced an intermediate increase in mass compared to other systems (2019)

MAIN TAKEAWAY:

The floating system yields largest/fastest-growing second-year oysters and intermediate size first-year oysters compared to other systems

OYSTER-GRO (MIDWATER CONDOS)

What the system looks like

- Wire cage structure, arranged with 3 separate compartments for bags horizontally, and 2 separate compartments for bags vertically (3x2 compartment layout)
- Bag compartments are accessed by opening a hinged door, secured with elastic and clip fastener
- Floatation fixed to the top of the cage, keeps cage submerged below the surface of the water



OYSTER-GRO (MIDWATER CONDOS)

Installation

- Maximum water depth is not a factor in site selection
- Minimum water depth is a factor in site selection
 - Need enough water depth to keep cages above substrate on a low tide
- Bottom substrate is not a primary factor in site selection
 - Would only be a factor for some anchoring systems
- System should be positioned with direction of prevailing winds considered to limit tension on the system
 - System is vulnerable to damage from storms (e.g. sustained high winds)
- A series of condos can be anchored with a floating main-line system, or condos can be anchored individually

OYSTER-GRO (MIDWATER CONDOS)

Maintenance

- Condo is flipped, so that floatation is on the surface of the water and cage compartments are out of the water
- Bags are removed from cage compartments, scrubbed of fouling agents/debris, flipped over, replaced within cage compartments
- Condo is flipped back into position with floatation on the surface and cage compartments submerged
- Depending on site water-depth, maintenance on a low tide may be helpful
- Bags are flipped bi-weekly at minimum, more frequent maintenance may be needed if fouling is consistently heavy
- Maintenance can be labor-intensive and time-consuming

OYSTER-GRO (MIDWATER CONDOS)

Costs

- Total gear cost for 15-cage/90-bag mid-water array
 - \$3,559.50 (cost in 2018)
- Cost relative to other growing methods used
 - Most expensive system
 - Little to no labor costs needed to make the system ready for deployment (can purchase gear "ready-made")
 - Anchoring system is the additional labor cost required, cost varies on type of anchoring system used



OYSTER-GRO (MIDWATER CONDOS)

Growth summary

- First-year oysters experienced the greatest increase in shell-height compared to other systems (2018)
- First-year oysters experienced a similar increase in shell-height compared to other systems (2019)
- First-year oysters experienced the greatest increase in mass compared to other systems (2018 and 2019)
- Second-year oysters experienced an intermediate increase in shell-height compared to other systems (both 2018 and 2019)
- Second-year oysters experienced an intermediate increase in mass compared to other systems (2018)
- Second-year oysters experienced the greatest increase in mass compared to other systems (2019)

MAIN TAKEAWAY:

The midwater system yields largest/fastest-growing first-year oysters and intermediate size second-year oysters compared to other systems

BOTTOM CONDOS

What the system looks like

- Wire cage structure, arranged with 3 separate compartments for bags horizontally, and 2 separate compartments for bags vertically (3x2 compartment layout)
- Bag compartments are accessed by opening a hinged door, secured with elastic and clip fastener
- “Feet”/“legs” attached to the bottom of the cage, keeps cage positioned just above bottom sediment



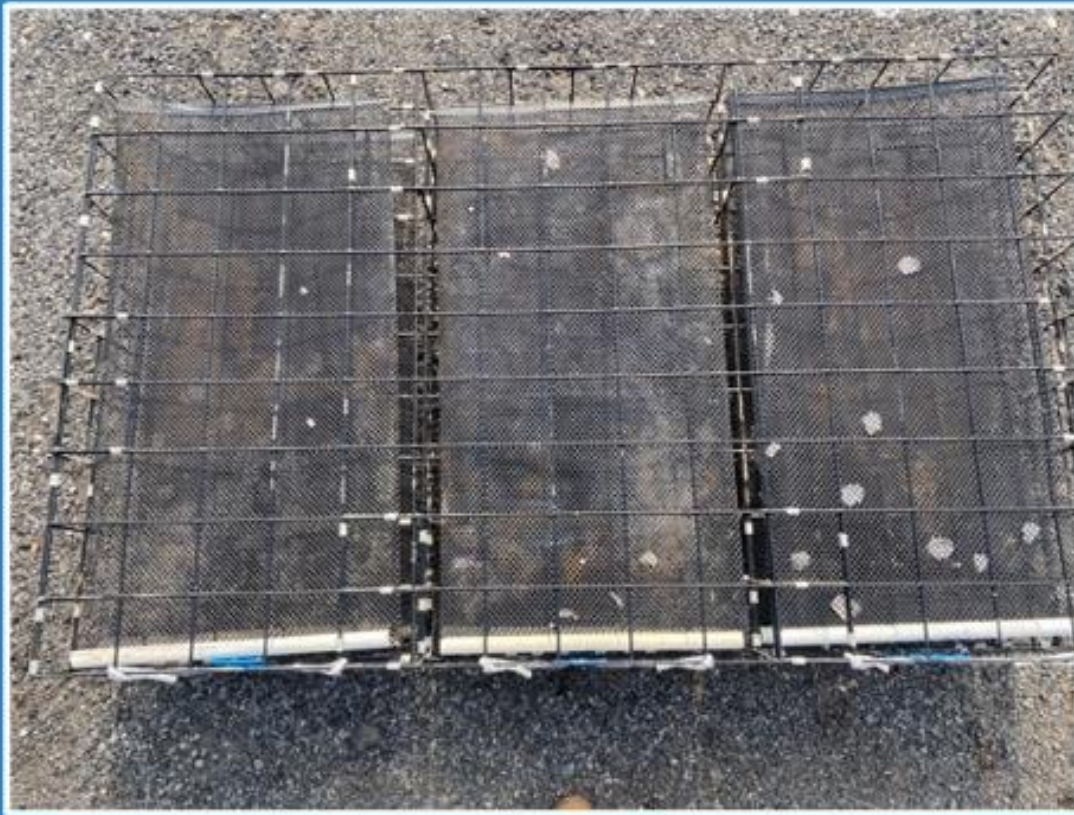
BOTTOM CONDOS

Installation

- Firm substrate needed for cage stabilization
- System is resilient to damage from storms



BOTTOM CONDOS



Maintenance

- Bags are removed from condo compartments, scrubbed of fouling agents/debris, flipped over, replaced within condo compartments
- Depending on site water-depth, maintenance on a low tide may be essential
- Bags are flipped bi-weekly at minimum
- Maintenance can be labor-intensive and time-consuming

BOTTOM CONDOS

Costs

- Total gear cost for 15-cage/90-bag bottom array
 - \$2,583.75 (cost in 2018)
- Cost relative to other growing methods used
 - Intermediate expensive system (more expensive than floating bag system, less expensive than midwater system)
 - No additional labor costs needed to make the system ready for deployment (can purchase gear “ready-made”)

