Experimental Design and Analysis

Scales in oceanic ecosystems

Daniel Vaulot

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Outline

- The marine Environment
- Marine Phytoplankton
- Spatial Scales
- Temporal Scales
- Time Series



Temperature



Marine Environment is highly dynamic



Currents

- Wind (Atmospheric Circulation)
- Coriolis effect
- Coast line



Currents

- Wind (Atmospheric Circulation)
- Coriolis effect
- Coast line



Currents

• Turbulence



Currents

• Turbulence





Classical view of marine foodwebs



Plankton diversity

- Phytoplankton
- Zooplankton
- Bacteria
- Viruses



Size classes



Complex processes

- Predation
- Symbiosis
- Mixotrophy
- Parasitism



Phytoplankton



Major groups



Diatoms and dinoflagellates: 20-200 µm





Picoplankton: 0.2-2 µm

- Very small eukaryotes (Ostreococcus)
- Cyanobacteria (Synechococcus)



Wide phylogenetic diversity





Not, F., Siano, R., Kooistra, W.H.C.F., Simon, N., Vaulot, D. & Probert, I. 2012. In Piganeau, G. [Ed.] Genomic Insights Gained into the Diversity, Biology and Evolution of Microbial Photosynthetic Eukaryotes. Elsevier.



Chlorophyll

Proxy of phytoplankton biomass



Chlorophyll

Can be measured from space



Chlorophyll

What can you see ?



Blooms

English Channel

Coccolithophorids





Blooms

New Zealand



Blooms

Baltic

Cyanobacteria





What factors control phytoplankton?



What controls phytoplankton ?

Positive

- Light
- Nutrients (Nitrogen, Phosphorus)
- Trace elements (Iron)

Negative

- Predation
- Parasites (e.g. viruses)
- Death

Species selection

- Temperature
- Salinity



Is phytoplankton uniformly distributed in the water column?



Water column



Euphotic layer



Chlorophyll maximum



Sampling the ocean

• Bucket sampling



Sampling the ocean

- Bottles on a Rosette
- CTD Conducitivity, Temperature, Depth





Sampling the ocean

• Filtration



Sampling the ocean

• Nets




Sampling the ocean

• Transects (Eulerian)



Sampling the ocean

• Grids (Eulerian)



Sampling the ocean

• Drifting buoy (Lagrangian)



Fig. 1. Location of the Lagrangian station in the northwest Atlantic Ocean over the 7 day period (Day 1 to Day 7). Position of the drifting buoy at every 6 h time point is indicated

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Temperate

What are the most important scales in the ocean?



Temporal variations



Annual scale - Spring bloom

- Diatoms
- Dinoflagellates





Lindemann C and St. John MA (2014) A seasonal diary of phytoplankton in the North Atlantic. Front. Mar. Sci. 1:37

Annual scale - Spring bloom

- Depends on latitude
 - Temperate
 - Tropical
 - Arctic



Trends in Microbiology

Multi-year scale - El Niño

Warm water accumulates over East Pacific



TAO Project Office/PMEL/NOAA

Multi-year scale - El Niño

- Blocks upwelling
- Phytoplankton decrease
- Lower fish capture (anchovy)



Multi-year scale - El Niño

• Year to year change in intensity



Climatic change

• ALOHA station





Climatic change

• ALOHA station









Daily scale

Unique to marine systems



Spatial and temporal scales



Benway HM. et al. 2019. Ocean Time Series Observations of Changing Marine Ecosystems: An Era of Integration, Synthesis, and Societal Applications. Frontiers in Marine Science



Long term stations



Bunse C., Pinhassi J. 2017. Marine Bacterioplankton Seasonal Succession Dynamics. Trends in Microbiology 25:494–505.

What kind of questions can be adressed by such long term series?



- What are the key periodicities ?
 - annual (what about equator ?)
 - tides (monthly)
 - daily
- Long term climatic trends
- What drives the year to year variability
- Recurring species ?

Chlorophyll time series

• North Atlantic Chl-a time series (57–628 N, 20–108 W) from 1967 to 1979



Chlorophyll time series

What can you see ?



Winder M., Cloern JE. 2010. The annual cycles of phytoplankton biomass. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3215–3226.

Chlorophyll time series

• Different environments have different frequencies





Winder M., Cloern JE. 2010. The annual cycles of phytoplankton biomass. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3215–3226.

