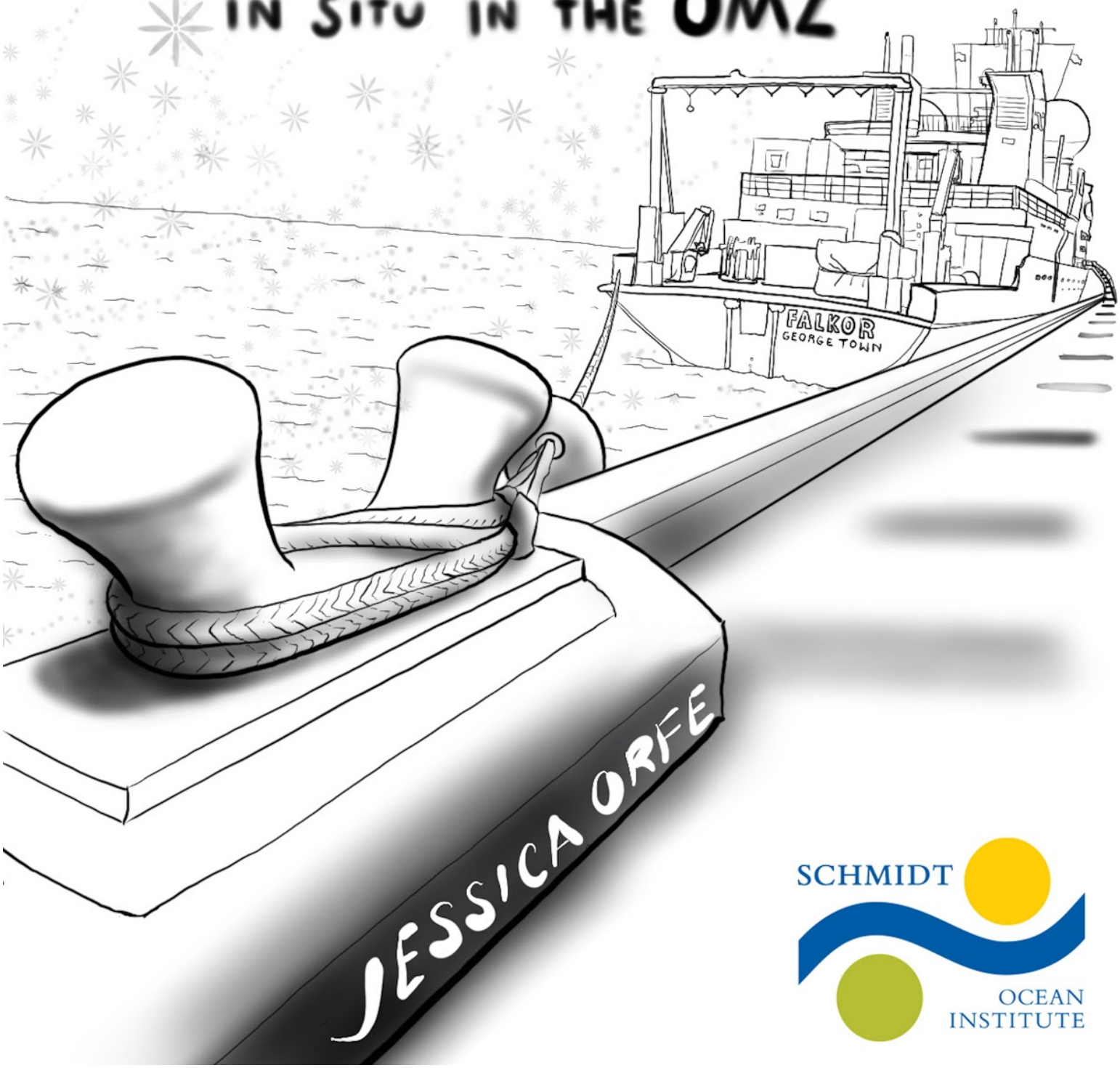
MICROBIAL MYSTERIES

IN SITU IN THE OMZ



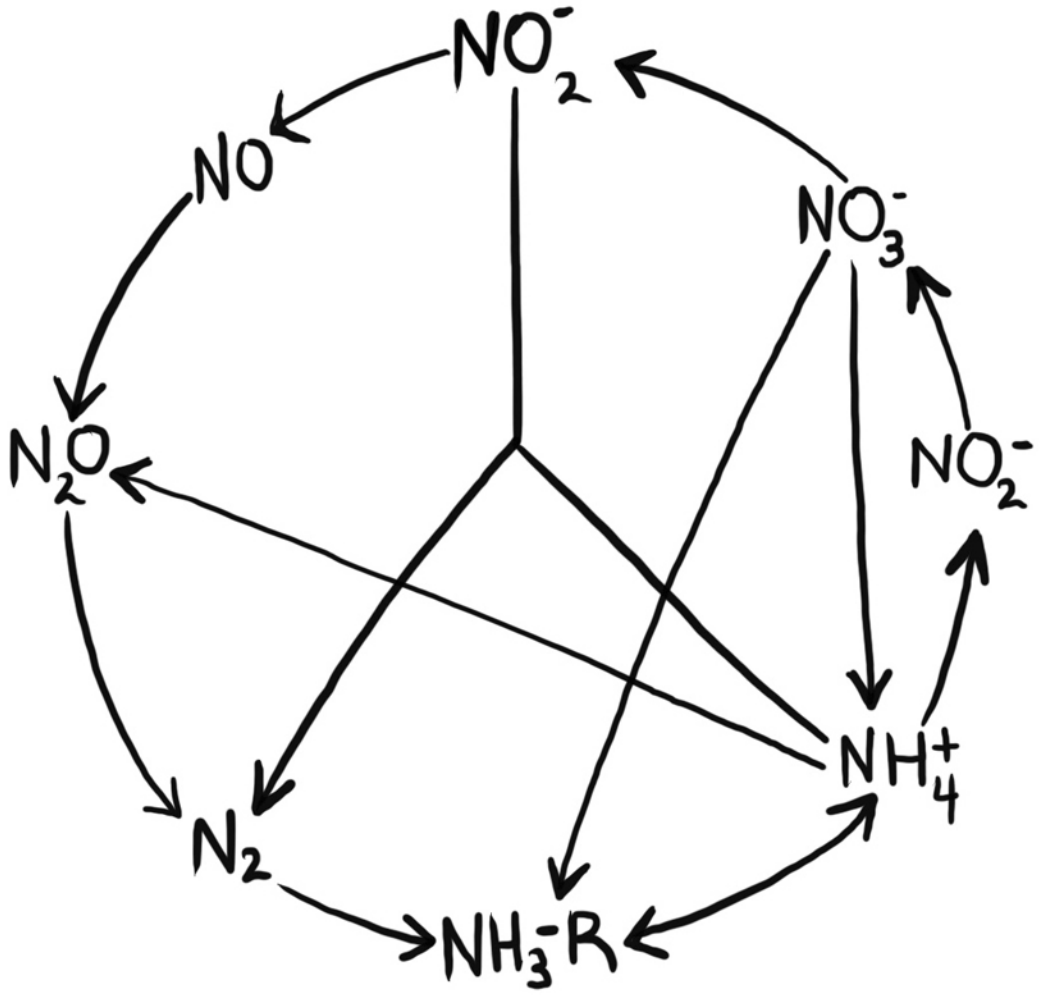
JESSICA ORFE

SCHMIDT OCEAN INSTITUTE

Welcome to "Microbial Mysteries: In Situ In The OMZ" the coloring book!

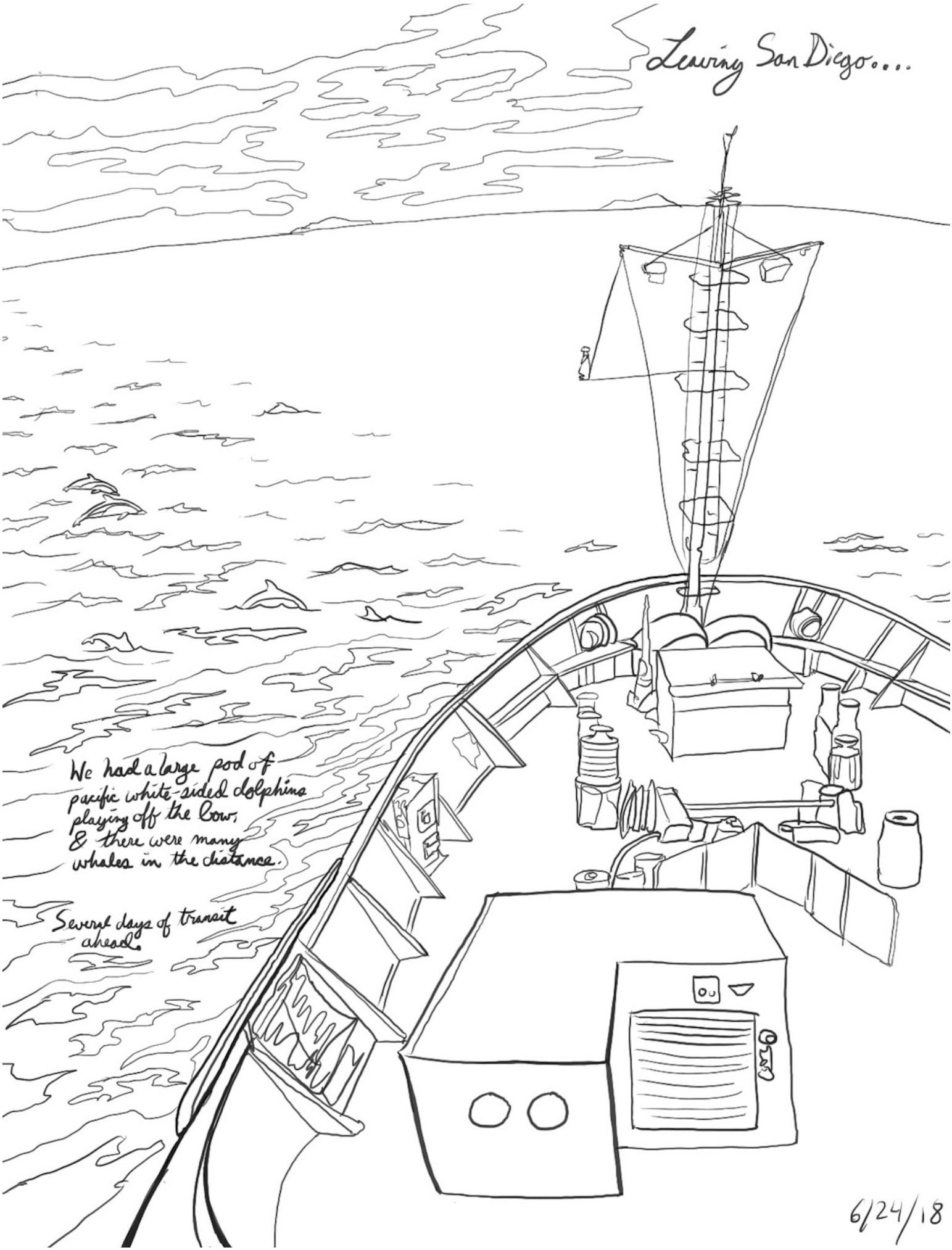
This visual narrative gives you a 'behind-the-scenes' look into the Solving Microbial Mysteries With Autonomous Technology expedition aboard the R/V Falkor. Led by Chief Scientist Andrew Babbin, an interdisciplinary team of scientists from MIT and Stanford set out to test new technologies – In Situ Incubators - and conduct research in the North Eastern Tropical Pacific, one of the ocean's largest oxygen minimum zones. Nutrients, energies, and microbial interactions in these zones have impacts beyond marine ecosystems, hence gaining insight into these oceanic processes is highly desired. Like any mystery, this 3-week expedition yielded results and questions. This book encourages you to ask questions, too!

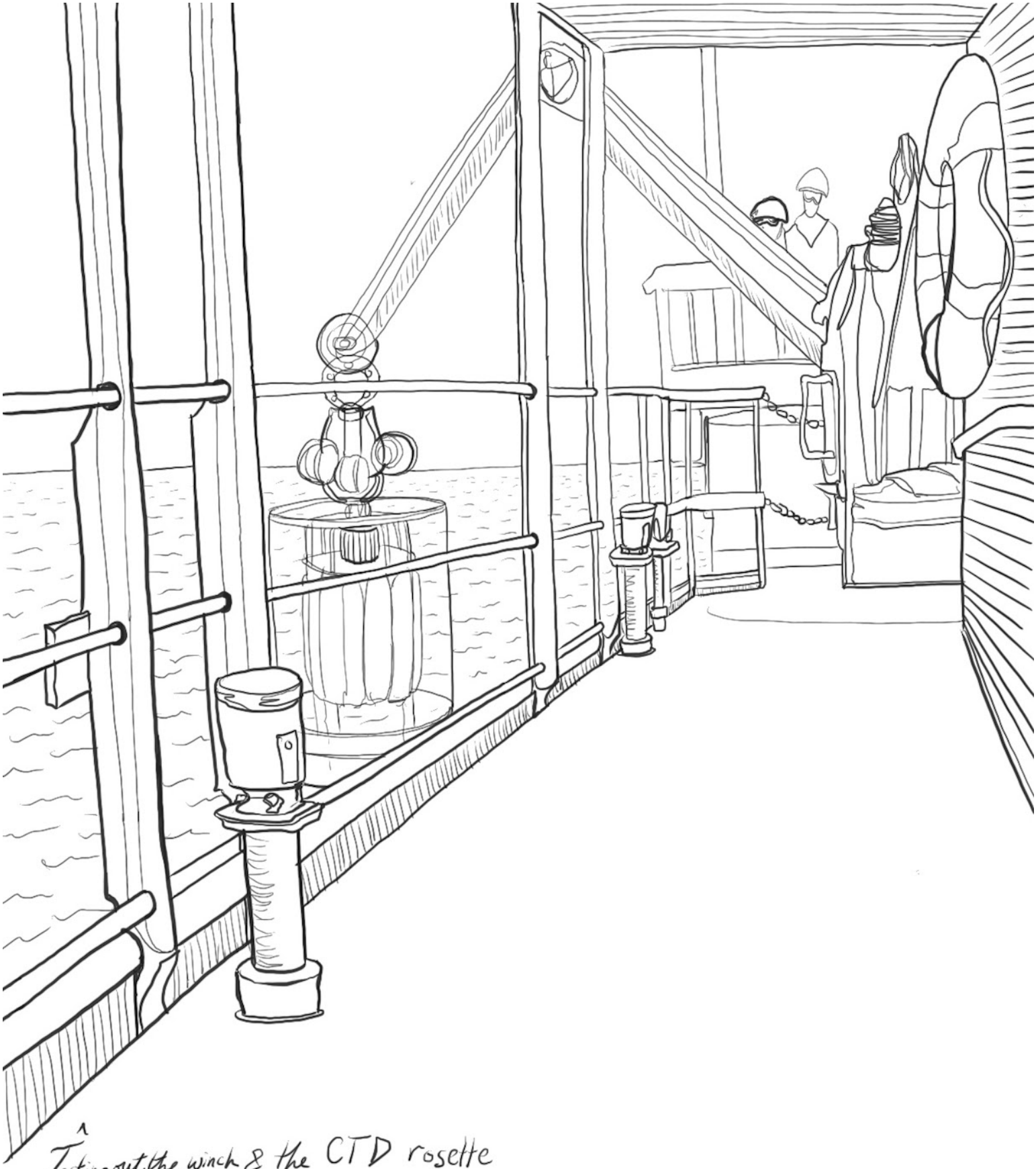
Use your imagination and creativity to add color to these illustrations, and enjoy!