BOTTOM CONDOS

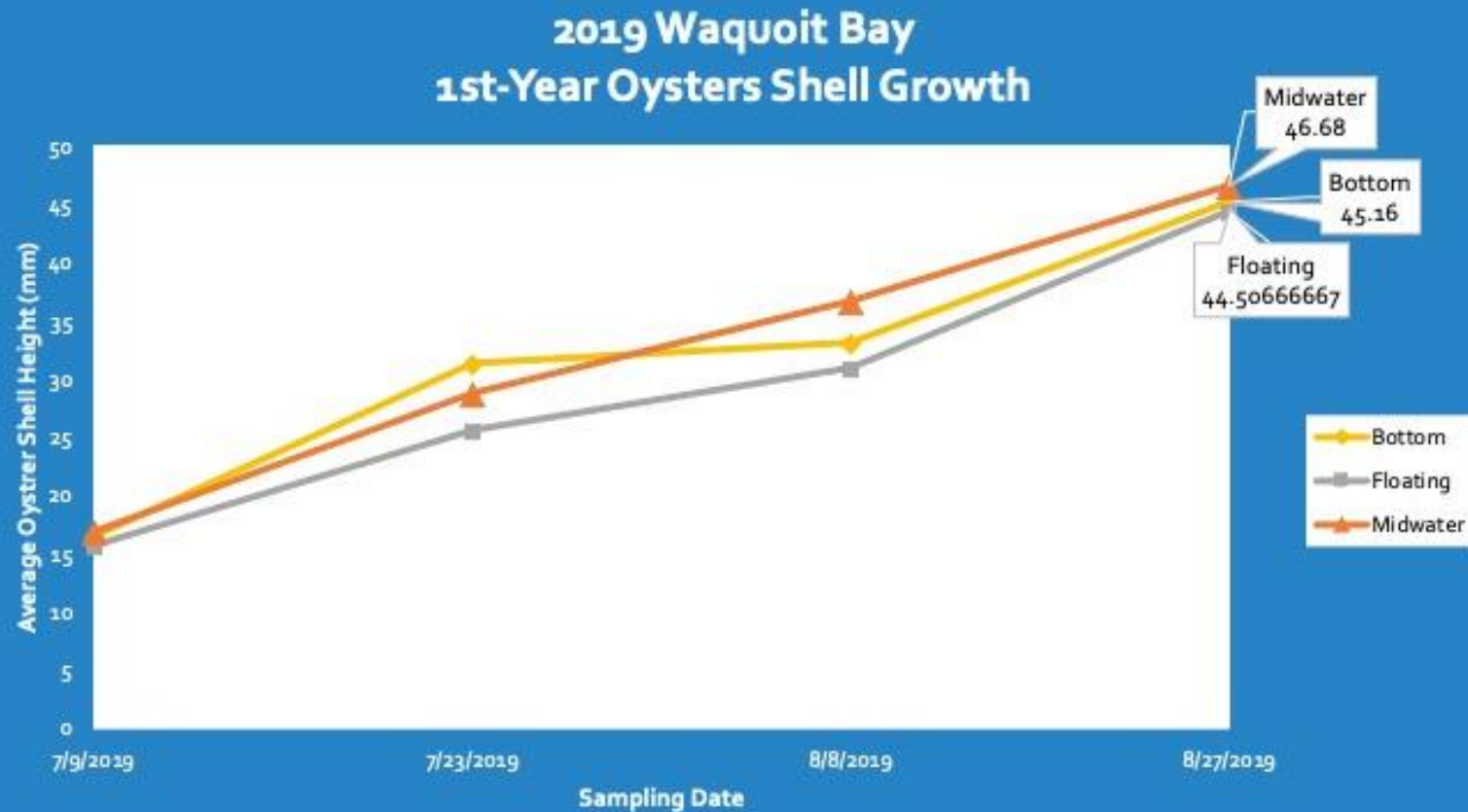
Growth summary

- First-year oysters experienced the smallest increase in shell-height compared to other systems (2018)
- First-year oysters experienced a similar increase in shell-height compared to other systems (2019)
- First-year oysters experienced a similar increase in mass compared to floating system (both 2018 and 2019)
- Second-year oysters experienced the smallest increase in both shell-height and mass compared to other systems (both 2018 and 2019)

MAIN TAKEAWAY:

The bottom condo system yields oysters of smaller or at best similar shell-height and mass compared to other systems.

OYSTER GROWTH DATA



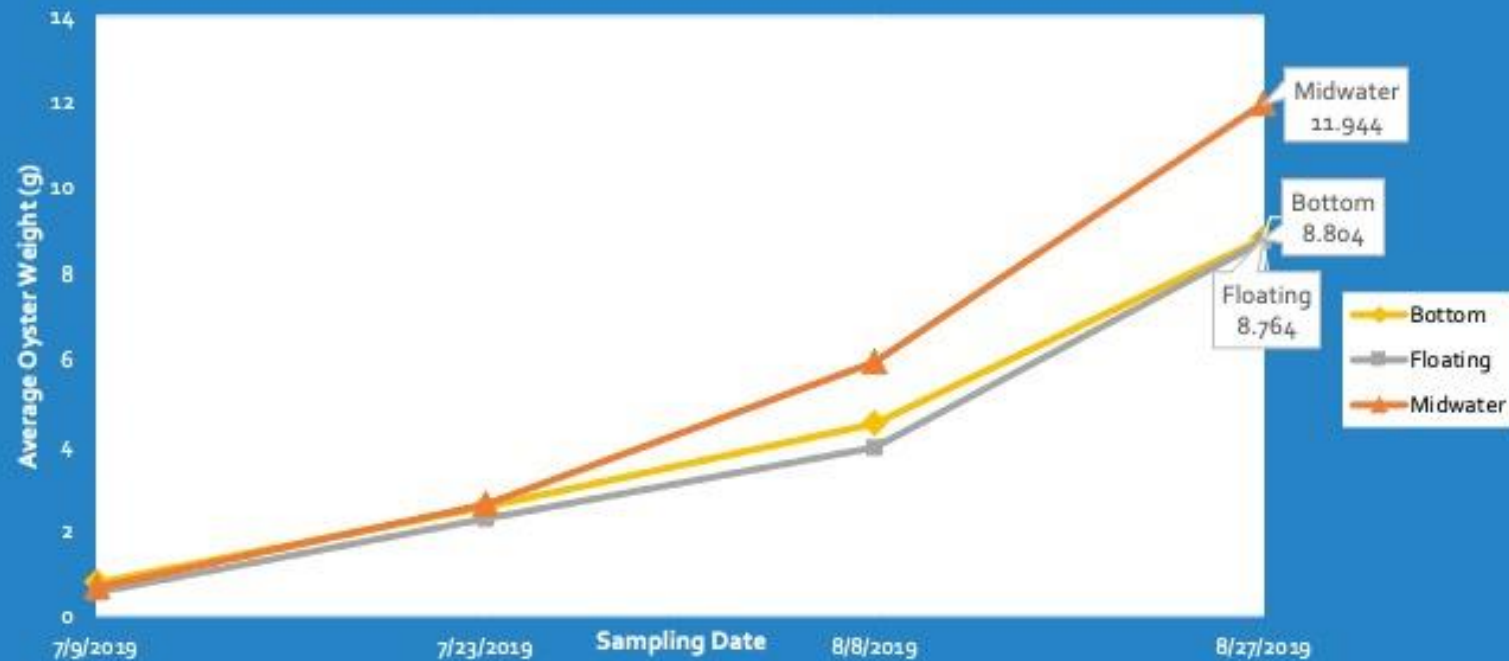
OYSTER GROWTH DATA

2019 Waquoit Bay
2nd-Year Oysters Shell Growth



OYSTER GROWTH DATA

2019 Waquoit Bay
1st-Year Oysters Weight



OYSTER GROWTH DATA

2019 Waquoit Bay
2nd-Year Oysters Weight





QUESTIONS?

Science Methods and Results

Daniel Rogers, Vivian Mara and Ginny Edgcomb



STONEHILL COLLEGE



The Big Questions

- ~~• Are there differences in oyster growth between gear types?~~
 - Thanks Chuck
- Does aquaculture activity change N_2 release (flux) from the sediments?
 - Is the amount of N_2 released enough to be included in management planning?
- Are the microbial communities in the underlying sediments changed?
- Are the activities of these communities changed in the presence of aquaculture?

Evaluating N removal and oyster aquaculture

- A story in three parts
 1. N_2 release (flux) from the sediments
 2. Characterizing the microbial community and activity
 3. Lessons learned, take home or extrapolating to future studies.



Δ
N

50 m

Waquoit Bay National Estuarine
Research Reserve

Control

Oyster
Gro'