Physiological and ecological drivers of early spring blooms of a coastal phytoplankter

Kristen R. Hunter-Cevera,¹ Michael G. Neubert,¹ Robert J. Olson,¹ Andrew R. Solow,² Alexi Shalapyonok,¹ Heidi M. Sosik^{1*}

Climate affects the timing and magnitude of phytoplankton blooms that fuel marine food webs and influence global biogeochemical cycles. Changes in bloom timing have been detected in some cases, but the underlying mechanisms remain elusive, contributing to uncertainty in long-term predictions of climate change impacts. Here we describe a 13-year hourly time series from the New England shelf of data on the coastal phytoplankter *Synechococcus*, during which the timing of its spring bloom varied by 4 weeks. We show that multiyear trends are due to temperature-induced changes in cell division rate, with earlier blooms driven by warmer spring water temperatures. *Synechococcus* loss rates shift in tandem with division rates, suggesting a balance between growth and loss that has persisted

Flow Cytobot

• Imaging and flow cytometry



Flow Cytobot

• Diatoms



Synechococcus

• Discovered in 1979 by John Waterbury - *Epifluorescence microscopy*







Fig. 1 Phase contrast photomicrograph of Synechococcus sp. (strain Syn-48) illustrating general cell morphology (scale bar, $5.0 \ \mu m$).

Waterbury, J.B., Watson, S.W., Guillard, R.R.L. & Brand, L.E. 1979. Nature. 277:293–4.

Cell multiplication

- Binary fission
- Typically once every day



Cell disappearance

- Virus
- Predation
- Cell death (UV, nutrient deprivation)





Growth rate vs Loss rate

$$rac{\mathrm{d}N}{\mathrm{d}t} = \mu_{net} * N$$
 $N = N_0 \exp^{\mu_{net} * t}$

 $\mu_{net} = \mu_{growth} - \mu_{loss}$

- Growth rate = division
- Loss rate = cell death, predation, viruses



Growth rate vs Loss rate

$$rac{\mathrm{d}N}{\mathrm{d}t} = \mu_{net} * N$$
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 $\mu_{net} = \mu_{growth} - \mu_{loss}$

- Growth rate = division
- Loss rate = cell death, predation, viruses



Synechococcus abundance



Hunter-Cevera et al. 2016. Physiological and ecological drivers of early spring blooms of a coastal phytoplankter. Science 354:326–329.



Temperature



Hunter-Cevera et al. 2016. Physiological and ecological drivers of early spring blooms of a coastal phytoplankter. Science 354:326–329.

Temperature anomaly



Figure S3. Daily temperature anomalies (°C) from daily climatological average for 2003 to available 2016. Red values indicate positive anomalies, while blue indicates negative anomalies.
What drives the Synechoccus bloom ?

Synechococcus vs. Temperature



Fig. 2. Multiyear trends showing spring temperature changes and Synechococcus bloom shifts from 2003 to 2016. The data are shown by day of the year (vertical axis), with values denoted by color. (A) Temperature. Markers indicate the day in each year when water temperature first exceeds 6° (triangles), 9° (circles), 12° (stars), or 15°C (squares). (B) Synechococcus cell concentration. Markers indicate the day in each year when cell concentration exceeds 8 × 10³ (triangles), 1.6 × 10⁴ (circles), 4.8 × 10⁴ (stars), or 9.6 × 10⁴ (squares) cells ml⁻¹. (C) Integrated division rate (cumulative summed division

What drives the Synechoccus bloom ?

Loss vs. Division rate





Study in the Mediterranean Sea



Yearly cycles



Giner et al. 2019. Quantifying long-term recurrence in planktonic microbial eukaryotes. 28:923-935. Molecular Ecology