MICROBIAL NITROGEN CYCLE by Andrew Babbin

Illustrations & Story by Jessica Orfe © 2018

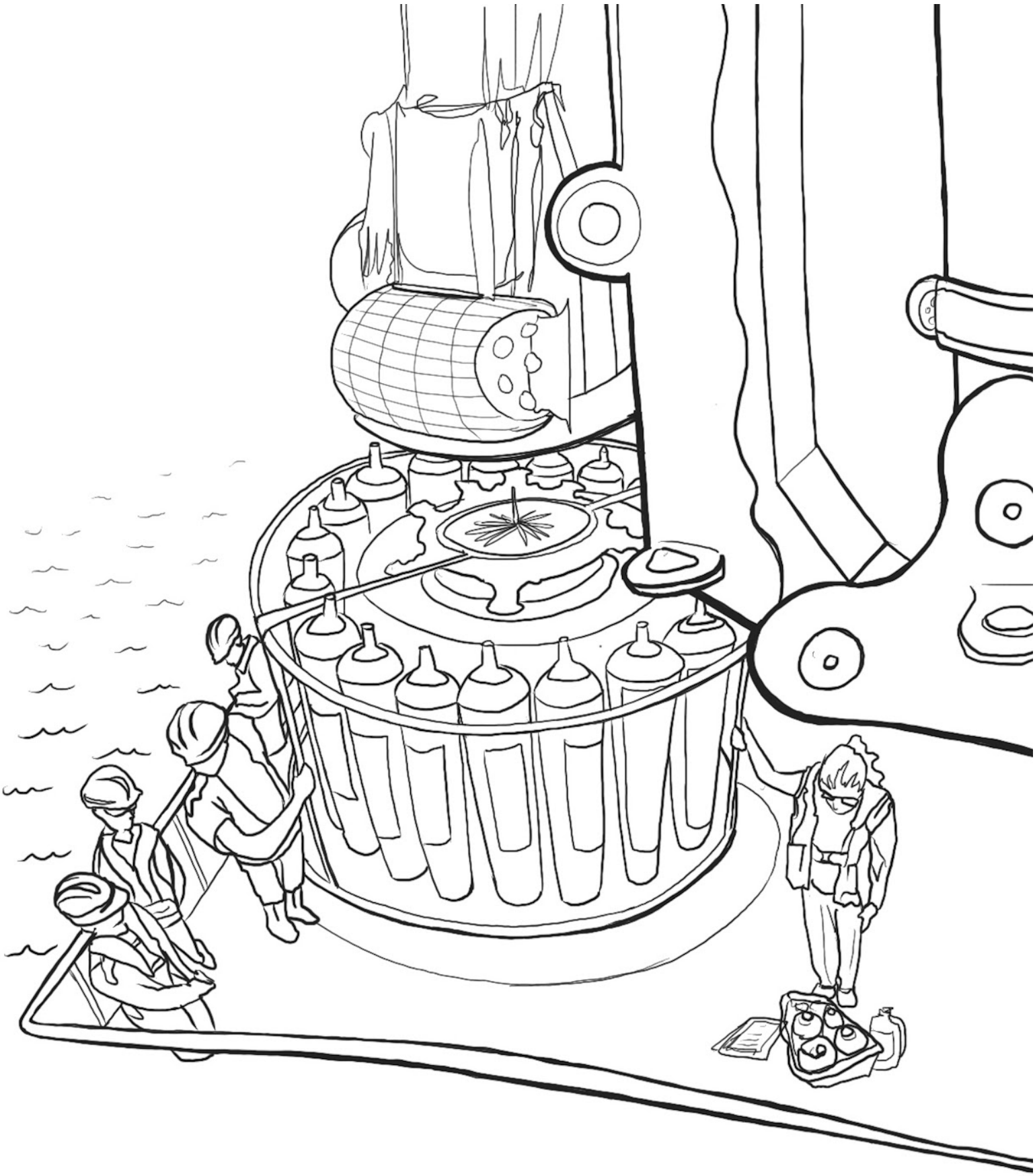

Leaving San Diego....
We had a large pod of pacific white-sided dolphins playing off the bow, & there were many whales in the distance.
Several days of transit ahead.
6/24/18



Testing out the winch & the CTD rosette
It works!
The water is so calm

6/26/18

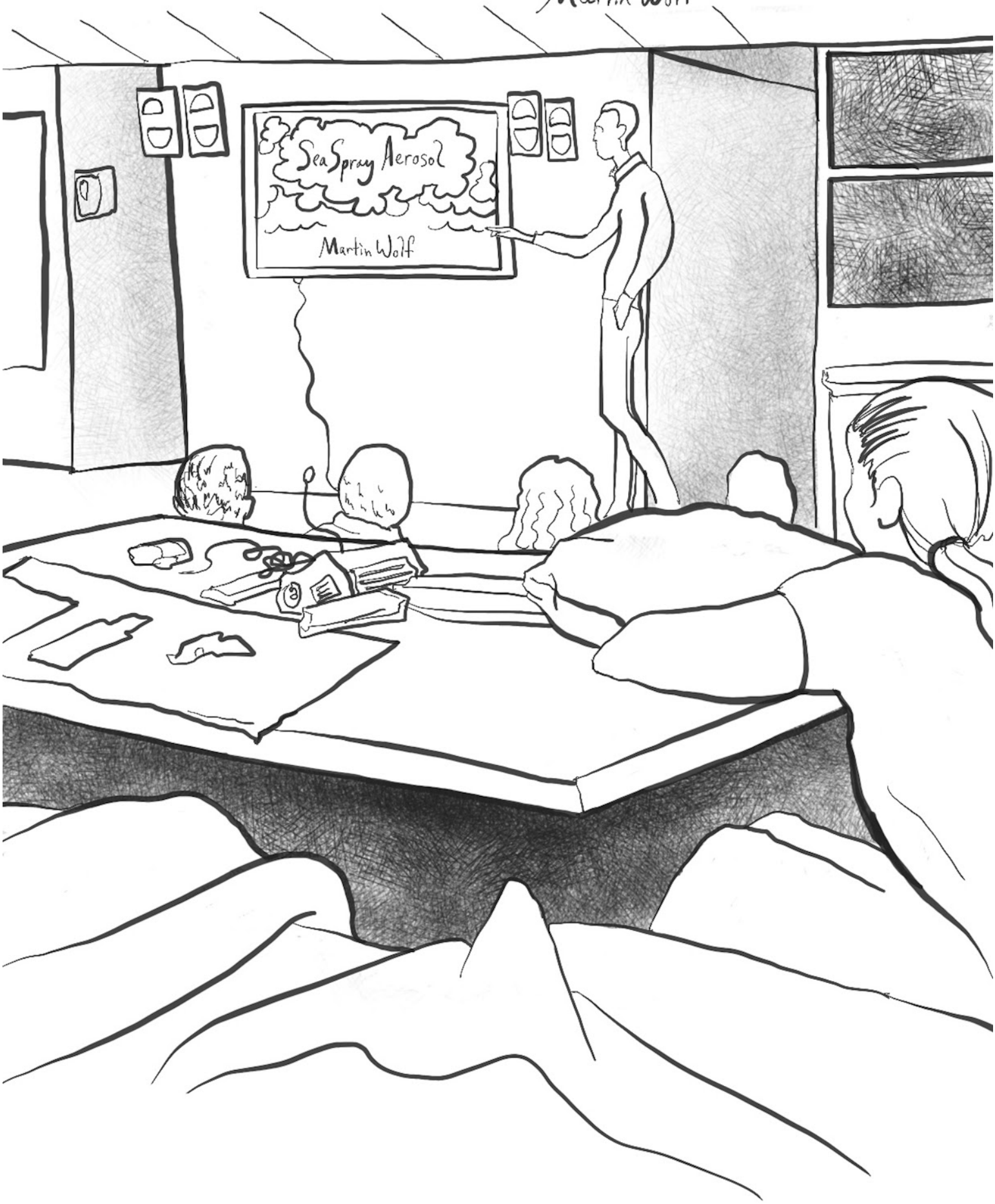
in the CONTROL Room to test deploy a CTD rosette



~ over 100 species of clouds

SEMINAR by Martin Wolf

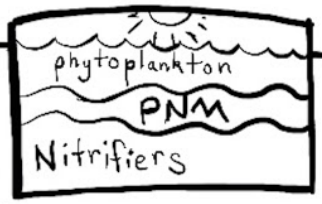
6/26/18



WETLAB
-setting up:
TUES, 6/26/18
A device for measuring
IODIDE
1st stock
spectrophotometer that way
for measuring Nitrogen
NED & SAN react w/Nitrogen to
turn it a shade of purple, which the device uses to measure Nitrogen

MORE PREP IN THE WET LAB 6/28/2018

6/28/18



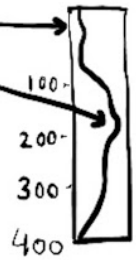
NOTES FROM CASCIOTTI LAB LECTURE:

NITROGEN CYCLE!

NITRITE

PNM ← euphotic zone, between SUN ZONE & NUTRIENT ZONE

SNM

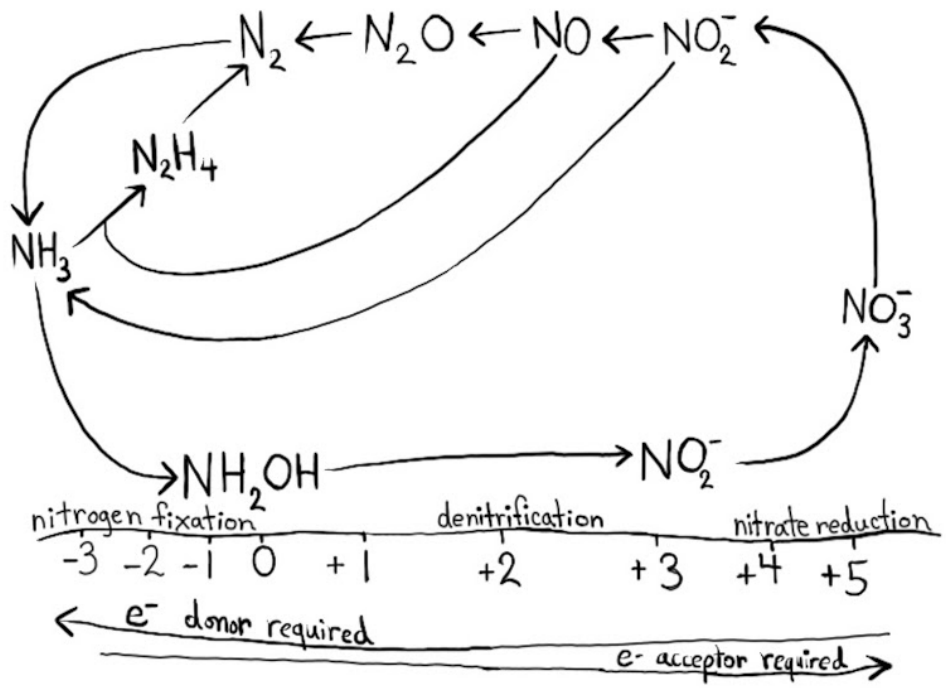


ODZs: Arabian Sea, *ETSP...
(Where we're going)

Nitrite does not occur in Most of the ocean

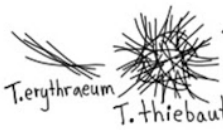
Q: Which microbes are responsible for PNM layer?

MICROBIAL NITROGEN CYCLE:



CYANOBACTERIA

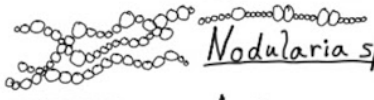
- or 'Cyanophyta'. photosynthetic prokaryotes
- phytoplankton capable of fixing nitrogen, ie, producing oxygen



Trichodesmium - often in filaments of ~100 cells, linked, that bundle into colonies (~10,000 cells) "Sea sawdust"

Richelia intracellularis - within Rhizosolena or Hemialus, a symbiont

(Diatoms)



Nodularia spumigena -

Aphanizimenon - (Cyanophycea) "lawngrass"

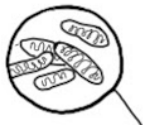
~they fix Nitrogen by means of heterocysts

~ phycocyanin - bluish pigment that captures light

~ diazotrophs (?) & diazocytes (?)

