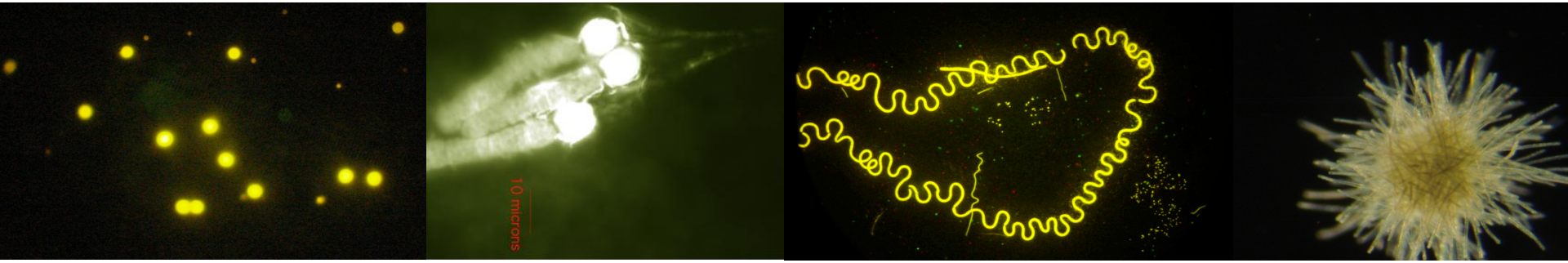
What are the necessary elements of standardized methods to measure the activity and diversity of diazotrophs?

