Marine Microbial Ecology

Source: Australian Government – Division of the Environment and Heritage – Australian Antarctic Division

http://www.aad.gov.au/

This site places an emphasis on the Southern Ocean, as reflected in some of the information below, but also contains good general information and movie clips related to marine microbes

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Why are marine microbes important?

These organisms comprise most of the living matter in the sea. Photosynthesis by phytoplankton takes up CO_2, producing the food that supports, directly or indirectly, the wealth of marine life for which Antarctica is renowned. However, only a small proportion of the carbon taken up flows directly on to organisms such as krill, fish and whales. Most of this carbon is cycled by microorganisms in the so-called microbial loop.

Marine microbes also have major effects on the world's climate. By absorbing carbon dioxide, they contribute to the uptake of CO_2 from the atmosphere, thereby moderating the global Greenhouse Effect. The Southern Ocean is one of the world's important 'sinks' where carbon is transported to the deep ocean by sinking particles. Some microorganisms also produce chemicals which, when ventilated to the atmosphere, form aerosol particles that can trigger the formation of clouds.

Microbial Components

Phytoplankton are single-celled algae. Like all plants, they use carbon dioxide and light to produce food in the process of photosynthesis. Most range in size from 1 - 100 micrometres (1 micrometer = 1/1000 millimetre, abbreviation = μm), although some needle-shaped species reach 4 mm in length. Phytoplankton are commonly subdivided into three size classes: picoplankton (<2 μm), nanoplankton (2 - 20 μm), microplankton (> 20 μm). For most of the year, nanoplankton dominate the Southern Ocean with superimposed blooms of microplankton in summer. Picoplankton are much less important in the Southern Ocean than in temperate waters. There are at least 360 different phytoplankton species identified in Antarctic waters, many of which can swim.

Protozoa are single celled animals that consume phytoplankton, bacteria and organic matter [see video images in Agents of death]. Their respiration releases much of the carbon dioxide incorporated by phytoplankton. However they also help remove CO_2 from the atmosphere by converting their microscopic food into their own cell mass, making it available for higher levels of the food web whose bodies and faecal pellets sink into the deep ocean.

Protists is the general term for single celled organisms, including phytoplankton and protozoa. Each litre of surface water from the Southern Ocean can contain from 0.5 million to 60 million protists.

Bacteria are abundant in the Southern Ocean. Typically there are about 600,000 cells per ml of seawater. They are vital components of the microbial community, breaking down particulate matter (cells and detritus), releasing nutrients for use by other organisms and releasing CO_2. They also take up dissolved organic matter, converting it to cell mass, and making it available to grazers.

Viruses are the most abundant biological agents in seawater. Concentrations in Antarctic waters range from 1 to 4 million particles per ml. They infect phytoplankton, protozoa and bacteria and may be responsible for up to 50% of deaths of marine bacteria. Bursting cells release their contents into the water, where they fuel bacterial growth. As each virus infects a particular species of microbe, viruses may be important in controlling the abundance and composition of microbial communities in Antarctic waters.

Microbial processes

Components of the microbial community are instrumental in several processes of the marine food web and global chemical cycles.

Photosynthesis: Phytoplankton absorb CO_2 and harness the energy of sunlight to manufacture sugars and other cell components, releasing oxygen. The sunlight is absorbed by chlorophylls and carotenoids, which can be used to identify the various groups of phytoplankton in the water.

Respiration: All organisms (from microbes to whales) oxidize intracellular carbon reserves to produce the energy necessary for cell function (growth, movement, chemical metabolism). This process of respiration releases to the atmosphere much of the CO_2 taken up by phytoplankton.

Feeding: The ocean has been likened to a vast very dilute jelly, containing a continuum of matter ranging from small molecules to large aggregates [see Aggregation]. Microbes are able to consume matter throughout this size range, changing the kind and size of these compounds. This alters the availability of these food sources to other organisms. Bacteria release enzymes that convert complex matter to simple molecules that can be absorbed across their cell membrane. Protozoa have various means of consuming cells and can graze on a large range of particles from molecules to cells larger than themselves [see video image Agents of death]. All protists are grazed by crustaceans and other zooplankton.