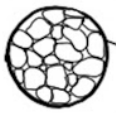
&

Heterotrophic Nitrogen-fixers:



Azotobacter

Azospirillum



Rhizobium

Mesorhizobium

Sinorhizobium

fixed nitrogen (N)

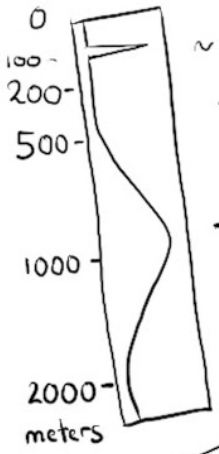
NITRATE OR AMMONIA

6/28/18

NITROUS OXIDE

300 x CO_2 greenhouse gas potential

· Ocean Microbes: account for 1/3 NO_2^- emissions



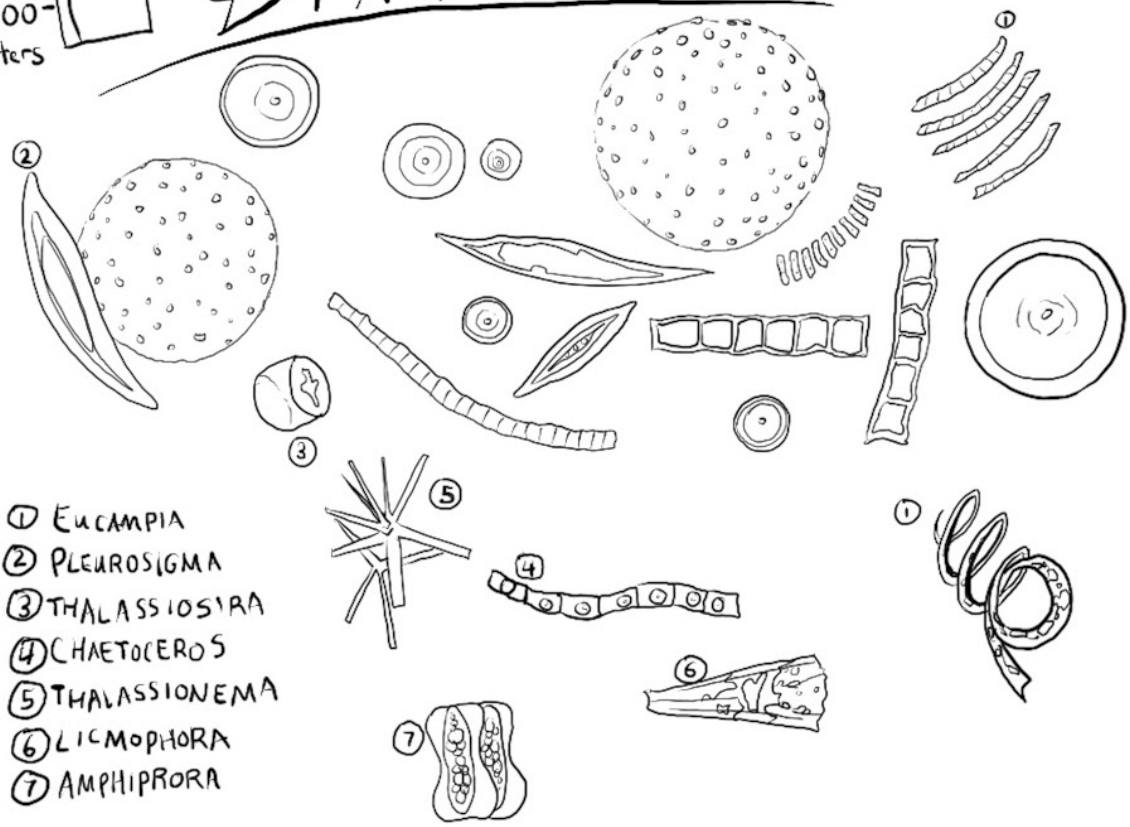
~ experiment involves driving out oxygen w/helium

- relevance - possible relation to global warming
  - relation of "DEAD" zones to N- high zones

- RUNOFF → large phytoplankton blooms → increase in N_2O gases

PHYTOPLANKTON:

DIATOMS:



1. EUCAMPIA
2. PLEUROSIGMA
3. THALASSIOSIRA
4. CHAETOCEROS
5. THALASSIONEMA
6. LICMOPHORA
7. AMPHIPRORA

~ DIATOMS tend to have RADIAL or BILATERAL SYMMETRY

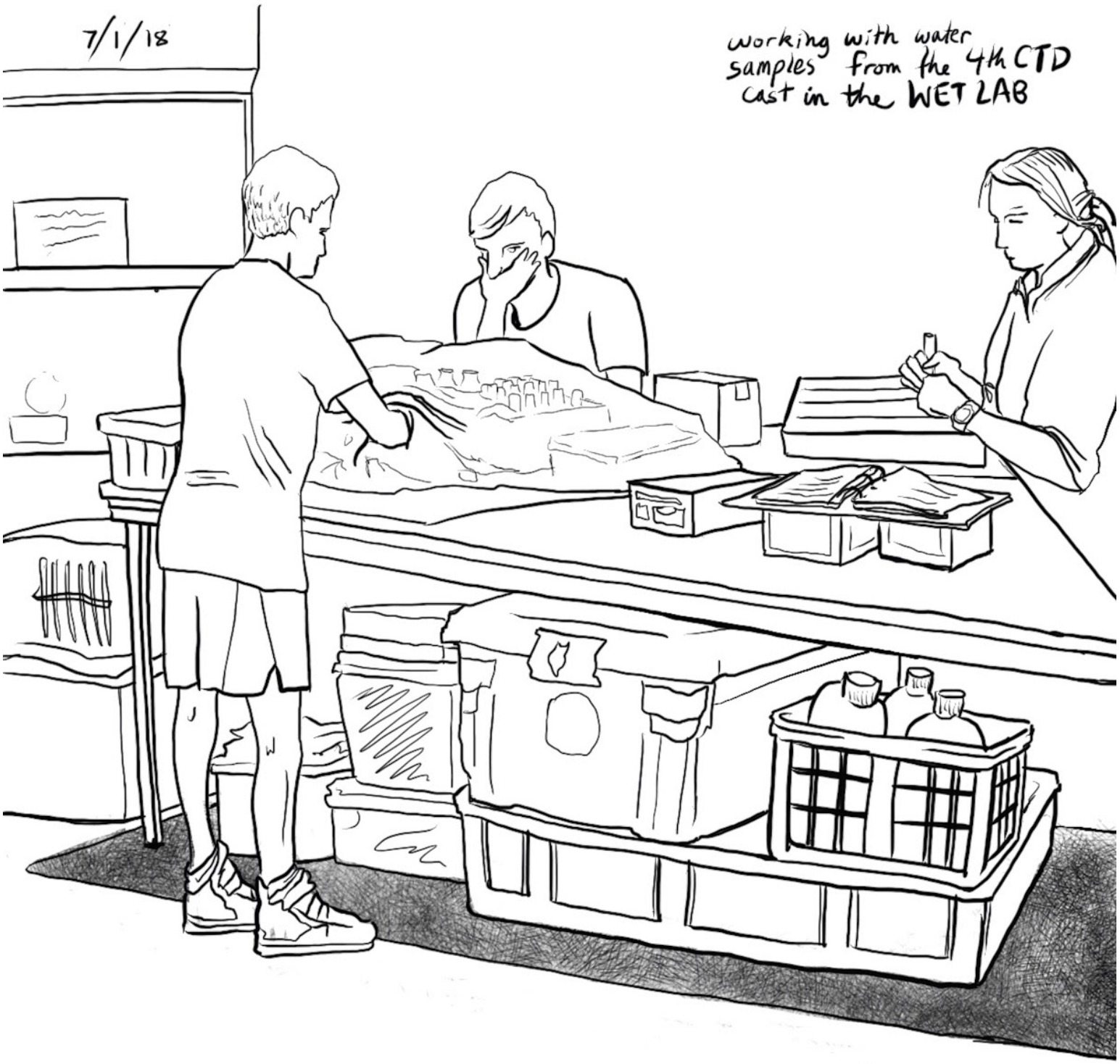
SAMPLING STATIONS - CTD Casts for Seawater @ different depths in the water column

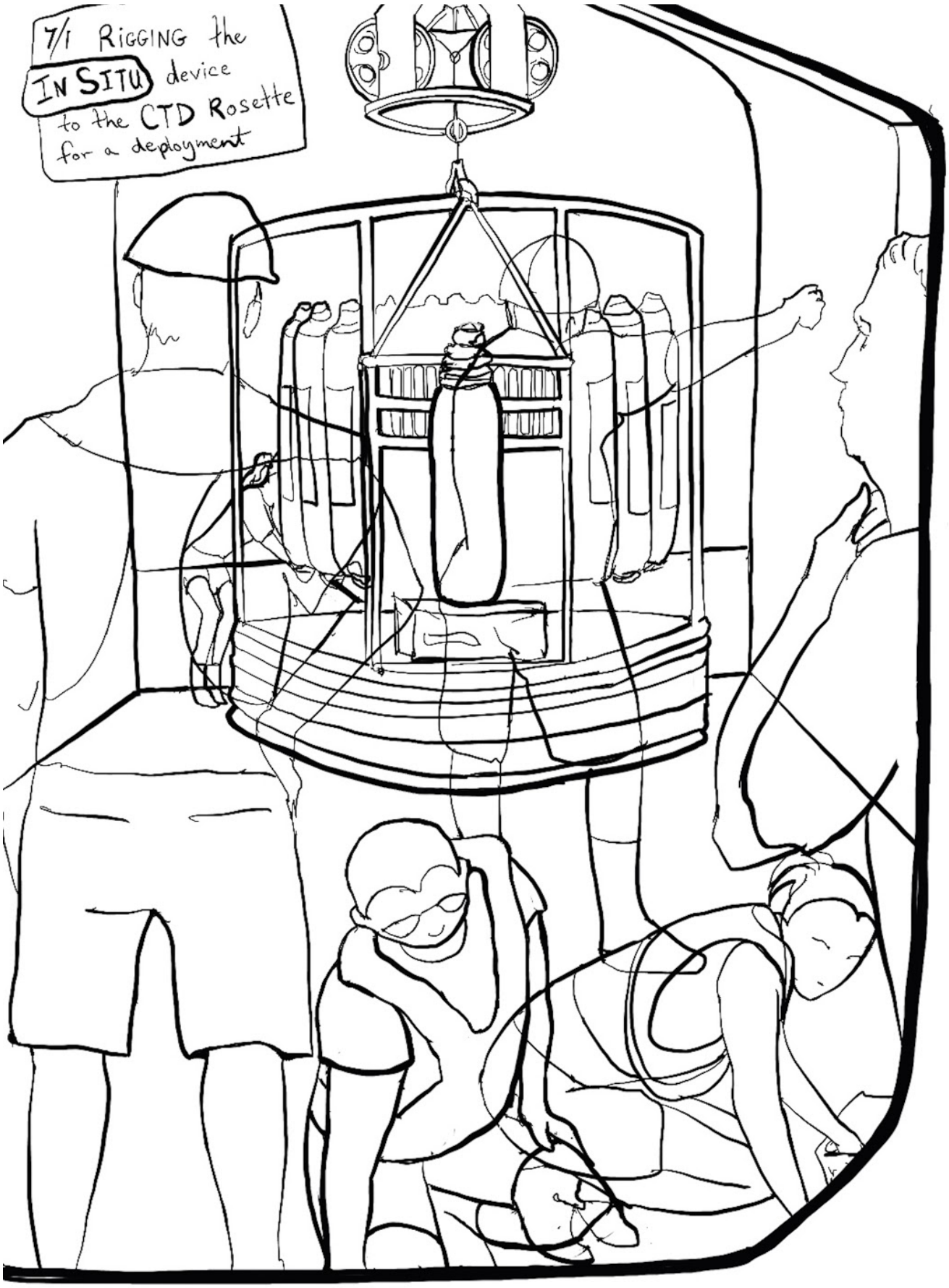
*CTD

LOCATION	CAST #	DATE	TIME
14N 102W	1	6/29	11 PM
14N 103W	2	6/30	7 AM
14N 103W	3	6/30	...
14N 104W	4	7/1	...
↓	5	7/2	...
↓	6	7/2	...
↓	7	7/3	...
...	↓		

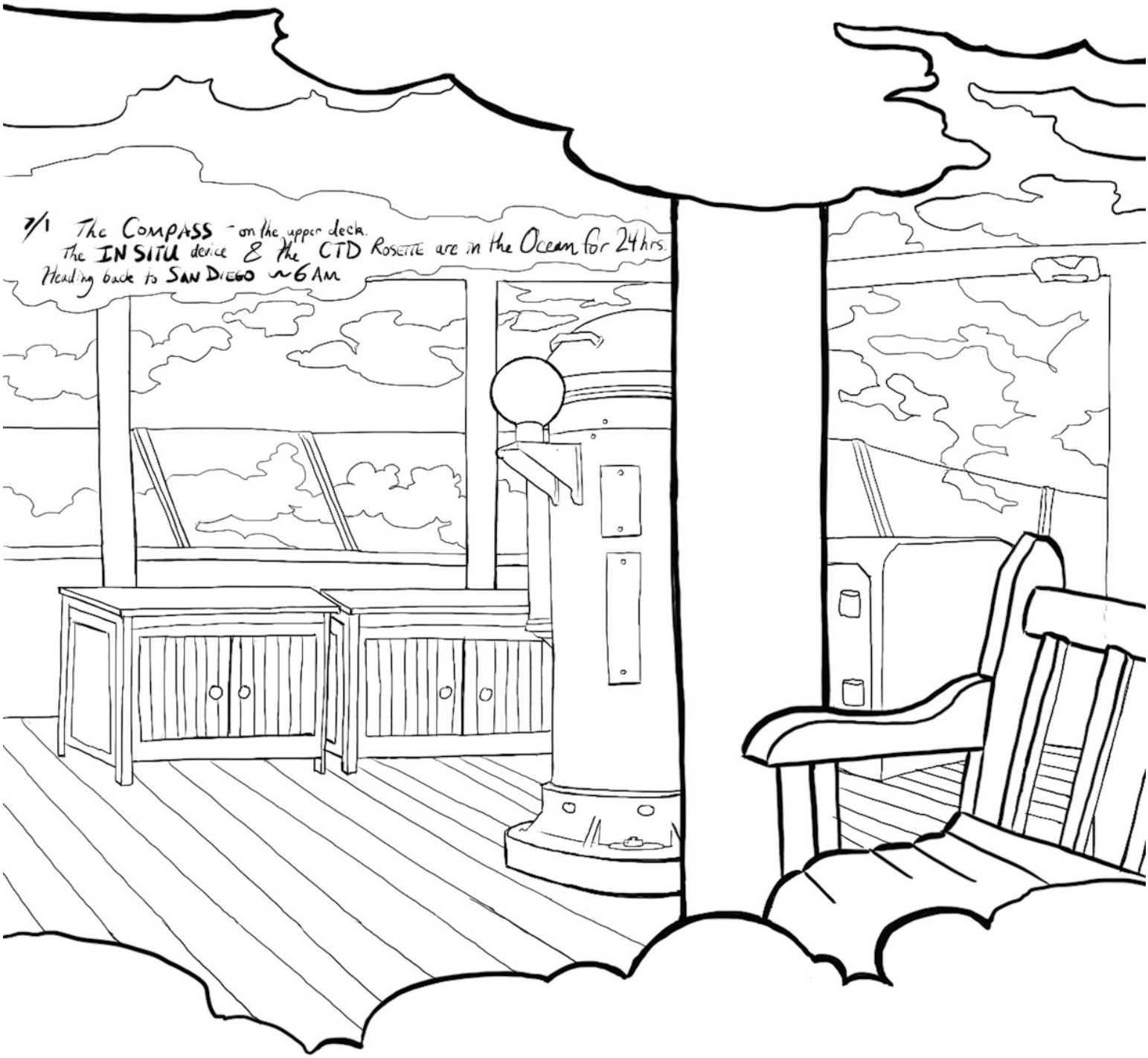
* CTD - "Conductivity, Temperature, Depth"

* *Due to weather conditions & other factors, the plans ^ are subject to change....


