Introduction:

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Zooplankton Ecologist and Biological Oceanographer

Founding PI, *California Current Ecosystem* Long Term Ecological Research site

Former Curator, Pelagic Invertebrate Collection, Scripps Institution of Oceanography

Lead PI, *Zooglider* project, an autonomous vehicle for optically and acoustically sensing zooplankton

Published > 170 peer-reviewed scientific papers

SCRIPPS INSTITUTION OF OCEANOGRAPHY UCSD

Today's Agenda

I. Phytoplankton biomass assessment by Chlorophyll *a*

II. Secchi disk as a proxy for beam attenuation coefficient

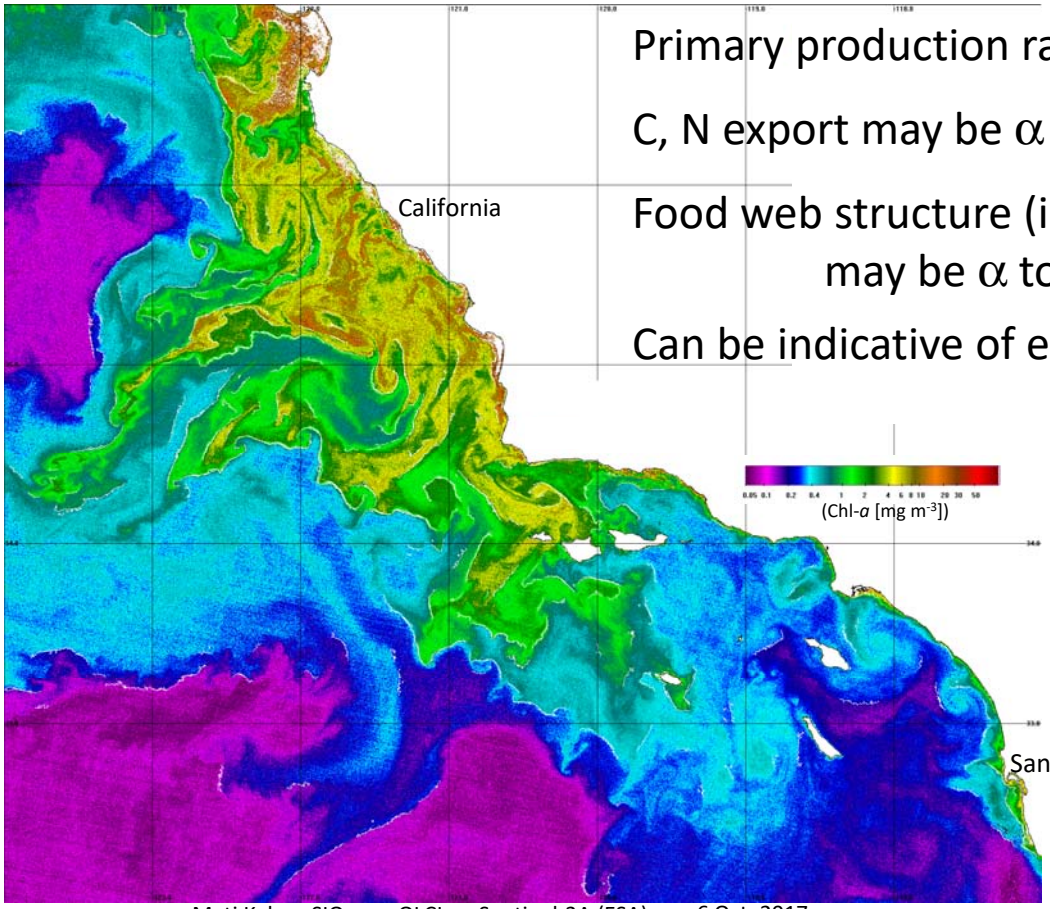
III. Neuston sampling by Manta net, for suspended microplastics

IV. Recognition of micro- and nanoplastics using epifluorescence microscopy

V. (Mesozooplankton sampling, splitting, and fixation)

VI. Archiving samples, curation, and databases

Remote Sensing estimates of phytoplankton biomass (as Chl-*a*)



Mati Kahru SIO OLCI on Sentinel-3A (ESA) 6 Oct. 2017

Primary production rate is often α to Chl-*a* biomass

C, N export may be α to Chl-*a* biomass

Food web structure (incl. larval fish feeding success) may be α to Chl-*a* biomass

Can be indicative of eutrophication

<u>Remote sensing:</u>

- Requires sea truth calibration
- Senses only near-surface phytoplankton
- Does not resolve phytoplankton community structure
- Only cloud-free, daytime images

I. Chlorophyll *a*

Primary photosynthetic pigment, used by all phytoplankton

(most algae also have accessory pigments)

Can be used as a proxy for biomass of phytoplankton

(requires knowledge of the conversion of C:Chl-*a* or N:Chl-*a*)

Measureable at very low concentrations using fluorometry

(can also measure spectrophotometrically, by HPLC,
and by remote sensing [usually ratio of blue:green absorbance])

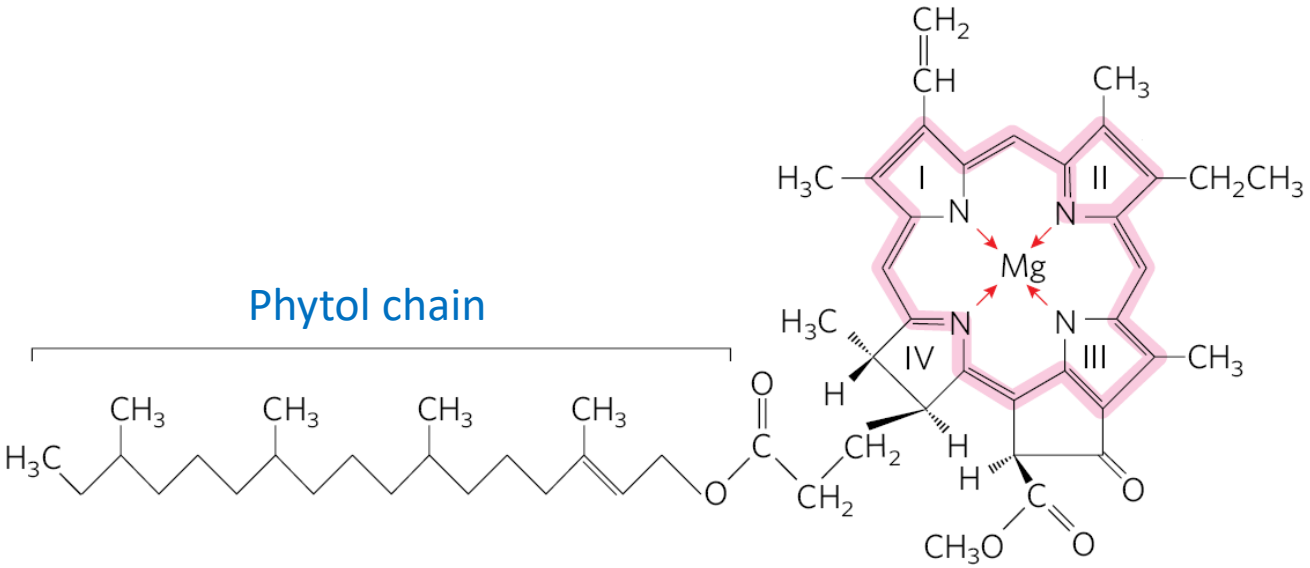
Difference between:

- **extracted** Chl-*a* (phytoplankton filtered, Chl-*a* extracted with organic solvents, measured in solution)
- ***in vivo*** Chl-a (measured in living cells)