Metabarcoding





Metabarcoding

	Α	В	С	D	E	F	G	Н	1	J	K	L	М	N	0	Р	Q	R	S	Т	U
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		Kingdom	supergroup	Dipoflogollata	Class	genus 🗸	Species									v ▼					
12	otu 0042	Eukapyota	Alveolata	Dinoflagellata	Dinophyceae	Convaulax	Convaulax, coinifora	-	0	0	0 0		0	250	239	0	0	0	4/4	0	-
44	otu_0043	Eukapyota	Onisthekente	Motozoo	Arthropoda	Tomora	Tomora turbinata		0	0	0 0		414	0	0	0	0	102	00		-
48	otu_0051	Eukanyota	Opisthokonta	Metazoa	Arthropoda	Pecticlina	Rectioling cimilie		0	0 60	0 0		414	479	1452	0	0	102	1749		5
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66	otu_0065	Eukaryota	Onisthokonta	Metazoa	Arthropoda	Paracalanus	Paracalanus aculeatus	4 ·	0	ő	0 0	1831	973	455	186	1644	0	- 110	0	0 1	6
72	otu_0071	Eukarvota	Stramenopiles	Ochrophyta	Bacillariophyta	Thalassiosira	Thalassiosira sp		0	0	0 0) 0	0.0	0	0	0	0	0	0	0	-
78	otu 0077	Eukarvota	Opisthokonta	Metazoa	Urochordata	Oikopleura	Oikopleura dioica	32	24 2	38 5	75 142	1 0	0	241	2208	97	246	0	590	0 3	2
79	otu 0078	Eukarvota	Opisthokonta	Metazoa	Cnidaria	Calcigorgia	Calcigorgia beringi		52	0	0 () 0	0	0	0	0	0	0	0	0	-
80	otu 0079	Eukarvota	Archaeplastida	Chlorophyta	Mamiellophycea	Micromonas	Micromonas commoda A	E 48	33	0	0 183	3 135	96	453	158	719	1006	388	0	1446	4
84	otu 0083	Eukarvota	Opisthokonta	Metazoa	Arthropoda	Acrocalanus	Acrocalanus gracilis		0	0	0 () (0	0	0	0	0	0	0	0	-
88	otu 0087	Eukarvota	Opisthokonta	Metazoa	Mollusca	Bathymodiolinae	Bathymodiolinae gen.		0	0	0 () (0	0	0	0	0	0	0	0	
95	otu_0094	Eukaryota	Opisthokonta	Metazoa	Arthropoda	Parvocalanus	Parvocalanus_crassirostri	s	0	0	0 () (0	0	161	0	0	0	0	0	
108	otu_0108	Eukaryota	Opisthokonta	Metazoa	Urochordata	Oikopleura	Oikopleura_dioica	31	15	0 40	0 540) 108	0	0	0	0	784	64	339	0 3	2
115	otu_0115	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Dinophyceae_XXX	Dinophyceae_XXX_sp.	1	51	0	0 () (0	1056	488	0	269	0	315	2079	4
119	otu_0119	Eukaryota	Opisthokonta	Metazoa	Arthropoda	Paracalanus	Paracalanus_sp.		31	0 192	25 855	5 0	0	0	371	0	113	179	0	0 1	1
127	otu_0127	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophycea	Micromonas	Micromonas_clade_B_wa	r 24	46	0	0 () (109	251	178	153	226	152	233	0	
136	otu_0136	Eukaryota	Hacrobia	Cryptophyta	Cryptophyceae	Geminigera	Geminigera_cryophila	34	47 2	99	0 289	9 135	52	247	146	194	430	201	109	341	2
141	otu_0141	Eukaryota	Archaeplastida	Chlorophyta	Trebouxiophyce	Nannochloris	Nannochloris_sp.		0	0	0 () (44	0	0	0	0	0	0	0	
146	otu_0148	Eukaryota	Opisthokonta	Metazoa	Arthropoda	Bestiolina	Bestiolina_sp.		0	0 70	06 83	3 558	0	0	0	0	0	0	0	0 5	1
148	otu_0150	Eukaryota	Archaeplastida	Chlorophyta	Trebouxiophyce•	Nannochloris	Nannochloris_sp.		0	0	0 () (1	1. 6	2 h	200	F 0	0	0	0	
151	otu_0153	Eukaryota	Opisthokonta	Metazoa	Arthropoda	Oithona	Oithona_davisae		0	0	0 () (NUI	TIDE	er (0	0	0	0	0	
171	otu_0173	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophycea	Ostreococcus	Ostreococcus_sp.		0	0	0 () (0	0	0	0	0	0	0	0	