7/1/18
working with water samples from the 4th CTD cast in the WET LAB


7/1 RIGGING the
IN SITU device
to the CTD Rosette
for a deployment

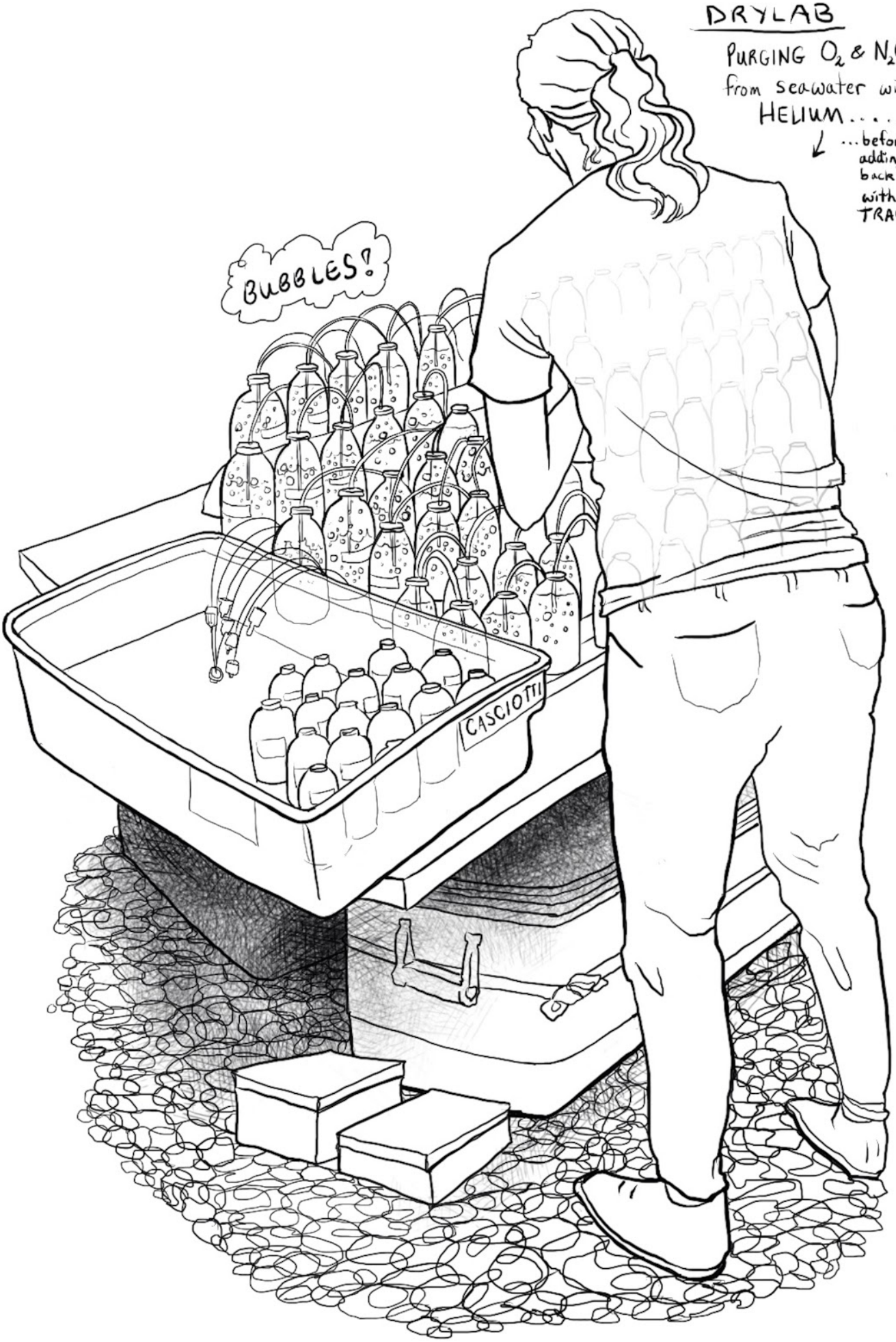
7/1
in the MESS:


7/1 The COMPASS - on the upper deck.
The IN SITU device & the CTD ROSETTE are in the Ocean for 24 hrs.
Heading back to SAN DIEGO ~6 AM

DRYLAB

PURGING O_2 & N_2O from seawater with HELIUM.....

...before adding N_2O back in along with a TRACER



7/2

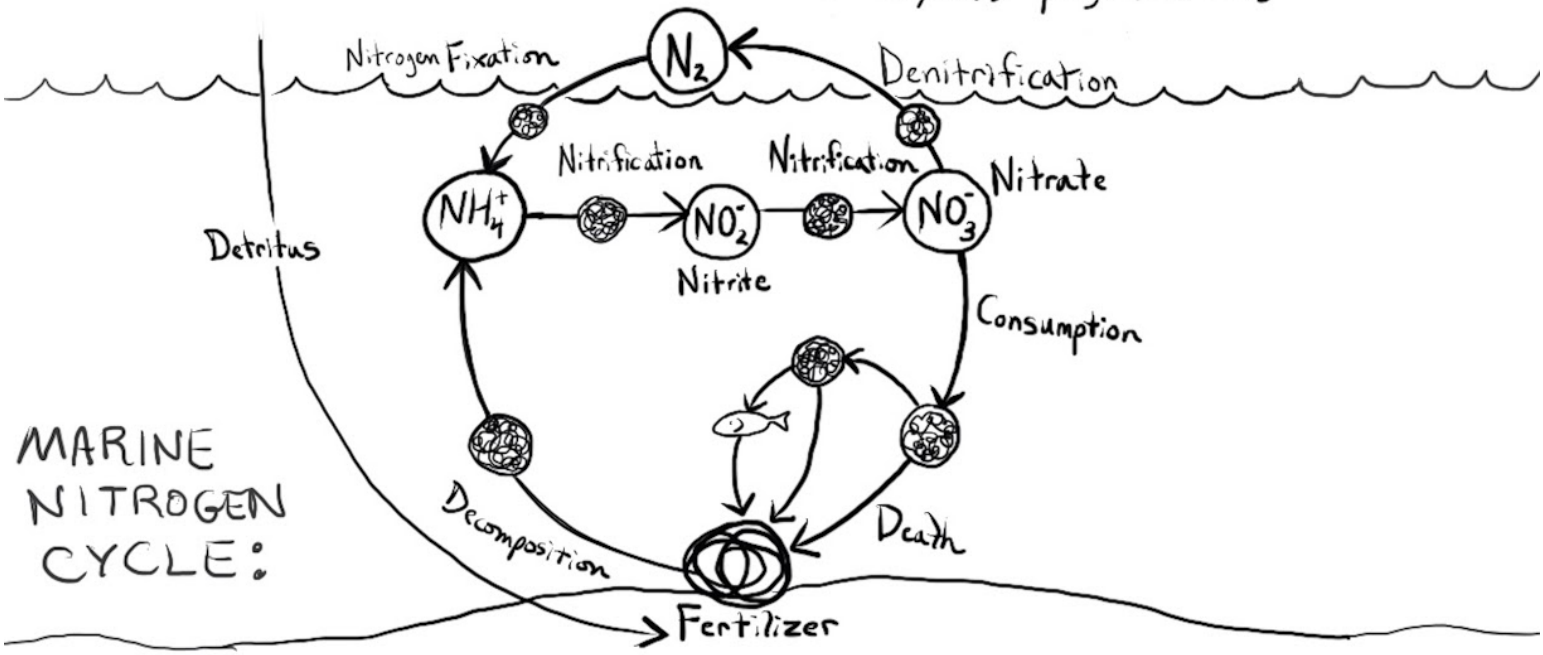
Injecting a TRACER - a heavy Isotope of Nitrogen

14°N 103°W

NITROGEN, NITRITE, NITRATE, & AMMONIUM

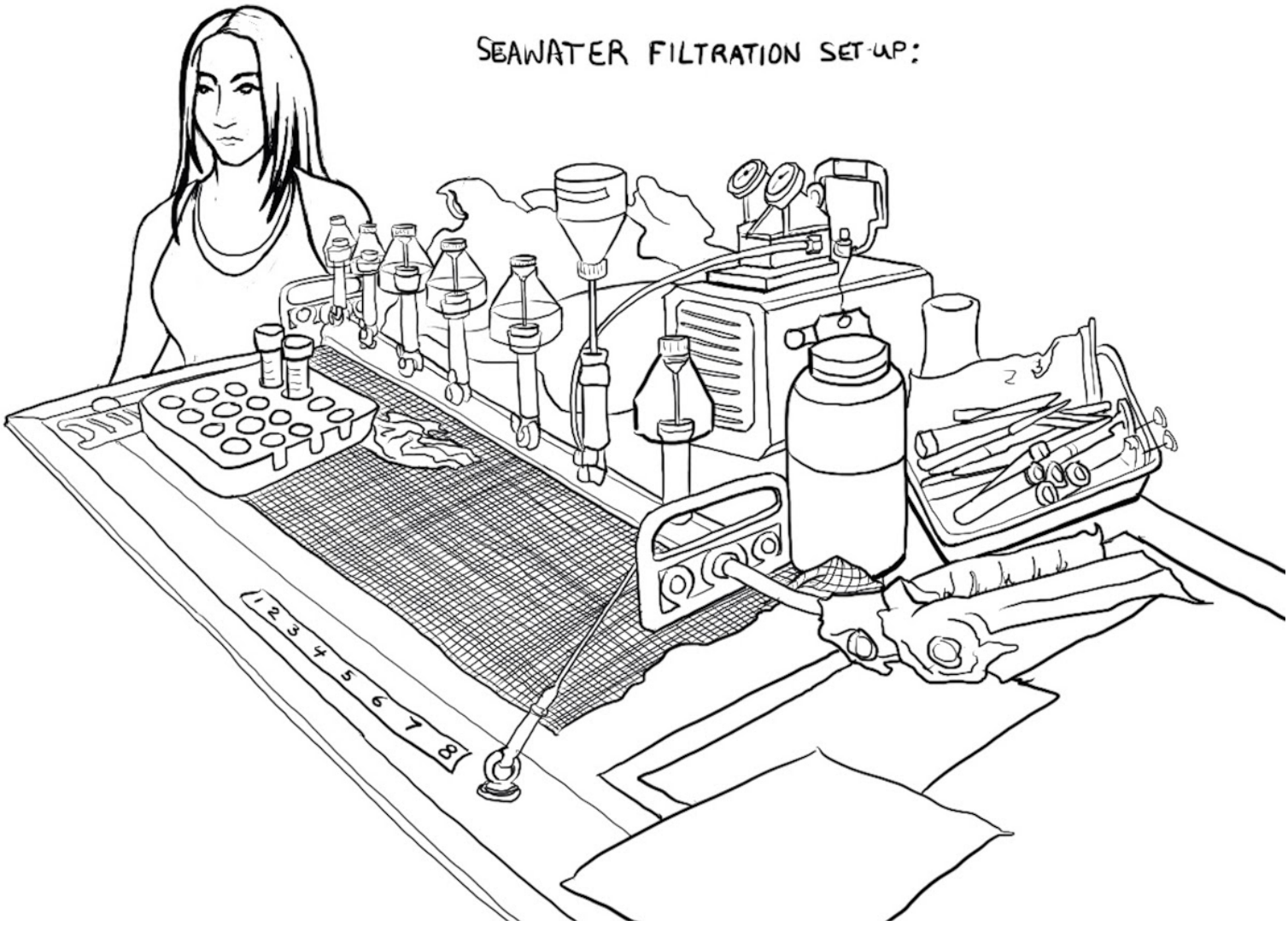
N element

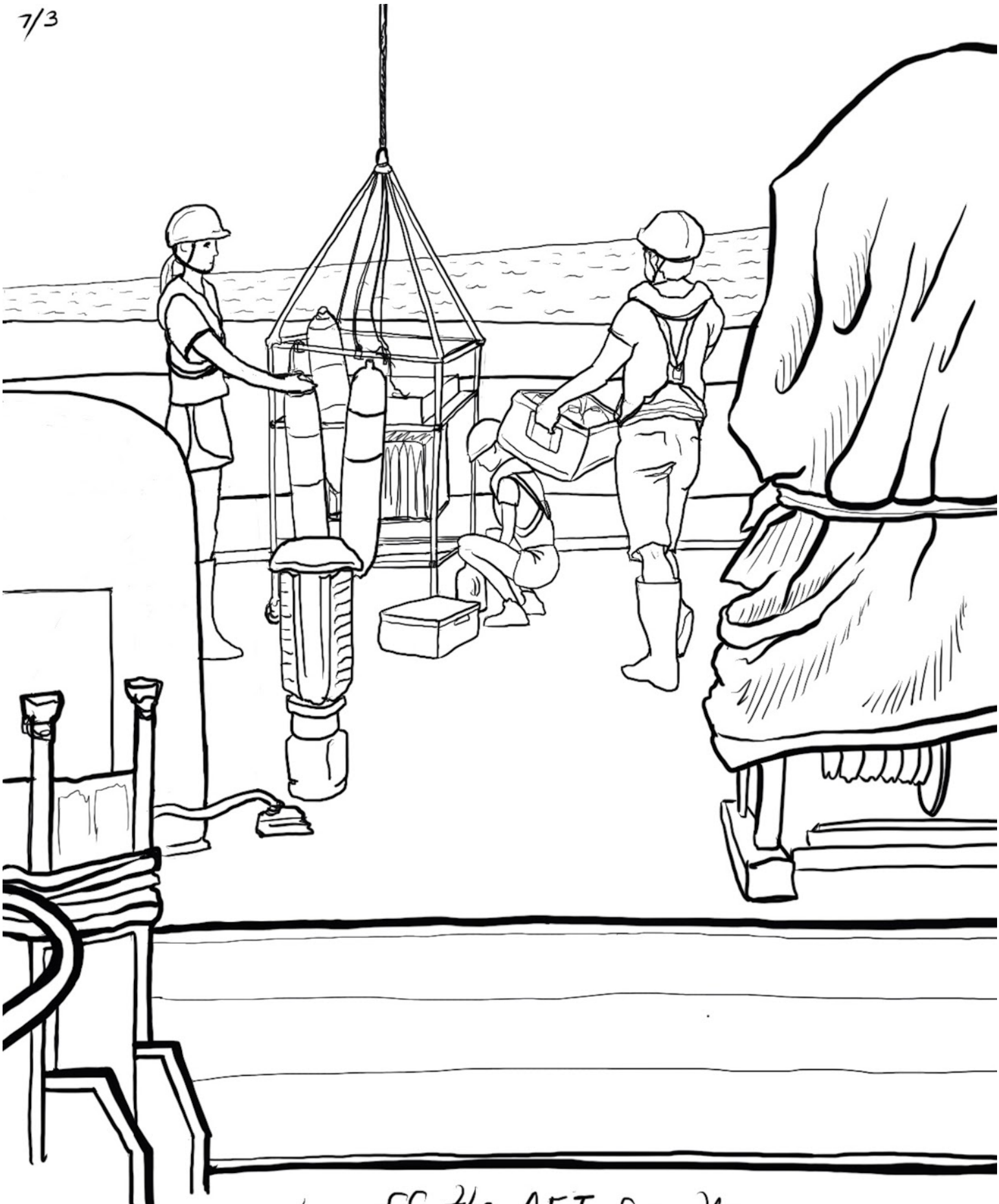
NO_2^-, NO_3^-, NH_4^+ chemical compounds - polyatomic ions