Size fractionation as a proxy for community structure

Structure of Chlorophyll *a*

Porphyrin ring



fluorescent

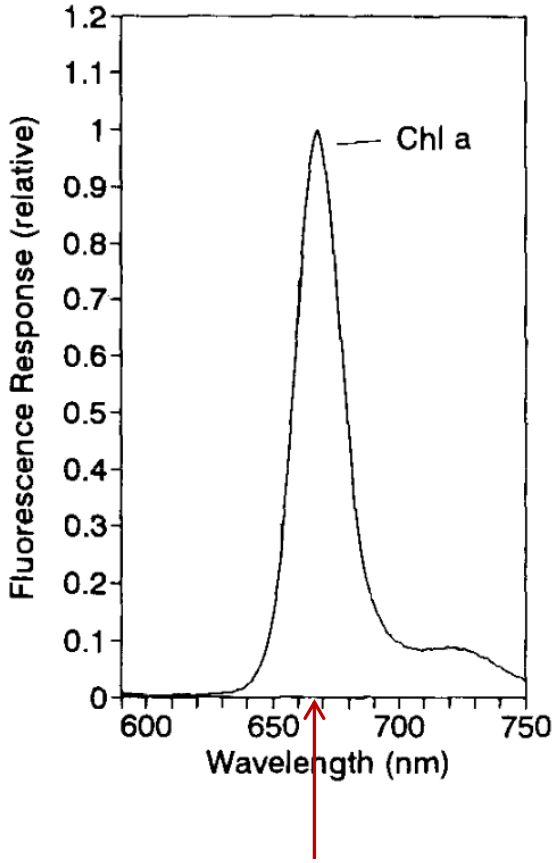
Lehninger, *Principles of Biochemistry*

Chlorophyll *a* fluorescence in 90% acetone

Excitation: ~ 440 nm (blue)

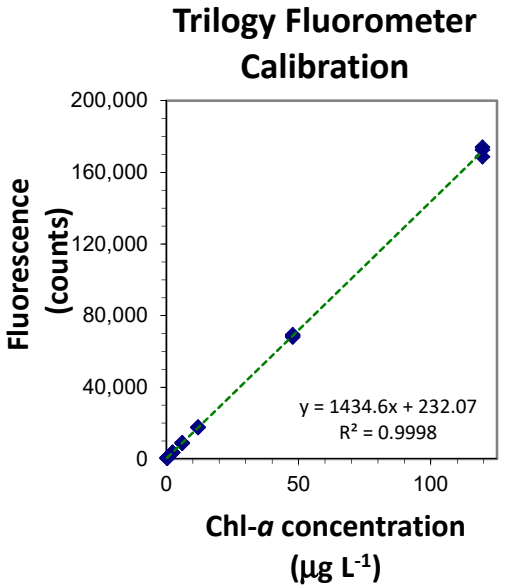
Fluorescence Emission peak:
~667 nm (red)

(Fluorescence assay is much more sensitive than spectrophometry)



Welschmeyer (1994) modified

Calibration relationship

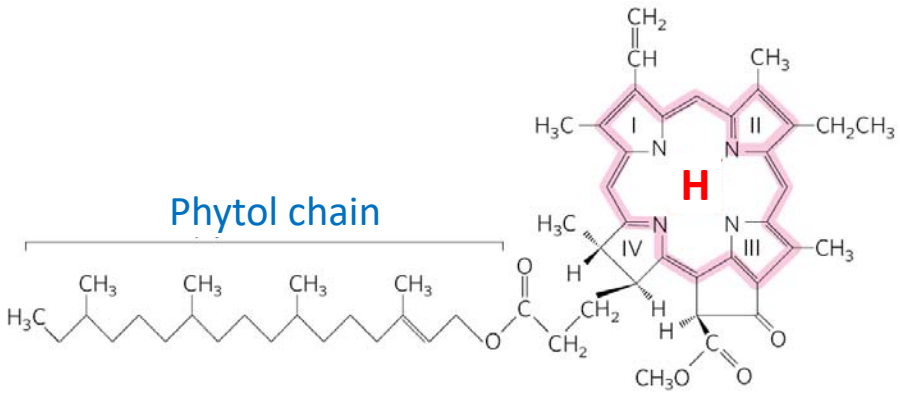


(in lab)

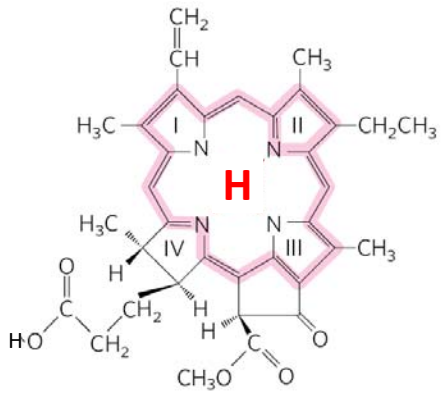
(purchase pure Chl-*a* for calibration e.g., from Sigma-Aldrich)

Common breakdown products of Chlorophyll *a*

Phaeophytin *a*



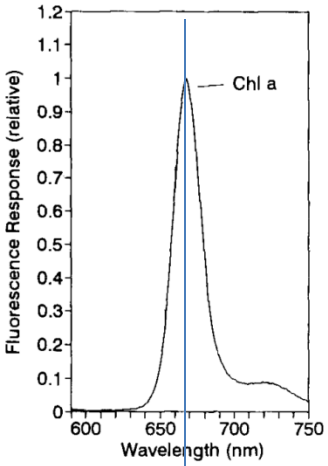
Phaeophorbide *a*



"Phaeopigments" = Phaeophytin + Phaeophorbide

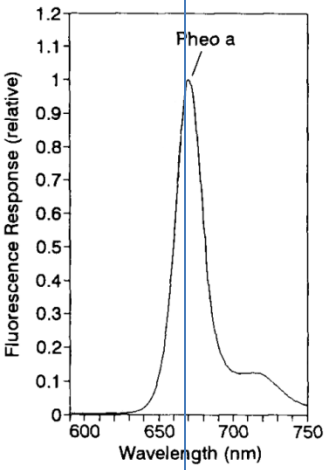
See Goericke et al. (2000) for more complete analysis

Fluorescence spectra of Chl-*a* and Phaeophytin-*a*



Chlorophyll *a* fluorescence in 90% acteone

blue light excitation



Phaeophytin *a* fluorescence in 90% acteone

Welschmeyer (1994) modified

Very similar fluorescence spectra

∴ Can analyze both Chl-*a* and phaeopigments

by measuring red fluorescence when excited with blue light

Measurement of Chl-*a* and Phaeopigment concentrations by difference

A. Measure total fluorescence (due to Chl-*a* + Phaeopigments) in 90% acetone
   (blue excitation, red fluorescence)

B. Acidify w/ 1N HCl to convert all intact Chl-*a* to Phaeopigments

C. Measure total fluorescence again

D. (Fluor. before acidification [F_0] – Fluor. after acidification [F_a])
   permits separation of amount of Chl-*a* from amount of Phaeopigments in the original sample*

*when corrected for the acid ratio *Tau*, for pure Chl-*a*

Laboratory assay for Chl-*a* and Phaeopigments by Fluorometry

Sample Collection and filtration

1. Collect water sample with Niskin bottle or other water bottle
2. Filter sample onto GFF glass fiber filter (nominal pore size ~ 0.7 µm)
3. Freeze filters at ≤ -20° C

Extraction of pigments in 90% acetone

4. Place filter in 90% acetone
5. Optional step: Disrupt cells with sonicator or tissue grinder
6. Place extract in the dark at ≤ -4° C for 24 h to extract pigments
7. Invert to mix, then centrifuge in clinical centrifuge to pelletize particulates

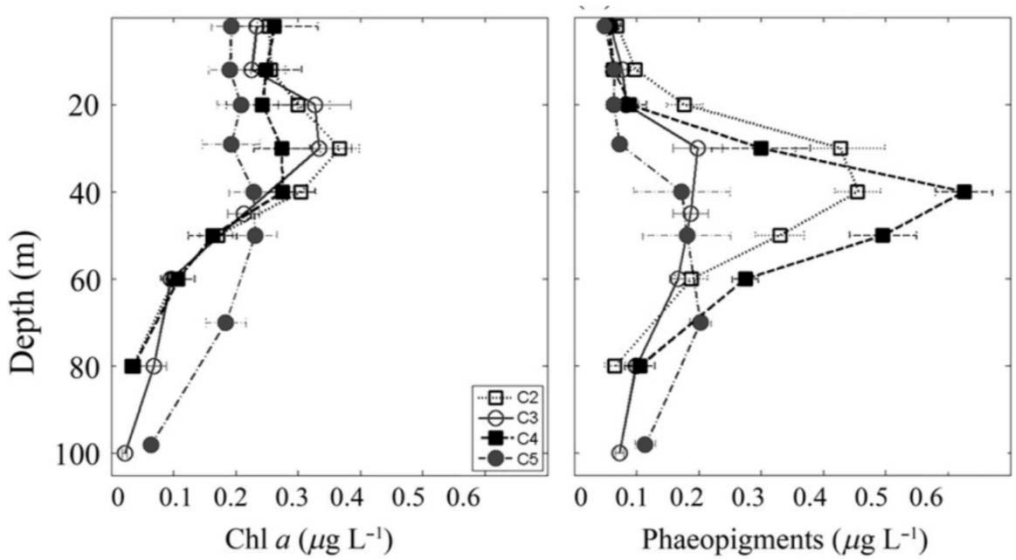
Analysis of pigments by Fluorometry

8. Analyze fluorescence on Trilogy fluorometer before acidification (F_o)
9. Add 2 drops of 1 N HCl
10. Analyze fluorescence on Trilogy fluorometer after acidification (F_a)
11. Calculate Chl-*a* and Phaeopigment concentrations by application of fluorometer calibration

