# CBT Oyster Denitrification -Presentation to Fisheries GIT

July 21, 2022

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## **Terms**

- Denitrification: the rate of conversion of N in organic matter, nitrate and ammonium to di-nitrogen gas (N<sub>2</sub>-N)
  - recognizing there are other (minor) processes that also may produce  $N_2$ - $N_3$
- "Lander": a lowered chamber that (mostly) seals up the interior of the device so that changes in water column chemistry reflect benthic processes. Similar in principal to other measures of benthic fluxes.
- Leakage: the exchange of exterior water with interior water due to an incomplete seal. Oyster reef topography guarantees a poor seal. Expressed as a proportion per hour (h<sup>-1</sup>)

# **Published Approaches**

#### **Whole Community**

- Ex-situ approach (Kellogg et al. 2013) Community transferred to trays by divers, recovered a month or so later, sealed up and fluxes measured in lab. Most Chesapeake data from Kellogg and Cornwell.
- In-situ approach with embedded rings (Humphries et al. 2016). Ring permanently embedded in bottom, chamber attached by divers and exchange measured over time.



#### **Community Components**

- Core incubations, often near but not in reefs (Piehler and Smyth 2011). We believe these give minimum rates.
- Oyster-only incubations.
  We discovered a majority of
  denitrification occurred in oyster
  clumps (Jackson et al 2018). Other
  studies show single oysters can
  denitrify.

# **Project Rationale**

#### Why Denitrification?

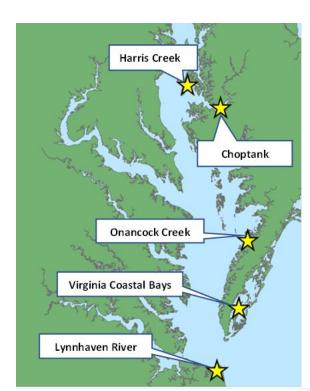
- Net loss to ecosystem of "fixed" nitrogen – of great value in a nutrient-stressed ecosystem
- Oyster-related denitrification rates are the highest rates observed in "nature"

#### Why a new approach

- Faster
- More efficient (manpower, \$)
- Less disturbance to the reef community
- Simpler
- Update BMP calculations

Sample Type	Reef	Location	Max DNF	
	Туре		Enhancement	
			(μmol N <sub>2</sub> -N m <sup>-2</sup> h <sup>-1</sup> )	Source
Reef	Subtidal	Choptank River, MD	1,486	Kellogg et al. (2013)
		Ninigret Pond, RI	~1,100	Humphries et al. (2016)□
		Harris Creek, MD	~600	Jackson et al. (2018)
Sediment-only	Intertidal	Bogue Sound, NC	~160	Piehler & Smyth (2011)*
			~250	Smyth et al. (2013)
			102	Smyth et al. (2016)
			NS	Onorevole et al. (2018)
		Middle Marsh, NC	~160	Smyth et al. (2015)
		Smith Island Bay, VA	~14	Smyth et al. (2018)
	Subtidal	Lake Fortuna and Sister Lake, LA	NS	Westbrook et al. (2019)
		Great Bay Estuary, NH	~16	Hoellein et al. (2015)

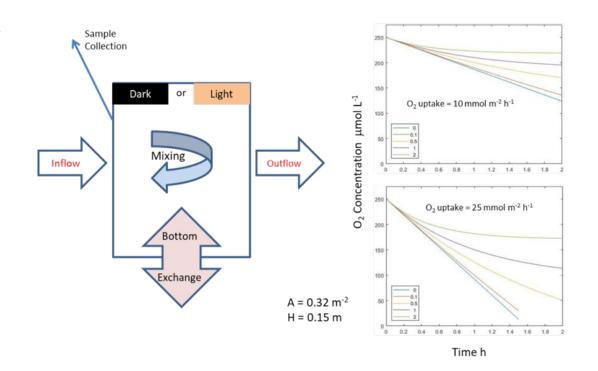
<sup>☐</sup> Based on median values

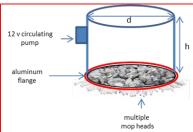


**Figure 8.2.** Map of Chesapeake Bay showing locations (yellow stars) where oyster reef denitrification rates included in the Panel's meta-analysis were measured. Some of the stars represent multiple sites in close proximity.

