

II. Phytoplankton

Phytoplankton fixes approximately **50 Gt of carbon in the euphotic zone of the oceans every year** (Longhurst et al., 1995; Field et al., 1998). **Micro-phytoplankton** relevant to this review consist of two species, **cyanobacteria** and **single celled algae**. Both are capable of photosynthesis and thus of converting CO₂ to sugars and O₂. In the early earth, cyanobacteria were responsible for the production of the oxygen in the atmosphere. In recent years remarkable facts about the role of ocean phytoplankton in the carbon dioxide cycle of the earth have been uncovered. This data was obtained with the Coastal Zone Color Scanner (CZCS) satellite launched by NASA. It was found that phytoplankton incorporated 45 to 50 billion tons of carbon into their cells each year. This **was twice the level of all previous estimates**. In addition, it was found that **land plants incorporated 52 billion tons of carbon each year, half of previous estimates**. In summary, although they amount to less than 1% of the photosynthetic biomass of the earth, **phytoplankton contribute almost half of the total photosynthetic activity. This difference is due to the fact the land plants have an average turnover of once every 10 years compared to once a week for the shows the power of phytoplankton compared to land plants.**

Plant Biomass and Productivity in Marine and Terrestrial Ecosystems

Ecosystem	Biomass (10 ¹⁵ g)	Net Primary Production (10 ¹⁵ g year ⁻¹)	Turnover time (years)
Marine	1-2	45-55	0.02-0.06
Land	600-1000	55-70	9-20

Although the biomass of phytoplankton is a fraction of that of land plants, their more rapid turnover allows them to have a net primary production of C comparable to land plants.

Two of the most popular approaches to Negative Emissions Technology (NET) are BECCS (Bioenergy with Carbon Capture) and CCS (Carbon Capture and Storage). Each approach has major drawbacks. Both require

the capture of carbon dioxide on a liquid solvent or solid sorbent and its subsequent release, a process which is very energy expensive with estimates of \$60 to \$1,000 per ton of CO₂ captured (National Academies, 2018). Both require some type of safe sequestration. In addition, BECCS has the additional problem of the requirement of large areas of land competing with farmland and food supplies (Stokstad, 2019).

There is a potential solution that avoids both the expense of capturing and releasing the CO₂ and the problems with safe sequestration. This alternative solution is the use of phytoplankton to capture the CO₂. When the phytoplankton die, they sink to the bottom of the ocean effectively burying the carbon dioxide.

The ocean phytoplankton have taken up one-third of the total CO₂ released in the past 200 years (Sabine, 2012). Two specific types of phytoplankton are utilized for this - **coccolithopores** and **diatoms**. After these adsorb the CO₂ it is safely and automatically sequestered when these species die and sink to the bottom of the ocean. This amounts to automatic and safe sequestration, something that is a major problem for CCS on land.

Coccolithopores

Coccolithophores are the most abundant type of calcifying unicellular micro-algae in the ocean. When the Atlantic Telegraph Company began the process of laying the transatlantic telegraph cable, Lieutenant Commander Joseph Dayman of the Royal Navy was a member of the expedition, aboard the HMS *Cyclops*. He took samples of the ocean floor along the route of the cable. His good friend Thomas Henry Huxley, Darwin's famous bulldog, examined the samples, many of which were white and felt like chalk (think white cliffs of Dover). When he examined them under the microscope, he noted many elaborate round objects that he called **coccoliths**, meaning spherical rocks. He thought they were inert minerals. Others called them coccospheres. Another man also named Henry, Henry Clifton Sorby, noted similar structures in English chalk. He proposed that these structures were made by living organisms. The organism inside the calcified coating was called a **coccolithopore** (Chimileski and Kolter, 2004). The following figures shows these beautiful organisms. Coccolithopores are almost exclusively marine and are found in large numbers throughout the sunlight zone of the ocean.



For the interested reader, Balch (2018) has written an excellent review of coccolithophores. I have drawn on it extensively, including lifting some whole paragraphs, for this review.

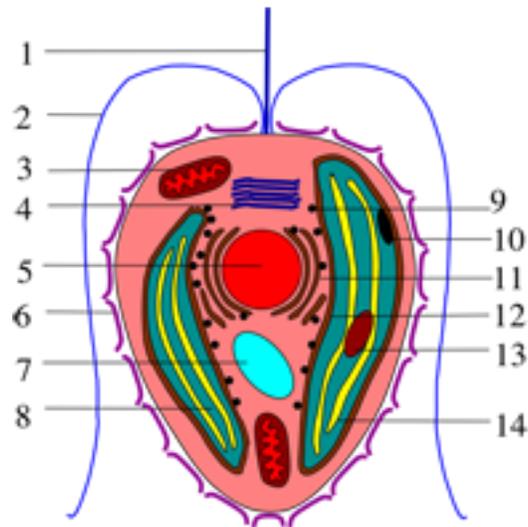
Some of the important characteristics of coccolithophores are:

- (a) They are ubiquitous in the global ocean, sometimes forming spectacular blooms that have length scales of thousands of kilometers (see section on blooms);
- (b) they are characterized by their ability to produce and excrete microscopic calcium carbonate coccoliths (plates) that surround coccolithophorid cells and eventually accumulate in seawater;
- (c) their plates are made of a dense mineral, calcite, that ultimately serves as ballast for sinking marine debris, thus linking coccoliths to the rate of the ocean biological carbon pump;
- (d) when coccolithophores calcify, for every mole of CaCO_3 that they produce, they consume 2 mol of bicarbonate and produce 1 mol of CO_2 thus linking them to ocean biogeochemistry and another important biogeochemical pump and the ocean alkalinity pump; and
- (e) their calcium carbonate coccoliths are highly optically refractive, indicating that they scatter light efficiently, which means that they can be easily seen from space using satellite remote sensing and can strongly affect the optical budget of the surface ocean (Balch, 2018).

To use this approach to reduce atmospheric CO_2 there are a number of issues that need to be known. In essence, it will involve producing blooms of coccolithophores in areas of the ocean that are relatively devoid of them. There are many things we need to understand to do this. The following reviews some of the issues involved.

Structure of coccolithophores

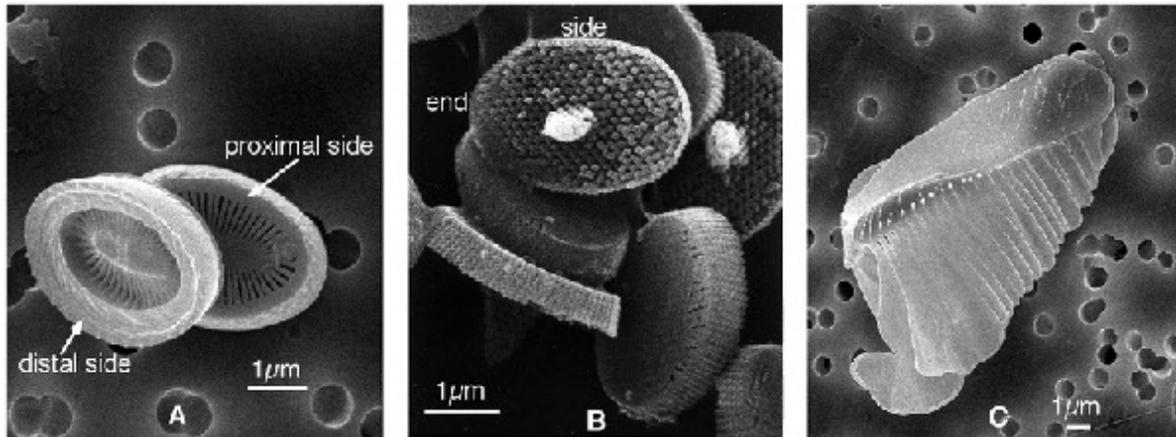
Coccolithophores are **haptophytes**. These are plants that thrive in water having greater than 0.5% NaCl. They have evolved convergently in numerous, related families. Because of their calcium carbonate coat, Coccolithophores are referred to as the **calcifiers**. They have the following internal structure:



Cell scheme. 1-haptonema, 2-flagella, 3-mitochondrion, 4-Golgi apparatus, 5-nucleus, 6-scales, 7-chrysolaminarin vacuole, 8-plastid, 9-ribosomes, 10-stigma, 11-endoplasmic reticulum, 12-chloroplast endoplasmic reticulum, 13-pyrenoid, 14-thylakoids.

Haptophytes uniquely possess a haptonema (see #1 above), a filament-like organelle that appears to be used for adhesion to other particles or substrata (Inouye & Kawachi 1994) and for capturing prey.

Two terms commonly used are holococcolith and heterococcolith. These characteristics can both occur in the same species. Heterococcoliths form crystal-units of variable shape and size, typically arranged in cycles with radial symmetry. Homococcoliths are formed of numerous minute (<0.1 um) crystallites all of similar shape and size.



Types of coccoliths: (A) **Heterococcoliths**; one in distal view (left) and the other, partially obscured, in proximal view. (B) **Holococcoliths**, formed of numerous minute crystallites; one in distal view (upper center) and one in latero-proximal view (lower right). These distinctions are important because the different types occupy different geographical and ecological areas.

Many coccolithophores have a haplodiplontic life cycle, alternating between a haploid motile form that is typically covered with holococcoliths (coccoliths constructed of simple rhombohedral calcite crystals) and a nonmotile diploid stage covered with heterococcoliths (more elaborate calcite scales). Both can divide asexually (Cros et al. 2000).

In addition to the hetero-, holo classification coccolithophores can also be classified on the basis of cross-section structure. Here there are three general types.

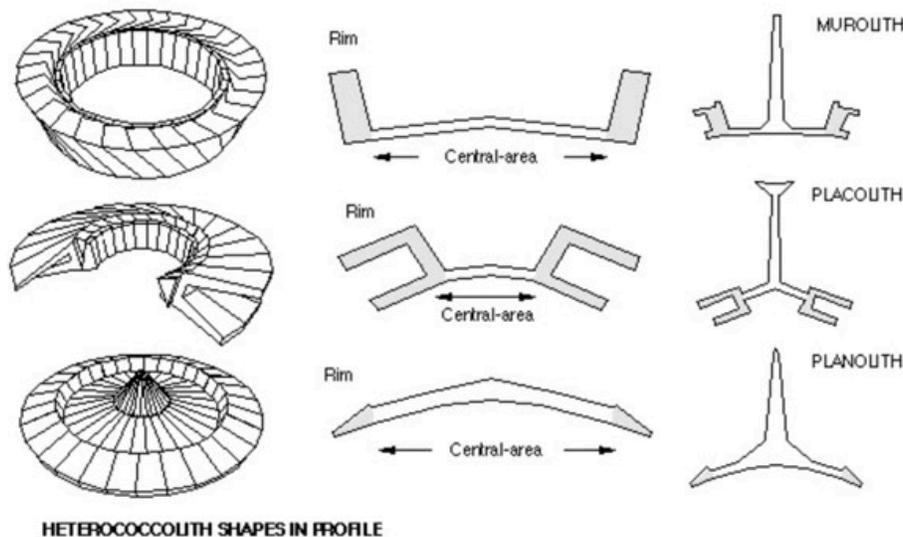
Murolith rim elevated but without well-developed shields

(e.g. *Zeugrhabdotus*, *Pontosphaera*). Latin murus = wall

Placolith rim has two, or more, well developed shields (e.g. *Coccolithus*, *Emiliana*, *Watznaueria*). (Lohmann 1902) Latin placo = flat plate see green photo above.

Planolith rim not significantly elevated (e.g. *Rhabdosphaera*, *Discoaster*). (Young 1992a) Latin planus = flat

These are illustrated as follows:



HETEROCOCCOLITH SHAPES IN PROFILE

International Nanoplankton Association

Autotrophy, heterotrophy and mixotrophy.

These terms also describe different types of coccolithophores. An **autotroph is a primary producer**, an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) using inorganic carbon from simple substances such as carbon dioxide and from water, generally using energy from light (photosynthesis) or inorganic chemical reactions (chemosynthesis). Autotrophs do not need a living source of carbon or energy and are the primary producers in the food chain, such as plants on land or algae in water.

A **heterotroph is an organism that cannot produce its own food**. It takes nutrition from sources of organic carbon, mainly plant or animal matter. Living organisms that are heterotrophic include all animals and fungi, some bacteria and protists, and many parasitic plants.

A **mixotroph** is an organism that can use a **mix of different sources of energy and carbon**, instead of having a single trophic mode on the continuum from complete autotrophy at one end to heterotrophy at the other. It is estimated that mixotrophs comprise more than half of all microscopic plankton.

Paradigms are shifting regarding the predominance of autotrophy versus mixotrophy in the marine environment. The traditional view of marine organisms was that they are either exclusively autotrophic or exclusively heterotrophic, whereas the newer, more nuanced view is that a significant fraction are mixotrophic, able to augment their carbon uptake through either photosynthesis or heterotrophic pathways (Mitra et al. 2014). This changing paradigm has ramifications for the survival of coccolithophores, especially the floriform species (such as *Florisphaera profunda*), which are associated with deep-photic-zone assemblages in low to middle latitudes (Young 1994).

The presence of deep-dwelling coccolithophores well below the zone rich in sunlight, has been taken as evidence that some coccolithophores are mixotrophic (Poulton et al. 2017).

Distribution of coccolithophores (Balch, 2018).

Holococcolith forms predominate in low-nutrient surface waters, whereas heterococcolith forms dominate in lower-light environments with higher nutrient concentrations (Cros & Estrada 2013).

There appears to be an annual cycle of hetero and holococcolith life stages: The heterococcolith phase is more abundant during winter months, whereas the holococcolith stage is more abundant during summer months (Supraha et al. 2016), and the summer is precisely the time when most bright coccolithophore blooms are observed.

Ecologically, coccolithophores have traditionally been thought to occupy four general niches within the ocean based on their biogeography (Young 1994): (a) placolith-bearing, bloom-forming species in coastal and upwelling waters at low and high latitudes; (b) umbelliform species assemblages in more oligotrophic, blue-water sites, in subtropical latitudes, and in the top 100 m; (c) floriform species in deep (150–200 m), stratified waters in low to middle latitudes; and (d) a group of miscellaneous, rarer species in environmental conditions other than those of the first three groups.

What Species is Most Prevalent?

The most widespread species on earth is a placolith, *Emiliana huxleyi* named after Thomas Henry Huxley. Although *E. huxleyi* is one of the most abundant coccolithophore species, the genus *Coccolithus*, which inhabits subpolar waters, has 30–80 times more PIC per cell and is capable of growing at approximately three-quarters of the growth rate of *E. huxleyi*; hence, *Coccolithus* spp. would be expected to now dominate calcification in subpolar waters (Daniels et al. 2014, 2016), and at typical concentrations, its response to climate change would have a disproportionate effect on total calcification.

Diatoms

Diatoms are a major group of microalgae found in the oceans, waterways and soils of the world. The **shells of diatoms are so heavy that when they die in the oceans, they typically sink to watery graves on the seafloor**, taking carbon out of the surface waters and locking it into sediments below. As such, they are an important part of the marine carbon cycle.

Living diatoms make up a significant portion of the Earth's biomass: **they generate about 20 to 50 percent of the oxygen produced on the planet each year**, take in over 6.7 billion metric tons of silicon each year

from the waters in which they live, and contribute nearly half of the organic material found in the oceans. The shells of dead diatoms can reach as much as a half-mile (800 m) deep on the ocean floor.



Photo of typical diatoms

Diatoms are unicellular: they occur either as solitary cells or in colonies, which can take the shape of ribbons, fans, zigzags, or stars. Individual cells range in size from 2 to 200 micrometers. In the presence of adequate nutrients and sunlight, an assemblage of living diatoms **doubles approximately every 24 hours** by asexual multiple fission; the maximum life span of individual cells is about six days. Diatoms have two distinct shapes: a few (centric diatoms) are radially symmetric, while most (pennate diatoms) are broadly bilaterally symmetric.