Floating
Bags

Bottom
Cages



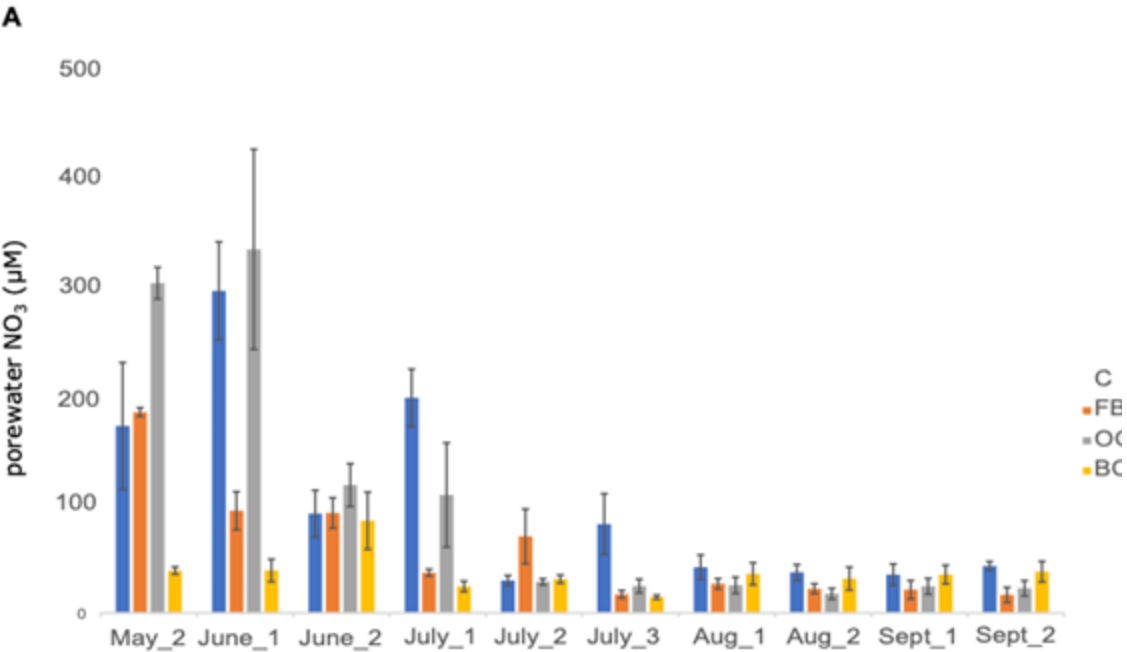
Waquoit Bay
Falmouth, MA



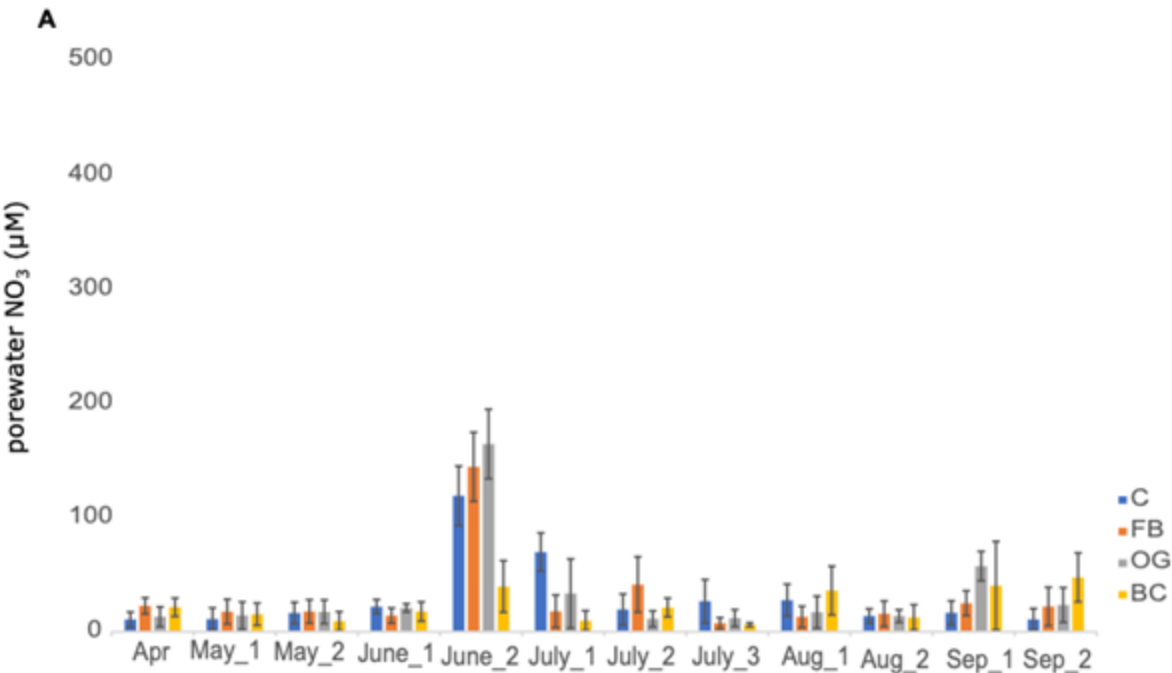
Porewater Data- Nitrate

- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

2018



2019

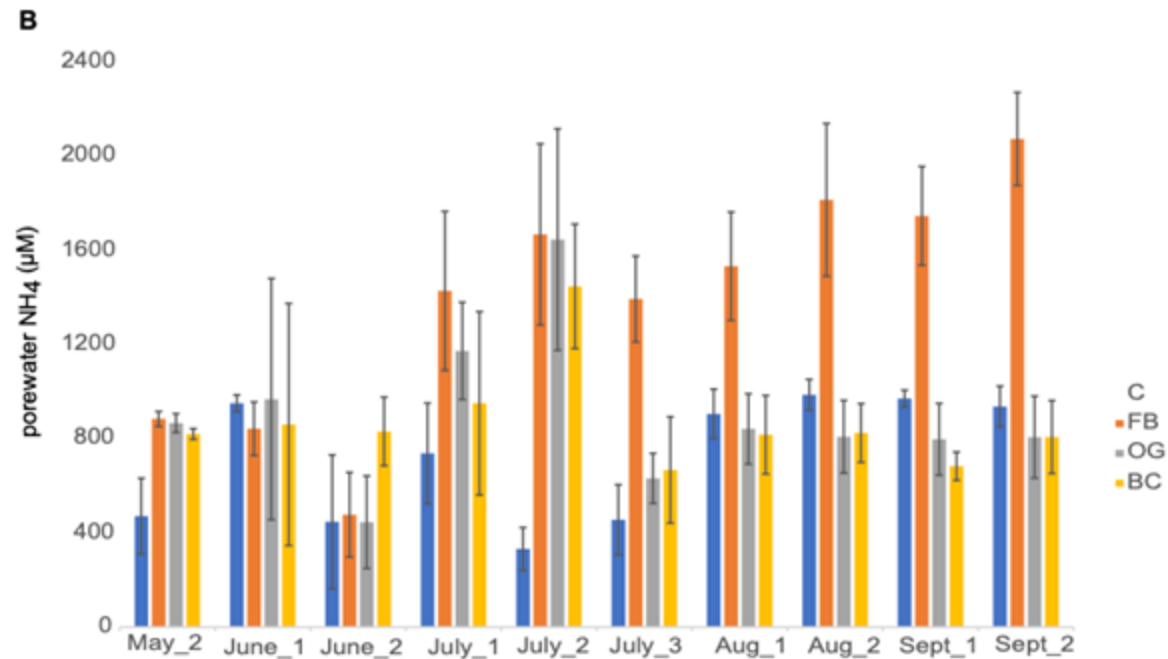


No significant difference between Control and treatment sites.

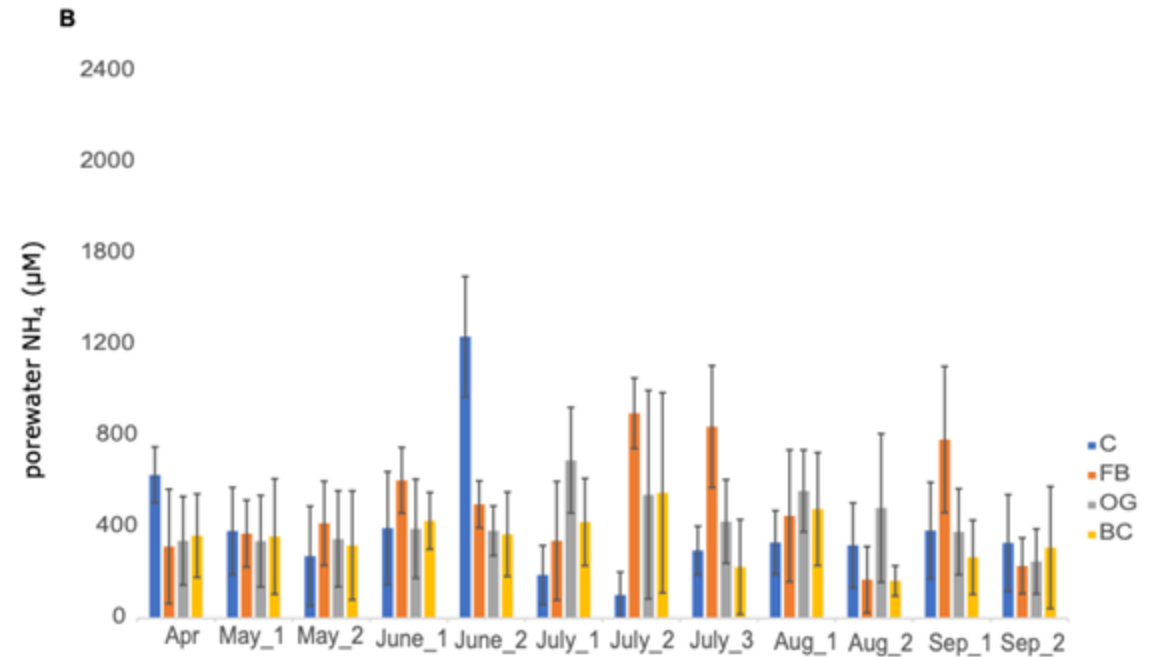
- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