<sup>\*</sup>Means not given; calculated by subtracting minimum reference rate from maximum reef rate

- You can't drop a lander on the surface of an oyster reef and get a good seal
- If we know how much leakage we encounter, we can solve for the bottom exchange
- A tracer, such as bromide, can be used to determine dilution
- High rates of leakage can be overcome by high rates of nutrient and gas exchange, such as in an oyster reef









Bottom flange for mop head attachment

Top view







Luer fitting

lifting ring (3)

o-ring port
for YSI

lifting handle (2)



circulation pump



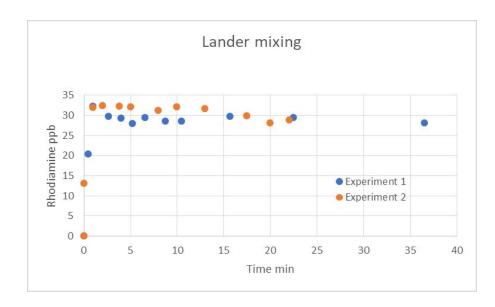
circular mop head



Best Estimate Oct 2021 - single dep		Unit cost	Sum	\$7,225.2
CHAMBER CONSTRUCTION				
mop replacement head	Amazon spin micro fiber mop	\$3.17	9	\$28.5
bilge pump for water circulation	Amazon: Johnson pump 500 gph	\$37.64		\$37.6
	We bought 14' for \$100 per foot from our	Ç57.04	-	Ç07.0
	hatchery. US plastics current cost is \$205.64			
24" White PVC Schedule 40 pipe	per foot, 20' minimum.	\$100.00	2	\$200.0
Acrylic top		Ţ0.00	_	7223.0
Aluminum angle for oyster tub		\$12.40	1	\$12.4
battery for bilge pump circulator	Amazon: Dakota lithium 12v 18 ah	\$179.99		\$179.9
Luer bulkhead fitting		\$1.00		\$2.0
lifting rings, handles, rope		\$100.00		\$100.0
battery for circulation pump	Amazon, Dakota lithium	\$179.99		\$179.9
	Material was on hand, costo fo 12 mm 24" x			
acrylic sheet for top	24" US Plastics	\$69.91	1	\$69.9
low voltage wire		\$10.00		\$10.0
tubing		\$0.27		\$10.8
Total chamber Cost		,		\$820.4
SAMPLING APPARATUS				
peristaltic sampling pump	Dyrabrest 0-140 mL	\$119.00	1	\$119.0
case for pump storage	Amazon: Sheffield 12626 field box	\$14.99		\$14.9
ammeter to determine if pump is w		\$24.58		\$24.5
battery pack for peristaltic sampling		\$199.99		\$199.9
Sampling gear cost				\$358.5
. , , ,				•
Essential Water Quality Gear				
	Xylem, university price. We have used 1,			
YSI Prosolo meter, ODO probe, case	, st now will use 2 for outside measurements	\$1,995.60	2	\$3,991.2
BOAT GEAR				
davit with block		\$400.00	1	\$400.0
winch		\$65.00	1	\$65.0
	for 3 point anchoring - 3 needed, 1 assumed			
anchors	with boat - 13 lb fluke anchor from Amazon	\$99.99	2	\$199.9
Boat gear total				\$664.9
EXTRA GEAR (optional)		\$220.04	-1	\$228.0
Go Pro Black 7	Amazon Suntia divo Esht 94 LED	\$228.04		
Underwater light	Amazon, Suptig dive light 84 LED	\$36.99	1	\$36.9
Extras Total				\$265.0
Labor				
	Per hour, billing in progress, best estimate	Per hour		
Machine Shop		\$75.00	15	\$1,125.0

## Mixing Is Fast

- Rhodamine dye added to filtered sea water in tank
- Mixing complete in << 2 minutes</li>