A unique feature of diatom anatomy is that they are **surrounded by a cell wall made of silica (hydrated silicon dioxide)**, called a **frustule** made up of two valves called thecae, that typically overlap one another. The biogenic silica composing the cell wall is synthesized intracellularly by the polymerization of silicic acid monomers. This material is then extruded to the cell exterior and added to the wall. These frustules have structural coloration due to their photonic nanostructure, prompting them to be described as "jewels of the sea" and "living opals". Similar to plants, diatoms convert light energy to chemical energy by photosynthesis. Unusually for autotrophic organisms, diatoms possess a urea cycle, a feature that they share with animals, although this cycle is used to different metabolic ends in diatoms (Wikipedia, 2020).

Diatoms sustain the marine food web and **contribute to the export of carbon from the surface ocean to depth**. They account for about **40% of marine primary productivity and particulate carbon exported to depth as part of the biological pump**. Diatoms have long been known to be abundant in turbulent, nutrient-rich waters.

Sinking speed

Diatoms vary widely in size, morphology and elemental composition, all of which control the quality, quantity and sinking speed of biogenic matter to depth. In particular, their silica shells provide ballast to marine snow and fecal pellets and can help transport carbon to both the mesopelagic layer and deep ocean.

The extent to which diatoms contribute to the export of carbon varies by diatom type, with carbon transfer modulated by the Si/C ratio of diatom cells, the thickness of the shells and their life strategies; for instance, the tendency to form aggregates or resting spores. Model simulations project a decline in the contribution of diatoms to primary production everywhere outside of the Southern Ocean (Treguer, P. et al., 2018).

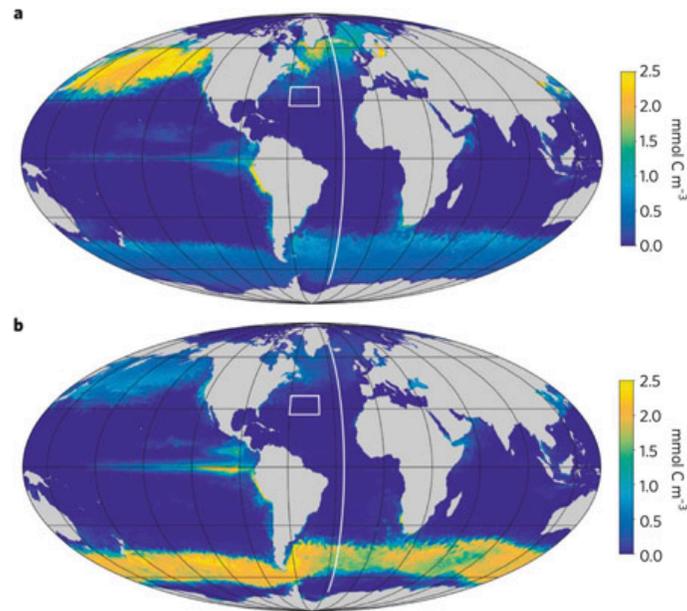
Diatoms are also believed to contribute ~40% of particulate organic carbon (POC) export, which can reach either the mesopelagic layer (the lower limit of which is ~1,000 m, coinciding with the nitrate maximum) or deeper, into the bathypelagic layer, which is also known as the 'CO₂ sequestration layer'. Carbon reaching bathypelagic depths is removed from the atmosphere for at least 100 years, and carbon will ultimately reach sediments at the seafloor and be buried.

The high sinking speed of most diatoms suggest they would be a good organism to concentrate on or fertilization experiments.

However, this may need to be modified if they produce toxic blooms (see below).

Diatom Distribution

Diatoms are an important component of phytoplankton biomass at high latitudes during spring (April–June in the Northern Hemisphere and October–December in the Southern Ocean) and in equatorial and coastal upwelling regions (see following figure).



Diatom concentrations at the surface for (a) April–June and (b) October–December.

They are present at lower levels in mid-ocean subtropical gyres where smaller phytoplankton such as cyanobacteria dominate but are found periodically at higher abundance even in these stratified regimes. In particular, **sympiotic diatom-diazotroph assemblages (DDAs) appear to allow diatoms to periodically be important components of plankton communities in oligotrophic regions.**

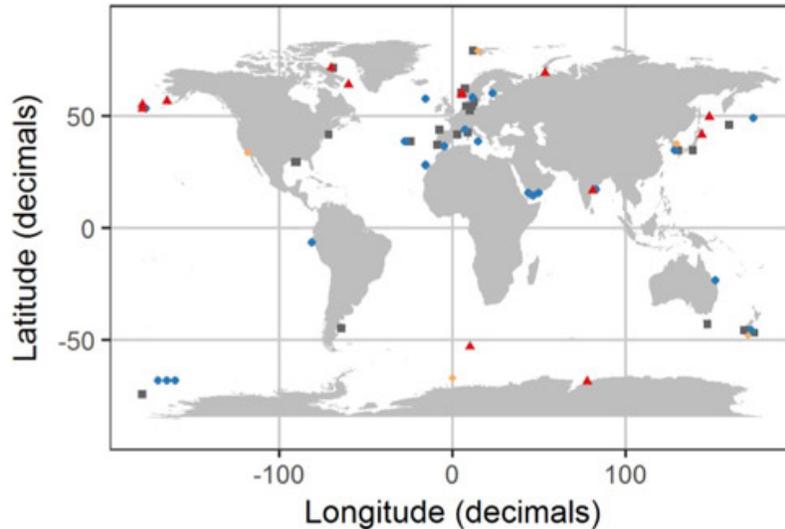
The distribution of diatoms is tightly coupled with the physics of the ocean, the supply of nutrients (**including iron**), as well as their interaction with predators, pathogens and parasites, and, in some cases, symbionts.

The diatom-specific traits of **high maximum growth rate, relatively low nutrient affinities and protection from grazers** allow them to occupy niches that are more turbulent and encompass deeper mixed layers, and higher nutrient concentrations than other phytoplankton. The requirement for silica further delineates their biogeography, especially for heavy versus light silicon users (Treguer, 2018).

Effect of Ocean Acidification on Diatoms

Bach and Taucher (2019) synthesized the literature from over a decade of ocean acidification (OA) experiments with natural diatom communities. They found that in 56% of the reports, diatom communities responded positively to high CO₂ while in 32% they responded negatively. Shifts among different diatom species were observed in 65% of the

experiments. The studies supported the hypothesis that high CO₂ favors larger species.



Response of the diatoms to OA in different experiments. **Blue circles** indicate positive response; **red triangles** indicate a negative response; **grey squares** indicate no response; **orange diamonds** indicate unknown direction of change.

In conclusion, there is growing evidence for the key role that diatoms play in the marine biological carbon pump in the modern ocean. In many regions of the world's ocean, they appear able not only to transfer particulate organic carbon to the mesopelagic layer but also to the CO₂ sequestration layer.

Metagenomics

In the 1900's there was a race to sequence the human genome. There were two teams involved, the NIH led consortium of hundreds of scientists across the country, each taking a different chromosome to work on, and the team of Craig Venter, using a shotgun approach, ignoring the individual chromosome approach. Here the human DNA was cut into relatively small pieces and using banks of machines, all the pieces were sequenced. Based on the sequences at the ends, high speed computers were used to align the pieces into long stretches. Both techniques worked but Venter's approach was far faster.

Once the human and many other species were sequenced, Venter turned to an examination of sea water, especially the Sargasso Sea, which was thought to be a desert devoid of many microorganisms. The advantage of Venter's technique was the even if the identity of individual microorganisms was not known, because they could not be grown in culture, the total DNA of a sample from the soil, the gut, or the ocean could be isolated and shotgun sequenced. The pieces could be assembled allowing the

identification of new species by virtue of having their entire genomes sequenced. This identified new species by their DNA and genes rather than appearance. This technique is called **metagenomics**.

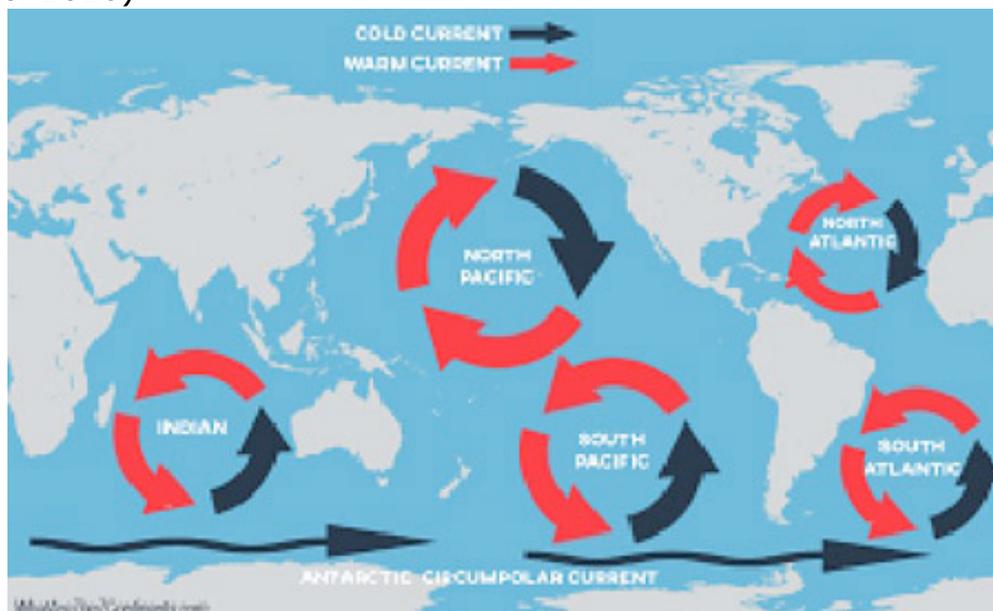
With this powerful tool in hand, Venter turned his attention to the ocean, i.e. the Sargasso Sea, considered a classic oligotrophic gyre. The presence of low wind, low nutrients, and high salinity help to justify why the Sargasso Sea was considered an ocean desert. Surprisingly, Venter's team found 1,800 species of microbes, including 150 new species of bacteria, and over 1.2 million new genes (Venter, et al. 2004; Microbewiki, 2020).

Gyres

The following from ocean gyre to oligotrophic gyres was taken verbatim from the Nat. Geo. Soc. <https://www.nationalgeographic.org/encyclopedia/ocean-gyre/>

If one of the approaches we wish to take is providing nutrients to parts of the ocean to encourage phytoplankton growth, one question is, What parts of the ocean to do this? That brings up the following discussion of gyres, oligotrophic gyres, and ocean deserts.

An ocean gyre is a large system of circular ocean currents formed by global wind patterns and forces created by Earth's rotation. The movement of the world's major ocean gyres helps drive the "ocean conveyor belt." The ocean conveyor belt circulates ocean water around the entire planet. Also known as thermohaline circulation, the ocean conveyor belt is essential for regulating temperature, salinity and nutrient flow throughout the ocean (Nat Geo Soc. 2020)



Ocean gyres are large system of circular ocean currents formed by global wind patterns and forces created by Earth's rotation. The five major circulation patterns formed by the currents on this map are the world's five major ocean gyres: North Atlantic, South Atlantic, Indian, North Pacific, and South Pacific. (The Indian Ocean Gyre is actually two, split slightly below the Equator.)

How a Gyre Forms

Three forces cause the circulation of a gyre: global wind patterns, Earth's rotation, and Earth's landmasses. Wind drags on the ocean surface, causing water to move in the direction the wind is blowing. The Earth's rotation deflects, or changes the direction of, these wind-driven currents. This deflection is a part of the Coriolis effect. The Coriolis effect shifts surface currents by angles of about 45 degrees. In the Northern Hemisphere, ocean currents are deflected to the right, in a clockwise motion. In the Southern Hemisphere, ocean currents are pushed to the left, in a counterclockwise motion. Beneath surface currents of the gyre, the Coriolis effect results in what is called an Ekman spiral where surface currents are deflected by about 45 degrees, each deeper layer in the water column is deflected slightly less. This results in a spiral pattern descending about 100 meters (330 feet).

Earth's continents and other landmasses (such as islands) also influence the creation of ocean gyres. The massive South Pacific Gyre, for instance, includes hundreds of kilometers of open ocean. It is bounded only by the continents of Australia and South America, as well as the Equator and powerful Antarctic Circumpolar Current. In contrast, the northern Indian Ocean Gyre is a much smaller ocean gyre. Unlike the South Pacific Gyre, its extent is determined largely by landmasses.

Types of Gyres

There are three major types of ocean gyres: tropical, subtropical, and subpolar. Subpolar gyres form in the polar regions of the planet. They sit beneath an area of low atmospheric pressure. Wind drives the currents in subpolar gyres away from coastal areas. These surface currents are replaced by cold, nutrient-rich water in a process called upwelling. The Northern Hemisphere has several subpolar gyres, bounded by islands such as Iceland, Greenland, and the Aleutians; and the northern reaches of Scandinavia, Asia, and North America.

Tropical gyres form near the Equator. The Coriolis effect is not present at the Equator, and winds are the primary creators of currents. For this reason, tropical gyres tend to flow in a more east-west (instead of circular) pattern. The Indian Ocean Gyre is actually two distinct tropical gyres—the northern and southern Indian Ocean Gyres. Most of the world's major gyres are subtropical gyres. These form between the polar and equatorial regions of Earth. Subtropical gyres circle areas beneath regions of high atmospheric pressure. These are placid ocean areas thousands of kilometers in diameter. Unlike coastal zones, these central regions are relatively stable. The ocean water generally stays in one place while the currents of the gyre circulate around it.

Movement of Gyres

Gyres are comprised of ocean currents that link up as they follow the coastlines of the Earth's continents. Each gyre has a powerful western boundary current and a weaker eastern boundary current.

The North Atlantic Gyre begins with the northward flow of the Gulf Stream along the East Coast of the United States. The Gulf Stream is the western boundary current of the gyre. The gyre then becomes the North Atlantic Current, which flows across the North Atlantic to Europe. Still flowing in a circular pattern, the current flows south as far as the northwestern coast of Africa, where it is known as the Canary Current—the gyre's eastern boundary current. The gyre is completed as the North Atlantic Equatorial Current crosses the Atlantic Ocean to the Caribbean Sea. This entire circle and the water within it are the North Atlantic Gyre.

Most ocean gyres are very stable and predictable. The North Atlantic Ocean Gyre always flows in a steady, clockwise path around the North Atlantic Ocean. Some gyres experience seasonal variation, however.

The temperature in an ocean gyre depends on many factors, including the current. The Gulf Stream and summer monsoon current are warm currents. They are heated by the warm tropical waters of the Caribbean Sea (Gulf Stream) and equatorial Indian Ocean (summer monsoon current). The North Atlantic Current and winter monsoon current are cool currents. They are cooled by Arctic winds and ocean currents (North Atlantic) and the winter monsoon blowing from the icy Himalayas (winter monsoon current).

Garbage Patches

Ocean gyres circle large areas of stationary, calm water. Debris drifts into these areas and, due to the region's lack of movement, can accumulate for years. These regions are called garbage patches. The Indian Ocean, North Atlantic Ocean, and North Pacific Ocean all have significant garbage patches. The garbage patch in the North Pacific Ocean is sometimes called the Pacific trash vortex or the Great Pacific Garbage Patch. Garbage patches are created slowly. Marine debris makes its way into the Great Pacific Garbage Patch, for instance, from currents flowing along the west coast of North America and the east coast of Asia. Some of the debris is also dumped from ocean vessels. The circular motion of the gyre draws in the debris, mostly small particles of plastic. Eventually, the debris makes its way to the center of the gyre, where it becomes trapped and breaks down into a kind of plastic soup.