Sometimes surprisingly high concentrations of Phaeopigments
in situ

Chl-*a*

Phaeopigments



Costa Rica Dome
Eastern Tropical Pacific

Décima et al. (2018) L&O

in comparison: Exponentially growing phytoplankton in culture
usually show negligible phaeopgiments

Size Fractionation of Chl-*a*

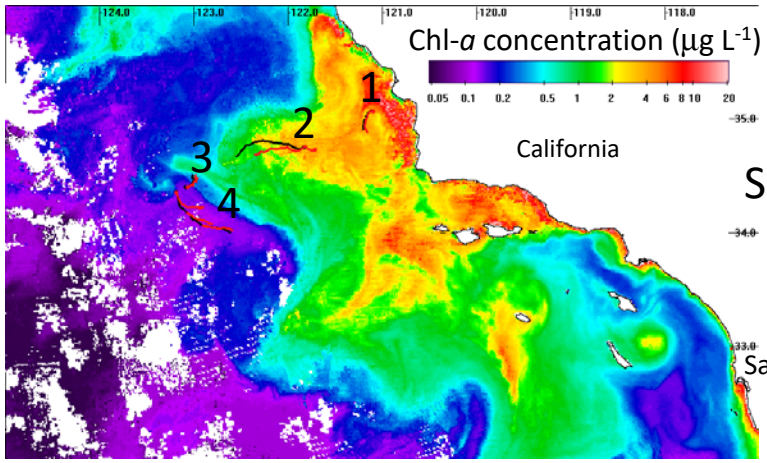
Approximation of changes in phytoplankton 'community structure'
Ignoring taxonomic, genetic, physiological, and functional differences
Considering only cell size

Simplified Size Fractionation of Chl-*a*

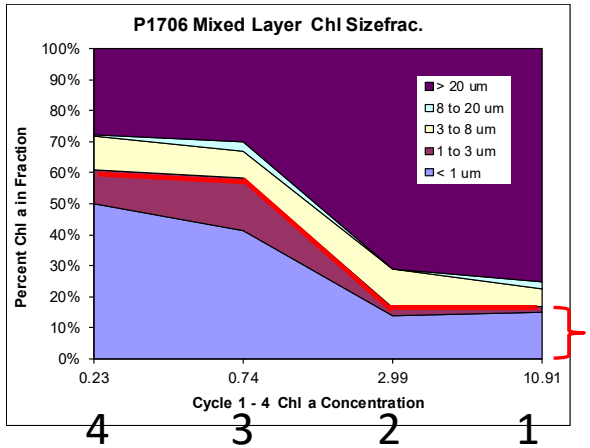
Filter an aliquot and analyze "Total" Chl-a retained on a GFF glass fiber filter
Filter another aliquot and analyze Chl-*a* retained on a 2.0-µm polycarbonate filter

By difference obtain: GFF to 2-µm "Picoplankton" – primarily *Synechococcus*, some *Prochlorococcus*
> 2-µm Nano- and Microplankton

Size Fractionation of Chl-*a*



Satellite-estimated total Chl-*a*



‘Cycle’ 4 3 2 1

Size-fractionated Chl-*a*
Filtered water samples

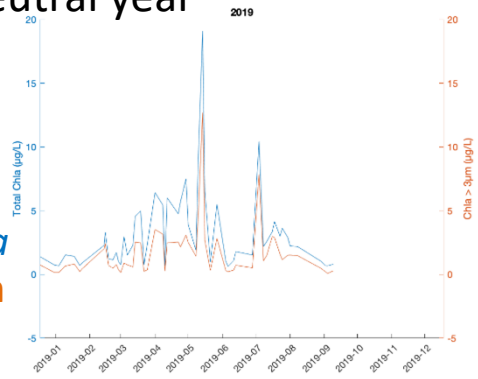
} < 3 µm

(mixed layer)

R. Goericke

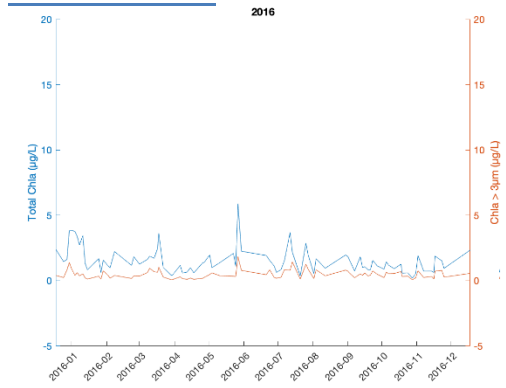
Example of the importance of separating
picoplankton from larger phytoplankton by size fractionation

Neutral year



Total Chl-*a*
Chl-*a* > 3 µm

El Niño



Picoplankton
Especially important for the microbial food web and recycling

Microphytoplankton
Often more important for the metazoan food web,
C export, sometimes Fisheries

Ocean Institute, CA Coastal time series

II. Secchi disk as a proxy for beam attentuation coefficient

Ocean optical transparency is affected by living plankton, detritus/marine snow, dissolved organic matter, and suspended inorganic matter (e.g., dust, sediments)

Attributable to absorption + scattering

Decreased transparency can be indicative of eutrophication, pollution

Transparency can be α to phytoplankton biomass

Transparency related to encounter volume with prey for sight-hunting fishes and other visual predators

Useful for defining the depth of the euphotic zone

Ocean Bio-optics

An advanced field far beyond my expertise

There are many types of instruments and approaches to characterize
suspended and dissolved substances

Relevant to calibration/validation of satellite remote sensing methods

One of the oldest and simplest methods:

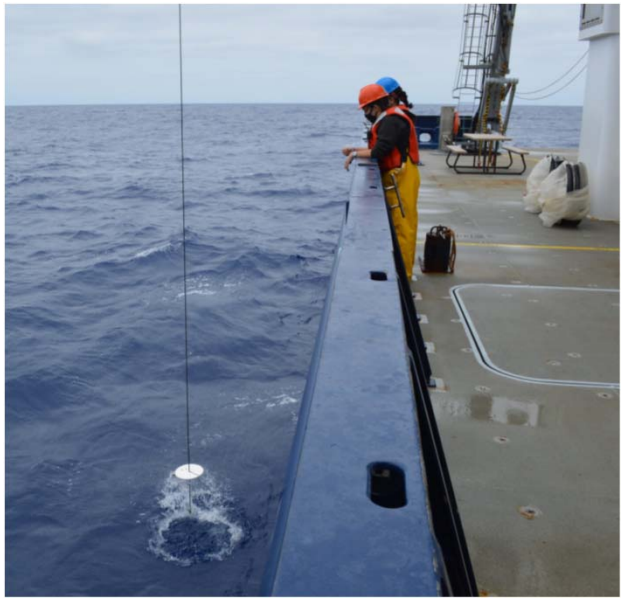
Secchi disk depth

The depth at which a 30 cm white (or alternating white/black) disk lowered into the ocean or a lake disappears from view, as viewed from the water's surface

Secchi Disk Depth as a proxy for beam and diffuse attenuation coefficients

$$Z_{SD} = \frac{\ln\left(\tau \frac{C_0}{C_T}\right)}{c + K}$$

Z_{SD} = Secchi Disk Depth
τ = air-water transmittance
C_0 = inherent contrast of disk
C_T = human threshold for disk
C = beam attenuation coefficient
K = diffuse attenuation coefficient