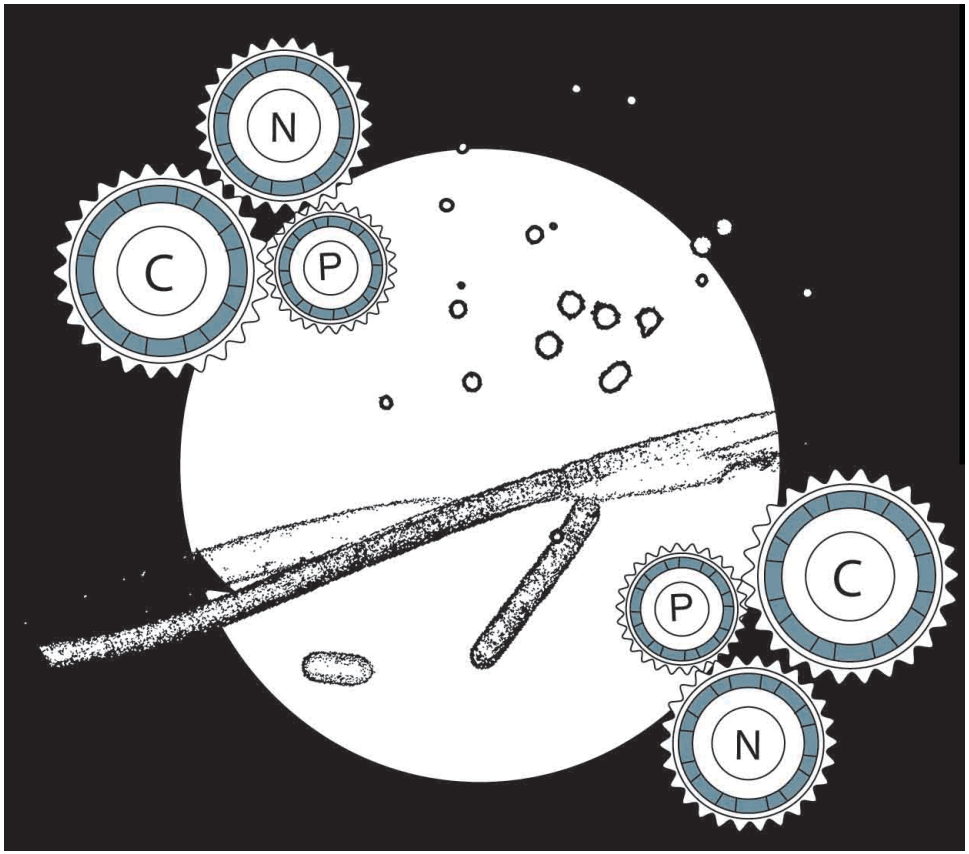


OCB
Ocean Carbon & Biogeochemistry

NSF

- $^{15}N_2$ Fixation
- QPCR

The Rate of Production: N_2-Fixation Measurements



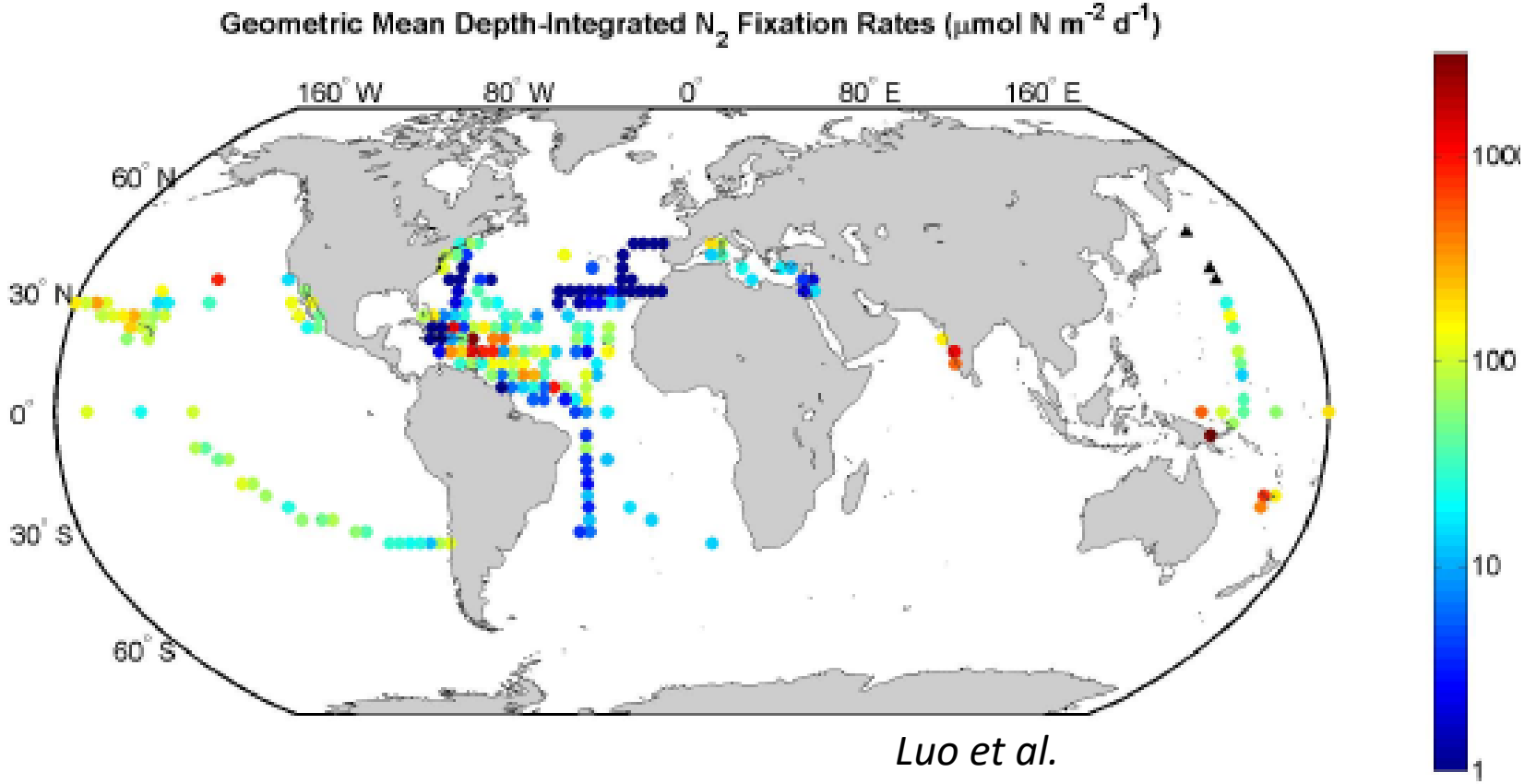
Angelicque E. White, Oregon State University
with input from the OCB Working group on N_2 Fixation

Objectives (today & later workshops)

- Define the current obstacles in measurement of NFR and establish a consensus path forward
- Develop recommendations for a standardized method for measurement of $^{15}N_2$ fixation
- Consider potential linkages to diversity metrics – e.g. does it matter who fixes N_2 or whether we can scale from diversity/abundance to rates ?