Oceanographers and ecologists are concerned about garbage patches. In particular, they are studying the degradation, or breaking down, of plastics. Unlike natural substances, such as wood or metal, plastic does not disintegrate into organic substances. It simply breaks down into smaller and smaller pieces of plastic. These tiny plastic particles are as small as the algae and plankton that form the basis of the entire ocean food web. Species

such as shrimp, birds, and fish consume these microplastics, which oftentimes kill them. The plastic chemicals can also be absorbed by predators of these species. The concentration of these chemicals increases through each trophic level of the food chain, a process known as biomagnification. Garbage patches generally accumulate far from any country's coastline, and it is nearly impossible to track the origin of marine debris. The tiny plastic particles that make up most of the patches are also very difficult and expensive to detect and remove. Few nations have accepted the responsibility of cleaning up the ocean's garbage patches.

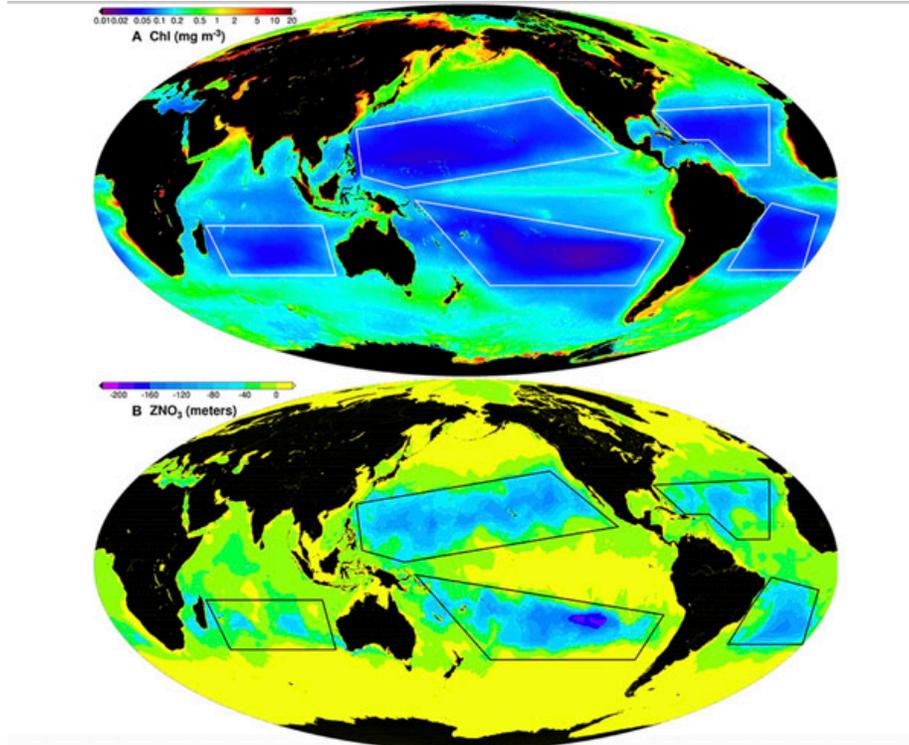
Horse Latitudes

Many ocean gyres are found in what are called the horse latitudes, between 30 and 35 degrees north and south. These areas are known to have calm waters and little precipitation or winds. Legend has it that the name refers to sailing ships that stalled in these latitudes, leading sailors to throw their horses overboard because they were afraid, they would run out of water.

Oligotrophic Gyres, Ocean Deserts, HNLCs

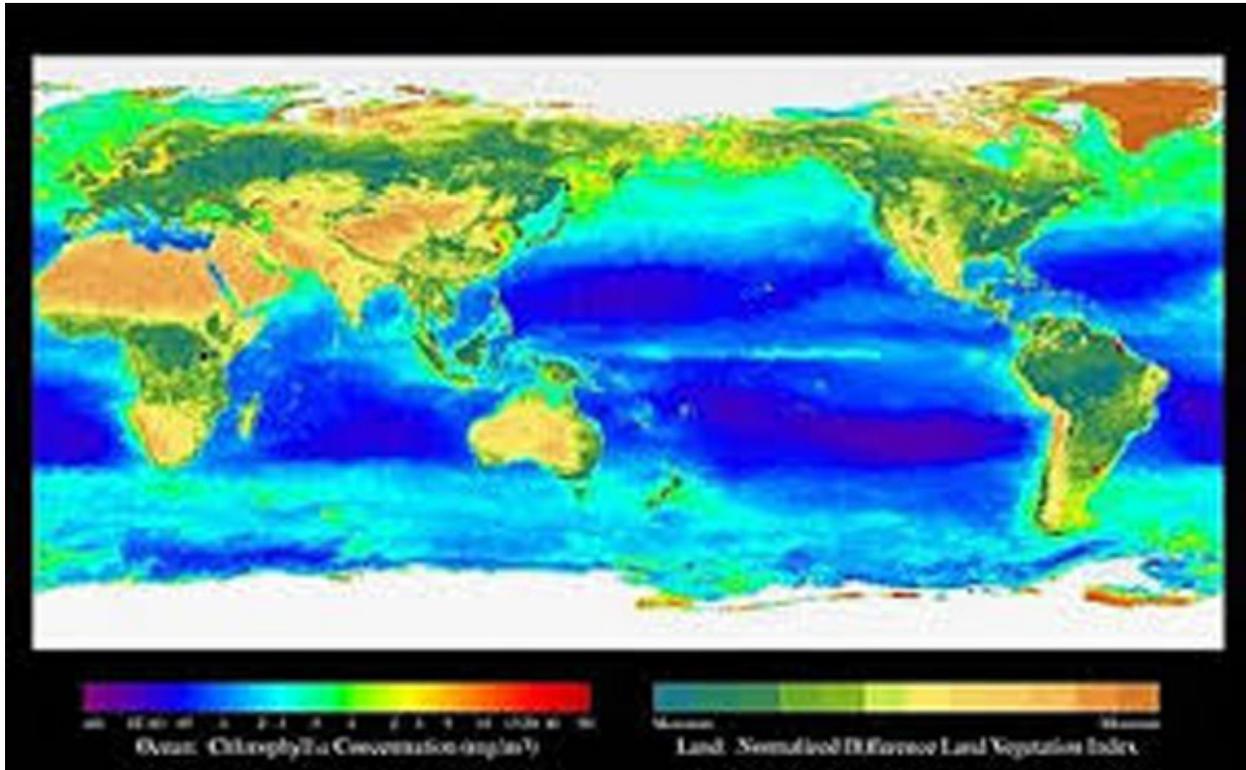
Scientists are only beginning to understand the biodiversity of ocean gyres. Their calm centers have traditionally been regarded as **oligotrophic**, or nutrient-poor, because they have few concentrations of the organic chemicals that support producers, such as algae and plankton, in the ocean food web. These have also been called ocean deserts and **High Nutrient Low Chlorophyll (HNLC)** areas.

In the ocean, the **subtropical gyres** north and south of the equator are regions in which the nutrients required for phytoplankton growth (for instance, nitrate, phosphate and silicic acid) are strongly depleted all year round. Their immense size (they occupy ~ **40% of the surface of the earth**) makes their contribution to the global carbon cycle very important. These areas are described as oligotrophic and exhibit low surface chlorophyll and some nitrogen depletion - see following figure (Signorini, et al. (2015).



Global satellite maps. Top: lack of chlorophyll in 5 different gyres. Bottom: Nitrogen content in surface water.

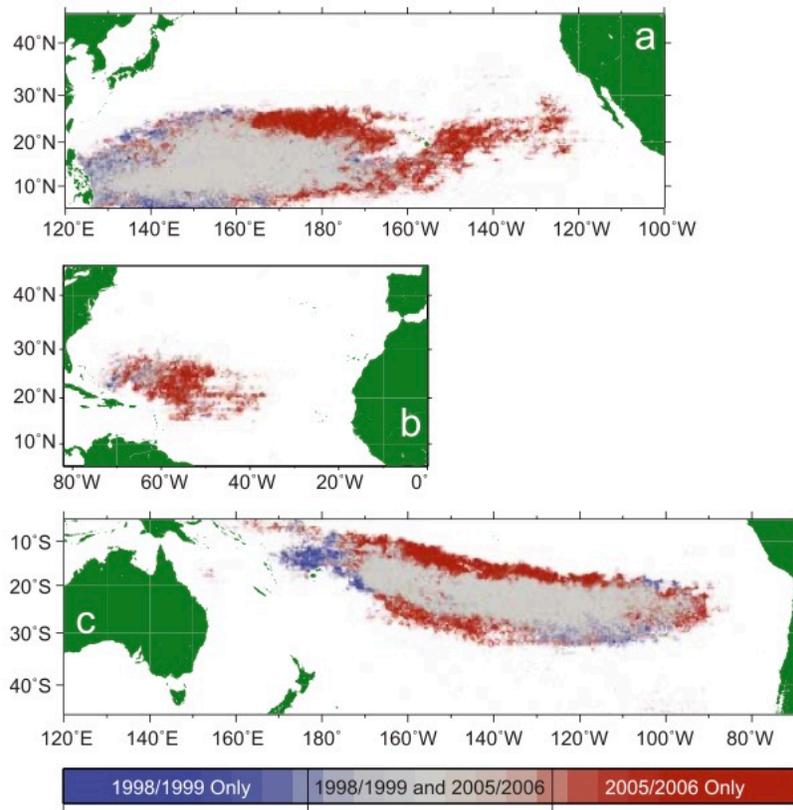
The **North Pacific Subtropical Gyre** (NPSG) is the largest contiguous ecosystem on earth. Oligotrophic subtropical gyres are regions of DOM accumulation (Polovina et al, 2011) and export. They are growing (Polovina, et al, 2008). The predicted growth in of gyres in the future, evidenced by a 56% increase of the **North Atlantic gyre** wintertime area between 1998 and 2006, may therefore increase net oceanic DOM production. However, experimental studies suggest that rising temperatures and ocean acidification will increase bacterial DOM consumption (Endres, et al, 2014; Engel et al, 2014), whereas the same drivers may reduce formation of colloids and microgels from DOM (Chen et al, 2015). Thus, whether or not the future ocean will experience greater accumulation of DOM or alterations in its chemical composition (Zark, et al 2015) is still unclear.



Satellite data provides a map of the area of the world, both land and sea, rich in chlorophyll which equates to plant mass. The gyres tend to be Chlorophyll poor.

Where are the ocean deserts?

Ocean desert are areas of the ocean deficient in chlorophyll and thus in photosynthetic plankton. The following maps (Polovina, et al, 2008) show these areas in three different oceans. (NOAA, 2009).



For (a) North Pacific and (b) North Atlantic **grey** represents the areas with surface chlorophyll less than or equal to 0.07 mg chl/m^3 for both the 2-month mean of December 1998 and December 1999 and the 2-month mean of December 2005 and December 2006. **Blue** represents the areas with surface chlorophyll less than or equal to 0.07 mg chl/m^3 for only the 2-month mean of December 1998 and December 1999. **Red** represents the areas with surface chlorophyll less than or equal to 0.07 mg chl/m^3 for only the 2-month mean of December 2005 and December 2006. For (c) the South Pacific, the colors represent the same areas based on the month of August.

They are located in the gyres. The desert areas expand and contract seasonally. During the winter months, the area is smaller compared to the summer months because winter storms and stronger winter winds **increase vertical mixing in the subtropical regions.**

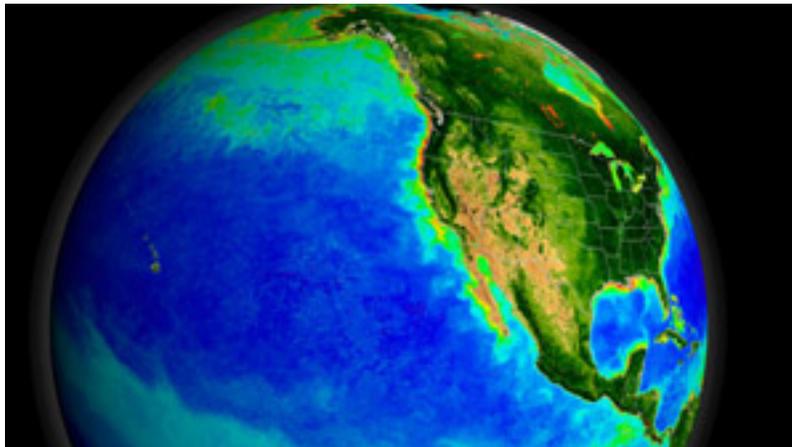
Since 1998, the least productive oceanic habitats or the desert areas in four of the world's major oceans have **been expanding at average rates between $0.8\%/yr$ and $4.3\%/yr$.** The rate of expansion is greater during the winter in three of these oceans. The North Atlantic has a much smaller desert area but it is expanding faster than the other oceans and especially during the winter. For example, the size during the first quarter in the North Atlantic has expanded by 56% between 1998 and 2006.

Natural blooms: where and why they occur.

If we plan to stimulate the growth of phytoplankton to sequester CO₂ we could learn how to do this by studying the cause of natural blooms.

A bloom can be considered as a phytoplankton population explosion. They occur when sunlight and nutrients are readily available to the plants, and they grow and reproduce to a point where they are so dense that their presence changes the color of the water in which they live.

Natural blooms of phytoplanktons, especially Coccolithophores, can be visualized from space and can cover hundreds of thousands of square kilometers.



Oceans are green when the photosynthetic properties are prominent,



and white when the calcite coatings reflect in the sunlight.

In studies of the Bay of Biscay, Smith et al, (2012) found that the most heavily calcified coccolithophore morphotype dominates when

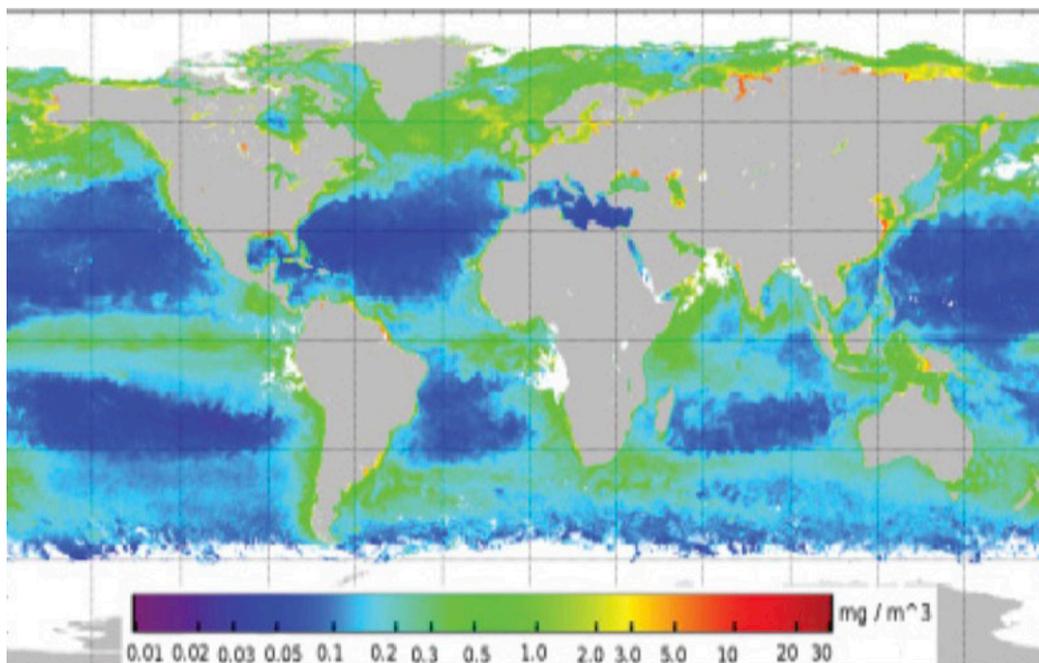
conditions are most acidic. While this is contrary to the other predictions, these results are most likely due to a unique subspecies of coccolithophore.