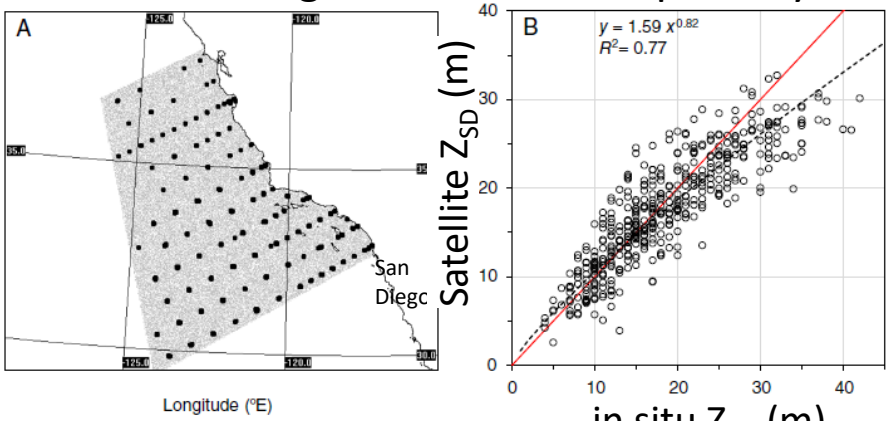


CalCOFI

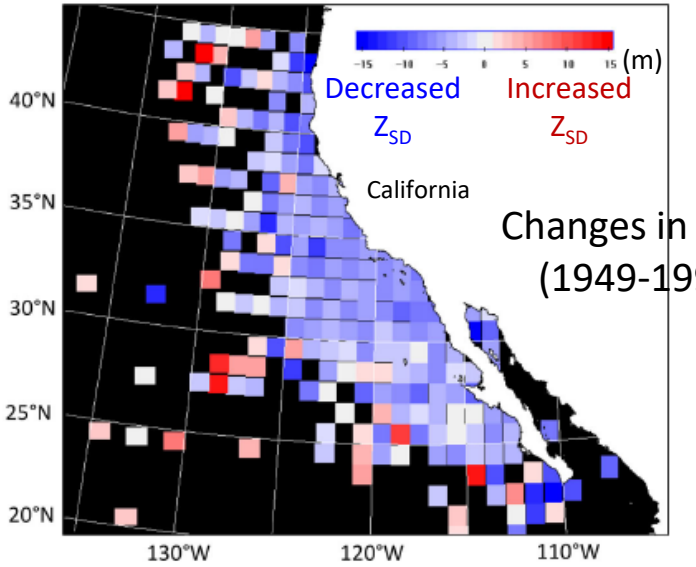
Euphotic zone depth (1% light level): $Z_{1\%} = 2.8 * Z_{SD}$

Aksnes and Ohman (2009) L&O

Multi-decadal changes in Ocean transparency, 1949 - 2021



Combination of Secchi disk + satellite measurements



Changes in Optical transparency (1949-1997) to (1998-2021)

Kahru, Lee & Ohman (2023) L&O

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III. Neuston sampling by Manta net, for suspended microplastics

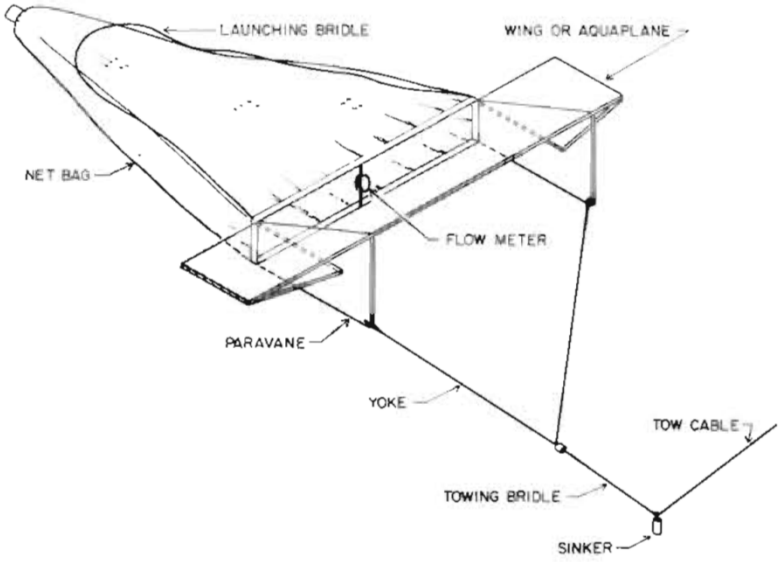
Many (but not all) types of microplastics are initially buoyant,
therefore collectible at the sea surface

Specialized sampling devices are required to sample the sea-air interface
(the "neustonic" layer)

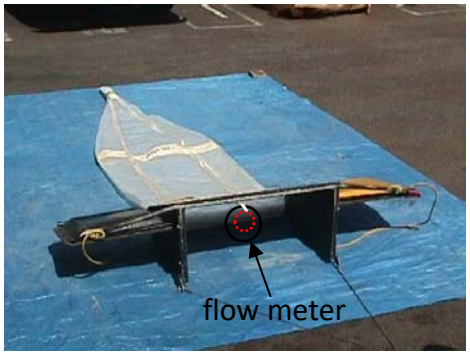
One such sampling device is the Manta net, designed to be towed half above,
half below the water's surface

Suitable for collecting "microplastics," usually defined as 0.33 - 5.0 mm

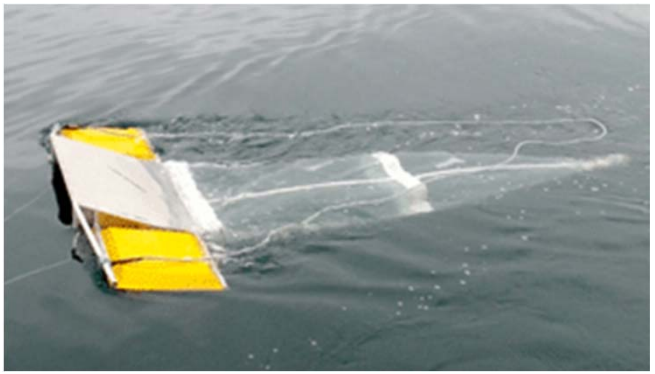
MANTA net – Neuston sampler (sea-air interface)



(Brown and Cheng 1981)