Porewater Data- Ammonium (extractable NH_4^+)

2018

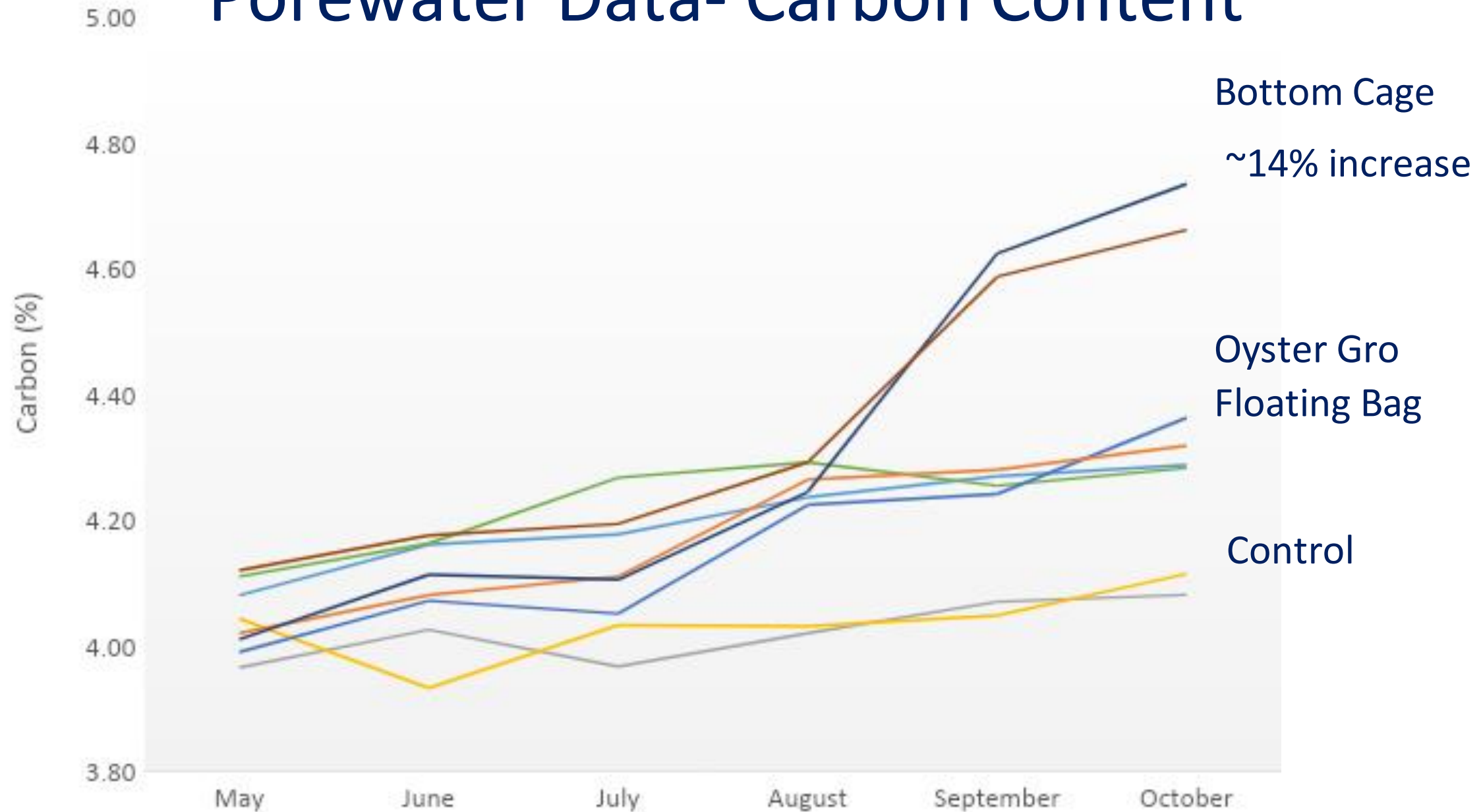


2019



Spikes in ammonium especially after July

Porewater Data- Carbon Content



Measuring N₂ release

- ▶ Flux core incubations
 - ▶ Monitor consumption of O₂
 - ▶ Monitor production of N₂ by MIMS
 - ▶ Yields rates/m²

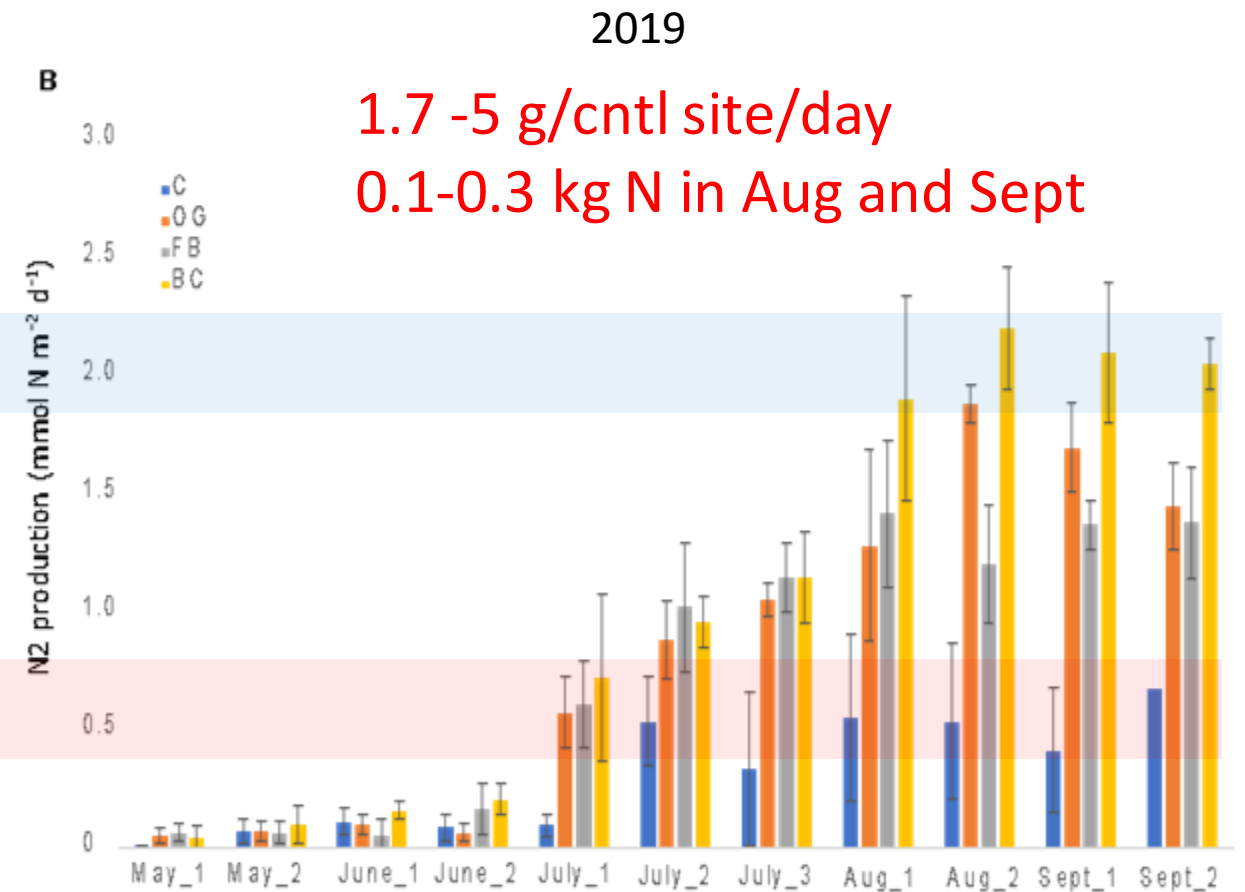
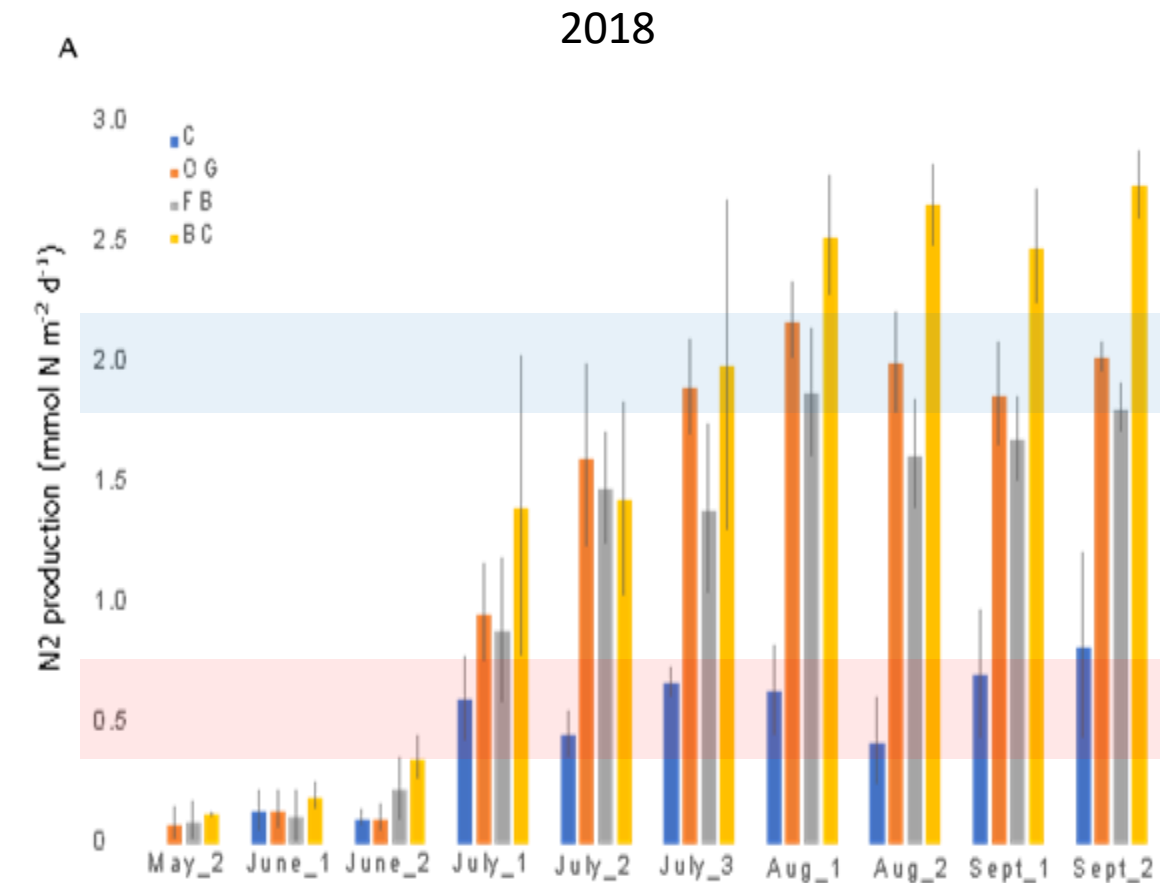