To further evaluate the impact of acidification on coccolithophore blooms, required knowledge of the temporal and spatial evolution of their blooms. Perrot, L. et al, (2016) determined from satellite radiance, the seasonal and interannual variability of coccolithophore blooms **for 18 years** (1998 to 2015) across the North-East Atlantic region covering the Bay of Biscay and the Celtic Sea. There was a high seasonal and interannual variability in the extent of the blooms. Although it has been shown that a small mixed layer depth, strong irradiance and high temperatures are factors which enhance coccolithophore development, other as yet undetermined environmental and biological factors are likely involved in this variability.

In the Sea of Marmara there are different phytoplanktons at different seasons. Diatoms tend to predominate in March–April, dinoflagellates in April–May, the dramatically colorful blooms of *E. huxleyi* predominate in May–June, and diatoms in July–August (Turkoglu, 1967).

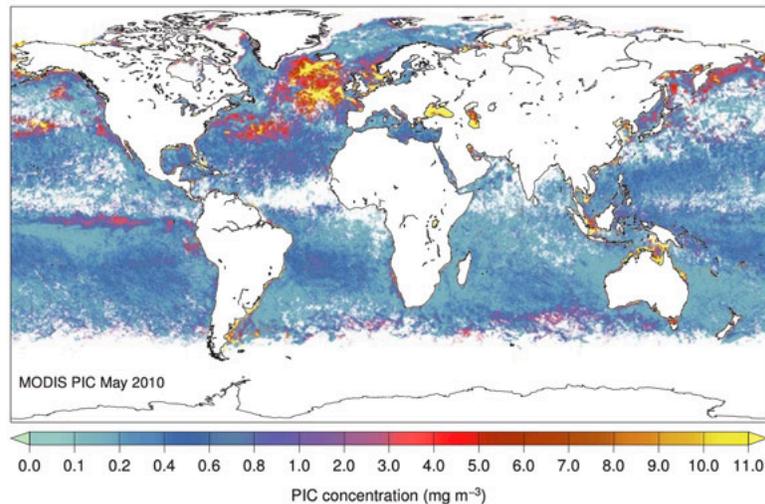
World-wide distribution of Coccolithophore Blooms.

Among different phytoplankton blooms, coccolithophore blooms are very important due to their wide coverage and frequent occurrence (Holligan et al., 1983), as well as their unique bio-optical and biogeochemical properties (Brown and Podesta, 1997; Balch, 2004).



Total coccolithophore density from the GlobColor satellite data. Note the paucity in mid-ocean compared to higher density close to shore and in arctic waters.

Also.



Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua satellite-derived particulate inorganic carbon (PIC) concentration (mg/m^3) averaged for the month of May 2010 highlights prominent coccolithophore blooms in the North Atlantic.

What Causes Coccolithophore Blooms?

The following is a listing of some of the proposed causes of coccolithophore blooms (Tyrrell and Merico, 2006).

High Light

The hypothesis is that high light conditions trigger *E. huxleyi* blooms was put forward by Nanninga and Tyrrell (1996). They suggested that the reason *E. huxleyi* outcompetes other phytoplankton at high light intensities is that *E. huxleyi* is uniquely tolerant of high light intensities, and is not prone to photoinhibition, in contrast to other phytoplankton.

Wind

Scientists at NASA believe the coccolithophore blooms are the result of **changing wind patterns in the region**. Weaker than normal winds fail to mix the water of the Bering Sea, resulting in the growth of coccolithophores instead of other types of phytoplankton. (NASA Earth Observatory, 2000). Anomalous wind triggered the largest recorded phytoplankton bloom in the oligotrophic North

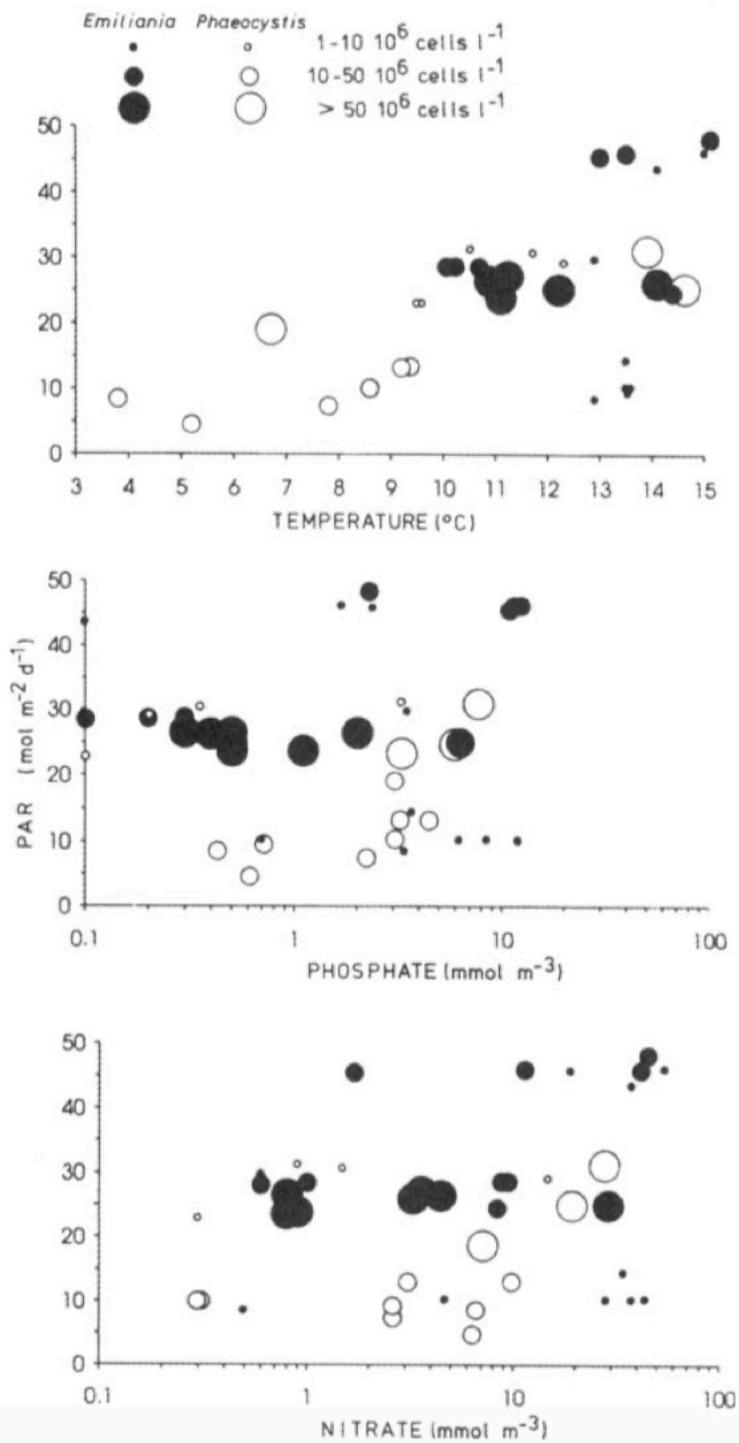
Pacific Subtropical Gyre. It peaked in 2010 and covered over 2 million km². (Chow et al 2019).

Low silicate Although sediment records show that *E. huxleyi* has been present at some concentration in the Black Sea for about 1600 years, phytoplankton sampling records show a decline in diatom numbers and an increase in *E. huxleyi* numbers since the 1960s (Mihnea 1997; Humborg et al. 1997). What has caused the recent shift away from diatoms and towards *E. huxleyi* (and other flagellates)? Since 1970-72, when the Iron Gates Dam was constructed across the River Danube (which provides the majority of the freshwater inflow to the Black Sea), the river load of dissolved silicate into the Black Sea has markedly decreased while river load of nitrogen has increased. This has depressed dissolved silicate levels in the Black Sea resulting in depressed diatom numbers (Humborg, et al. 1997).

Phosphate more limiting than nitrate

Several studies have noted anomalous N:P ratios during *E. huxleyi* blooms. The role of temperature, phosphate and nitrate is shown in the following figure (Egge and Heimdal 1994). Temperatures range from 10°C to 16°C.

Most *E. huxleyi* blooms (**large black circles**) occurred at lower phosphate concentrations while the same cannot be said for lower nitrate concentrations. *E. huxleyi* is known to be able to synthesize the enzyme alkaline phosphatase (Riegman et al. 2000), which allows uptake of some fractions of dissolved organic phosphates and should therefore impart an advantage to *E. huxleyi* when inorganic phosphate is limiting. Recent examination of nutrient data from the eastern Bering Sea during the years of the *E. huxleyi* blooms there suggests that those blooms occurred when N was limiting but P was abundant, suggesting that low phosphate is not an absolute requirement for the occurrence of these blooms.



Emiliania huxleyi and *Phaeocystis* sp. in enclosure experiments in 1988--1992.
 (Egge, J.K. and Heimdal, B.R., 1994)

Low dissolved carbon dioxide

The chemical reaction for calcification is:



The synthesis of calcium carbonate coccoliths therefore releases CO_2 as a by-product, and **this extra CO_2 is probably available for photosynthesis**. This indirect means of obtaining CO_2 from HCO_3^- could potentially give *E. huxleyi* an advantage over other phytoplankton if the rate of supply of external CO_2 is limiting for growth. **The reutilization of CO_2 from the production of CaCO_3 seems to mitigate the potential problem that the coccolithophores might have limited utility in the uptake of atmospheric CO_2 because they themselves produce CO_2 .**

Despite the enormous uptake by phytoplankton, it is unlikely that CO_2 ever falls much below about 10 pMol/kg in the open ocean, even following intense spring blooms such as those that occur annually in the northeast North Atlantic (Tyrrell and Taylor 1995). It is likely that carbon availability never sets a limit to the final amount of phytoplankton growth in a season, but it is possible that it sets a limit to the instantaneous rate of growth at the height of blooms.

High carbonate saturation state (carbonate ion concentration)

A wide range of experiments, mostly on other marine calcifiers but also on *E. huxleyi*, suggest a possibly important role for carbonate ion concentration (carbonate saturation state) in determining where blooms can occur.

Coral reefs are restricted to low latitudes, most probably because of a dependence on the **carbonate saturation state of seawater (Omega)** (Kleypas et al. 1999a), which attains highest values between about 0-30° of latitude, and then falls away towards the poles. Calcium carbonate saturation state is

$$\text{OMEGA} = [\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}] / K'_{\text{sp}}$$

where K'_{sp} is the stoichiometric solubility product which takes different values for the different mineral phases, aragonite (coral reefs, pteropods) and calcite (coccoliths, foraminifera shells), but for which the geographical trend is the same. Calcium concentration varies little throughout the ocean, so variability in Omega is mostly due to variability in CO_3^{2-} . Aragonite and calcite are crystal forms of CaCO_3 .

It is rather important to answer the question of sensitivity of coccolithophores to carbonate ion because the emission of CO_2 into the atmosphere from burning of fossil fuels is, following diffusion of large

amounts of the CO_2 into the surface ocean, rapidly acidifying the surface ocean and decreasing carbonate ion concentration and Omega there. It is estimated that $\text{Omega}_{\text{arag}}$ averaged ~ 4.6 in the tropics 100 years ago, is currently ~ 4.0 , and is projected to drop to ~ 2.8 by 2100 (Kleypas et al. 1999a). Both coccolithophores and coral reefs could become substantial casualties of the rise in atmospheric and ocean carbon. We need to understand whether coccolithophores and coral reefs will be driven out of most or all oceanic environments by rising CO_2 and consequent falling CO_3^{2-} .

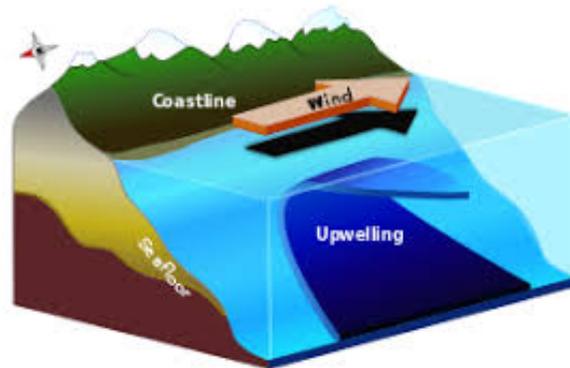
Grazers (microzooplankton and/or jellyfish) The respective numbers of different species of grazers at a location may influence the viability of that location for different species of phytoplankton. The major grazers of *E. huxleyi* were micro zooplankton (Holligan et al. 1993a) and therefore variations in the density of micro zooplankton could be important in determining where blooms of *E. huxleyi* can form.

Olson and Strom (2002) measured phytoplankton growth and microzooplankton grazing rates, by means of seawater dilution techniques inside and outside an *E. huxleyi* bloom area in the southeastern Bering Sea. They observed that "a reduced microzooplankton grazing is a key component in the formation and temporal persistence of *Emiliana huxleyi* bloom".

Upwellings derived from various Wikipedia entries. Another important factor is the increase in coastal upwelling events due to climate change. Upwelling is an oceanographic phenomenon that involves **wind-driven** motion of dense, cooler, and usually nutrient-rich water towards the ocean surface, replacing the warmer, usually nutrient-depleted surface water. The nutrient-rich upwelled water stimulates the growth and reproduction of primary producers such as phytoplankton. Due to the biomass of phytoplankton and presence of cool water in these regions, upwelling zones can be identified by cool sea surface temperatures (SST) and high concentrations of chlorophyll.

The increased availability of nutrients in upwelling regions results in high levels of primary production and thus fishery production. Approximately 25% of the total global marine fish catches come from five upwellings that occupy only 5% of the total ocean area. Upwellings that are driven by coastal currents or diverging open ocean have the greatest impact on nutrient-enriched waters and global fishery yields.

The following figure illustrates one mechanism of producing upwellings.



If the wind blows parallel to the coast, then Ekman transport can produce a net movement of surface water 90° to the right. This may result in coastal upwelling.

Ekman Transport. As the wind blows it casts a friction force on the ocean surface that drags the upper 10-100m of the water column with it. However, due to the influence of the Coriolis effect, the ocean water moves at a 90° angle from the direction of the surface wind. The direction of transport is dependent on the hemisphere: in the northern hemisphere, transport occurs at 90° clockwise from wind direction, while in the southern hemisphere it occurs at a 90° counterclockwise.

The major upwellings in the ocean are associated with currents that bring deeper, colder, nutrient rich waters to the surface. Because of this it is not surprising that upwellings can produce phytoplankton blooms (Menschell, et al 2016). The following are the five types of upwellings.

1. Coastal upwelling is the best-known type of upwelling, and the most closely related to human activities as it supports some of the most productive fisheries in the world.

Deep waters are rich in nutrients, including nitrate, phosphate and silicic acid, themselves the result of decomposition of sinking organic matter (dead/detrital plankton) from surface waters. When brought to the surface, these nutrients are utilized by phytoplankton, along with dissolved CO_2 and light energy from the sun. As a result, upwelling regions result in very high levels of primary production of carbon fixed by phytoplankton. Coastal upwelling exists year-round in some regions, known as major coastal upwelling systems, and only in certain months of the year in other regions, known as seasonal coastal upwelling systems.

2. Equatorial Upwelling at the equator is associated with the Intertropical Convergence Zone (ITCZ) which actually moves, and consequently, is often located just north or south of the equator. Although there are no Coriolis forces present along the equator, upwelling still occurs just north and south of the equator.