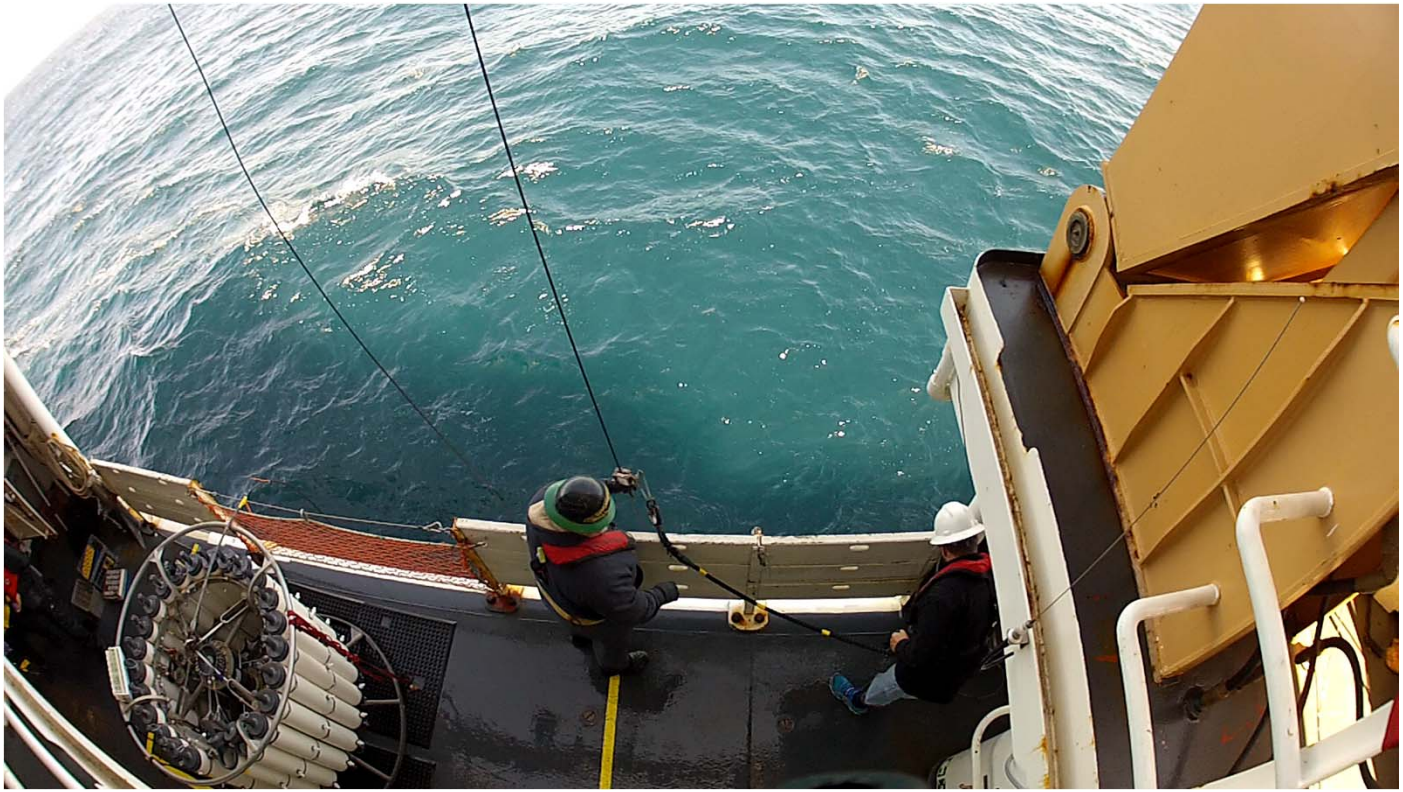


buoyant wings



Towed for 15 mins at a ship speed of ~1.5 knots.
Net is towed by a wire yoke with one short bridle and one long bridle in order to angle the net away from the ship.
Net mouth area = 0.133 m^2 and is made of 505 µm square mesh nylon with a 333 µm mesh codend

Manta Deployment



SWFSC/CalCOFI

Manta Under Tow

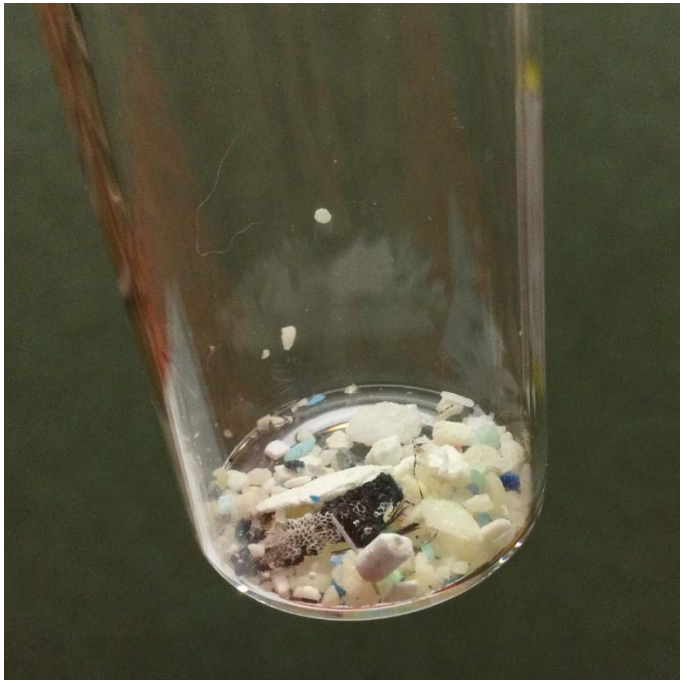
SWFSC/CalCOFI

Manta Recovery

SWFSC/CalCOFI

Manta net-collected sample
Central North Pacific Subtropical Gyre

Plastic microdebris
sorted from 1 Manta sample



Miriam Goldstein
SIO

IV. Recognition of microplastics using epifluorescence microscopy

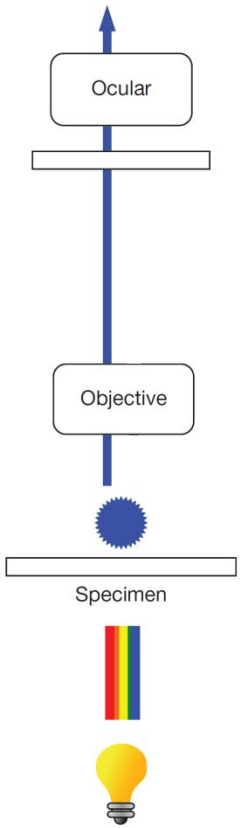
Analysis of microplastics

Analytical Chemical methods: FTIR, Raman Spectroscopy, GCMS/Pyrolysis, etc.

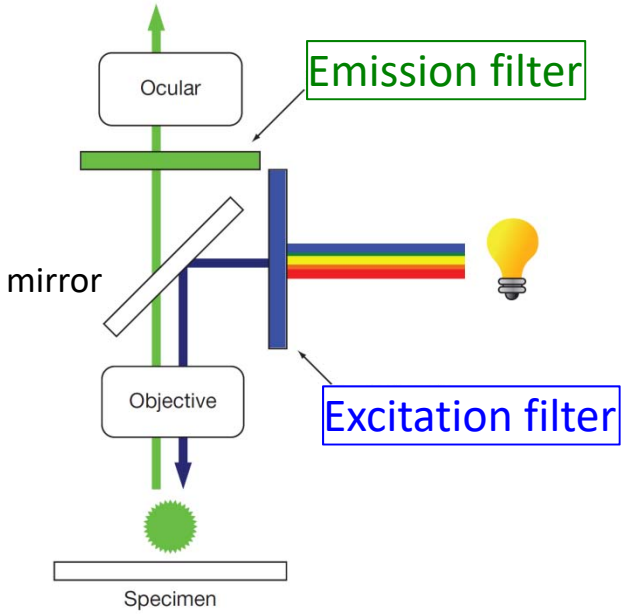
Epifluorescence microscopy: Visual differentiation of microplastics from other inorganic material, and from organic material

$\Rightarrow$ Particularly useful for very small, nanoplastic particles

Transmitted Light Microscopy

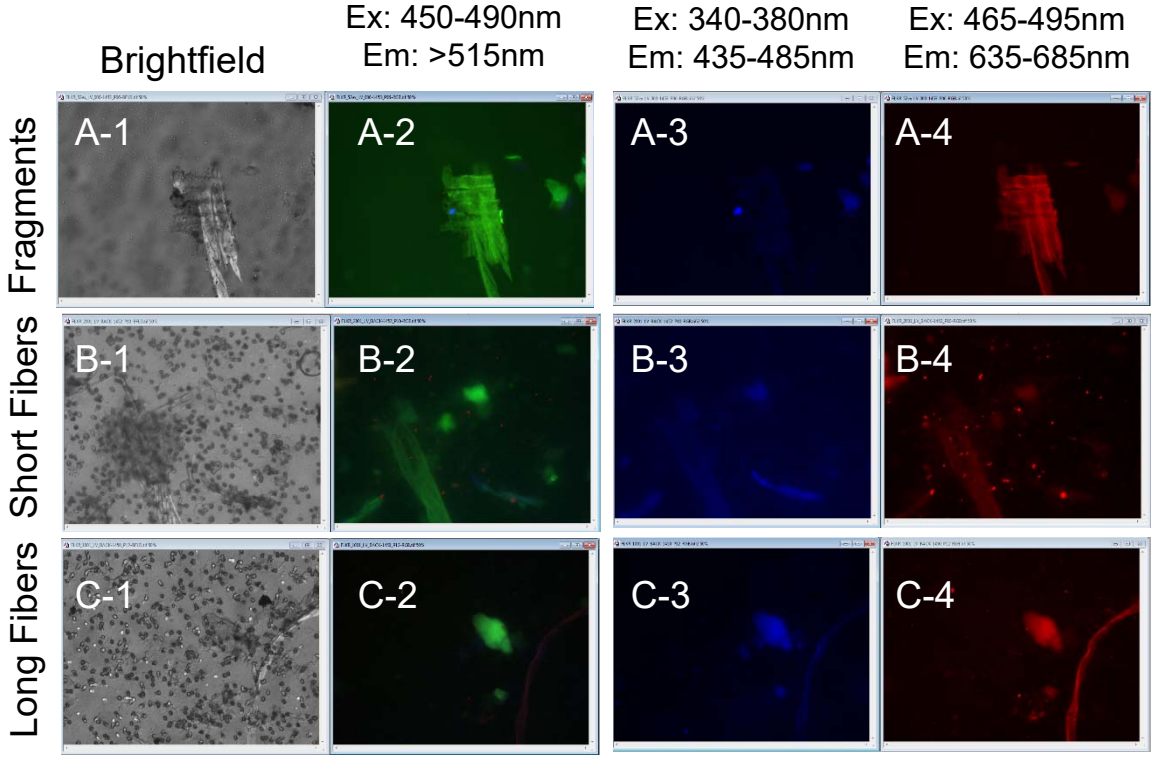


Epifluorescence Microscopy



microscope stage

Microplastics and Nanoplastics – or naturally occurring matter?