Figure 2. Membrane Inlet Mass Spectrometer designed and built by the Rogers' Lab. This instrument can measure gases with a ± 0.1 mbar resolution. This instrument is used for measuring the flux of O₂ and N₂ from a sample of MIMS.

- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

N₂ release Data

14 -17 g/site/day
0.8-1 kg in N Aug and Sept
at each farm



Farm size ~250 m²

Increase in net N₂ production from late July/August through September

Molecular Methods

iTAG (which microbes are present at the time of sampling)

- Collect the total DNA pool
- Describes community structure
- Moderate expense

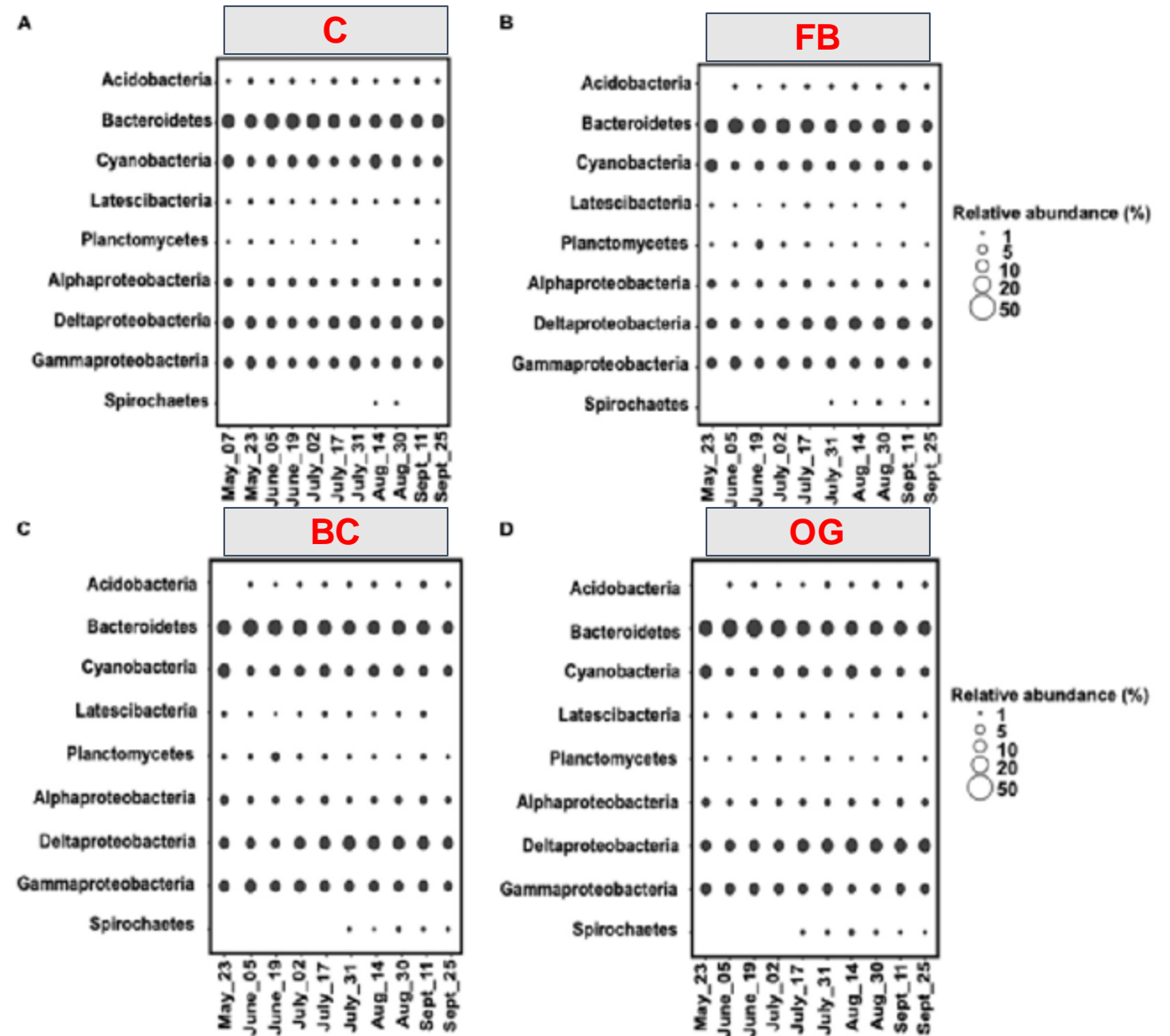
Metatranscriptomes (which genes are expressed at the time of sampling)

- Collect the total RNA pool
- Proxy for microbial activity
- Expensive and technically challenging