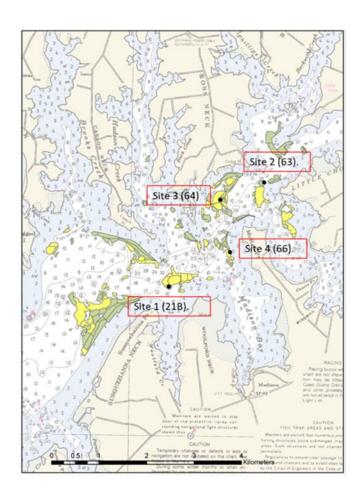


Activity #	Description
1	Collect gas sample. Using the always flowing peristaltic pump connected to the chamber,
	speed up pumping rate and overfill 7 mL glass tubes. Add preservative and store under
	water. Duplicate samples collected on first run.
2	Collected nutrient sample. Fill 30 mL plastic syringe, filter (0.4 mm, 25 mm diameter), fill
	3 sample vials with 5 mL of sample. Vials for soluble reactive P, ammonium, and both
	bromide and nitrate+nitrite in one vial. Keep samples on ice until frozen at laboratory.
3	Chlorophyll a. Fill a 60 mL syringe from pump, filter 50 ml through a 25 mm diameter
	glass fiber (GFF) filter, remove and store in aluminum foil, freeze at laboratory











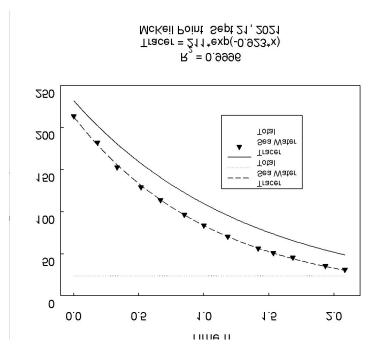


Run Video?

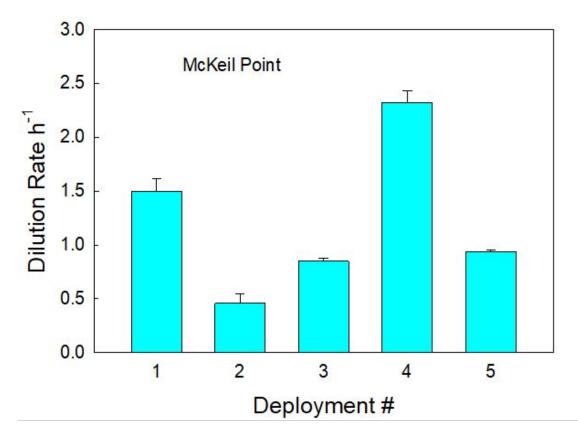
## Bromide Works Well As A Tracer

Time course of bromide change in a > 2 hour experiment in the Little Choptank River (9/21/2021), after a spike of > 200 mg  $L^{-1}$  Br<sup>-</sup> above sea water values. These data suggest a leakage rate of 0.92 h<sup>-1</sup>; the regression fit is excellent ( $R^2 = 0.9996$ )

$$C = C_0 * e^{-R*t}$$

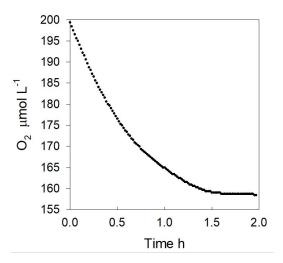


Br does not adsorb to particles, rhodamine disappears quickly onto particles (filtered by oysters). Note: varying pumping rate had minimal effect.



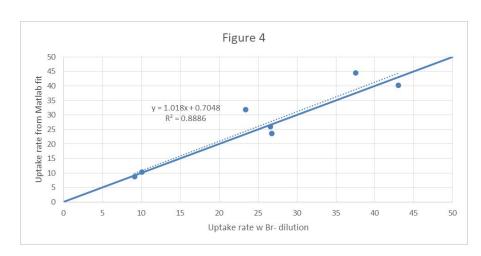
As expected, each deployment can have a different seal to the bottom and a different leakage rate

- Oxygen fluxes were estimated from YSI continuous data (below)
- The equation presented here can use bromide leakage rates and a point to point look at O<sub>2</sub> data to estimate fluxes
- The oxygen curve can also be fit to the equation and both the flux rate and leakage rate estimated.
- Both approaches yield similar data

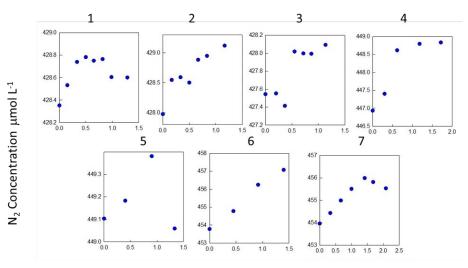


$$R = \frac{h(c_{in}(0) - c_{in}(t))}{\frac{V}{F} \left(1 - e^{-\frac{F}{V}t}\right)}$$