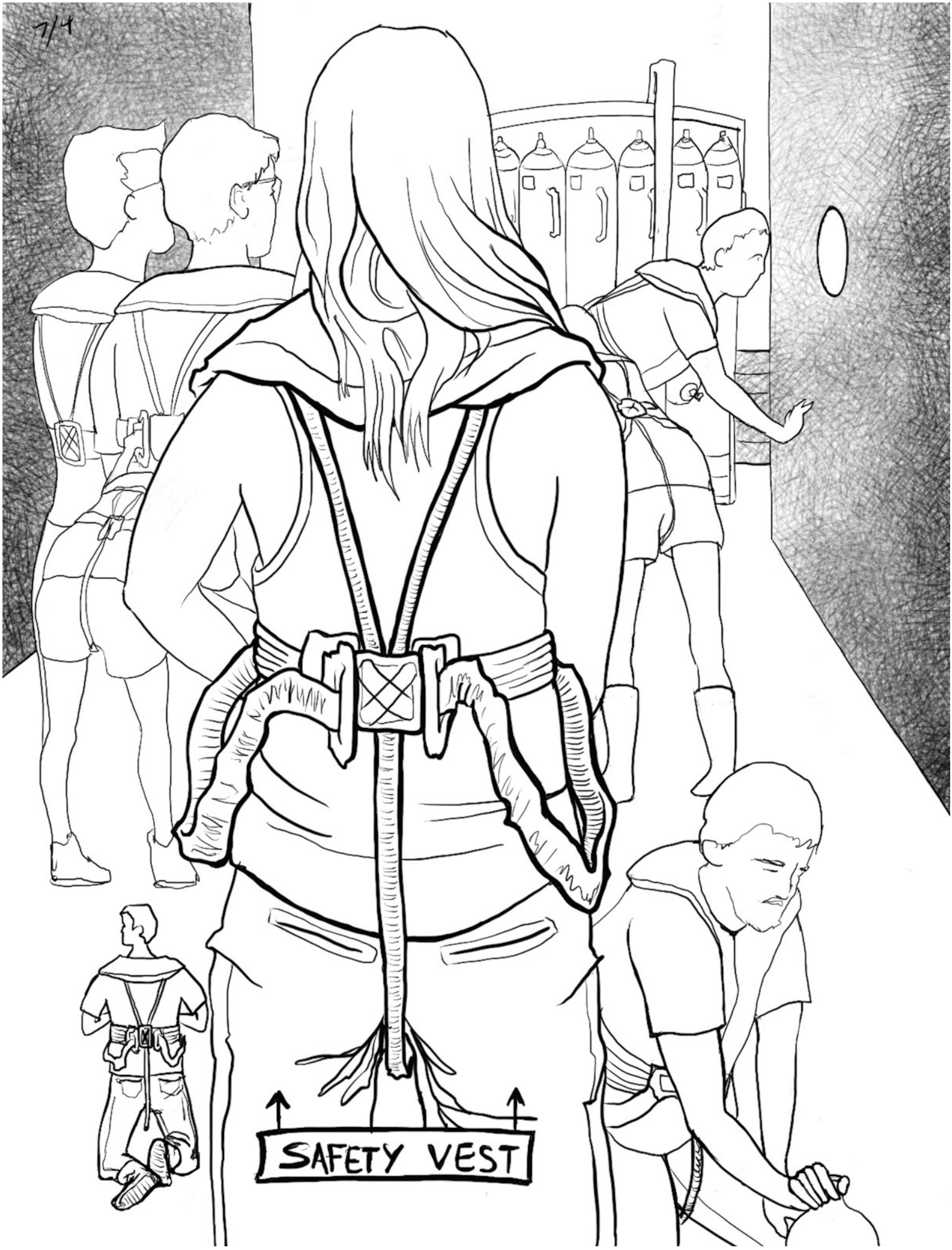
MARINE NITROGEN CYCLE:

7/3 0.2 MICRON-pore filters for purifying seawater
~Ammonia-oxidizing archea~





GETTING water off the AFT from the IN SITU device


SAFETY VEST

-Adding a standard reagent [NED & SULFANILAMIDE] to seawater. This chemical compound bonds with Nitrite and forms a supermolecule that the photometer can detect and use to measure Nitrite.

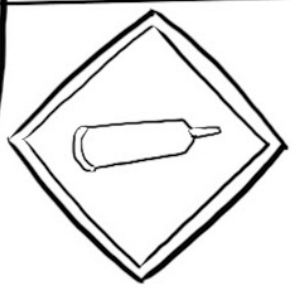
*NED - N-1-naphthyl-ethylendiamine

GHS- HAZARD PICTOGRAMS & Related Hazard Classes

EXPLODING BOMB
Explosives
self reactive

CORROSION
skin corrosion/burns
eye damage

FLAME OVER CIRCLE
oxidizing gases, liquids, solids



GAS CYLINDER
Gases under pressure

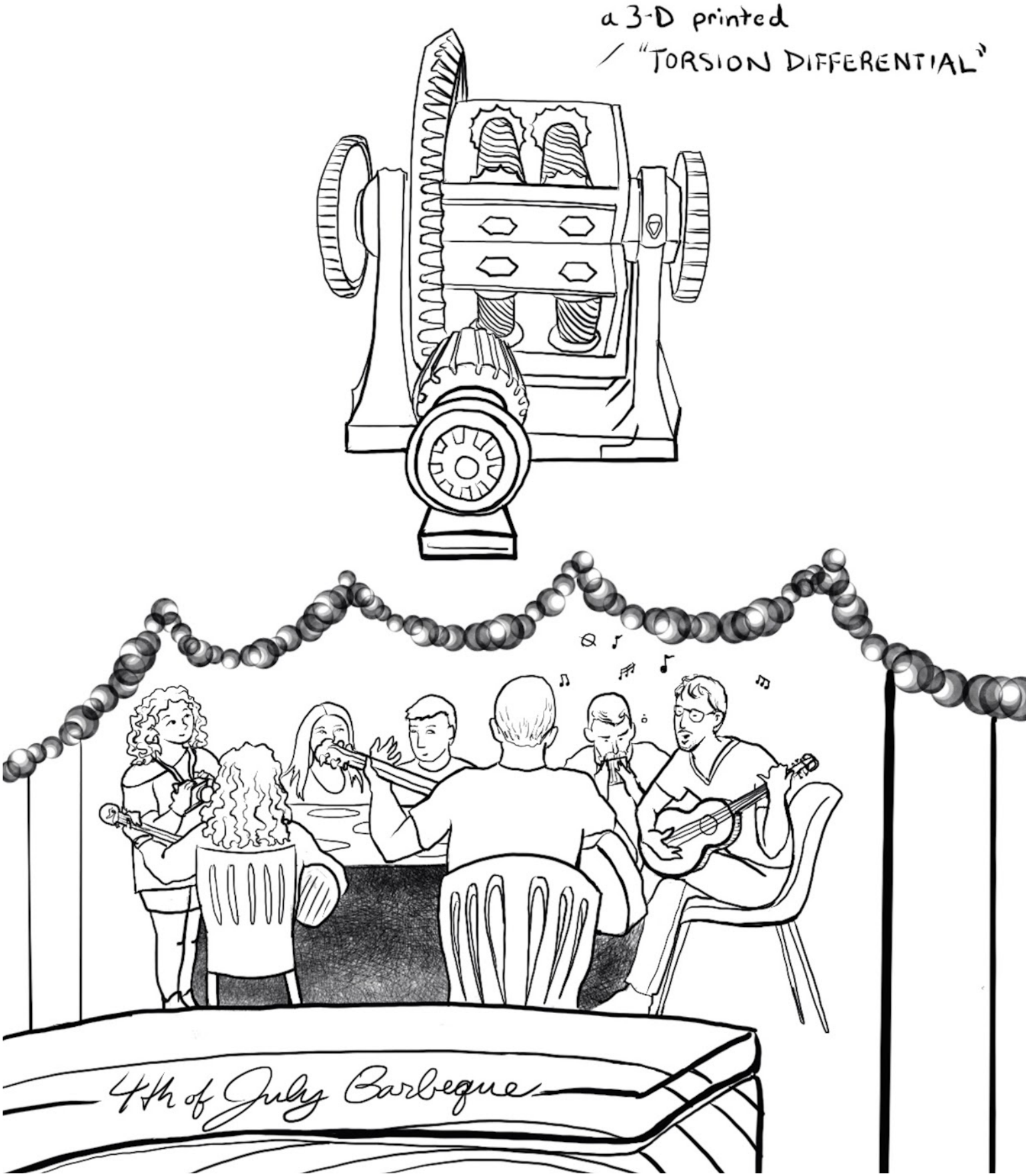
ENVIRONMENT
Aquatic toxicity

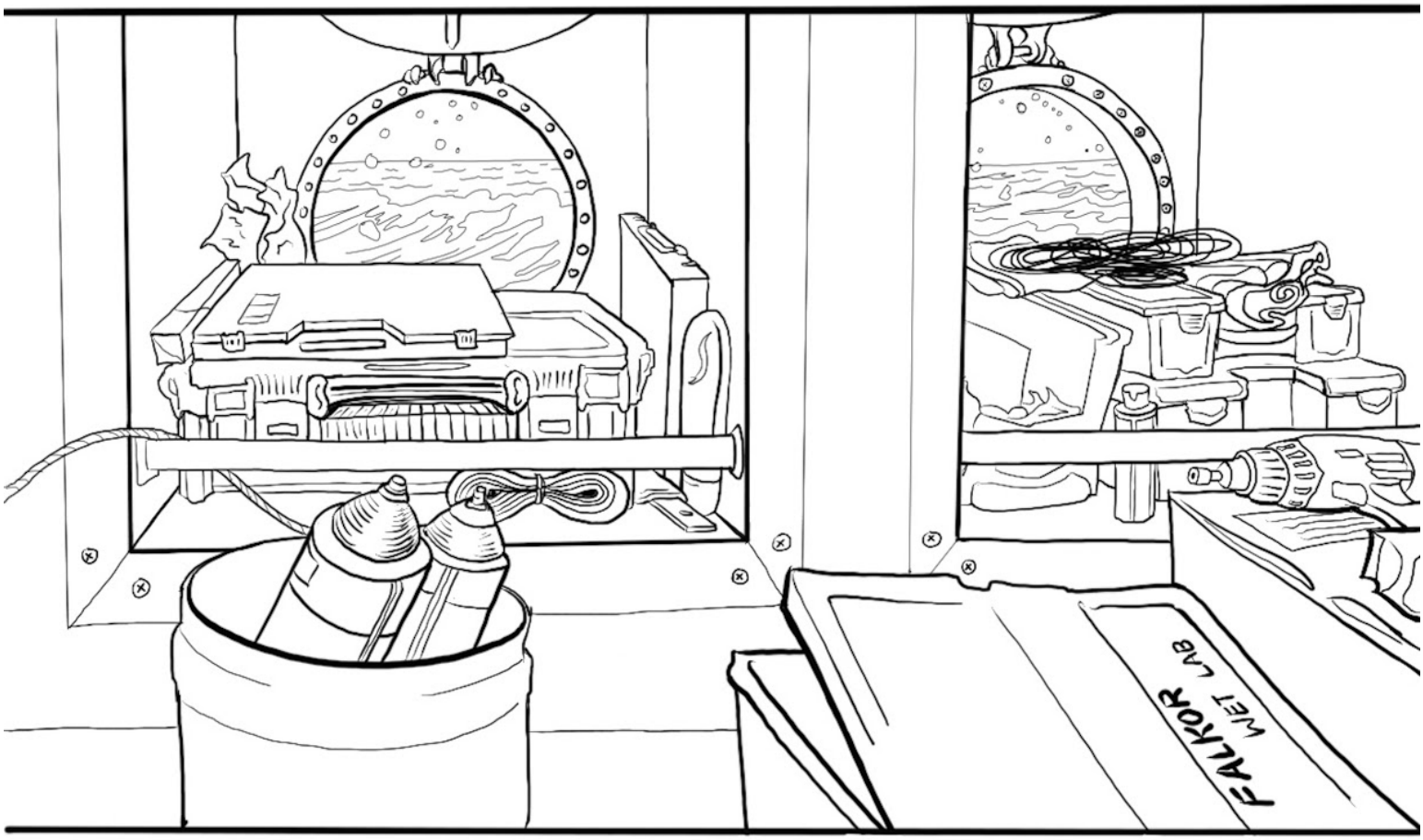
SKULL & CROSSBONES
acute toxicity (fatal or toxic)

EXCLAMATION MARK
Irritant (eye & skin)
Skin sensitizor
Acute toxicity
Narcotic effects
Respiratory tract irritant
Hazardous to ozone