3. Southern Ocean A Large-scale upwelling is also found in the Southern Ocean. Here, strong westerly (eastward) winds blow around Antarctica, driving a significant flow of water northwards.

4. Tropical Some models of the ocean circulation suggest that broad-scale upwelling occurs in the tropics, as pressure driven flows converge water toward the low latitudes where it is diffusively warmed from above.

5. Other sources Local and intermittent upwellings may occur when offshore islands, ridges, or seamounts cause a deflection of deep currents, providing a nutrient rich area in otherwise low productivity ocean areas. Examples include upwellings around the Galapagos Islands and the Seychelles Islands, which have major pelagic fisheries.

Due to the biomass of phytoplankton and presence of cool water in these regions, upwelling zones can be identified by cool sea surface temperatures (SST) and high concentrations of chlorophyll.

Artificial upwelling (see below) is produced by devices that use ocean wave energy or ocean thermal energy conversion to pump water to the surface. Ocean wind turbines and ocean wave devices have been shown to produce plankton blooms. (Wikipedia 2020, Upwelling)

Coccolithophore Blooms and Dimethyl sulfide.

Coccolithophores also affect the atmosphere and climate by emitting dimethyl sulfide (DMS) into the atmosphere (Tyrrell and Merico, 2004; Andreae, 1990), where it is converted to the sulfur aerosols and cloud condensation nuclei (CCN) and influence the climate and the Earth's energy budget (Charlson et al., 1987; Andreae, 1990).

The Great Calcite Belt (GCB)

The Great Calcite Belt is a region of elevated surface reflectance in the Southern Ocean (SO) covering ~16% of the global ocean and is thought to result from elevated, seasonal concentrations of coccolithophores. The GCB sits between approximately 38° S and 60° S and can be observed around the entire Southern Ocean (Balch et al. 2011). Competition between diatoms and coccolithophores in the Southern Ocean also means that instead of being a net sink for CO₂ (e.g., in the diatom-rich waters), the **coccolithophore-rich waters of the GCB become net sources of CO₂** because of the calcification process (Balch et al. 2016)

Harmful Algae Blooms (HABs)

Harmful algal bloom species are a small subset of algal species that negatively impact humans or the environment. HABs can pose health hazards for humans or animals through the production of toxins or bioactive compounds. They also can cause deterioration of water quality through the buildup of high biomass, which degrades aesthetic, ecological, and

recreational values. It is widely believed that the frequency and geographic distribution of marine HABs have been increasing worldwide.

Increased nutrient loading has been acknowledged as a likely factor contributing to the increased occurrence of high biomass HABs. Research has shown that changes in nutrient quality (e.g., organic versus inorganic nutrients), not quantity, may favor proliferation of some harmful algal species. The majority of these HAB species are microalgae (microscopic, single-celled algae), that live suspended in the water. Optimal growth occurs when environmental conditions such as nutrient and light availability, temperature, and salinity are optimal for cell growth. Other biological (e.g., vertical migration, grazing, viral infection, and parasitism) and physical (e.g., transport) processes determine if enhanced cell growth will result in biomass accumulation.

Massive fish kills are perhaps the most commonly observed impact of HABs on wildlife, but HABs can detrimentally affect many aspects of marine ecosystems. Algal toxins have caused deaths of whales, sea lions, dolphins, manatees, sea turtles, birds, and wild and cultured fish and invertebrates (Landsberg, 2002).

High biomass blooms are a common type of event that can cause hypoxia or anoxia (low or no dissolved oxygen), which suffocates fish and bottom-dwelling organisms and can sometimes lead to hydrogen sulfide poisoning. High biomass blooms can also directly inhibit growth of beneficial vegetation by blocking sunlight penetration into the water column. Macroalgal blooms also reduce sunlight penetration and can overgrow or displace seagrasses and corals.

Seasonal variability in PIC, to indicate changes in coccolithophore biomass, is identified across much of the global ocean. Blooms, which typically start in February–March in the low-latitude ($\sim 30^\circ$) Northern Hemisphere and last for ~ 6 – 7 months, get progressively later (April–May) and shorter (3–4 months) moving poleward. A similar pattern is observed in the Southern Hemisphere, where blooms that generally begin around August–September in the lower latitudes and which last for ~ 8 months get later and shorter with increasing latitude (Hopkins, J. et al, 2015)

What organisms cause toxic blooms?

Some haplophytes such as Prymnesiophytes, can cause harmful algal blooms. Some of these are mixotrophic; *Prymnesium parvum*, for example, can graze on *Rhodomonas salina* (Brutemark & Graneli 2011). Recent studies show that the growth of the predator *Prymnesium parvum* is related to the nutritional (N:P) status of its prey, *Rhodomonas salina*; low phosphorus concentrations accelerate toxin production and induce greater feeding rates (Lundgren et al. 2016). High aeration also promotes toxin production by *Prymnesium parvum* (Vidyarathna et al. 2014). Of note, *E. huxleyi* does not produce any toxins.

If one of the pathways we choose to fight climate change is to artificially induce blooms, the issue of bloom harm needs to be addressed. As waters warm more than usual due to climate change, harmful algae blooms will increase. These warm waters favor the formation of harmful blooms in a number of ways. For example, the tiny nanoplankton and picoplankton species such as the more toxic cyanobacteria and some coccolithophores prefer warmer waters. High temperature levels at the surface prevent mixing of the water column, which allows harmful blooms to become thicker and grow faster. It is generally accepted that warm waters favor the proliferation of tiny phytoplankton bloom species and allow them to survive much easier in the surface waters. It is known that algal blooms absorb solar radiation, which makes the water even warmer than usual and facilitates more algal blooms. On the other hand, climate change might lead to more drought seasons, making freshwater saltier. So, marine algal bloom species could spread to freshwater and brackish water ecosystems (Turkoglu, M. (2016).

Phytoplankton species need dissolved CO₂ to proliferate. Higher CO₂ levels—first in the air and then water—might lead to rapid phytoplankton increase, particularly **picoplanktonic species that float on the surface layer of the water**. Moreover, climate change might affect precipitation dynamics, leading to alternating periods of drought and intense storms. The main source of nutrients is rain and river water discharge into aquatic ecosystems, supporting more algal blooms.

In Summary It seems unlikely that any single factor can explain all blooms. More probably a combination of conditions is required. Wind, high light, limiting iron and limiting silicate (to restrict diatoms) are probably essential, and high carbonate saturation state may also be critical, but much work remains to be done. (Tyrrell, & Merico, 2004)

Nutrients required for growth.

The temporal variability of coccolithophore growth in nature has been attributed to a host of environmental factors, both physical and biological: mixing (Young 1994), temperature-dependent growth optima (Balch 2004), limitation by micronutrients and trace nutrients (Balch et al. 2016, Perrin et al. 2016), competition with other phytoplankton species (Boyd et al. 2010), grazing (Harvey et al. 2015), and viral infection (Brussaard 2004).

Two recent studies have documented phenotypic variability related to temperature (Blanco-Ameijeiras et al. 2016, Zhang et al. 2014). Both studies demonstrated that the temperature maxima for *E. huxleyi* strains are related as much to the conditions that the cultures were originally isolated from as to the phenotypic plasticity of species exposed to new environmental conditions associated with climate change.

Silica Durak et al. (2016) recently demonstrated that silicon plays a role in calcification in certain coccolithophore species, which has profound consequences for our understanding of coccolithophore ecology. The bloom-forming coccolithophores *E. huxleyi* and *Gephyrocapsa oceanica*, however, have no apparent silicate requirement. Regardless, such a silicate requirement for certain coccolithophores for growth, calcification, and coccolith integrity could have major impacts on where these species grow, their biogeochemistry, their paleo-variability, and their optical properties. Moreover, these observations help explain the apparent competitive advantage that bloom-forming coccolithophores with no silicon requirement, such as *E. huxleyi*, have over diatoms in low-silicate waters and the typical succession of coccolithophores following diatom-dominated blooms, a paradigm that dates back to Margalef (1978).

Iron Changes in the iron supply to oceanic plankton are thought to have a significant effect on concentrations of atmospheric carbon dioxide by altering rates of carbon sequestration, a theory known as the '**iron hypothesis**'. For this reason, it is important to understand the response of pelagic biota to increased iron supply. Boyd et al (2000) report the results of a mesoscale iron fertilization experiment in the polar Southern Ocean, where the potential to sequester iron-elevated algal carbon is probably greatest. Increased iron supply led to elevated phytoplankton biomass and rates of photosynthesis in surface waters, **causing a large drawdown of carbon dioxide and macronutrients, and elevated dimethyl sulphide levels** after 13 days. This drawdown was mostly due to the proliferation of **diatom** stocks. Moreover, satellite observations of this massive bloom 30 days later, suggest that a sufficient proportion of the added iron was retained in surface waters. These findings demonstrate that iron supply controls phytoplankton growth and community composition during summer in these polar Southern Ocean waters, but the fate of algal carbon remains unknown and depends on the interplay between the processes controlling export, remineralization and time scales of water mass subduction (Boyd et al, 2000).

The above study was relevant to diatoms. Recent work examining the effects of trace metals (specifically iron) on coccolithophore growth has reinforced the interpretation that coccolithophores (exemplified by *E. huxleyi*) are able to maintain growth at lower levels of inorganic iron than other phytoplankton (such as diatoms) and have lower cell quotas for iron (Hartnett et al. 2012).

One aspect aiding coccolithophores in the uptake of iron is a mutualistic relationship with marine bacteria (e.g., the genus *Marinobacter*) in which the bacteria produces a siderophore called vibrioferrin. Photolysis of the iron-vibrioferrin chelate can increase bacterial iron uptake by 70% and subsequently increase the algal uptake of iron by 20 times (Amin et al. 2009). Community composition also affects iron cycling. Regenerated iron sustains much of the phytoplankton growth in the sea, and grazing activity

by meso- and microzooplankton also enhances the uptake of iron by phytoplankton, including coccolithophores (Nuester et al. 2014). The degree of enhanced uptake of regenerated iron thus depends not only on the type of grazer but also on the type of prey (Nuester et al. 2014)—that is, iron released during consumption of diatoms by the meso-zooplankton *Acartia tonsa* is taken up twice as fast by the diatom *Thalassiosira pseudonana* as the regenerated iron released during the consumption of the coccolithophore *E. huxleyi*.

Ocean acidification (OA) may secondarily affect the uptake of iron by coccolithophores (and by most phytoplankton, for that matter). A decrease in pH will decrease the concentration of hydroxide (which typically forms complexes with iron) and increase the concentration of ionic Fe(II) (Marchetti & Maldonado 2016). All else being equal, when free Fe(II) increases, primary production (of coccolithophores as well as other phytoplankton) should also increase; however, most dissolved iron in the ocean is attached to strong ligands that are organic in nature (Gledhill & van den Berg 1994). Thus, decreasing pH will be expected to increase the solubility of Fe(III) (the labile dissolved inorganic form, here called Fe'). The effect of pH on ligand binding is critical to the availability of Fe' to cells. In general, the accessibility of Fe' to phytoplankton is reduced at lower pH as a function of the redox chemistry of the binding ligands (Marchetti & Maldonado 2016).

CO₂ and reduced pH in mesocosm experiments, adversely affect the growth of a coastal *E. huxleyi* variety, addition of the siderophore desferoxamine B (DFB) partially offsets the negative effect of OA (Segovia et al. 2017). Moreover, under low pCO₂ **the addition of DFB induced a striking increase of *E. huxleyi* abundance to bloom levels**, once again showing that the effects of multiple stressors involving OA and iron or OA and temperature can have stronger effects than OA on its own (Gao et al. 2012, Segovia et al. 2017).

Iron appears to be a key trace nutrient responsible for the growth of coccolithophores in the Southern Ocean (Balch et al. 2011). Silicate (plus nitrate and phosphate) upwelled in the Circumpolar Deep Water, along with iron, sustains dense diatom populations. However, when silicate is exhausted (and nitrate and phosphate are still at relatively high concentrations), non-silicate-requiring coccolithophores can thrive on the ambient iron levels and outcompete diatoms, resulting in elevated growth of coccolithophores (Balch et al. 2016). This is important is we wish to focus on diatoms because of their rapid sinking rate. To avoid a switching to coccolithophores it may be necessary to add silica to the iron.

The effect of the iron cycle on coccolithophore blooms is likely more complex than originally proposed (Archer & Johnson 2000), especially because most of the biologically available iron is now known to be bound to a wide variety of ligands, and there are also many more sources of iron than

previously considered, e.g., preformed, regenerated, sedimentary, and scavenged iron, as well as hydrothermal vents and dust (Tagliabue et al. 2017).

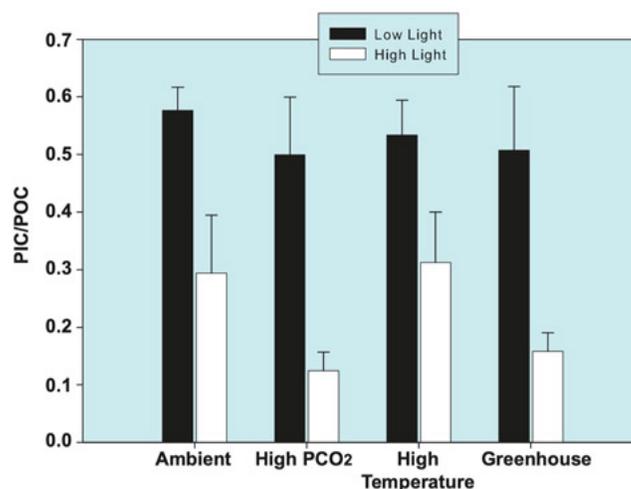
Despite the above, it should be noted that ***E. huxleyi* distribution is not at all correlated with regions of iron deficiency in the oceans** (Tyrril and Merico, 2004).

Effect of Ocean Acidification on Phytoplankton

Studies have shown a negative effect on the calcium carbonate shells of mollusks, coral and other marine fauna, due to the acidification of the oceans, as a result of the increased adsorption of CO₂ from the atmosphere. Wouldn't this acidification destroy a proposal to use phytoplanktons to adsorb atmospheric CO₂, develop calcium carbonate plates, and sink to the bottom of the ocean?

The literature on the effect of ocean acidification on phytoplankton growth, especially coccolithophores, and calcification is mixed. Some report a decrease in calcification with increasing acidification (Riebesell U, et al. (2000), Zondervan I, et al (2002), Delille B, et al. (2005), Engel A, et al. (2005), Sciandra A, et al. (2003), Feng Y, et al. (2008), Hoppe CJM, et al (2011), Beaufort, et al (2011), some report an increase in calcification (Iglesias-Rodriguez MD, et al. (2008), Shi D, et al (2009), Smith, H.E.K. et al. (2012) and some find no effect (Feng Y, et al. (2008). Using data from the Continuous Plankton Recorder, one study showed that coccolithophore occurrence in the North Atlantic increased from ~2% to more than 20% from 1965 through 2010. (Rivero-Calle, S. et al, 2015)

The following study (Feng et al, 2008) examining the role of high versus low light offers some clarification of the differences and indicated a decrease in calcification of *Emiliana huxleyi* in high but not low light.



Results of an experiment examining the interactions between pCO₂, temperature and light as they affect calcification of acclimated cultures of the coccolithophorid *Emiliana huxleyi*. The **degree of calcification** is expressed as

the cellular PIC/POC ratio (particulate inorganic carbon to particulate organic carbon). Treatments include an **ambient control** (20°C and 375 ppm pCO₂), **high pCO₂ alone** (750 ppm), **high temperature alone** (24°C), and **“greenhouse”** (both pCO₂ and temperature increased together). All four treatments were run at both **high light (open bars)** and **low light (black bars)**. This experiment suggested that neither pCO₂, light, nor temperature affected cellular calcification at low light. However, increased light greatly decreased the PIC:POC ratio in all treatments, while increased pCO₂ further interacted with light to decrease calcification even more at high light. (Feng et al. 2008).