Filtered sample on polycarbonate filter (dark dots are filter pores)

Brightfield and epifluorescence images

A) Plastic fragment, B) Thick and thin short plastic fibers, C) Long fiber and TEP.

Brandon, Freibott & Sala (2019) *L&O Letters*

N.B. Not all plastics fluoresce

Importance of Nanoplastics (> 5 µm)

Suspended at the sea surface; NOT collected by nets
Can be separated from non-plastic particles by epifluorescence microscopy

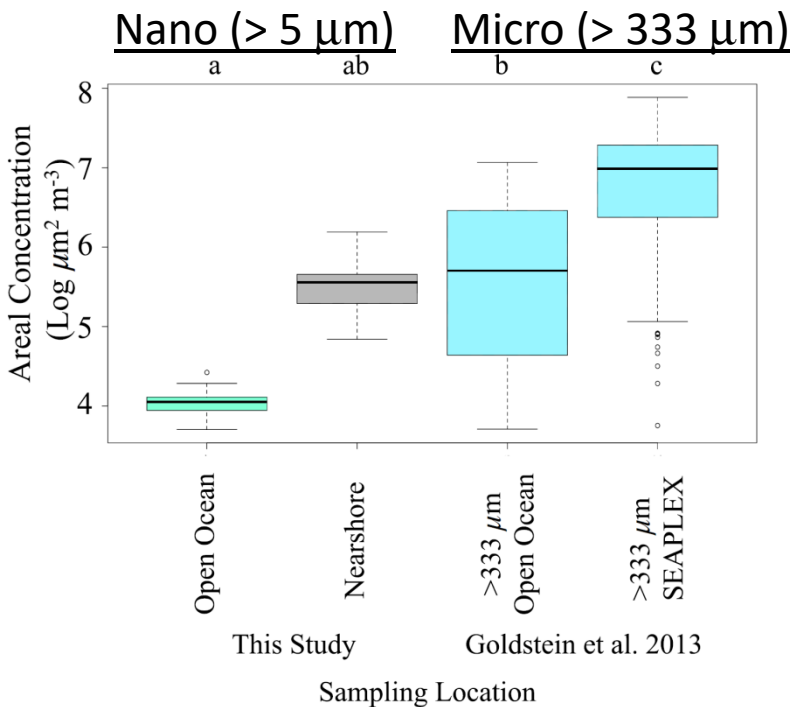
Comparison by Number

Nano (> 5 µm) vs. Micro (> 333 µm)

Nanoplastics present at **10^5 - 10^7**
higher concentrations than Microplastics
(by number)

Brandon, Freibott & Sala (2019) *L&O Letters*
California Current System
termed > 5 µm plastics *'mini-microplastics'*

Comparison by Surface Area



Sampling for Nanoplastic Particles

Collection by **metal** buckets at sea surface

Extend buckets from metal cables

Filter through glass tubing – no plastics – onto (5.0 μm) polycarbonate filters

Work under fume hood; minimize contamination by airborne fibers,
which are ubiquitous !

Freeze filters in glass petri dishes

Analyze by epifluorescence microscopy, or other methods

Brandon, Freibott & Sala (2019) *L&O Letters*

Shifting Baselines and the need for systematic, sustained measurements

Microplastics in sediment cores

Particles sorted visually from sediment cores
Analyzed by FTIR

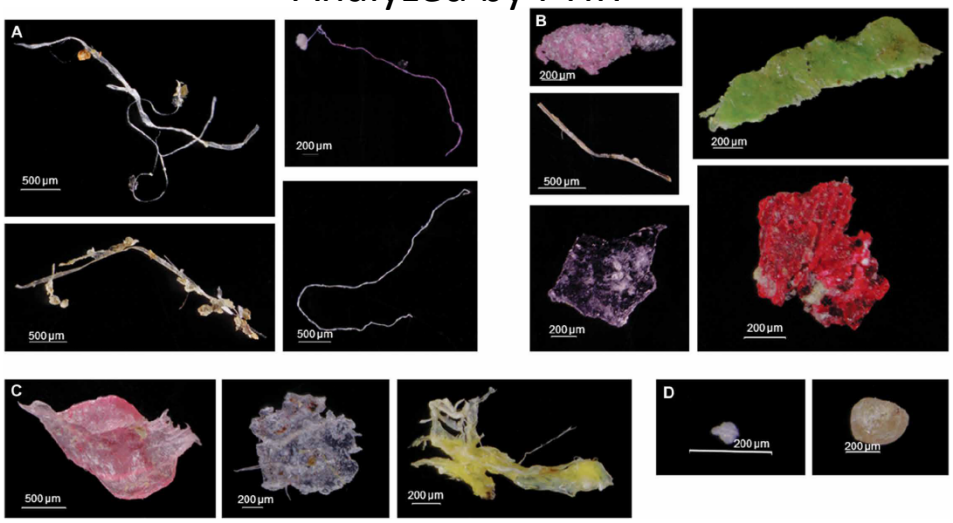


Fig. 2. Plastic particles from box core. Examples of (**A**) fibers, (**B**) fragments, (**C**) film, and (**D**) spherical particles.

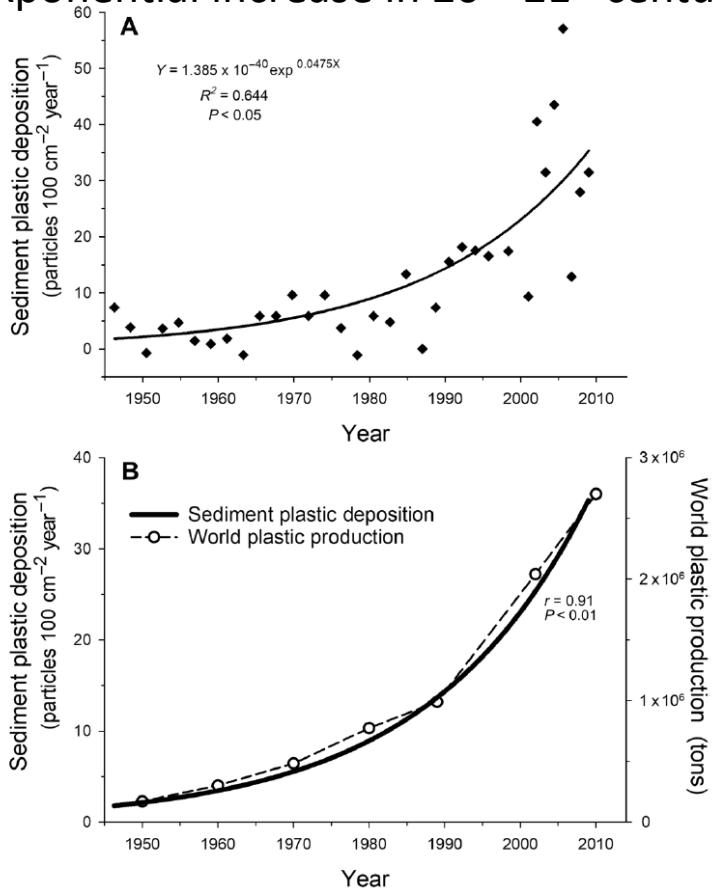
Brandon *et al.*, *Sci. Adv.* 2019; **5** : eaax0587 4 September 2019

Polymers identified by FTIR

PET – polyethylene terphthalate
LDPE – low density PE
PS – polystyrene
PVC – polyvinyl chloride
HDPE – high density PE
nylon

Santa Barbara Basin, California
Brandon, Jones, & Ohman (2019) *Science Advances*