173	otu_0175	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Dinophyceae_XXX	Dinophyceae_XXX_sp.		0	54 58	51 () (5	sed	uer	nce	S 0	0	0	0 1	4
175	otu_0177	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Cerataulina	Cerataulina_pelagica		0	0	0 () (6	24			~ 0	0	0	0	
177	otu_0179	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Cyclotella	Cyclotella_choctawhatche	2	0	0	0 (0 0	47	67	0	0	0	0	0	0	
190	otu_0192	Eukaryota	Alveolata	Dinoflagellata	Dinophyceae	Gyrodinium	<u>Gyrodinium_gutrula</u>		0 1	31 1	76 (0 0	0	0	0	0	0	118	0	0	8
191	<u>otu_0193</u>	Eukaryota	Rhizaria	Radiolaria	RAD-B	RAD-B-Group-IV_X	RAD-B-Group-IV_X_sp.		0	20	0 5	0	0	0	0	656	68	0	0	0	_
193	otu_0195	Eukaryota	Opisthokonta	Metazoa	Arthropoda	Acrocalanus	Acrocalanus_gracilis		0	0	0 (0 0	0	0	0	0	0	1252	0	0	_
194	otu_0196	Eukaryota	Opisthokonta	Metazoa	Porifera	Unclassified_Halichondrida	Halichondrida_sp.		0	0	0 0	0 0	0	0	0	0	0	0	0	0	
198	otu_0200	Eukaryota	Opisthokonta	Metazoa	Arthropoda	Othona	Oithona_similis		0	0	0 0	0 0	0	0	0	0	0	0	0	0	
199	0001	Eukaryota	Alveolata	Dinotiagellata	Dinophyceae	vvoloszynskia	woloszyńskia_nalophila		0	0	0 0		0	0	0	0	0	0	0	0	_
205	otu_0207	Eukaryota	Archaepiastida	Chiorophyta	Mamiellophycea	Ostreococcus	Ostreococcus_sp.	2	0	10	0 0		0	0	10	0	100	100	0	0	0
208	010_0210	Eukaryota	Rnizana	Cercozoa	Filosa-impricate	Novel-clade-2_X	Novel-clade-2_X_sp.	3,	29	40	0 0		58	0	18	000	123	123	0	0	24
209	000_0211	Eukaryota	Opisinokonia	Metazoa	Chidana Filoso Theosfilos	FOISKalla	TACIDIA lineana X an		0	0	0 0		0	0	0	209	0	0	0	0	
217	otu_0219	Eukaryota	Ruil/alla	Ochrophyto	Pagillarianbuta	The lessioning	Thelessinging biopide	-	0	0	0 0		0	0	0	0	0	0	0	0	-
219	otu_0221	Eukaryota	Stramonopiles	Ochrophyta	Pacillariophyta	Cyclotello	Cyclotella, choctowheteba		0	0	0 0		0	0	0	0	0	0	0	0	
224	otu_0220	Eukanyota	Opictbokopto	Motozoo	Arthropodo	Oithono	Oithono dovisoo	-	0	0	0 0		0	0	0	0	0	0	0	0	
220	otu_0228	Eukanyota	Opisthokonta	Metazoa	Arthropoda	Adomia	Artomia calina	-	0	0	0 0		0	0	0	0	0	0	0	0	-
227	otu_0229	Eukanyota	Archaenlastida	Chlorophyta	Mamiellonbycog	Ostrancoccus		-	0	0	0 5	7 0		0	0	0	0	- 0	120	0	-
1/4	000 0201	Lunaryoid	riciaculasilua	Onorobinta	mannenounvcea	0340000003	Concococcus claue D		v	0	0 01	u U	0	0	0	0	0	0	123	0	

How to determine periodicity?



How to determine periodicity ?

• Autocorrelation





$$r_{xy} = rac{\sum_{i=1}^n (x_i - ar{x})(y_i - ar{y})}{\sqrt{\sum_{i=1}^n (x_i - ar{x})^2} \sqrt{\sum_{i=1}^n (y_i - ar{y})^2}}$$

How to determine periodicity ?

• Autocorrelation



Group periodicity

• Autocorrelation function



Group periodicity



Species periodicity





Giner et al. 2019. Quantifying long-term recurrence in planktonic microbial eukaryotes. 28:923-935. Molecular Ecology

What did we talked about ?

- Spatial scales
- Time scales
- Sampling the Ocean
- Time series
 - Chlorophyll periodicity
 - Bloom dynamics
 - Which species are periodic ?