This suggests a significant effect of OA on the calcification of *Emiliana huxleyi* since most growth is in the high light regions of the photic zone.

Another study that appears to resolve these conflicts is that of Beaufort, et al (2011). Instead of relying on laboratory studies, or studies of individual species, or localized areas, they quantified the calcite mass of dominant coccolithophores in the present ocean and **over the past forty thousand years**, at multiple different sites, and found a marked pattern of **decreasing calcification with increasing partial pressure of CO₂** and concomitant decreasing concentrations of CO₃²⁻. They found that *Emiliana*, *Gephyrocapsa* and *Reticulofenestra* dominated coccolithophore communities numerically for more than 20 million years. In their large data set temperature and salinity were not strongly correlated with coccolith mass (calcification). The only correlations that were highly significant in all subsets of the data were those linking coccolith mass to [CO₃²⁻], [HCO₃⁻] and calcite saturation state (ω Ca). Both *Gephyrocapsa* and *Emiliana* showed a decrease in coccolith mass of about 25% from the Last Glacial Maximum to the near-present, paralleling an increase in CO₂ of about 100 parts per million by volume. Different strains of *Emiliana* apparently accounted for much of the confusing picture. The highly calcified *E. huxleyi*, reported by Iglesias-Rodriguez, et al, (2008) may be **a genetic variant with an adaptation enabling it to calcify heavily in the relatively acidic upwelling waters.**

Most of the studies of the effect of ocean acidification on plankton growth centered around answering the questions of the effect of global warming on plankton growth, often with an eye to the fairly distant future. This has little effect of our goal of manipulating coccolithophores to lower atmospheric CO₂ levels since the current level of ocean acidification, while already having some disturbing consequences, is not sufficient to counter efforts to use of coccolithophores to increase the uptake of atmospheric CO₂.

How can genetics help?

Krueger-Hadfield et al. (2014) provided a discussion of high genetic diversity among *E. huxleyi* populations. High genetic diversity usually equates to a robust species. There may also room for selection of subspecies with especially desirable traits.

The role of viruses (Brussaard 2004, Balch, 2018).

The control by *Emiliana huxleyi* viruses has recently been invoked over a vast range of spatial scales in the sea. For example, the giant virus Ehv infects individual cells and takes over the host plant's lipid metabolism (Rosenwasser et al. 2014). Indeed, recent evidence suggests that genetically distinct viruses that infect the same *E. huxleyi* host show large phenotypic variability in their rates of host infection, such that one will outcompete another with a shorter lytic cycle (Nissimov et al. 2016) and dominate the host infection cycle. At the other size extreme, the demise of an entire coccolithophore population within a large-scale mesoscale eddy in the Greenland Sea (Lehahn et al. 2014) has also been attributed to viral infection. Remotely sensed imagery of the feature demonstrated its mesoscale size as well as the several-week boom-and-bust cycle. Ship samples collected within the feature showed plentiful large viruses (Ehv) of the coccolithophore *E. huxleyi*, suggesting that the bloom's rapid demise was related to viral infection.

Experiment to test the role lysis and microzooplankton grazing on processes such as cleavage of dimethylsulfoniopropionate (DMSP) or cell growth, demonstrated that micro-zooplankton grazing was a stronger modulator than viral infection/lysis in regulating coccolithophores (Evans et al. 2003, 2007; Kimmance et al. 2007). Although earlier work has shown that viral infection can rapidly clarify coccolithophore cells (Vaughn et al. 2010), causing a profound optical clearing and making them difficult to see from space, further work is needed on the impact of viruses on coccolithophore optical properties.

The Distribution of Coccolithophores

There are two important aspects relevant to the distribution of coccolithophores and all other phytoplankton – distribution and diversity. **Distribution** asks where are the largest amounts of coccolithophores located in the world's oceans? **Diversity** refers to how many different species are there in a given area? This is a critical variable since if we examine only one species it might indicate that coccolithophores were, for example, primarily located in subarctic oceans. This would hide the fact that the greatest diversity of coccolithophores is in the equatorial latitudes.

Pacific In an early examination of the subject by Okada and Honjo, (1973) they reported that in the Subarctic Zone, corresponding to the Pacific Subarctic Current, the specimens were almost exclusively a cold-water subarctic variety of *Emiliana huxleyi*. *Rhabdosphaera clavigera* were dominant in the Transitional Zone, which coincided with the North Pacific Current. The Central zones were dominated by *Umbellosphaera irregularis*. The North Equatorial Current and Equatorial Countercurrent comprise the Central North Zone where the largest variety of species was observed. The

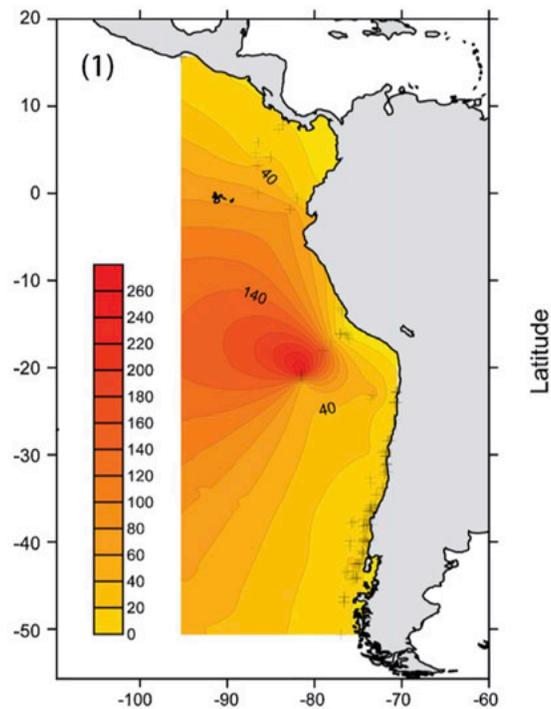
Equatorial zones including the South Equatorial Current and were characterized by an abundance of three placolith-type species: *Gephyrocapsa oceanica*, *Cyclococcolithina leptoporus* and *Cyclococcolithina fragilis*. The Central South Zone coincides with the northern portion of the South Pacific circulation. In summary, ***Emiliana huxleyi* was prominent in the cold subarctic waters.** By contrast, the equatorial waters were dominated by species diversity. Is this still the pattern years later?

This appears to be the case (Hattori et al, 2004). They examined vertical distributions of coccolithophores in the depth range of 0–50 m in the western subarctic Pacific and western Bering Sea in summer, 1997. Thirty-five species of coccolithophores were collected. Overall, *Emiliana huxleyi* var. *huxleyi* was the most abundant species, accounting for 82.8% of all coccolithophores.

Gravalosa et al (2008) examined the horizontal distributions of coccolithophores in sea surface water samples collected in the spring of 2001, in the Pacific sector of the Southern Ocean. A total of fifteen species of coccolithophores were identified, showing cell abundances of up to 67×10^3 cells/l down to 63°S . *Emiliana huxleyi* was the most abundant taxon, always accounting for more than 85% of the assemblage. The second most abundant species was *Calcidiscus leptoporus*, with values lower than 7%. Cell density increased significantly in both the Subantarctic and Polar Fronts ($151\text{--}155 \times 10^3$ cells/l). Although temperature at high latitudes was the main factor controlling the biogeographical distribution of coccolithophores, at the regional level (Southern Ocean) the frontal systems, and consequently nutrient distribution, played a crucial role.

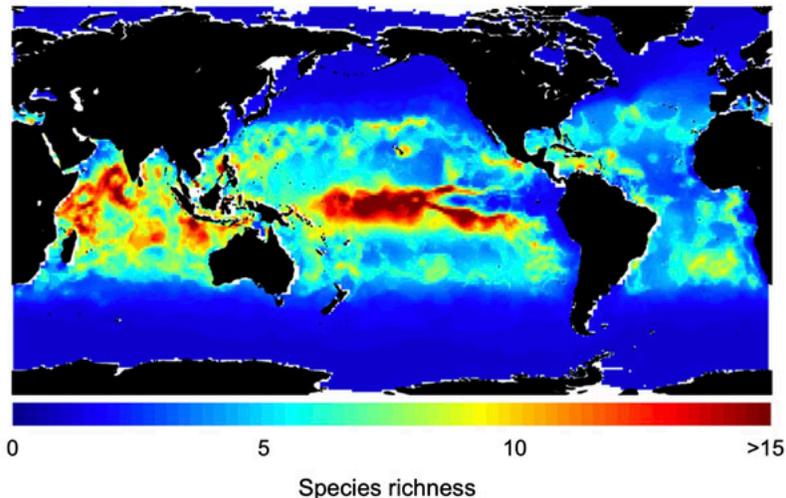
Saavedra-Pellitero, M. et al (2010) examined the coccolithophore distribution in the Equatorial and Southeastern Pacific. While the distribution was dependent upon the species, there was a significant concentration of many different species off the coast of Peru (see following figure).

Coccoliths per field of view (in LM)



Distribution map showing the number of coccoliths per field of view in light microscopy in the Southeastern Pacific Ocean.

Diversity of Coccolithophores As mentioned previously, there are two important aspects of coccolithophore distribution: density and diversity. Obrien et al (2016) examined the diversity of coccolithophores. Light and temperature were the strongest predictors of coccolithophore diversity. This **diversity was highest in the equatorial low latitudes**, where coccolithophores were a relatively dominant component of the total phytoplankton community. As shown in the following color map, the global diversity pattern was dominated by the Pacific Ocean, which shows a clear latitudinal gradient with diversity peaking at the equator, whereas in the Atlantic Ocean diversity is highest in the subtropics.



Annual mean distribution of coccolithophore diversity based on the results of the neural network analysis.

They found a unimodal relationship between coccolithophore diversity and biomass, as has previously been observed for total phytoplankton assemblages. In contrast, diversity shows a negative relationship with total chlorophyll. They projected an increase in the diversity of coccolithophore assemblages by the end of this century.

Atlantic In the Atlantic, Balch, et al,(2019) found the lowest concentrations of coccolithophores were consistently found in equatorial waters. **Highest concentrations of coccolithophore cells and coccoliths were associated with temperate, sub-polar environments.** Peaks in Particulate Inorganic Carbon (PIC) and Biogenic Silica (BSi) were observed within, and above the Sub-antarctic Mode Water (SAMW), in waters with low phosphate and silicate concentrations. The results of this study suggest that coccolithophore-rich SAMW in the Southern Ocean is being conditioned after its formation, such that by the time it upwells in the Atlantic equatorial region, the water is no longer conducive to coccolithophore growth.

It is interesting that the large wealth of data obtained in the past 40 plus years since the 1997 report of Okada and Honjo, verified their report of the **predominance of *Emiliana huxleyi* in the subpolar regions and high species richness in the Pacific equatorial region.**

Are Phytoplankton Decreasing?

If phytoplankton are decreasing it adds a second reason for trying to stimulate their growth. This is because, **if they die, we Die.**

This concern is valid because **phytoplankton are at the very bottom of the food chain**. As such they ultimately provide the food for every higher marine organism, especially fish. Over a billion humans rely primarily on fish for food and the rest rely on them to some degree. In addition, krill rely on phytoplankton and whales, penguins, seals, and many sea birds rely on krill. If phytoplankton die, then krill also die.

Phytoplankton account for half of all the CO₂ absorbed by photosynthesis. Each day, more than a 100 million tons of carbon in the form of CO₂ are fixed into organic material by these microscopic plants of the upper ocean, and each day a similar amount of organic carbon is transferred into marine ecosystems by sinking and grazing (Behrenfeld, et al, 2006). Put differently, **phytoplankton account for just as much CO₂ sequestration as all the plants on all the land surface of the earth**. Thus, **if phytoplankton are decreasing it is serious problem**. Are they? What is the evidence?

I will examine some of the critical papers on this subject in detail. In 2010 Boyce et al. published a paper in *Nature* was entitled *Global phytoplankton decline over the past century*. They combined available **ocean transparency** (C_T) measurements and *in situ* chlorophyll observations (C_I) to estimate the time dependence of phytoplankton biomass at local, regional and global scales **since 1899**. They observed **declines in eight out of ten ocean regions** and estimated **an alarming global rate of decline of 1% per year**. Their analyses further revealed inter-annual to decadal phytoplankton fluctuations superimposed on long-term trends. These fluctuations are strongly correlated with wind energy, climate indices, whereas long-term declining trends are related to **increasing sea surface temperatures**.

Estimated changes in marine phytoplankton using satellite remote sensing (1997–present) have been variable (McClain, 2009), with reported global decreases (Gregg & Conkright, 2020) and increases (Gregg et al, 2005; Antoine et al, 2005), and large interannual (Behrenfeld, et al. 2006) and decadal scale variability (Martinez et al, 2009). Despite differences in scale and approach, it was clear that long-term estimates of phytoplankton abundance are a necessary, but elusive, prerequisite to understanding macro-ecological changes in the ocean (Behrenfeld, et al 2006; Martinez et al, 2009; Falkowski, et al, 1998; Raitos, 2005).

Phytoplankton biomass is commonly inferred from measures of total chlorophyll pigment concentration ('Chl'). As Chl explains much of the variance in marine primary production (Ryther, & Yentsch, 1957) and captures first-order changes in phytoplankton biomass - it is considered a reliable indicator of both phytoplankton production and biomass (Henson, et al 2010).

Shipboard measurements of upper ocean Chl have been made since the early 1900s, first using spectrophotometric and then fluorometric

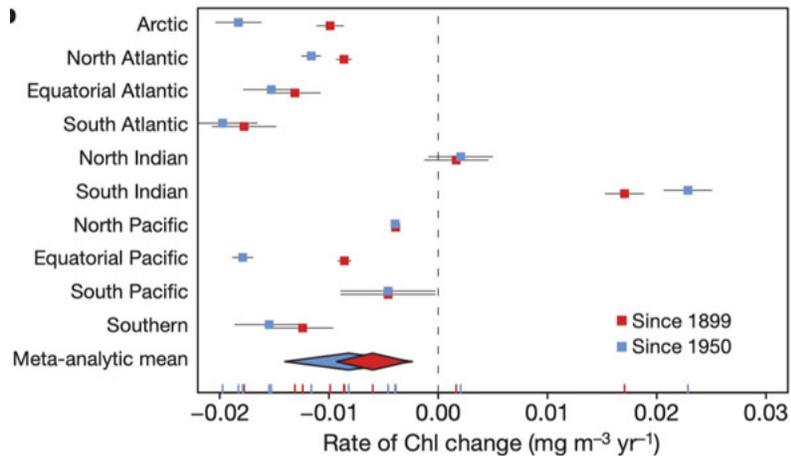
analyses of filtered seawater residues, and more recently through *in situ* measurements of phytoplankton fluorescence (C_I) (Jeffrey, et al, 1997). Additionally, measurements of upper ocean transparency using the standardized **Secchi disk** are available from 1899 to present and can be related to surface Chl (C_T) through empirically based optical equations (Falkowski & Wilson, 1992; Lewis, et al, 1988). The technique consists of lowering a standardized white disk on a rope marked in feet and half-feet. When the disk disappears, the depth measured to half a foot is the Secchi disk reading. Although the Secchi disk is one of the oldest and simplest oceanographic instruments, Chl concentrations derived from Secchi depth observations are closely comparable to those estimated from direct *in situ* optical measurements or satellite remote sensing (Lewis, et al, 1988). The authors compiled publicly available *in situ* Chl (C_I) and ocean transparency measurements (C_T) collected in the upper ocean over the past century. The blended data consisted of **445,237** globally distributed Chl measurements collected between 1899 and 2008.