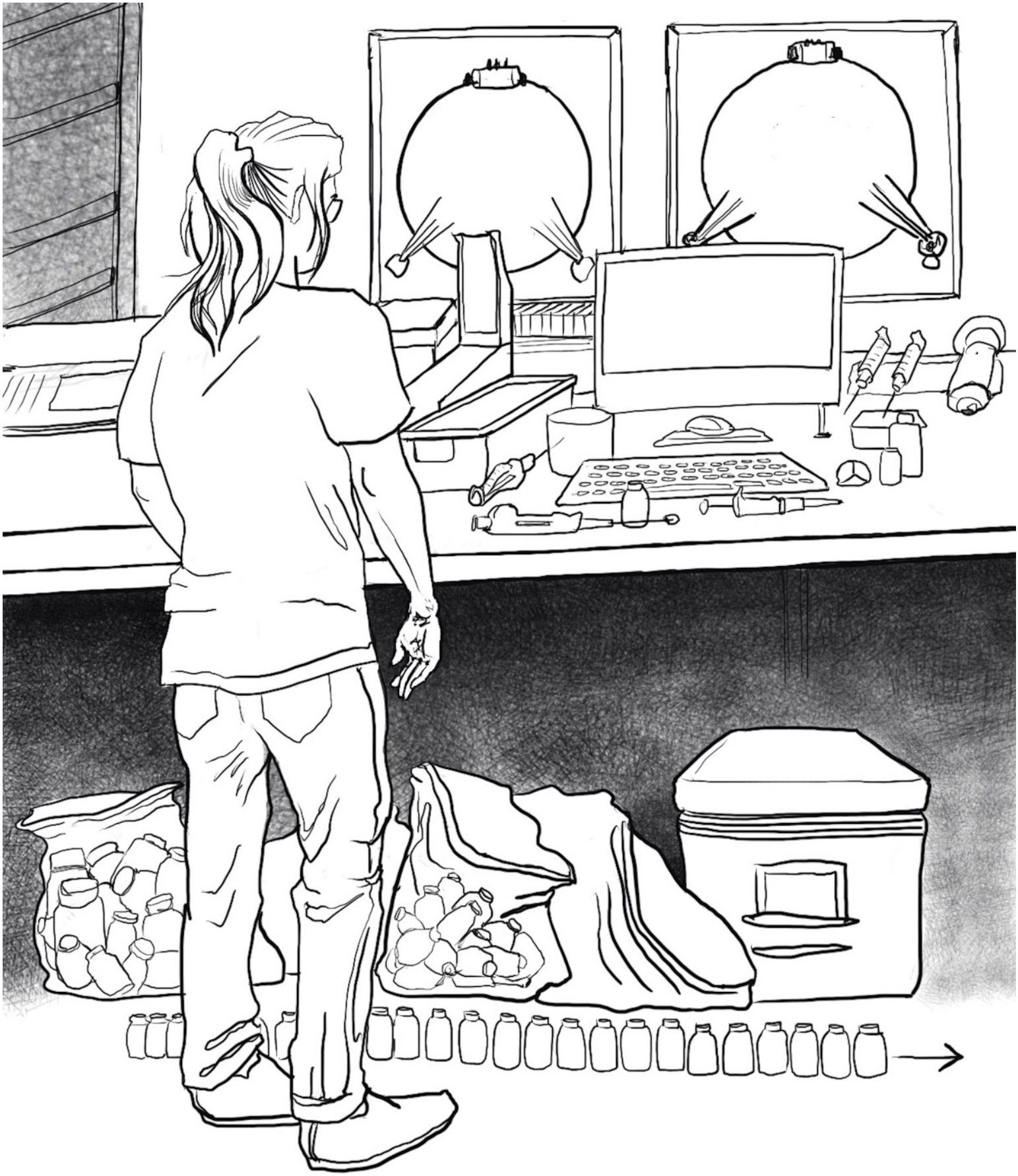
HEALTH HAZARD
Carcinogens
Mutagenicity
Reproductive toxicity
Respiratory sensitizer
Target organ toxicity
Aspiration toxicity

FLAME
Flammables, Pyrophorics
Self-heating
Emits flammable gas
self-reactives
organic peroxides


a 3-D printed
"TORSION DIFFERENTIAL"
4th of July Barbeque



7/5 - 14°N 106°W
WORK ZONE across from the WET LAB ^



RUNNING IODATE through a SPECTROPHOTOMETER
~after adding to seawater ← { 1% sulfuric acid
sulfanilamide
potassium iodide

current plan:

STATION 9 - PROCESS 2

7.6.18 Friday

5:00 Profile CTD

9:00 GoPro - INCUBATORS (IN SITU)

11:00 Incubator #1 (14hr)

↳ on deck ~ 1:00

7.7.18 Saturday (+'Frank' @ 9:00, 11:00)

3:00 Experiment CTD

6:30 Incubator #2 (14hr)

↳ on deck ~ 20:30

22:30 Incubator #3 (14 hr)

↳ on deck ~ 12:30

7.8.18 Sunday

14:30 Incubator #4 (14hr)

↳ on deck ~ 4:30

*NUTS:

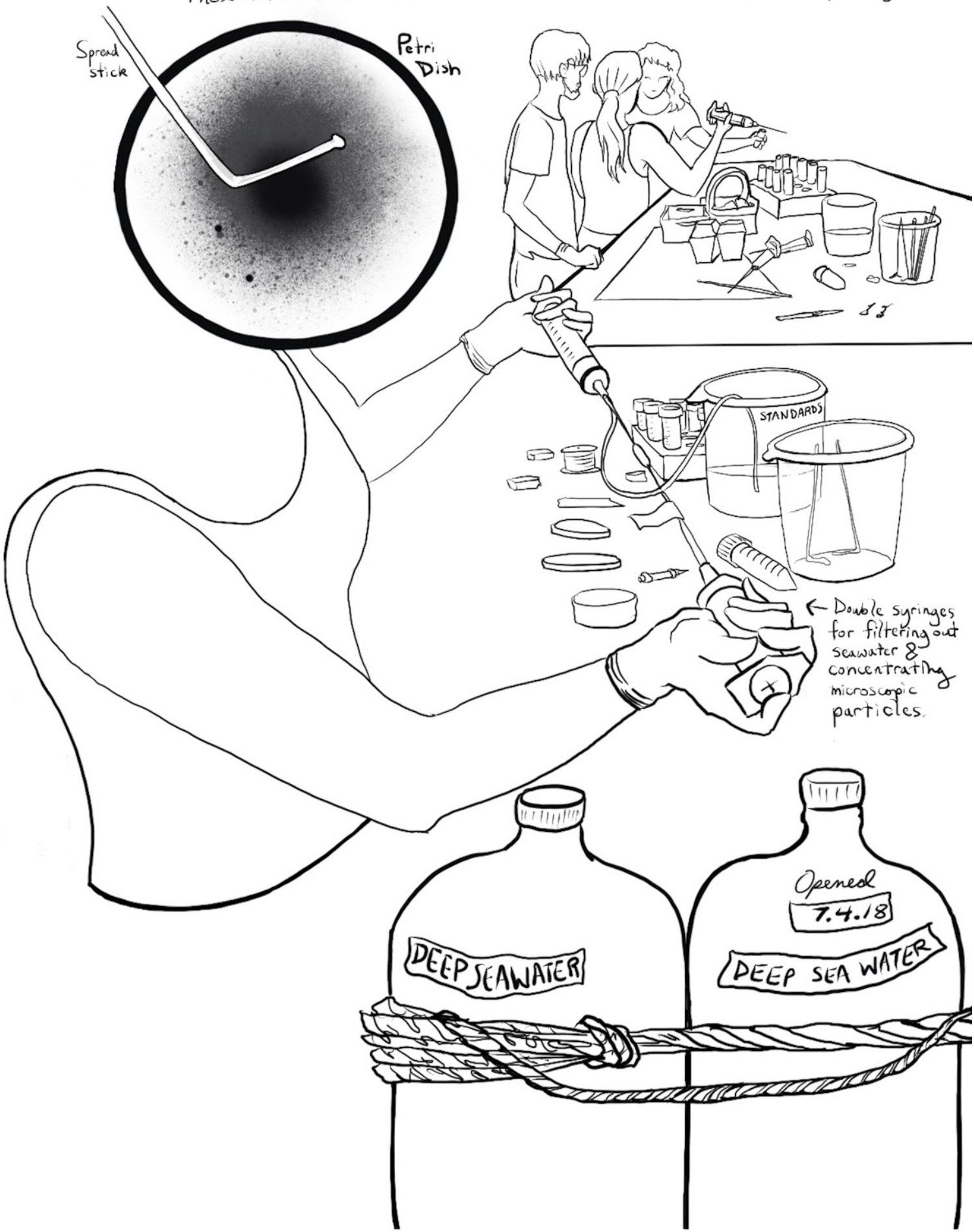
Nitrates
Nitrites
Ammoniums
Phosphates

- collecting for all 4 nutrients ('nuts') at these stations
→ Nutrient profiles

...plans subject to change

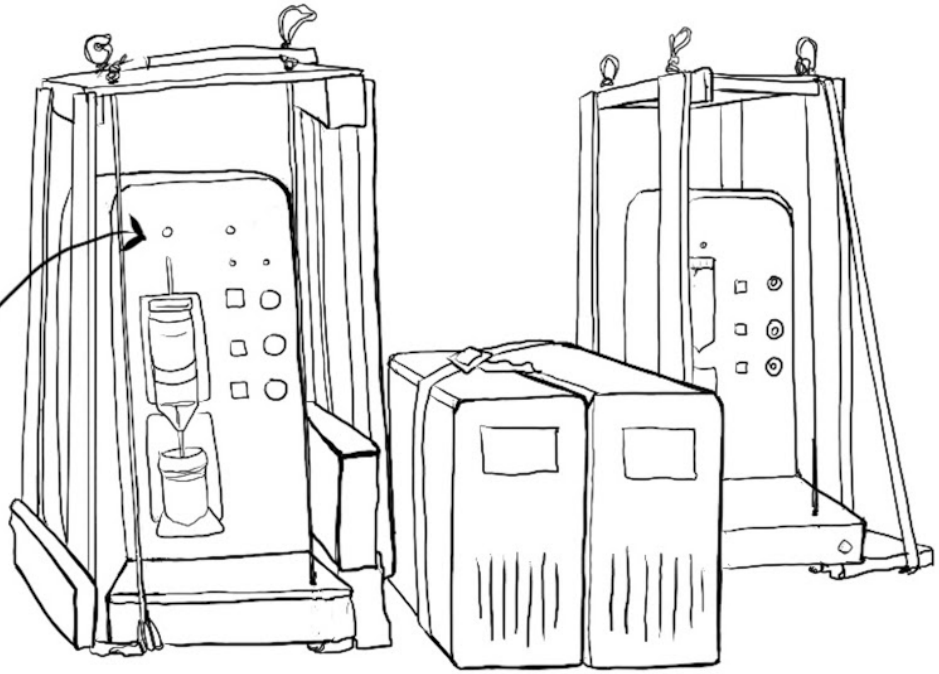
- running seawater samples through the spectrophotomer for NITRATE.

7/6 Seawater filtered & concentrated using 0.2 micron-pore filters is swabbed onto auger in a petri dish to allow for cultures to grow. These will be frozen & taken back to onshore labs or sent off for DNA sequencing.

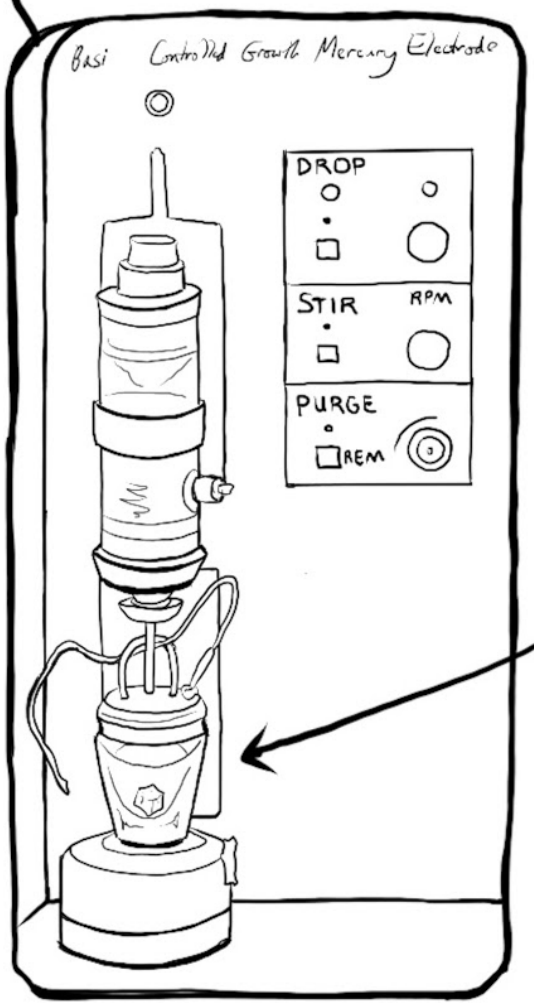


1/1

IODATE-measuring devices, stabilized with ingenuity



CGME- Controlled Growth Mercury Electrode

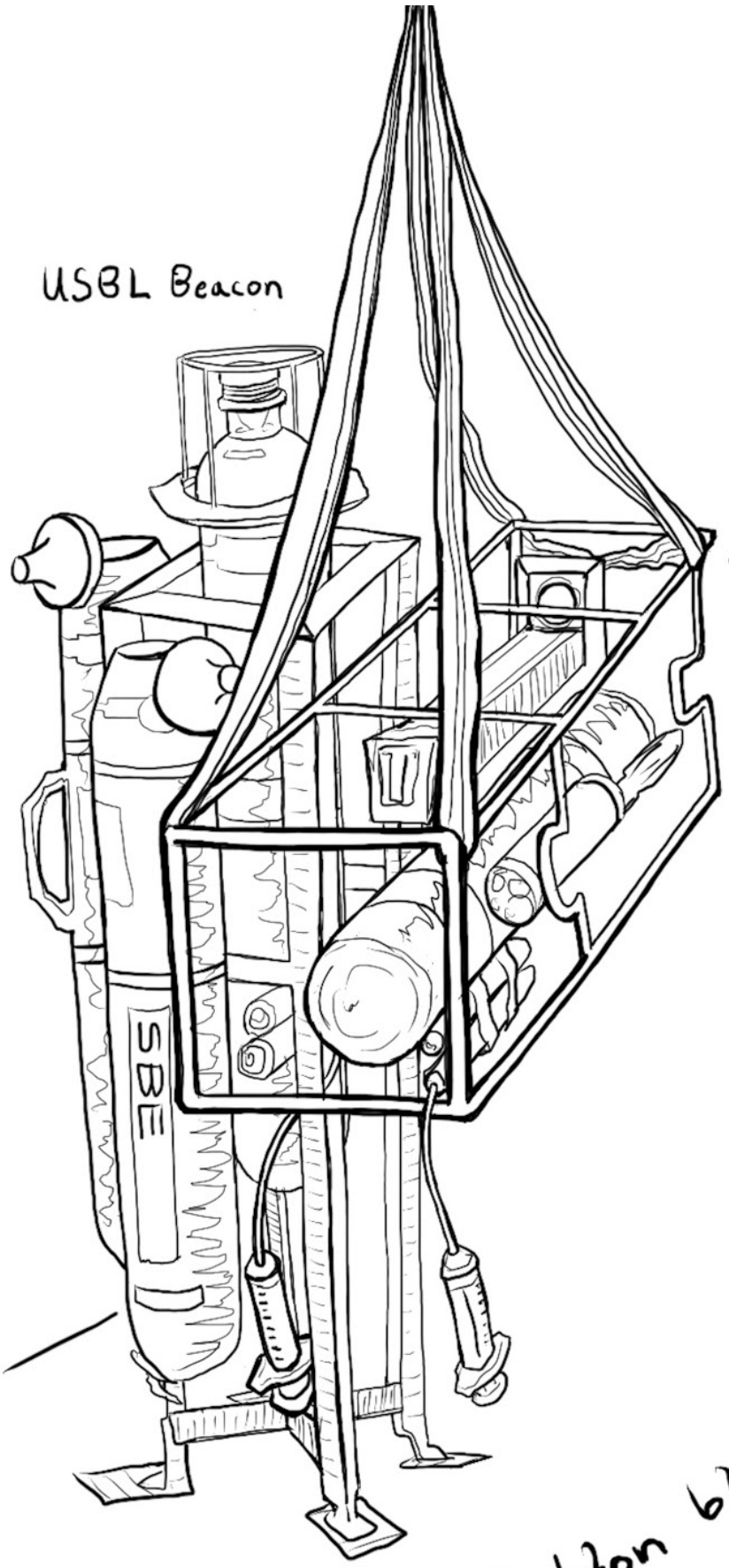


7/7

meet FRANK:

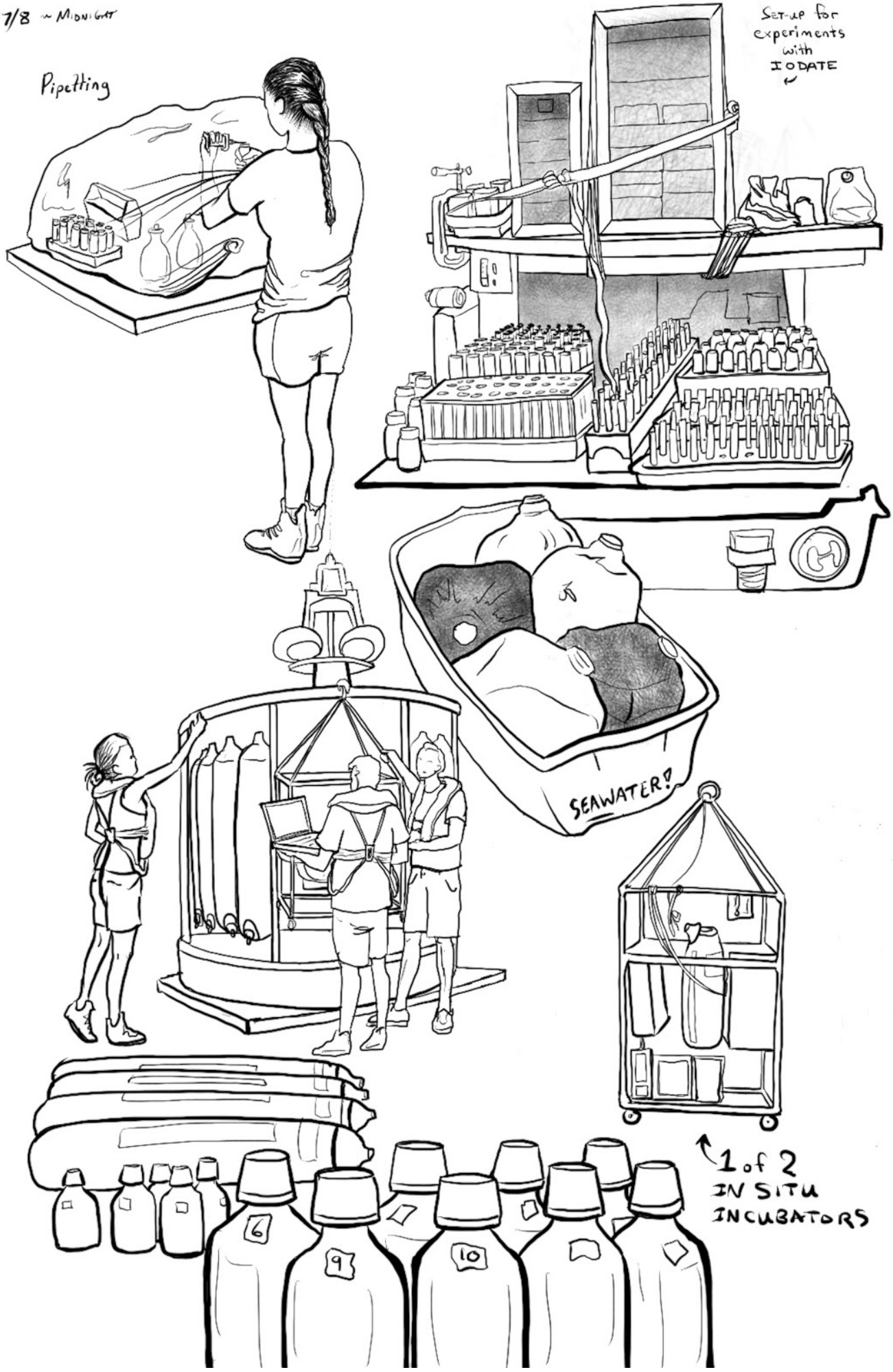
FRANK GETS cast off the STERN . . . . .

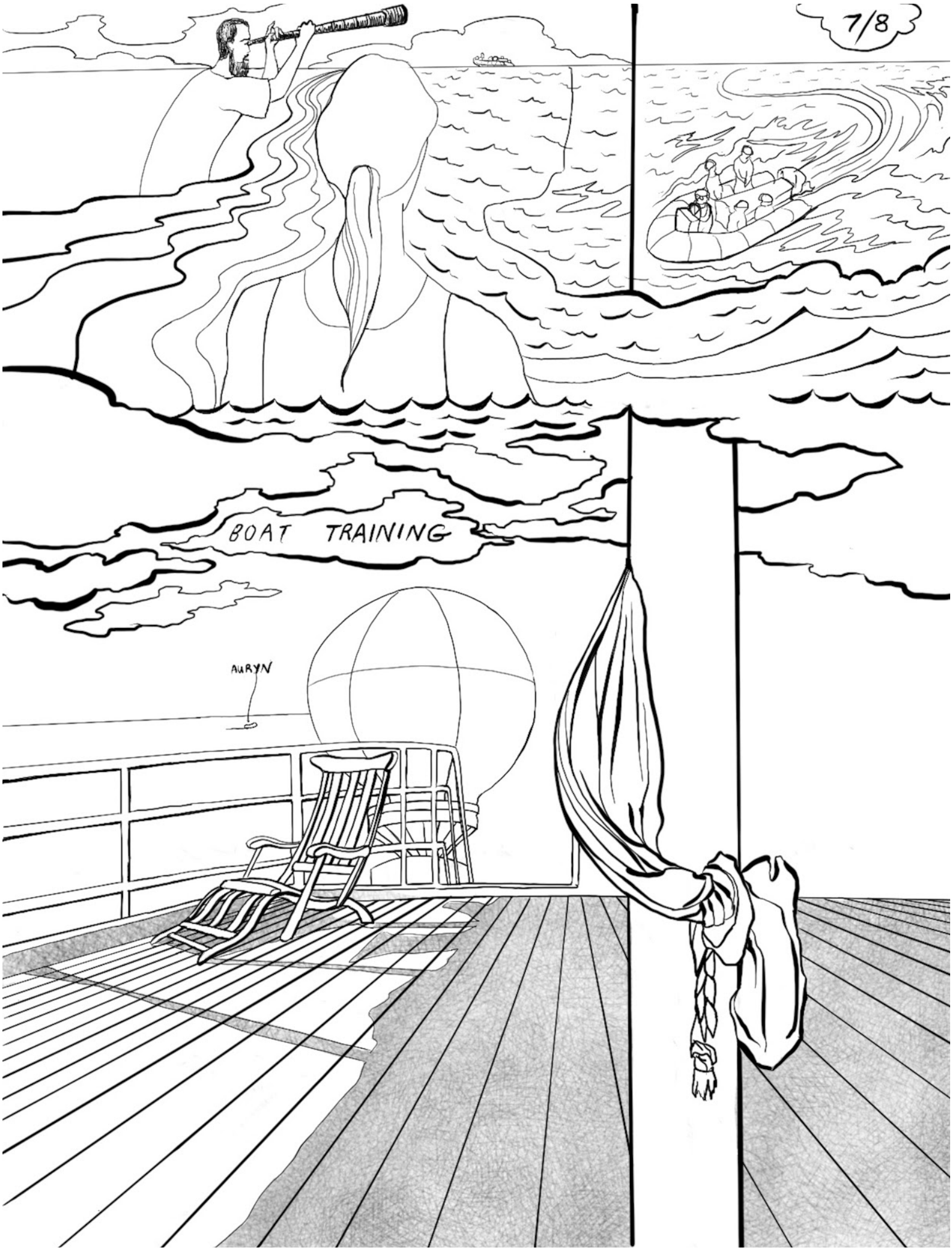
-3 Niskin bottles are triggered shut simultaneously at a desired depth, remotely, from the control room



SIDENOTE:
We are currently experiencing a **phytoplankton bloom!**

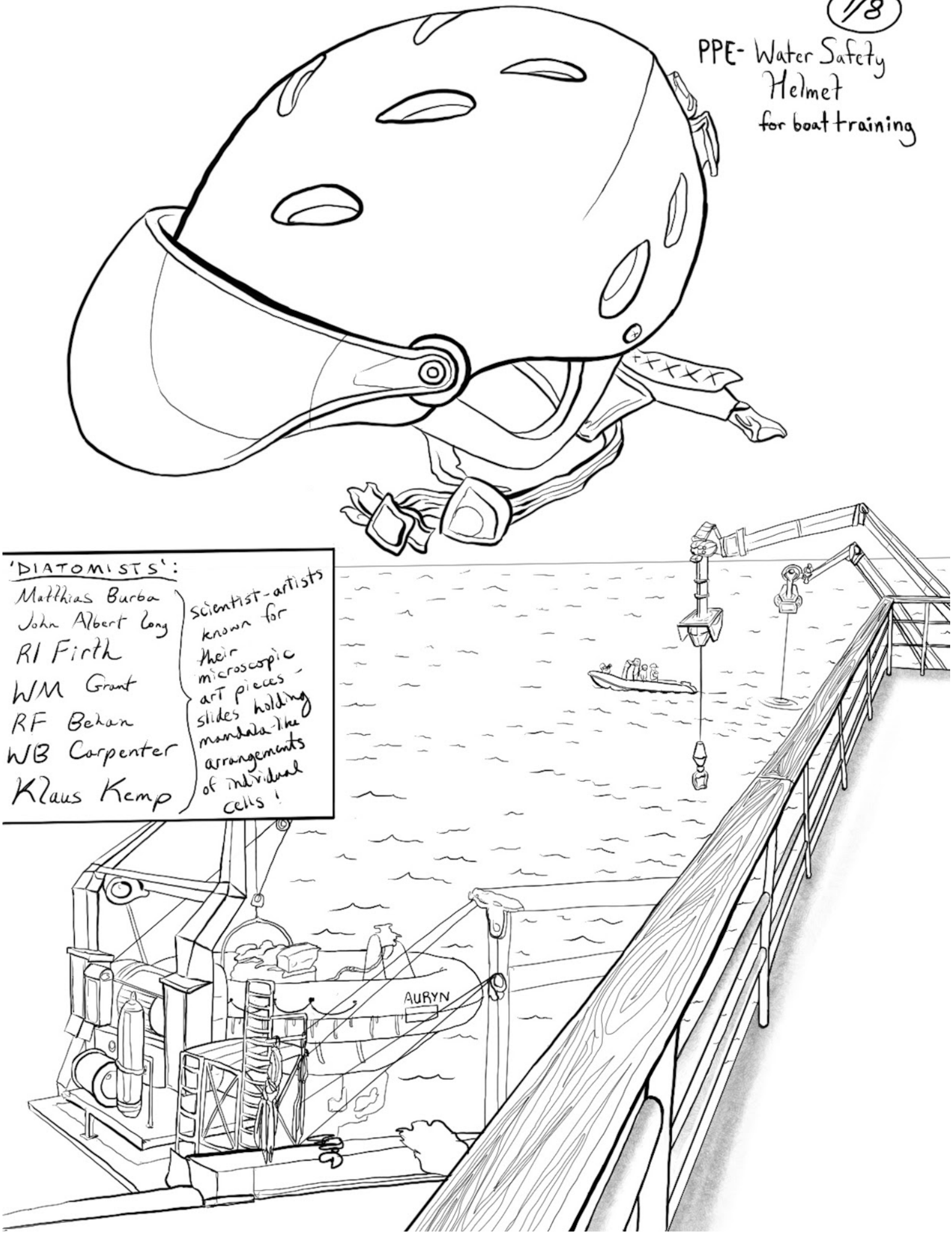
7/8 ~ MIDNIGHT


Pipetting
SET-UP for experiments with IODATE
SEAWATER?
1 of 2 IN SITU INCUBATORS
6
9
10