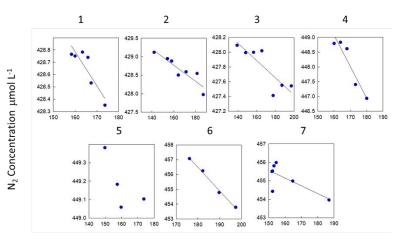
- R =  $O_2$  exchange rate mmol m<sup>-2</sup> h<sup>-1</sup>
- h = chamber height m
- c = O<sub>2</sub> concentration mmol L<sup>-1</sup> at time 0 and time t (h)
- V = volume m<sup>3</sup>
- F = leakage rate h<sup>-1</sup> from Br<sup>-</sup>



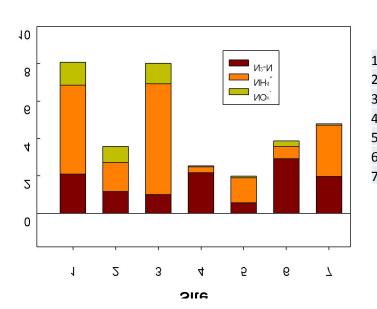
## $N_2$ -N Flux = $O_2$ Flux<sub>(YSI)</sub> \* $\Delta N_2/\Delta O_{2(mass spec)}$

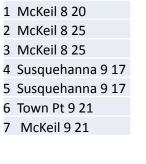


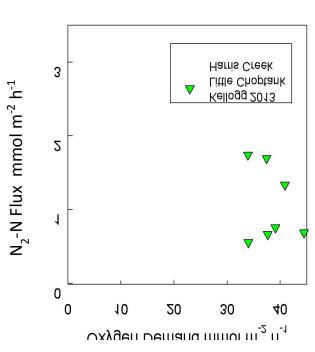
Time From Start of Incubation h

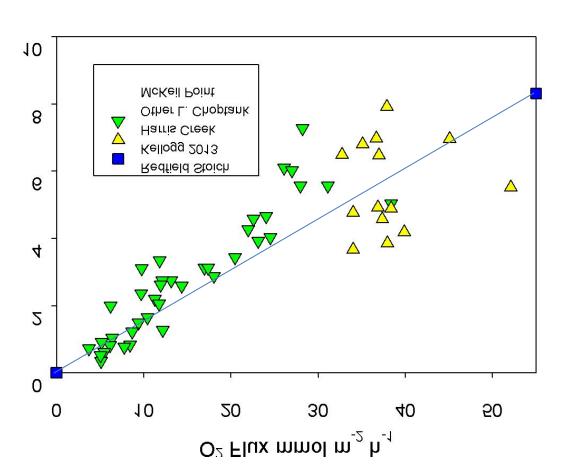


O<sub>2</sub> Concentration μmol L<sup>-1</sup>



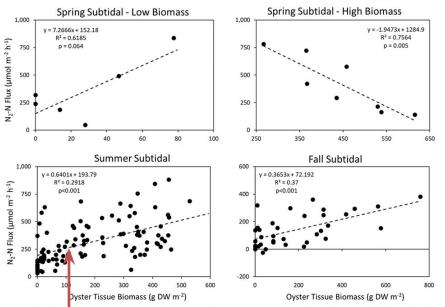






Current oyster BMP plans would credit the biomass of 112-123 g dw m<sup>-2</sup> at Harris Creek and other sites for ~250 umol m<sup>-2</sup> h<sup>-1</sup> of N<sub>2</sub>-N efflux, minus background (maybe 50-75, so ~200 umol m<sup>-2</sup> h<sup>-1</sup>.

Our Little Choptank data from McKeil Point averaged 2.1±0.6 mmol m<sup>-2</sup> h<sup>-1</sup>



**Figure 8.3.** Final I near regressions of spring, summer and fall data oyster reef denitrification rates plotted as a function of oyster tissue biomass.