Local models suggested that Chl has declined more rapidly with increasing distance from land. This agrees with results derived from satellite data, documenting declining phytoplankton in the open oceans, and **expansion of oligotrophic gyres**, probably due to intensifying vertical stratification and ocean warming (Behrenfeld, 2006; Polovina, et al, 2008).

In the North and South Pacific, North and South Atlantic, outside the equatorial zone, the areas of **low surface chlorophyll waters have expanded** at average annual rates from 0.8 to 4.3%/yr and replaced about 0.8 million km²/yr of higher surface chlorophyll habitat with low surface chlorophyll water. It is estimated that the **low surface chlorophyll areas** in these oceans combined **have expanded by 6.6 million km² or by about 15.0%** from 1998 through 2006. In both hemispheres, evidence shows a more rapid expansion of the low surface chlorophyll waters during the winter. The North Atlantic, which has the smallest oligotrophic gyre is expanding most rapidly, both annually at 4.3%/yr and seasonally, in the first quarter at 8.5%/yr. (Polovina, et al, 2008).

By contrast, in the shelf regions, Chl trends switched from negative to positive in more recent years (since 1980), consistent with reported Chl increases due to intensifying coastal eutrophication and land runoff (Gregg, et al, 2008).

To estimate regional Chl trends, Boyce et al. (2010) divided the global ocean into ten regions, in which similar variability in phytoplankton biomass was observed in response to seasonality and climate forcing. The following figure shows the Chl trends in these regions.



Mean instantaneous rates of Chl change estimated for each region, with 95% confidence limits. Diamonds indicate the global meta-analytic mean rate of Chl change, with 95% confidence intervals. Trends were estimated using all available data (**red symbols**) and data since 1950 only (**blue symbols**). Individual estimates are displayed as tick-marks on the x axis. All estimates were statistically significant ($P < 0.05$), except for the North Indian region. (Boyce et al, 2010).

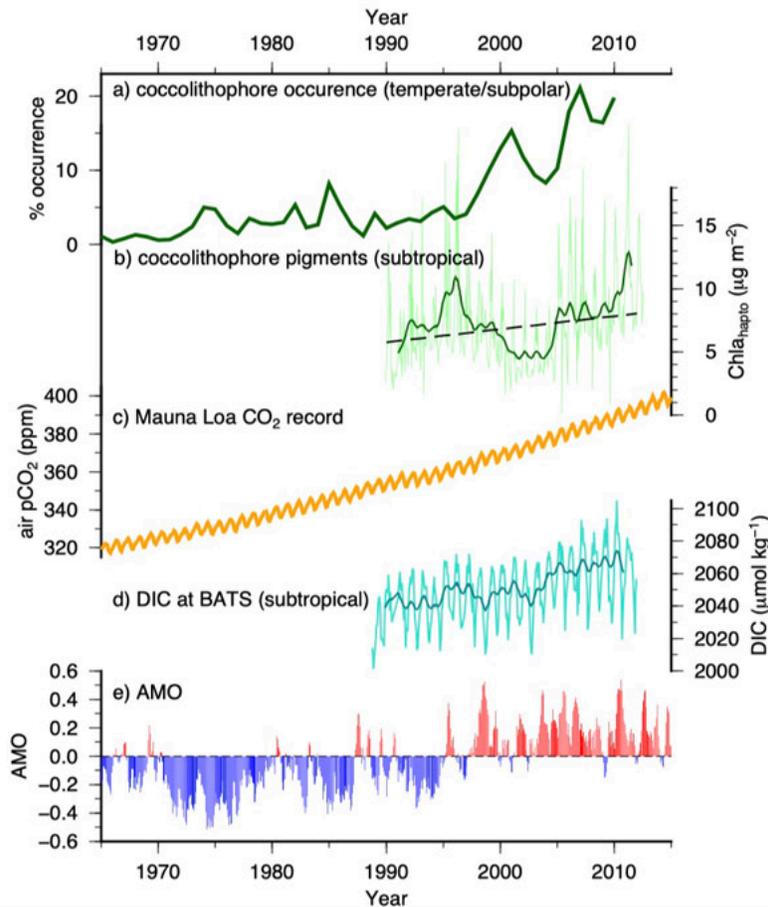
Chl trends revealed phytoplankton **declines in eight out of the ten regions**. The South Indian Ocean showed the greatest increase while the North Indian Ocean showed a very modest increase. Strong seasonality in polar regions reflected pronounced variability in mixing, irradiance and ice cover, whereas weak seasonality in equatorial regions was a function of a near constant solar irradiance. Long-term trends in phytoplankton could be linked to changes in vertical stratification and upwelling, aerosol deposition, ice, wind and cloud formation, coastal runoff, ocean circulation or trophic effects. Sea Surface Temperature (SST) was the strongest single predictor of Chl.

In a comment on the paper, Mackas (2011) stressed that although his concern was not a denial of any long-term change, the trends described by Boyce et al. (2010) included an important potential negative bias as a result of combining two different measures, (C_T and C_I). He recommended that this bias should be removed to improve the estimation of phytoplankton decrease.

In a second comment, Rykaczewski & Dunne (2015) had the same concern of bias in that transparency (C_T Secchi-disk measurements) of Chl consistently overestimates Chl concentrations compared to *in situ* measurements (C_I). Since these were heavily weighted to the earlier measurements this would result in an upward bias in estimate of

phytoplankton loss. They estimated that the median bias between the two sampling methods was about 35%.

McQuatters-Gollop et al (2011) submitted the third comment in the same issue of Nature. They noted that eight decades of data on phytoplankton biomass collected in the North Atlantic by the Continuous Plankton Recorder (**CPR**) survey (Reid, et al, 2003) showed an increase in an index of chlorophyll (Phytoplankton Color Index) in both the Northeast and Northwest Atlantic basins (Edwards, et al, 2001; Head et al, 2010; Reid et al, 1998; Raitsos, et al, 2005; McQuatters-Gollop, 2007).



Time-series data from 1960 to 2015 on (a) CPR coccolithophore annual occurrence in the temperate/subpolar North Atlantic, (b) 140m depth-integrated chlorophyll a from coccolithophores (haptophytes) at BATS in the subtropical North Atlantic ($\mu\text{g m}^{-2}$) with a 2-year Gaussian filter (dark green) and a linear regression (dashed line), (c) global atmospheric CO₂ concentration from Mauna Loa (ppm), (d) dissolved inorganic carbon (DIC) at BATS ($\mu\text{mol kg}^{-1}$) with a 2-year Gaussian filter (darker turquoise), and (e) the Atlantic Multidecadal Oscillation (AMO) index.

Other long-term time series, including the Hawaii Ocean Time-series (HOT) (Saba, V. S. et al. 2010), the Bermuda Atlantic Time Series (**BATS**) (Saba, V. S. et al. 2010), and the California Cooperative Oceanic Fisheries Investigations (CalCOFI) (Kahru, 2009) also indicate increased phytoplankton biomass over the last 20–50 years.

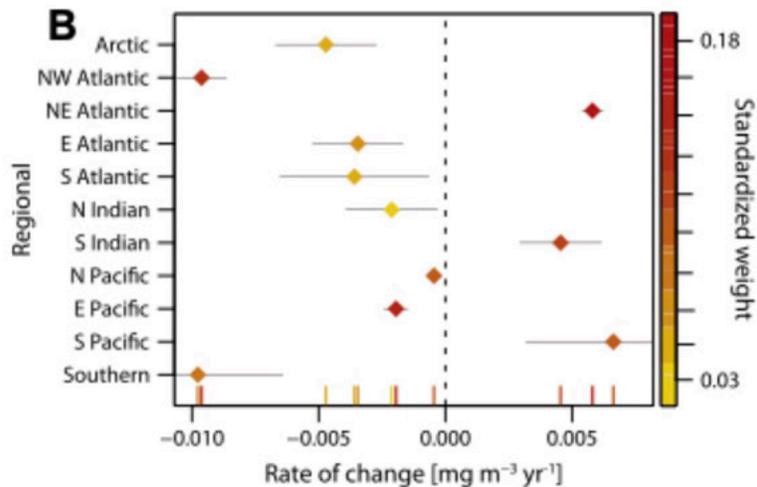
In the same issue Boyce, et al (2011) responded to these comments. They stated that in regard to the issue of bias, although they could not entirely discount the possibility that changes in sampling methods may have introduced such bias, extensive sensitivity analyses detailed in their response showed that this was not responsible for the observed Chl declines. Furthermore, the accuracy of C_T (transmission) as a proxy of surface Chl (C_I) has been independently verified. The first two comments suggested that a systematic bias between C_T and C_I combined with an unbalanced temporal sampling effort may have influenced the direction of Chl trends. However, several lines of evidence indicate that this is not the case. We adjusted C_T using the corrective algorithm suggested by the Mackas equations and re-estimated Chl trends. This improved the agreement between C_T and C_I and did not change the direction of Chl trends in any of the regions.

Boyce et al (2010) further stated that while McQuatters et al.(2011) Continuous Plankton Recorder (CPR) color index data indicated that phytoplankton abundance in the North Atlantic had increased rather than decreased, there are were important differences between the CPR data and those used in the Boyce et al, (2010) analysis, which may explain some of the observed discrepancies. As McQuatters et al mention, the CPR retains the largest phytoplankton cells (.270 mm), and the vast majority of phytoplankton cells—which are much smaller—are not sampled quantitatively. Thus, a CPR-derived color index may not be strictly comparable to direct Chl (C_I) or transparency (C_T) measurements. Additionally, the CPR data set almost exclusively contains measurements sampled north of 40° latitude and many observations from inshore areas, which is contrary to our approach. The suggested phytoplankton increase across the Atlantic is also not supported by an independent analysis of *in situ* and satellite data collected over similar timescales (Gregg, et al, 2003).

McQuatters et al. also observed that some shorter-term (<20 yr) localized time series show increases rather than decreases in Chl. We do not dispute this but suggest that comparing such series to the longer-term (>50 yr), basin-scale trends we report may be misleading. Ours (Boyce et al,2010) and others (Behrenfeld, et al, 2001;2006; Martinez, 2009; Henson, et al, 2010) analyses demonstrate that large-scale, long-term data sets are needed to isolate low-frequency trends from the yearly to decadal fluctuations that are often driven by climate oscillations. Comparisons of ours and other long-term regional estimates indicate broad agreement (Falkowski & Wilson,1992; Gregg, 2003; Gregg, e al, 2002). Furthermore, as we included the cited BATS, HOTS and CalCOFI time series in our analysis,

the important contributions that these data make are fully accounted for. As shown both in our paper and in the CPR time series (McQuatters, et al. 2011; Reid, 1998), phytoplankton has increased in some areas and thus it should not be surprising that some time series reproduce this trend.

Following this exchange, Boyce et al, (2014) published a follow-up paper entitled *Estimating global chlorophyll changes over the past century*. They used a new, publicly available database of historical shipboard oceanographic measurements to estimate long-term changes in chlorophyll concentration (Chl) from 1890 to 2010. This work built upon their earlier analysis (Boyce et al., 2010) by taking published criticisms into account, and by using recalibrated data, and novel analysis methods. This revealed statistically significant Chl declines over 62% of the global ocean surface area where data were present in 8 of 11 large ocean regions. While Chl increases have occurred in many locations, weighted syntheses of local- and regional-scale estimates confirmed that average chlorophyll concentrations have declined across the majority of the global ocean area over the past century. The following figure shows how this re-analysis gave only moderately different results.

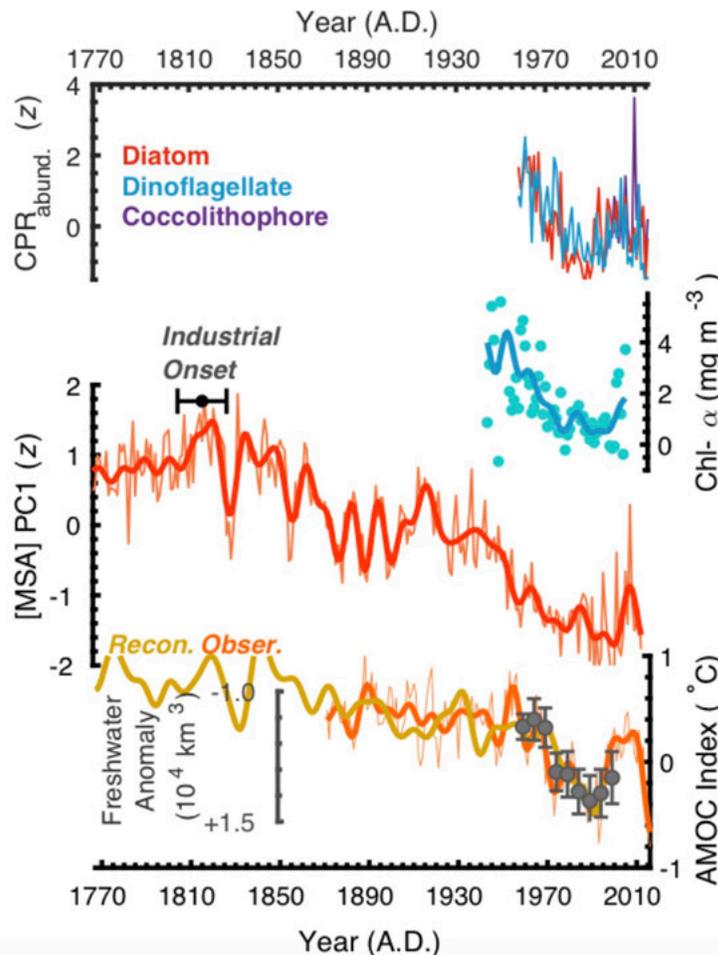


Mean instantaneous rates of Chl change estimated for each region, with 95% confidence limits in 2014 study for comparison with the above 2010 study.

Sensitivity analyses indicated that these changes did not arise from any bias between data types, nor did they depend upon the method of spatial or temporal aggregation, nor the use of a particular statistical model. The wider consequences of this long-term decline of marine phytoplankton are presently unresolved but, will need to be considered in future studies of marine ecosystem structure, geochemical cycling, and fishery yields.

Markedly extending the time scale also indicates support for decline in diatoms in the industrial era. Osman et al (2019). They utilized a continuous,

multi-century record of subarctic Atlantic marine productivity. This was based on ice cores we show that a marked $10 \pm 7\%$ decline in net primary productivity has occurred across this highly productive ocean basin over the past two centuries. They used a marine productivity proxy, using the signal of the planktonic derived aerosol methane sulfonic acid, which is commonly identified across an array of Greenlandic ice cores. This allowed measurements back to 1770.



Multi-century decline of subarctic Atlantic productivity.

From top: standardized (z-score units relative to ad 1958-2016) indices of **Continuous Plankton Recorder (CPR)-based** diatom, dinoflagellate and coccolithophore relative-abundances.

Next, North Atlantic [chlorophyll-α] reconstruction from Boyce et al. (2010); ice core-based [MSA] PC1 productivity index.

3rd down The “Industrial Onset” range shows the estimated initiation of declining subarctic Atlantic productivity.

Bottom reconstructed (Rahmstorf et al., 2015) and observed sea-surface temperature-based Atlantic Meridional Overturning Circulation (i.e., AMOC)

index, alongside 5-year averaged subarctic Atlantic freshwater storage anomalies (relative to A.D. 1955) from Curry and Mauritzen (2005; Science).

Oxygen is also involved.