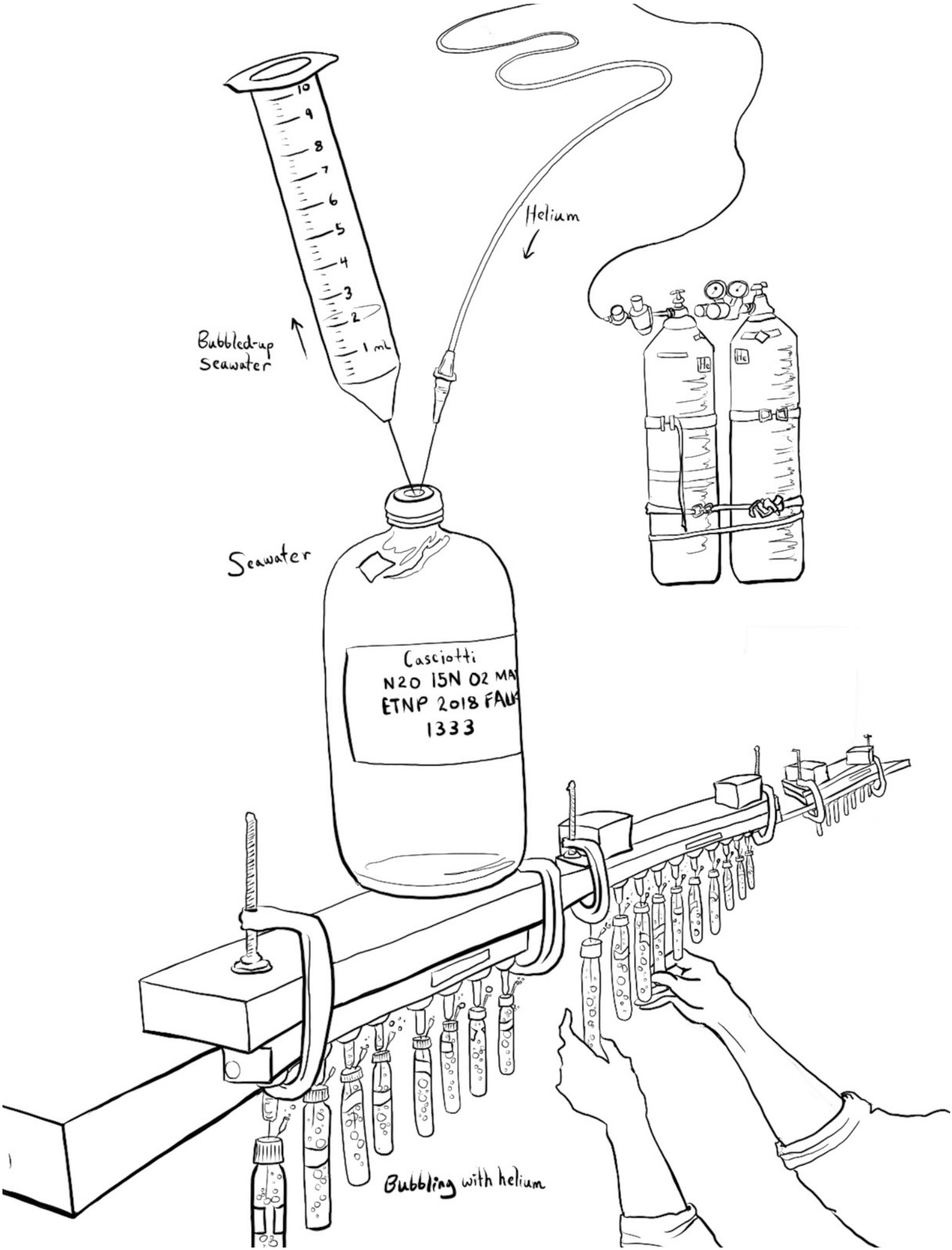

7/8
BOAT TRAINING
AURYN

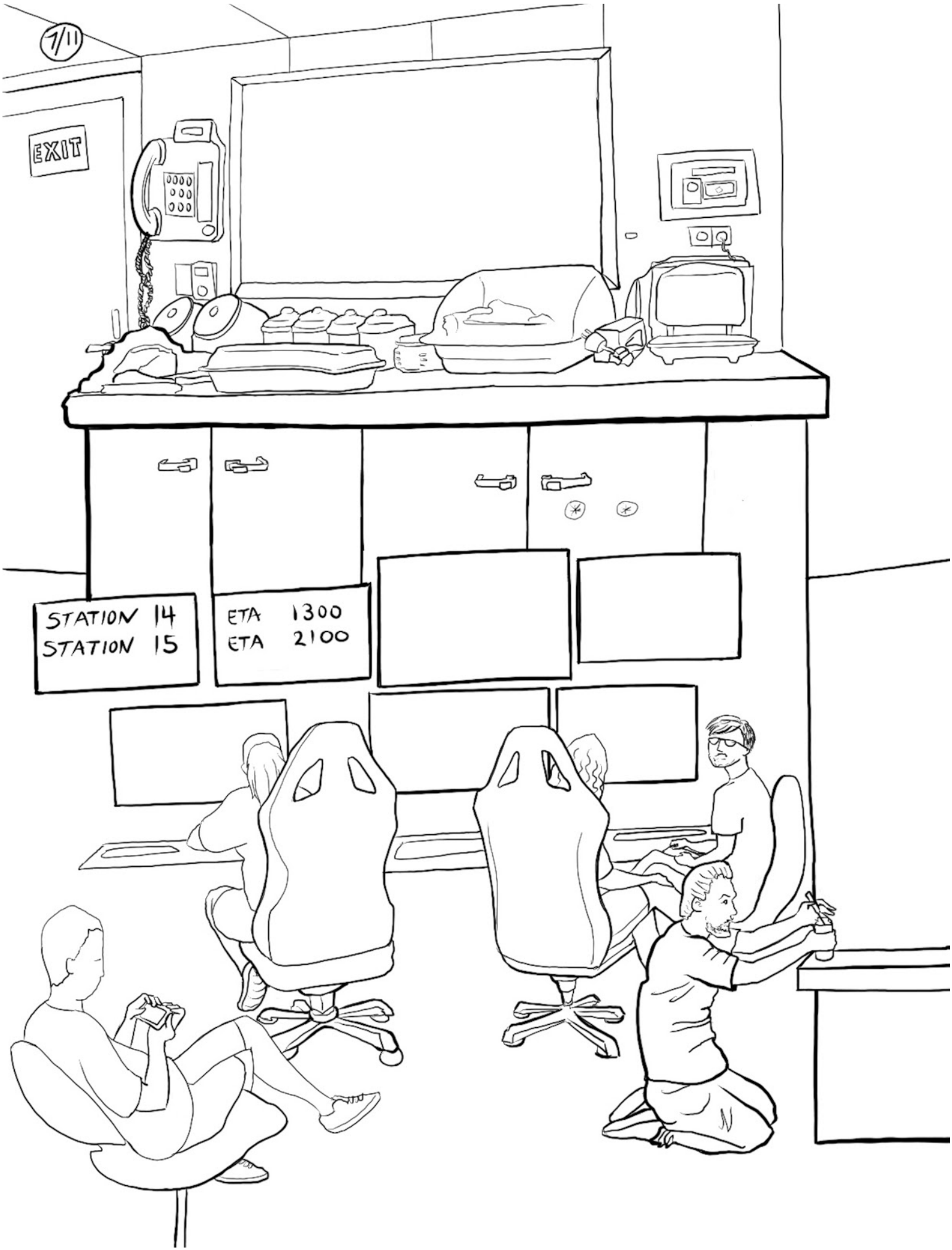
7/8 ON THE AFT
OPERATING the WINCH^
PREPARING BAGUETTES>
from PATE FERMENTE

PPE- Water Safety Helmet
for boat training



7/9
STATION 10
(No more In Situ Incubator casts)
13°N 110°W
10
R/V FALKOR


Helium
Bubbled-up seawater
Seawater
Casciotti
N20 15N 02 MA
ETNP 2018 FAL
1333
Bubbling with helium


7/11
EXIT
STATION 14
STATION 15
ETA 1300
ETA 2100

attempting to capture the flurry of water collecting with a gesture sketch.
7/12
STATION
16

7/15

BACTERIA:

Look up the BLANKS

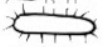
- e. coli - (freshwater)

- volvox - (colony-forming)

- planktomycede Anammox -

- plasmids (HGT) -

-



- pseudomonas -

- trichodesmium - nitrogen-fixing

- prochlorococcus - photosynthetic
  500 nanometers - thrive in low-oxygen, low-light environments

- Richelia intracellalaris -

- Nodularia spumigena - (fresh/coastal)

- Aphanizimenon - (fresh/coastal)

MARINE BACTERIA:

CHEATSHEET - PARTIAL, NOT COMPLETE :)

NITROGEN FIXERS

- Trichodesmium - colonial cyanobacteria, "sawdust," uses nitrogenase enzyme (nifH)
  - can "fix" Nitrogen during daylight
  - ↳ inactive in rich oxygen zones
- Heterocystus cyanobacteria - (eg. genus Richelia, which usually lives in diatoms)
- Crocosphaera - prefers tropical waters (75°F), fixes nitrogen at night, photosynthesizes during the day
  - 2-4 μm (microns) wide
- UCYN-A - discovered in 1998 near Hawaii
- Alphaproteobacteria & gammaproteobacteria - diverse group. some carry nifH gene

MARINE BACTERIA INVOLVED IN NITRIFICATION

- Ammonium oxidizing bacteria (AOB) - convert ammonium to nitrite ('ammonium Oxidation')
  - most are betaproteobacteria & gammaproteobacteria
- Nitrite oxidizing bacteria - convert nitrite to nitrate (nitrite oxidation)

MARINE PRIMARY PRODUCERS THAT DO NOT FIX NITROGEN

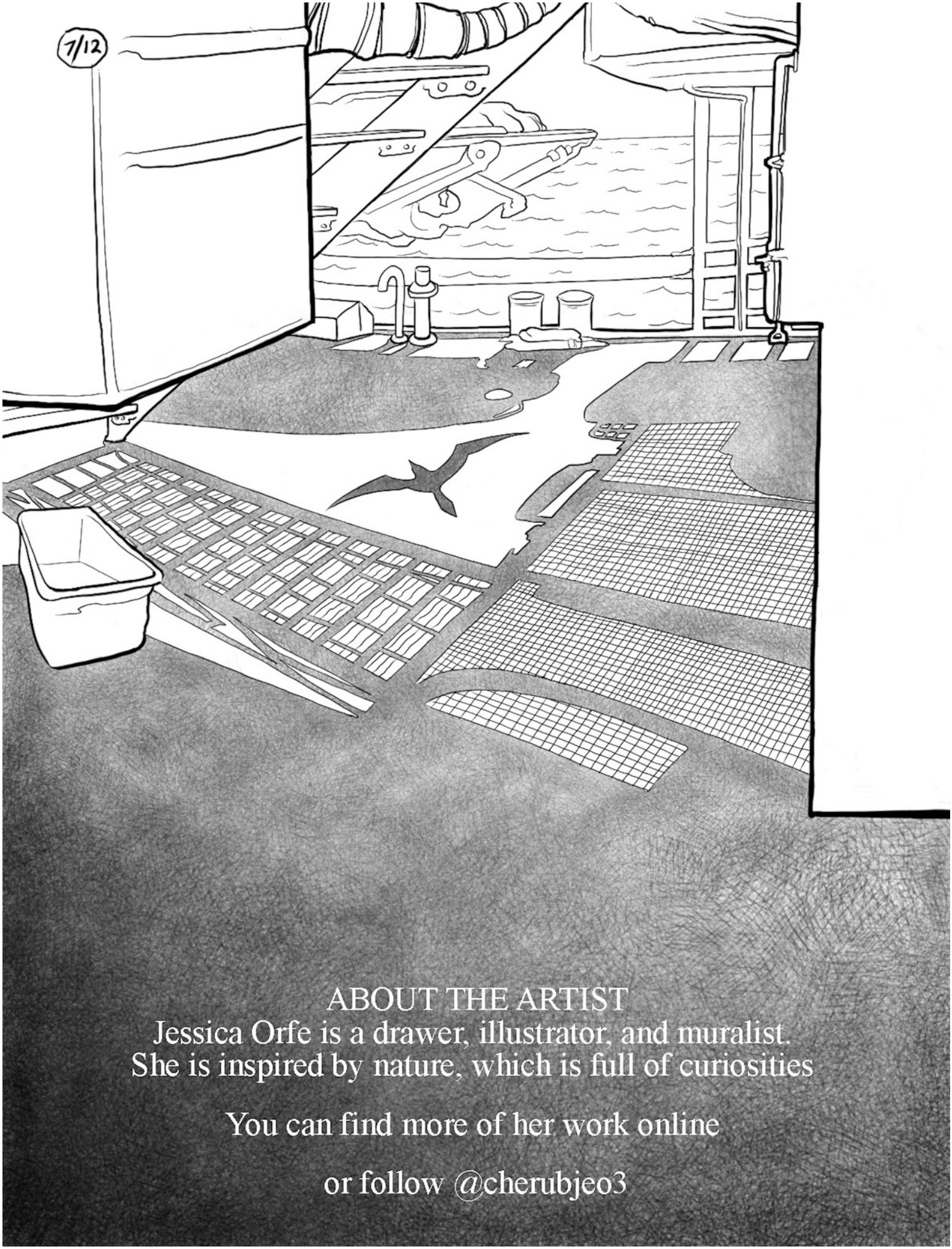
- Prochlorococcus - 0.5 - 0.8 μm
  - obligate photoautotrophs that use photosynthesis but need Nitrogen as a nutrient
- Synechoccus - 0.8 - 1.5 μm
  - obligate phototrophs that require Nitrate as a nutrient

MARINE ARCHEA:

~ a separate DOMAIN from BACTERIA

- Ammonium-Oxidizing Archea (AOA) - perform ammonium oxidation:
  $(NH^4 \rightarrow NO_2)$
  - may produce nitrous oxide
  - use enzyme ammonium monooxygenase (amoA)

~ Sourced from CMORE SOEST



ABOUT THE ARTIST

Jessica Orfe is a drawer, illustrator, and muralist.
She is inspired by nature, which is full of curiosities

You can find more of her work online

or follow @cherubjeo3

John Albert Long

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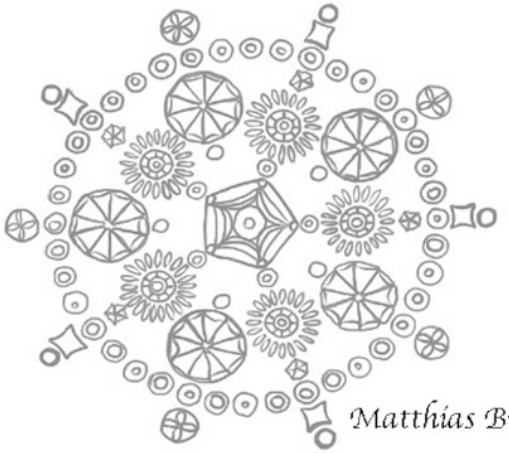
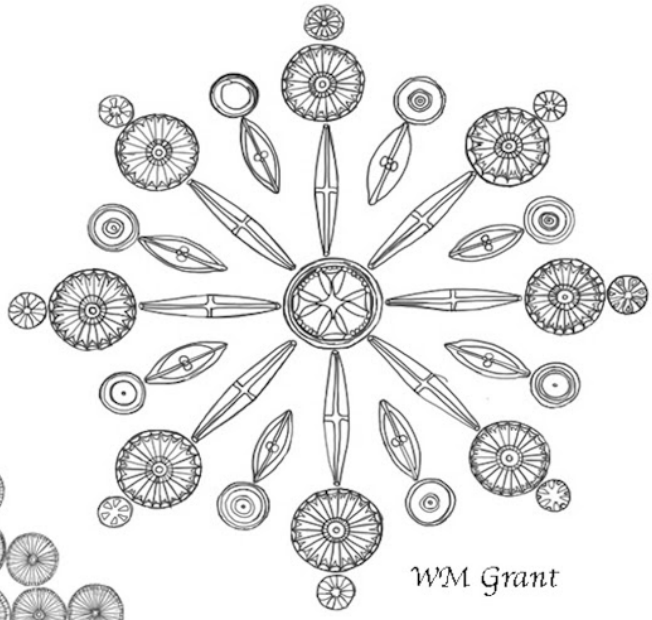
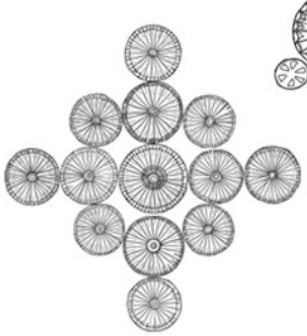
RI Firth



Matthias Burba



WM Grant



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