# Lander-Tray Comparison

### Tray

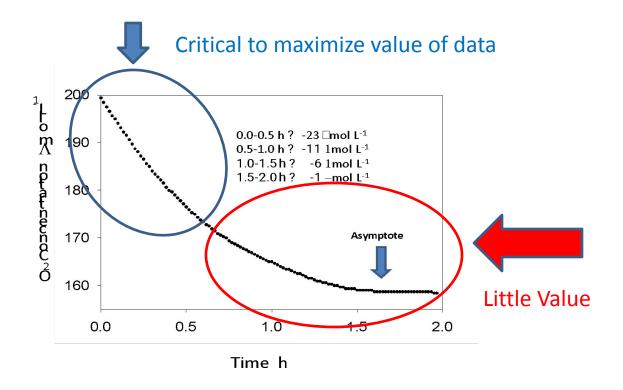
- Known oyster biomass in tray, but may differ from actual biomass
- Disruption of sediment layers
- Require divers
- May take 4-5 hours after sampling before incubation
- Need rapid sampling
- Requires temperature control, supply of water at appropriate salinity
- Great for manipulations

#### Lander

- Dependent on ORP biomass from a hopefully recent assessment
- Minimal disruption
- Can be done by 2 personnel in boat
- Requires good weather for anchoring
- Need rapid sampling
- May not be as sensitive to low rates

Table 3. Denitrification cost comparison, lander versus tray approach. This is an estimate for each individual rate measurement, including oxygen and nutrients.

Cost Category	Lander – This	Tray Approach
	Study	Kellogg et al. 2013
Personnel	812	1,832
Boats & Logistics	79	164
Analytical	671	407
Gear (amortized - 25	54	75
deployments)		
Per Measurement	1,615	2,477
With 26% overhead (State of	2,035	3,121
MD)		
With 55 % overhead (Federal)	2,503	3,839



After looking at the data, it is clear we need higher frequency data, but don't need to go to the asymptote. Many incubation could be 0.5-0.75 hours, with  $\rm O_2$  monitoring informing the time course.

# Now For Something Entirely Different

Large, Very Large Substrate





Oyster Castles – Shoreline Protection

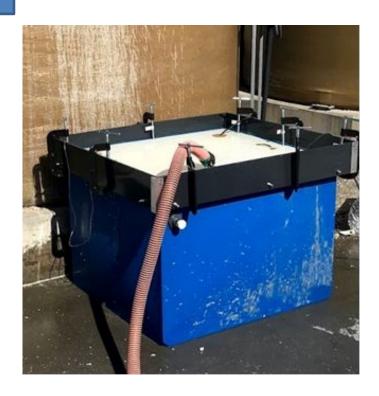


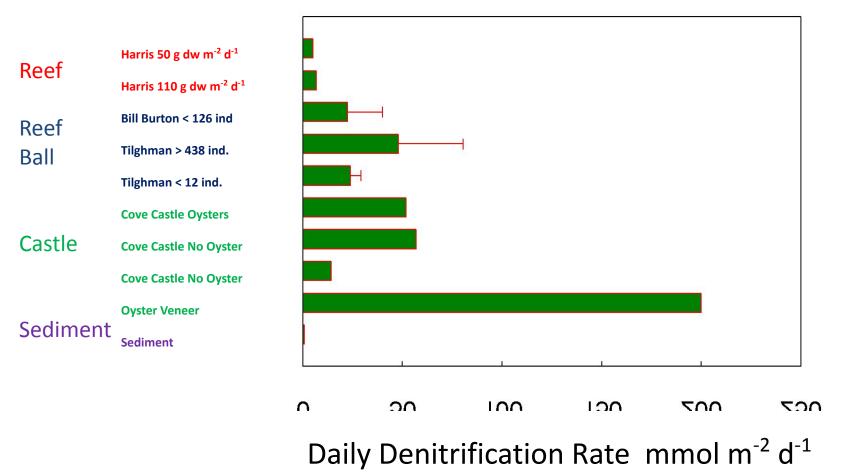
Reef Balls – Fish Habitat



Photo of two of the four castle segments incubated. The circulating pump in the upper right corner keeps the water homogenous during incubation.

Incubation tank for oyster castle incubations. All bubbles are excluded during incubation under the while lid which is clamped down. We have used this setup for incubations of reef balls with the Chesapeake Bay Foundation.





As we move towards adding/improving infrastructure to enhance coastal resilience, there may be a place, or better yet an opportunity, to increase bivalve populations.

The highest areal rate of denitrification we have observed is the fouling community (bryozoans, barnacles, mussels) in Baltimore Harbor.

Areal rates of denitrification on structures are very high – the question is how much area can there be?