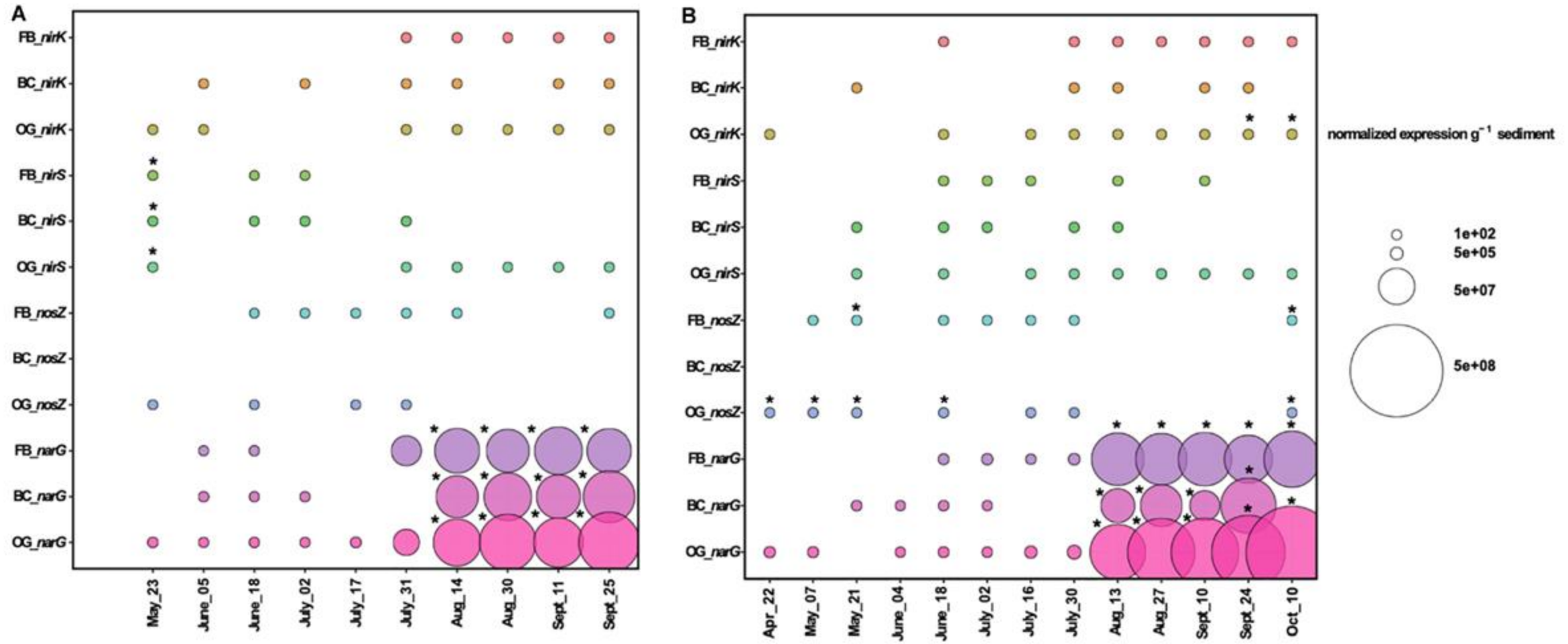
RT-qPCR (targets specific genes of interest)

- Uses the total RNA pool
- Quantifies specific target genes.
- Quick, inexpensive if protocol developed

- Similar patterns in all treatments
- Similar patterns across growing season



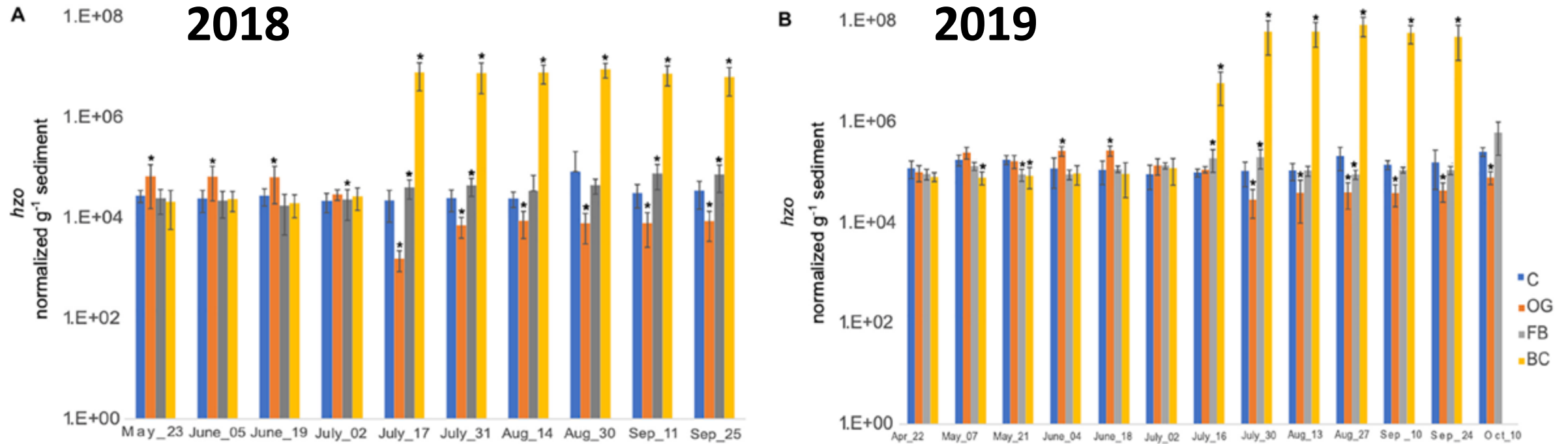
Nitrogen cycling: Denitrification (N₂ release)



Detection of higher expression of denitrification genes (*nirK*, *nirS*, *nosZ* and *narG*) under the treatments when compared to the control

- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

Nitrogen cycling: anammox (N_2 release)

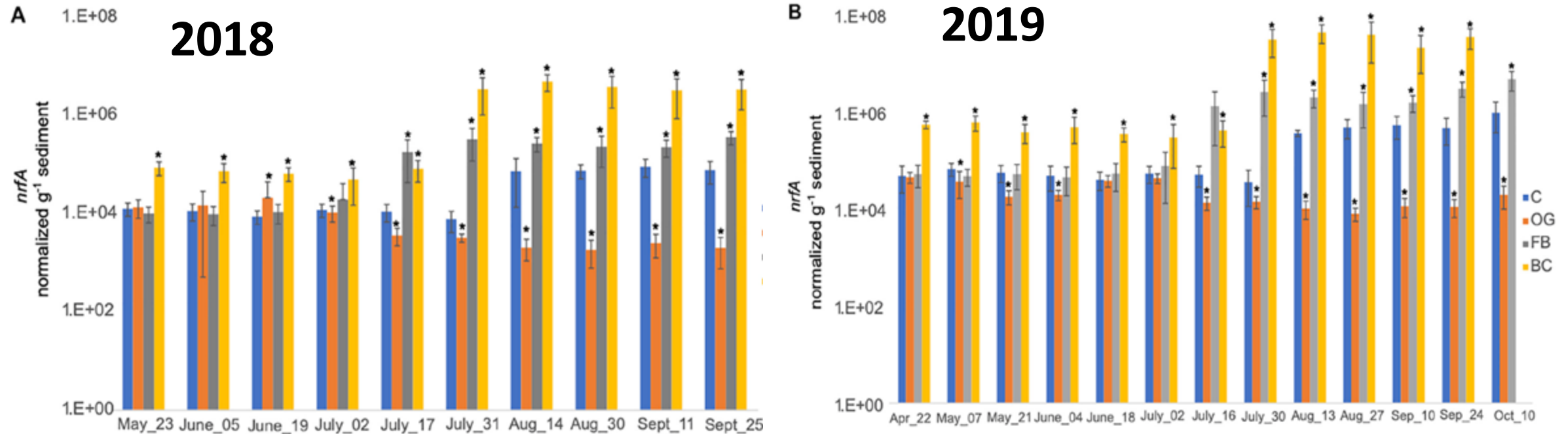


***hzo* gene: marker for anammox**

- High *hzo* expression in the Bottom Cages compared to the control
- Similar *hzo* expression between Floating Bags and the control
- Decreased expression (below the control levels) in the Oyster Gro' treatments

- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

Nitrogen cycling: DNRA (ammonium retention)