Exponential increase in 20th-21st centuries



V. (Mesozooplankton sampling, splitting, and fixation)

Sampling	**Analysis**
Nets	Taxonomic enumeration
Pumps	Size spectra
Water bottles	Functional groups or 'Traits'
In situ Optical imaging	Optical imaging
(multi-frequency) Acoustics	DNA Metabarcoding
Continuous Plankton Recorder	eDNA
Autonomous gliders, floats	Transcriptomics (gene expression)
	Experimental incubations

Mesozooplankton splitting, and fixation – Community structure

i) 50% in buffered formaldehyde for enumeration by microscopy or digital scanning (e.g., digital Zooscan)

ii) 50% in 95% non-denatured ethanol for molecular genetics

Mesozooplankton splitting, and freezing – **Rate determinations** and **Biomass**

iii) Aliquot frozen in liquid N_2 or at -80° C for enzymatic assays, grazing determinations, molecular probes of diet, biomass

VI. Archiving samples, Curation, and Database access

Physical samples are needed for verification, and for unanticipated uses by present scientists and by posterity

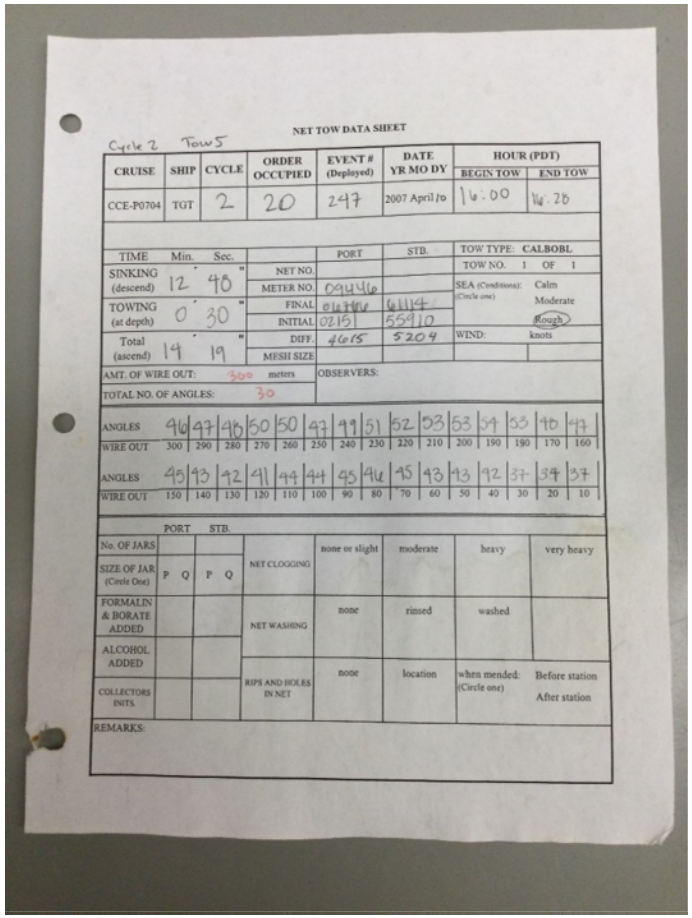
Plankton (and other) samples require complete, comprehensive labeling, completed in the field

Samples should be archived in a safe location, with controlled temperature (and humidity, if possible) and seismic restraints

Samples should be curated to ensure that preservation fluids are replenished, adverse pH changes do not occur

Electronic databases are needed to ensure that sample metadata (dates, times, locations, sampling methods, depths, volumes filtered, fixatives, preservatives, etc.) are readily accessible

Field Tow sheets – permanent record of field notes



NET TOW DATA SHEET

Cycle 2 Tow 5

CRUISE	SHIP	CYCLE	ORDER OCCUPIED	EVENT # (Deployed)	DATE YR MO DY	HOUR (PDT) BEGIN TOW	END TOW
CCE-P0704	TGT	2	20	247	2007 April 20	16.00	16.28

TIME	Min.	Sec.		PORT	STB.
SINKING (descend)	12	40 "	NET NO.		
			METER NO.	094446	
TOWING (at depth)	0	30 "	FINAL	61174	
			INITIAL	55910	
Total (ascend)	19	19 "	DIFF.	5204	
			MESH SIZE		

TOW TYPE: CALBOBL

TOW NO. 1 OF 1

SEA (Conditions): (Circle one) Calm / Moderate / Rough

WIND: knots

AMT. OF WIRE OUT: 300 meters

TOTAL NO. OF ANGLES: 30

OBSERVERS:

ANGLES	46	47	48	50	50	47	49	51	52	53	53	54	53	40	47
WIRE OUT	300	290	280	270	260	250	240	230	220	210	200	190	180	170	160
ANGLES	45	43	42	41	44	44	45	46	45	43	43	42	37	34	37
WIRE OUT	150	140	130	120	110	100	90	80	70	60	50	40	30	20	10

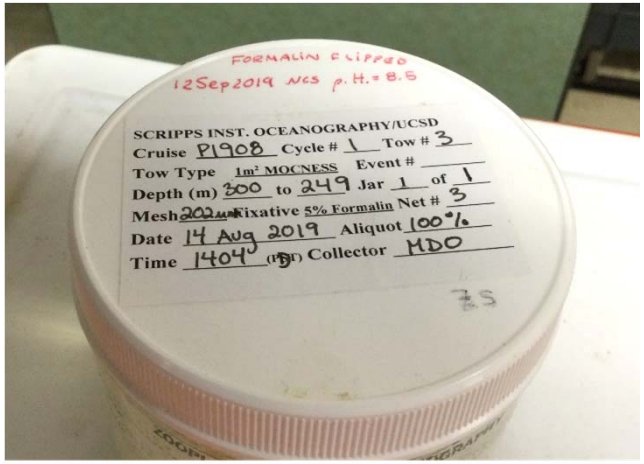
	PORT	STB.					
No. OF JARS			NET CLOGGING	none or slight	moderate	heavy	very heavy
SIZE OF JAR (Circle One)	P Q	P Q					
FORMALIN & BORATE ADDED			NET WASHING	none	rinsed	washed	
ALCOHOL ADDED							
COLLECTORS INITS.			RIPS AND HOLES IN NET	none	location	when mended: (Circle one)	Before station / After station

REMARKS:

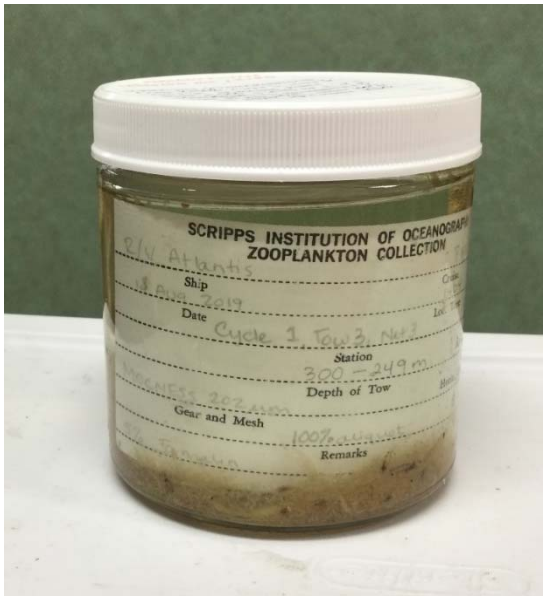
Pelagic Invertebrate Collection

Clear, permanent labeling of each sample

Jar Lid



Inside label (waterproof stock)