About 70 % of the atmospheric oxygen is produced in the oceans due to the photosynthetic activity of phytoplankton. However, the rate of oxygen production depends on water temperature and hence can be affected by the global warming. Plankton consists of two different taxa: phytoplankton and zooplankton. Phytoplankton produce oxygen in the daytime sunlight while zooplankton consume it at night. The surplus is called the net oxygen production. The photosynthesis rate and the respiration rate depend on the water temperature differently (Hancke and Glud 2004).

Sekerci, Y. and Petrovski, S. (2015) published a mathematical model of a coupled plankton–oxygen dynamics where the rate of oxygen production slowly changed with time in response to ocean warming. They showed that a sustainable oxygen production is only possible in an intermediate range of the production rate. If, in the course of time, the oxygen production rate becomes too low or too high, the system's dynamics changes abruptly, resulting in the oxygen depletion and plankton extinction. The depletion of atmospheric oxygen on global scale can obviously kill most of life on Earth. While the level of temperature rises for this to happen is not immediately likely, if we do not eventually get CO₂ levels under control, it is another possible catastrophic consequence of the global warming.

So just when it seemed the issue of: Are phytoplankton decreasing? was settled in the affirmative, Rousseaux and Gregg, (2015) published a paper entitled *Recent decadal trends in global phytoplankton composition*. They collected world-wide data that was grouped into 28 seas and oceans and binned in 10-year intervals. The research focus was to detect and describe significant decadal variations in ocean color (Chl) and to establish if a significant trend on a timescale of a century is present for each of the 28 seas or oceans. Their analysis suggested that since the early 20th century chlorophyll concentrations have decreased in the Indian Ocean and have fluctuated in the Pacific; they increased in the Atlantic Ocean, the Mediterranean, the Chinese Sea, and in the sea's west and north-west of Japan. **No global trend of a uniform increase or decrease in chlorophyll concentration was found**, and phytoplankton abundance was identified.

Consistent with an increase in phytoplankton in some areas is a report by Rivero-Calle et al, (2015) entitled *Multidecadal increase in North Atlantic coccolithophores and the potential role of rising CO₂*. Data from the Continuous Plankton Recorder showed that coccolithophore occurrence in the North Atlantic increased from ~2 to more than 20% from 1965 through 2010. They examined more than 20 possible environmental drivers of this

change and concluded that CO₂ and the **Atlantic Multidecadal Oscillation** were the best predictors. This led them to hypothesize that in some cases higher **CO₂ levels might be encouraging growth**. A compilation of 41 independent laboratory studies supported this hypothesis. This study showed a long-term wind driven increase in coccolithophores and suggested that increasing CO₂ and temperature have accelerated the growth of a phytoplankton group that is important for carbon cycling.

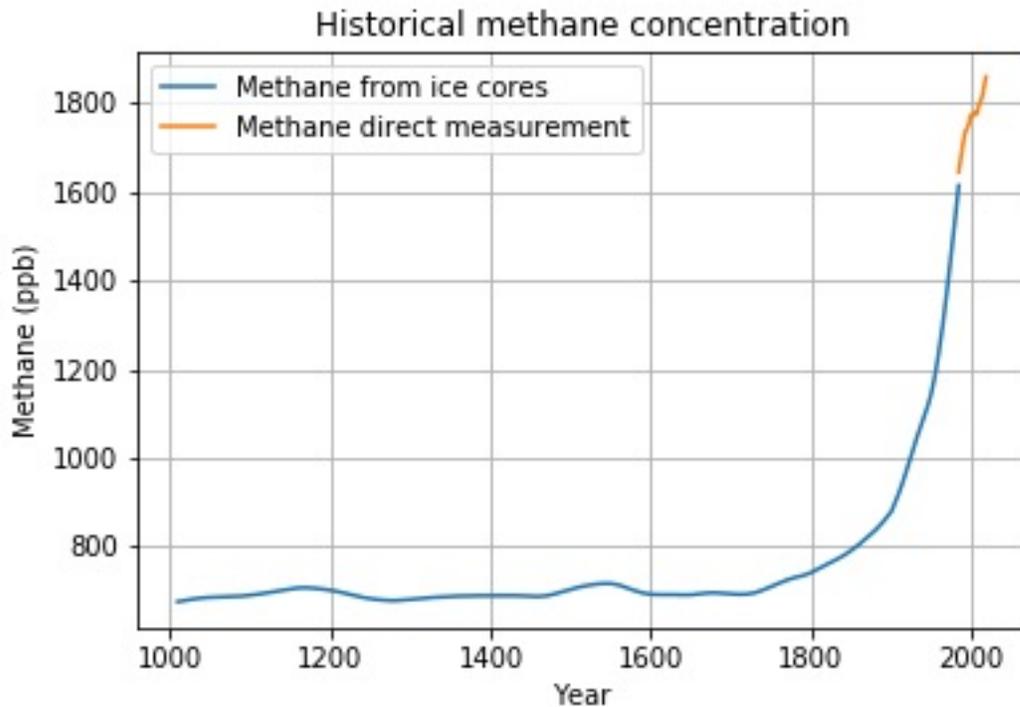
The sum of all these studies is that global warming has a negative effect on phytoplankton in many but not all areas of the world, and that the further back the data the greater the evidence for decreasing phytoplankton.

A potential explanation of why some phytoplankton are not affected by increased temperature was provided in a report by Padfield, et al. (2016) entitled *Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton*. They examined the evolution of elevated thermal tolerance in the phytoplankton, *Chlorella vulgaris*. Initially, population growth was limited at higher temperatures because respiration was more sensitive to temperature than photosynthesis meaning less carbon was available for growth. **Tolerance to high temperature evolved after 100 generations via greater down regulation of respiration relative to photosynthesis**. By down-regulating respiration, phytoplankton overcame the metabolic constraint imposed by the greater temperature sensitivity of respiration and more efficiently allocated fixed carbon to growth. Rapid evolution of carbon-use efficiency provides a potentially general mechanism for thermal adaptation in some phytoplankton.

Evidence that there is a limit to the resilience of phytoplankton and other organisms to rising CO₂ levels comes from studies of the cause of the great Permo-Triassic mass extinction 252 million years ago. This extinction spanned a period of 60,000 years and was believed to be due to an increase in atmospheric CO₂ secondary to Siberian Trap volcanism that acidified the ocean. Based on boron isotope data, Clarkson et al (2015) showed that during the second extinction pulse a rapid and large injection of carbon caused an abrupt acidification event **that drove the preferential loss of heavily calcified marine biota**. Rothman et al (2014) suggest that a contributing factor was a burst of methane released as a result of rapid growth of a methanogenic bacteria *Methanosarcina*. This is consistent with the important role of methane in global warming.

Methane

The concentration of methane, CH₄, in the atmosphere is rising. This is shown in the following figure.



Concentration of methane in parts per billion, from the year 1000 to present.

Over a 20year period, **methane traps 84 times more heat per mass unit than carbon dioxide** and 32 times the effect when accounting for aerosol interactions. Global methane concentrations rose from 722 parts per billion (ppb) in pre-industrial times to **1866 ppb by 2019**, the highest value in at least 800,000 years. It remains in the atmosphere for 12 years.

A UN FAO (Food and Agriculture Organization) study, the Stern Report (UNFAO, 2006), stated that **livestock generate more** greenhouse gases as measured in CO₂ equivalents **than the entire transportation sector**.

Livestock, cows and sheep, account for 9 percent of anthropogenic CO₂, **65 percent of anthropogenic nitrous oxide and 37 percent of anthropogenic methane**. A senior UN official and co-author of the report, Henning Steinfeld, said "Livestock are one of the most significant contributors to today's most serious environmental problems."

Approximately 5% of the methane is released via the flatus, whereas the other 95% is released via burping.

A peer reviewed NASA report published in the journal *Science* has indicated that the contribution of methane to global warming has been underestimated (Shindell, G, 2009). Nicholas Stern, the author of the 2006 Stern Review on climate change has stated "people will need to turn vegetarian if the world is to conquer climate change". President of the National Academy of Sciences, Ralph Cicerone, (an atmospheric scientist),

has indicated the contribution of methane by is a "serious topic". Cicerone states "Methane is the second-most-important greenhouse gas in the atmosphere now. The population of beef cattle and dairy cattle has grown so much that methane from cows now is big. This is not a trivial issue."

Since the chance of getting the entire world to become vegetarian is remote, more attainable goal is to **prevent livestock from emitting methane**. There are two ways to do this (see What to do? below): use of Zelp to eliminate methane by belching or add red algae to the livestock food to eliminate the production of methane by gut bacteria.

Ocean Methane and the "Methane Paradox"

While the ocean is believed to contribute only a few percent of atmospheric methane, it is important to know where that methane is coming from. Very shallow coastal waters contribute at least 50 percent of the total methane emissions from the ocean, despite making up only 5 percent of the ocean area (Ivanoff et al 1993; Weber et al 2019). Pohlman et al (2017) showed that in the Svalbard region CO₂ uptake rates in shallow water was twice that of surrounding waters and ~1,900 times greater than the release of methane. The negative radiative forcing expected from this CO₂ uptake is up to 231 times greater than the positive radiative forcing from the methane emissions. Surface water characteristics (e.g., high dissolved oxygen, high pH, and enrichment of ¹³C in CO₂) indicate that upwelling of cold, nutrient-rich water from near the seafloor accompanies methane emissions and stimulates CO₂ consumption by photosynthesizing phytoplankton. This finding is important because there is concern that continued warming of the Arctic Ocean in coming decades **may trigger the release of teragrams (1 Tg = 10⁶ tons) of methane** from thawing subsea permafrost on shallow continental shelves and dissociation of methane hydrate on upper continental slopes. The findings of Pohlman et al (2017) challenge the widely held perception that areas characterized by shallow-water methane seeps will always increase the global atmospheric greenhouse gas burden.

The world's upper ocean is supersaturated with methane. Why? Evidence shows that a portion of the methane comes from *in situ* production in oxygenated waters, however that seems to contradict the fact that methanogenesis is a strictly anaerobic process. This phenomenon has been termed the '**oceanic methane paradox**'. Repeta, et al (2016) concluded that aerobic bacterial degradation of phosphonate esters in dissolved organic matter may explain the marine methane paradox. Alternatively, Hatton, et al (2020) suggest that marine zooplankton, their excreted fecal material and other sedimenting particles may provide oxygen free microsites in pelagic waters. They identified the presence of methane producing microorganisms (methanogens) within marine zooplankton fecal pellets and sedimenting particles. Methanogens can use a range of substrates, including carbon dioxide and formate. In addition, some of the fecal pellets contain members

of the genus *Methanobolus* which are thought to utilize one-carbon (C1) compounds, including dimethylsulphide (DMS) and methylamines (MAs). Potential sources of these two compounds are dimethylsulphoniopropionate (DMSP) and glycine betaine (GB), which are produced by marine phytoplankton to maintain their osmotic balance in seawater. It is likely that when zooplankton eat phytoplankton, they consume at least some of the DMSP or GB, which is then packaged into their fecal pellets. DMSP and GB are thought to be converted into DMS and MAs respectively by microbial activity. Grazing therefore represents a pathway for these C1-compounds to enter into the zooplankton gut and fecal pellets, where they may be substrates for methanogenesis.

Aerosol particles generated from either DMS or MAs may contribute to the pH of natural precipitation and play a role in climate control due to their influence on cloud albedo and reflection of solar radiation. Therefore, zooplankton fecal pellets could be instrumental sites both in the production of a greenhouse gas and the removal of climatic feedback gases.

If zooplankton are so important to the methane and oxygen cycle, what are they? Many are copepods. Although copepods are only a few 100 micrometres to a few millimetres in size, they are the most species-rich group of the crustaceans (around 14,000 species) and make up the largest share of marine zooplankton. They represent an important food source for fish and other pelagic animals.

Combined, these studies indicate that continued accumulation of CO₂ in the atmosphere has the potential to **cause mass extinction of life by three drivers – increased CO₂ and temperature, increased methane, and decreased oxygen.**

Nitrous Oxide (see Wikipedia, 2020 for refs.)

Nitrous oxide, commonly known as laughing gas or nitrous, is an oxide of nitrogen with the formula N₂O. At room temperature, it is a colorless non-flammable gas, with a slight metallic scent and taste. At elevated temperatures, nitrous oxide is a powerful oxidizer similar to molecular oxygen. It is soluble in water.

It has significant medical uses, especially in surgery and dentistry, for its anesthetic and pain reducing effects. Its colloquial name "laughing gas", coined by Humphry Davy, is due to the euphoric effects upon inhaling it, a property that has led to its recreational use as a dissociative anesthetic. It is also used as an oxidizer in rocket propellants, and in motor racing to increase the power output of engines.

Nitrous oxide occurs in small amounts in the atmosphere but has been found to be a major scavenger of stratospheric ozone, with an impact comparable to that of CFCs. It is estimated that 30% of the N₂O in the atmosphere is the result of human activity, chiefly agriculture and industry.

Being the third most important long-lived greenhouse gas, nitrous oxide substantially contributes to global warming (Thompson et al, 2019; Wikipedia, 2020). Nitrous oxide has significant global warming potential as a greenhouse gas. On a per-molecule basis, considered over a 100 year period, nitrous oxide has **298 times the atmospheric heat trapping ability of carbon dioxide**, however, because of its low concentration (less than 1/1,000 of that of CO₂), its contribution to the greenhouse effect is less than one third that of carbon dioxide, and also less than water vapor and methane. On the other hand, since 38% or more of the N₂O entering the atmosphere is the result of human activity, and its concentration has increased 15% since 1750, control of nitrous oxide is considered part of efforts to curb greenhouse gas emissions.

Greenhouse gas trends A 2008 study by Nobel Laureate Paul Crutzen suggests that the amount of nitrous oxide release attributable to agricultural nitrate fertilizers has been seriously underestimated, most of which presumably, would come under soil and oceanic release in the Environmental Protection Agency data. (Crutzen, et al, 2008)

Nitrous oxide is released into the atmosphere through agriculture, when farmers add nitrogen-based fertilizers onto the fields, through the breakdown of animal manure. Approximately 79 percent of all nitrous oxide released in the United States came from nitrogen fertilization. Nitrous oxide is also released as a by-product of burning fossil fuel, though the amount released depends on which fuel was used. The total amount of nitrous oxide released that is of human origins is about 40 percent (see Wikipedia, 2020 for refs).

Ozone layer depletion Nitrous oxide has also been implicated in thinning the ozone layer. A 2009 study suggested that N₂O emission was the single most important ozone-depleting emission and it was expected to remain the largest throughout the 21st century.

Pelagic mesocosms

A mesocosm (meso- or 'medium' and -cosm 'world') is any outdoor experimental system that examines the natural environment under controlled conditions. In this way mesocosm studies provide a link between field surveys and highly controlled laboratory experiments. Mesocosms also tend to be medium sized to large. A mesocosms range in size from 1 to > 10,000 L).

In contrast to laboratory experiments, mesocosm studies are normally conducted outdoors. Mesocosm studies may be conducted in an enclosure that is small enough that key variables can be brought under control or by field-collecting key components of the natural environment for further experimentation. Extensive mesocosm studies have been conducted to evaluate how organisms or communities might react to environmental change, through deliberate manipulation of environmental variables, such as

increased temperature, nutrients, carbon dioxide or pH levels. A detailed description of the methodology for the use of mesocosms has been provided by Riebesell et al (2011).

The following photos show some examples.



Mesocosm studies in ocean acidification research - upper left: PeECE III study in the Espegrend Marine Biological Station, Bergen, Norway (Riebesell et al., 2008a); upper right: mesocosm facility at Jangmok on the southern coast of Korea (Kim et al., 2008); lower panel: free-floating mesocosms deployed in the Baltic Sea (Riebesell et al., unpubl.).

Mesocosms remain one of the few tools we currently have that can provide us with plausible, testable hypotheses about future biological responses to acidification, nutrition and other variables.