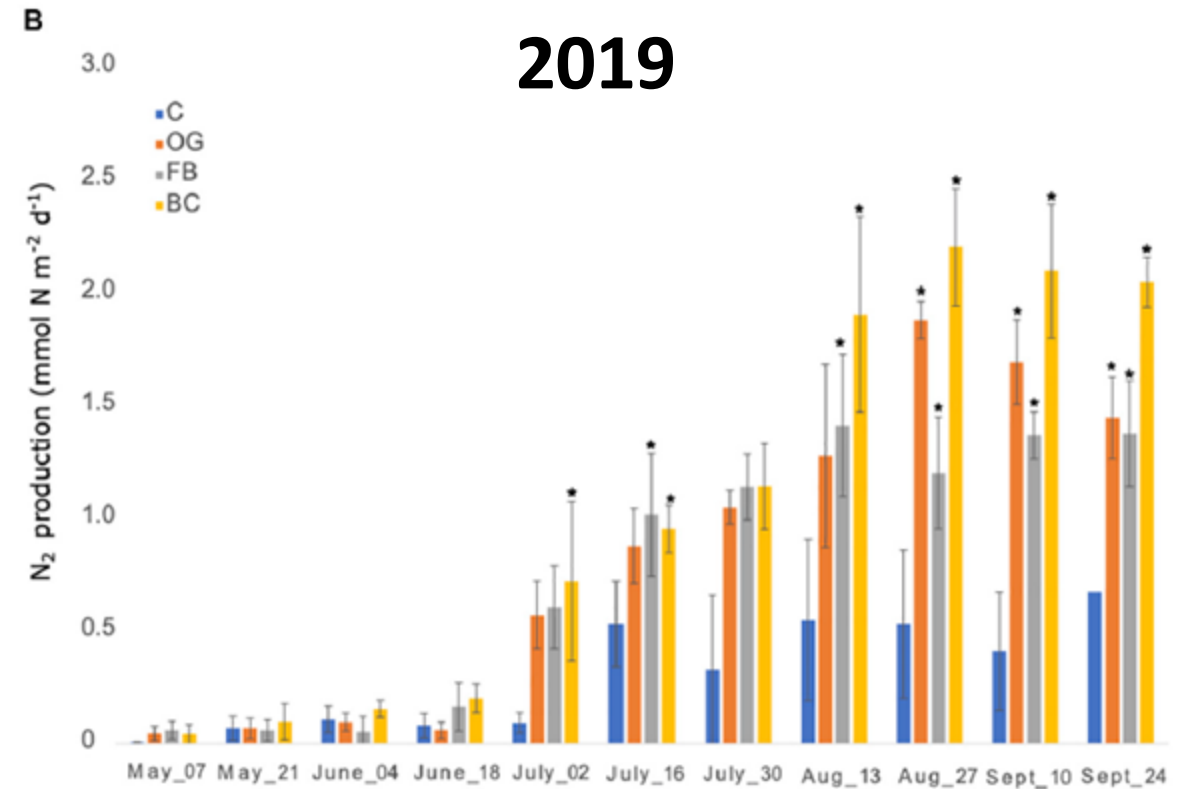
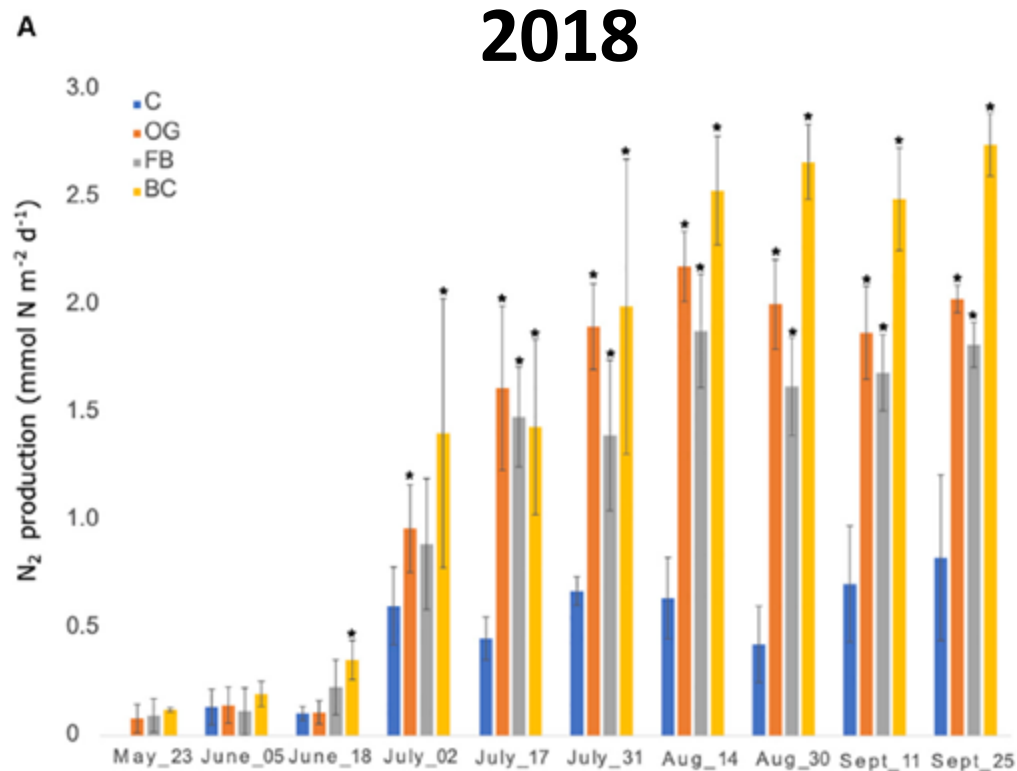
nrfA gene: marker for DNRA

- High expression *nrfA* under the Bottom Cages when compared with the control
 - Similar *nrfA* expression between Floating Bags and the control
- Decreased expression (below the control levels) in the Oyster Gro' treatment

- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

Circling back to our big questions

- Does aquaculture activity change N_2 release from the sediments? **YES**



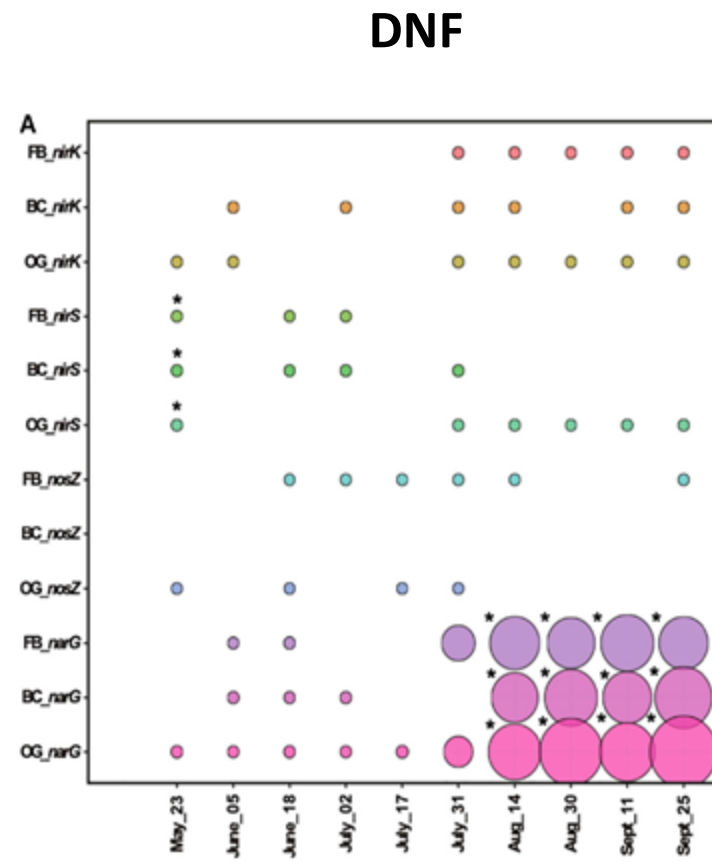
Circling back to our big questions

- How are the underlying sediments altered microbiologically or chemically?
- one of our original aims was to identify a quick gene marker or a “dipstick” = gene whose expression is correlated with N_2 release and would tell users how much N_2 was being released at their site

Circling back to our big questions

- How are the underlying sediments altered microbiologically or chemically?
- one of our original aims was to identify a quick gene marker or a “dipstick” = gene whose expression is correlated with N_2 release and would tell users how much N_2 was being released at their site
- it turned out it wasn't so easy because DNF is a complex process sometimes carried out cooperatively by different microbial groups
- affected by many environmental factors, and competes with other N cycling processes for nitrate

- How are the underlying sediments altered microbiologically or chemically?