Digital bar coding of specimens and samples
for tracking and management

Integrated with Collection database

Pelagic Invertebrate Collection

Permanent Sample Archive

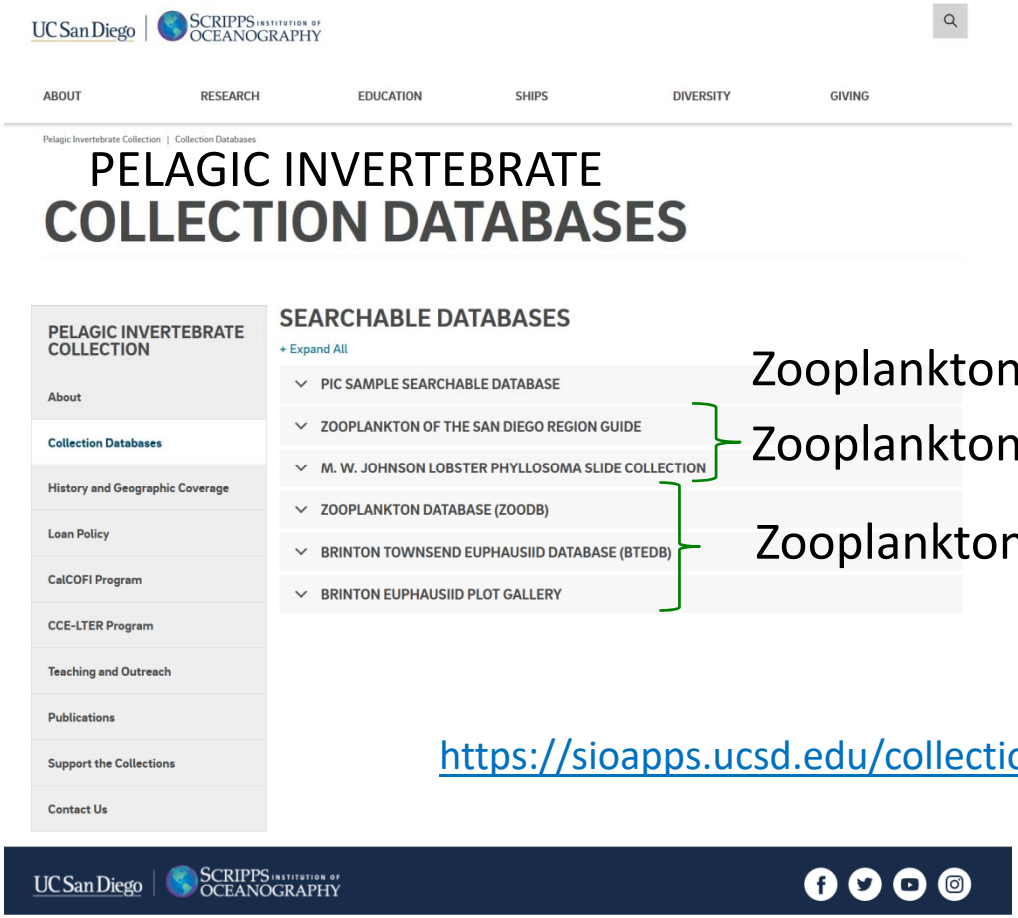
Pelagic Invertebrate Collection

Compact mobile carriages
for efficient use of space

Pelagic Invertebrate Collection

‘Freezer farms’
for storage of DNA,
tissue extracts, and
and specimens for
molecular and
biogeochemical analyses

Critical importance of Digital Databases



Zooplankton sample holdings

Zooplankton guides and images

Zooplankton enumeration data

https://sioapps.ucsd.edu/collections/pi/

Final Comments

How to handle spills and other adverse incidents in the future?

Of Paramount Importance:

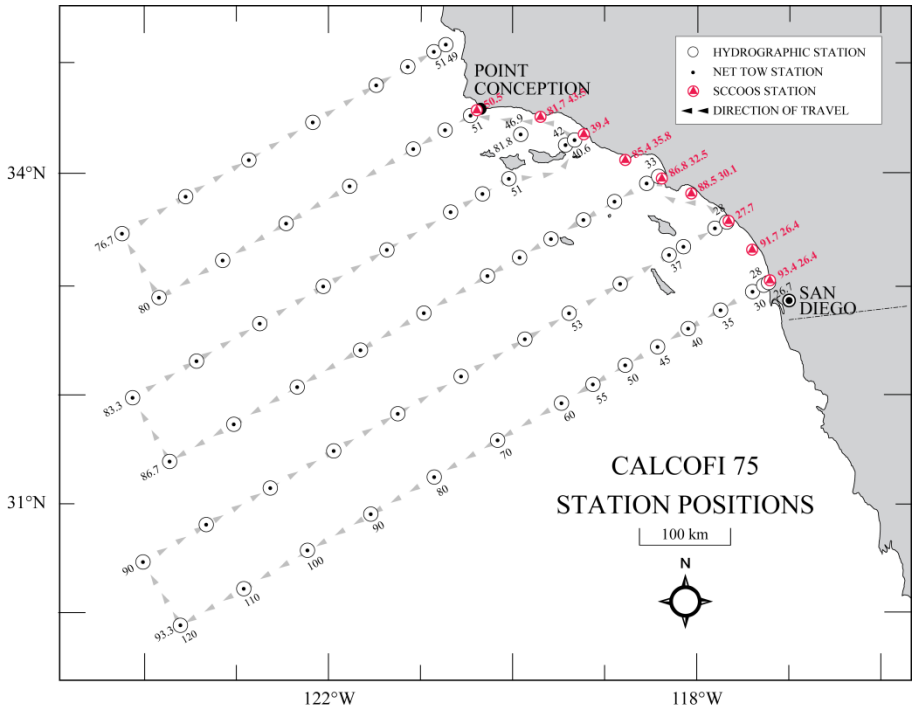
Ongoing, Baseline measurement program

- Systematic, regular sampling
- Rigorous methods, calibrated instruments
- People trained and proficient in analytical methods

A more limited, but sustainable sampling program is likely to be more useful than irregular ‘spot’ sampling

CalCOFI* Sampling Pattern

Occupied 4X y^{-1}



Consortium:

University of California (Scripps Inst. Oceanography)

National Marine Fisheries Service

California Department of Fish and Wildlife

Founded in 1949

**California Cooperative Oceanic Fisheries Investigations*

Today's Presentation

I. Phytoplankton biomass assessment by fluorometric analysis of Chl-a and Phaeopigments

II. Secchi disk as a measure of optical attenuation

III. Neuston sampling by Manta net, for suspended microplastics

IV. Recognition of micro- and nanoplastics using epifluorescence microscopy

V. (Mesozooplankton sampling, splitting, and fixation)

VI. Archiving samples, curation, and databases

fin

References for NARA training presentation by Mark D. Ohman, 20 June 2023

Aksnes, D. L., and M. D. Ohman. 2009. Multi-decadal shoaling of the euphotic zone in the southern sector of the California Current System. Limnology and Oceanography **54:** 1272-1281

Brandon, J. A., A. Freibott, and L. M. Sala. 2020. Patterns of suspended and salp-ingested microplastic debris in the North Pacific investigated with epifluorescence microscopy. Limnology and Oceanography Letters **5:** 46-53 doi 10.1002/lol2.10127.

Brandon, J. A., W. Jones, and M. D. Ohman. 2019. Multidecadal increase in plastic particles in coastal ocean sediments. Science Advances **5:** doi 10.1126/sciadv.aax0587.

Brown, D. M., and L. Cheng. 1981. New net for sampling the ocean surface. Marine Ecology Progress Series **5:** 225-227

Décima, M., M. R. Stukel, L. Lopez-Lopez, and M. R. Landry. 2019. The unique ecological role of pyrosomes in the Eastern Tropical Pacific. Limnology and Oceanography **64:** 728-743 doi 10.1002/lno.11071.

Goericke, R., S. L. Strom, and M. A. Bell. 2000. Distribution and sources of cyclic pheophorbides in the marine environment. Limnology and Oceanography **45:** 200-211

Goldstein, M. C., A. J. Titmus, and M. Ford. 2013. Scales of Spatial Heterogeneity of Plastic Marine Debris in the Northeast Pacific Ocean. Plos One **8:** doi 10.1371/journal.pone.0080020.

Kahru, M., Z. Lee, and M. D. Ohman. 2023. Multidecadal changes in ocean transparency: Decrease in a coastal upwelling region and increase offshore. Limnology and Oceanography**:** doi 10.1002/lno.12365. -

Lehninger, A. L., D. L. Nelson, M. M. Cox, and A. A. Hoskins. 2021. Principles of biochemistry, 8th ed. W.H. Freeman (epub)

Welschmeyer, N. A. 1994. Fluorometric analysis of Chlorophyll-*a* in the presence of Chlorophyll-*b* and pheopigments. Limnology and Oceanography **39:** 1985-1992 doi 10.4319/lo.1994.39.8.1985.

Web sites:

California Current Ecosystem (CCE) Long-Term Ecological Research (LTER) site

https://ccelter.ucsd.edu/ - landing page

https://ccelter.ucsd.edu/cce-calcofi-methods-manual/ - methods

CalCOFI methods:

https://calcofi.org/samp hods/ - CalCOFI sampling and analytical methods

Scripps Pelagic Invertebrate Collection

https://sioapps.ucsd.edu/collections/pi/ - portal to several databases