The current state of N_2-Fixation rate measurements

~ a wide array of methods



Luo et al.

Current methods for N_2fixation rates at sea

1. <u>The traditional bubble method:</u>

   add a bubble → incubate

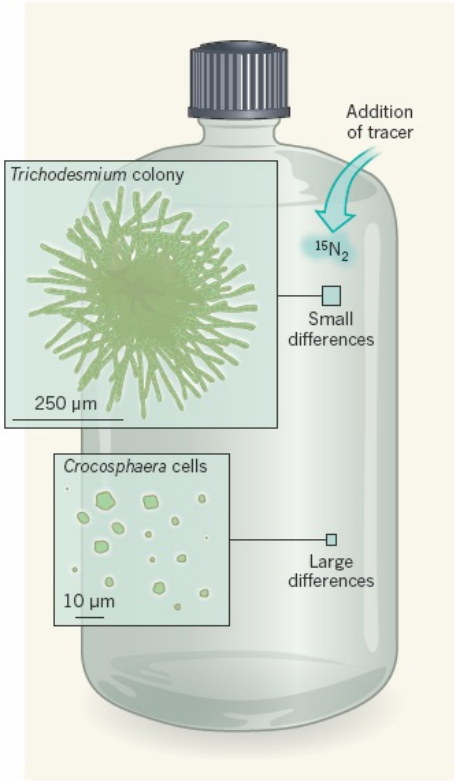
2. <u>The bubble release method:</u>

   add bubble→ mix, release → measure enrichment → incubate

3. <u>Enriched seawater method (s):</u>

   add bubble to degassed or filtered seawater→ agitate until dissolution→ measure enrichment in parallel bottles→ inoculate seawater →incubate

4. ARA/Concentration of biomass-ARA



Areas of Inconsistency in the Application of Methods

- Experimental setup: timing and duration of incubations, incubation temperature and light control, incubation volume, collection of PN at t_0, t_a, and t_f to evaluate variability in isotopic composition
- Dissolved $^{15}N_2$ gas
  - Not quantified
  - measured on MIMS with one point calibration
  - GC-IRMS
  - Variable percent saturation (but see Kiel notes)
- Change in δ^{15}N-PN with organic/inorganic additions
- Detection limits – generally not reported and when reported a wide range of metrics are used (e.g. mass spec precision v. error propagation)

Is there a one-size fits all protocol or at least a set of recommendations

- Bubble release v. equilibrated additions (Klawoon et al. 2015 discuss pros/cons including trace metal contamination, cost, time, preparation, achievable N-atom%, other recent papers are also informative)
- Reporting of detection limits – few groups report propagated error
- https://www.oceanbestpractices.net/
- https://www.us-ocb.org/n-fixation-working-group/
- See google drive for Kiel notes, papers, etc.

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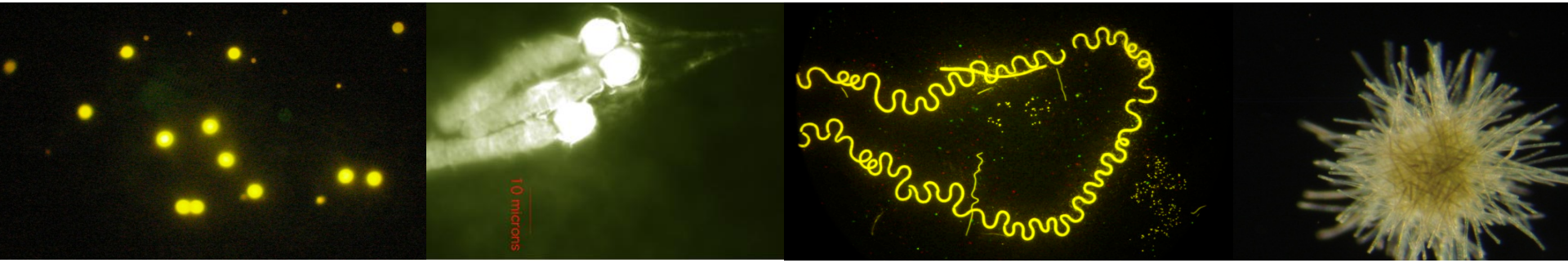

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