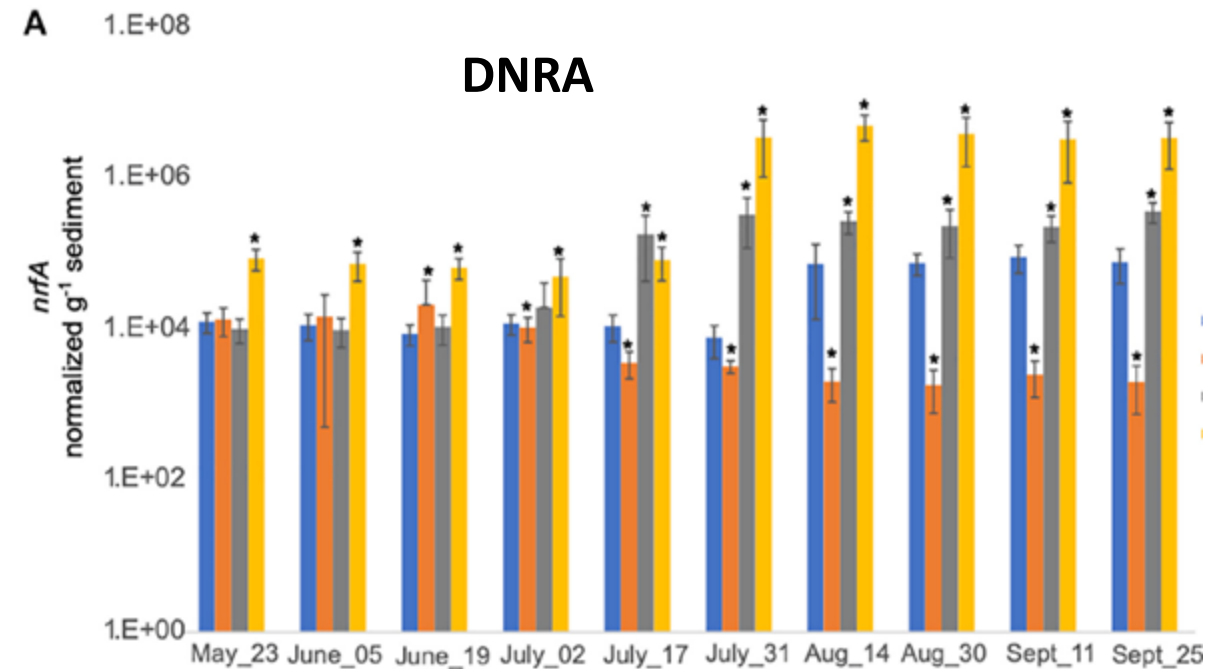
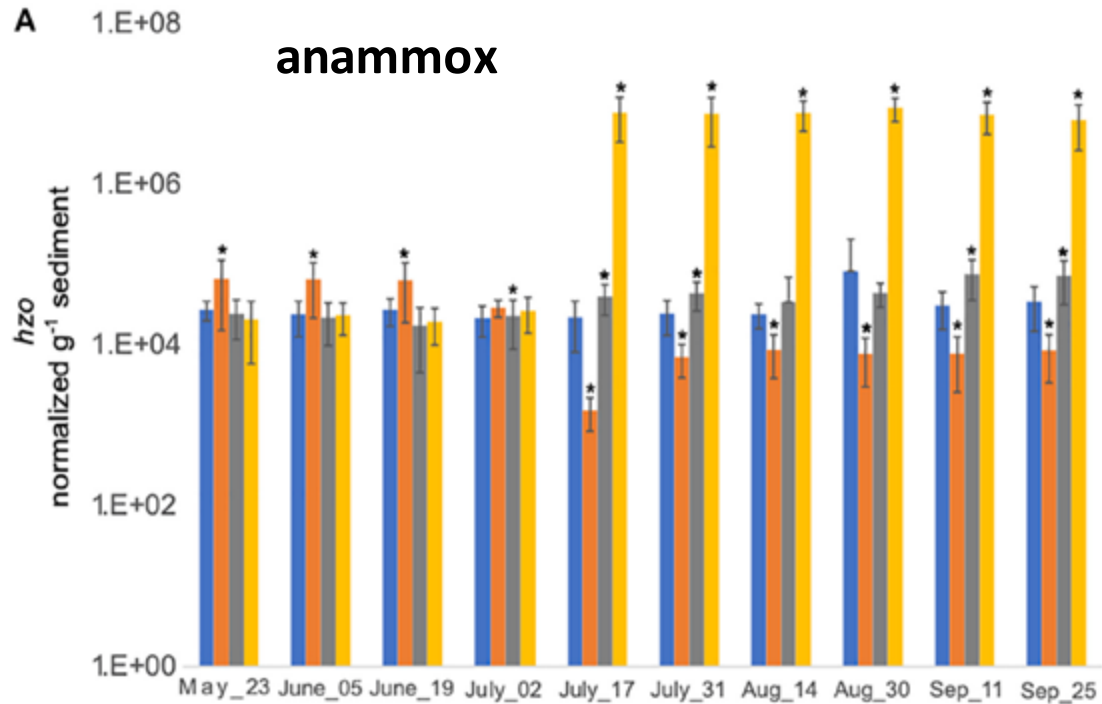


Expression of genes associated with denitrification stimulated!

- Control
- Oyster Gro'
- Floating Bag
- Bottom Cage

Circling back to our big questions

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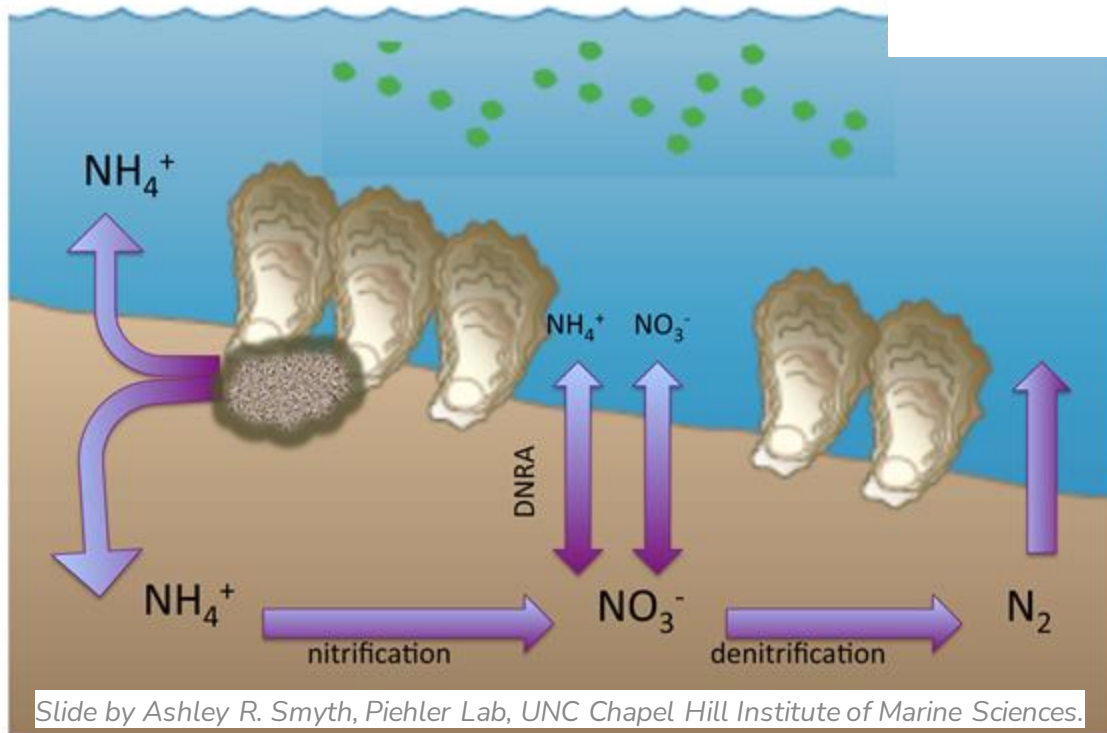
Anammox contributes to N₂ but much less so than denitrification. It is stimulated under BC because of the organic matter accumulation

DNRA is sensitive to O₂, so it is repressed under the OG gear which aerates the surface sediments ("piston pump" activity). It is stimulated under the BC relative to the control where sediments are more sulfidic

Circling back to our big questions

- Is there enough N_2 generated to be included in the N management planning?

OYSTER AND THE NITROGEN CYCLE



microbial N_2 released from under oyster aquaculture is about 10% of the amount of N removed in oyster biomass

Aug-Sept: ~1 kg of N per farm (250m²)

vs.

0.1-0.3 kg of N_2 at the control site

Lessons Learned



Relaying oysters at the end of the season

- Choice of gear will depend on priorities: ease of management, cost, hydrodynamics, wind and wave exposure, and whether N removal is a priority
- If N removal is a priority BC give most benefit but NOT if conditions go too sulfidic (> 2 ppm)
- If sediments are already organic-rich (approaching 7-8% total organic carbon), FB and OG gear may be better choices for N benefits, and consider site rotation!

Implications of the science for management

- Denitrification dominates but it is possible to push sediments to DNRA if organic matter and sulfide accumulate too much, which is counter-productive
- Hydrodynamic setting, the method and the stocking density can all affect nitrogen cycling
- Site Selection
 - Measure sulfide prior to farm installation
 - Measure organic matter content



Sulfide test kit
www.Lamotte.com
m

Implications of the science for management

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Implications of the science for management

\$200

- Denitrification dominates but it is possible to push sediments to DNRA if organic matter and sulfide accumulate too much, which is counter-productive
- Hydrodynamic setting, the method and the stocking density can all affect nitrogen cycling
- **Site Selection**
 - Measure sulfide prior to farm installation
 - Measure organic matter content (~\$20/sample)



Sulfide test kit
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Argument for site rotation in some areas?

Conclusions

- Increase in sediment N removal in each of the three systems compared to the control
- Bacterial community structure controlled by season and not by aquaculture method
- N removal consistent with upregulation of genes associated with denitrification
- You can push the system toward DNRA and increase retention of N, decreasing your N removal benefits

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