Microbial loop: The processes discussed above operate simultaneously in a microbial community, whose collective metabolism is called the microbial loop. Most of the carbon in the marine ecosystem is cycled through this loop, strongly influencing the quality, quantity and size distribution of food available to higher organisms.

Aggregation: There are several processes by which particles can aggregate. Particulate and dissolved organic matter can spontaneously aggregate in seawater, a process aided by mixing. Grazing protozoa and higher organisms repackage matter into faecal pellets. Mucilage produced by algae provides a substrate that can be colonized by other cells. Aggregates support a rich and diverse microbial community within which the close proximity of organisms enhances recycling of matter via the microbial loop. Such aggregates are often called **marine snow**.

Sedimentation: There is a continuous 'rain' of particles from the sunlit upper waters to the ocean depths where there is insufficient light for photosynthesis and hence respiration rules. Much of the matter is recycled *en route*. Sedimentation to the deep ocean is the principal global process by which CO_2 is biologically removed from the atmosphere for geological time scales.

Succession: The composition and abundance of marine microbes varies greatly due to physical and environmental factors including, light, temperature, salinity, depth, nutrient concentrations, the nature, extent and persistence of sea ice, the depth and speed of vertical mixing of the water column, and grazing pressure.

Large areas of the Southern Ocean are unproductive. This is thought to be due to both strong vertical mixing that carries cells out of the sunlit portion of the water column and low concentrations of micronutrients (especially iron) that limit phytoplankton growth. Most microbial production occurs close to the Antarctic continent. Here they bloom in or on the bottom of the sea ice during spring, or occur as brief, spectacular water column blooms near the margin of the sea ice as it retreats southward during spring and summer.

In spring, as sunlight returns to Antarctic waters, phytoplankton concentrations begin to increase. *Phaeocystis antarctica*, a flagellate around 6 μm diameter that forms gelatinous colonies up to 2 cm in diameter, is often the first species to bloom in ice-edge waters. Subsequent blooms are often comprised of large (>20μm) diatoms, which are superimposed upon a background of nanoplanktonic (2 - 20μm) flagellates and diatoms. Towards the end of the season, phytoplankton abundance declines and protozoan and bacterial concentrations increase to consume the remainder of the summer's production. However, at many sites around the Antarctic coastline there is little interannual consistency in the timing, abundance or successional sequence of marine microbes.

Production by phytoplankton over the entire Southern Ocean can vary 25% between years and, at a single location, can vary by a factor of 5 - 10 between years. Small-scale variation in the physical and biological environment causes significant differences in the composition and abundances of protists (phytoplankton and protozoan) communities over distances of meters. The composition of and abundance viruses and bacterial communities can vary over distances of centimetres. Thus, while patterns are apparent in the overall community structure and function, the Antarctic marine microbial community constantly changes in response to an ever-changing environment.

How are microbes studied?

Various techniques have to be used for studying microbes, for no one technique can measure everything. All are selective and have particular advantages and disadvantages.

Microscopy

Light microscopy

Both conventional compound microscopes and inverted microscopes are used to examine samples. These are used in transmitted light, Normaski differential interference contrast, phase contrast and epifluorescent modes to distinguish cellular organelles, appendages, and stained or autofluorescent cells (red autofluorescence typically denotes cellular chlorophyll).

Images are obtained using fine grained photographic film in microscope-mounted cameras or broadcast-quality video.

Living organisms are routinely examined at sea using microscopes that are isolated from the ship's vibrations. Live material can also be examined using microscopes in a cold (0°C) room.

Light micrographs (LM) of various organisms are shown below.

Light microscopy allows direct identification of microplankton but does not have sufficient resolution to identify most nanoplankton (especially those smaller than 10 μm). It allows cell behaviour (swimming, feeding etc.) to be studied as well as direct counts of phytoplankton. However, plankton counting is extremely time consuming, and "a task which cannot be completed without ruin of mind and body" (Haeckel, 1890).

Fluorescent stains are used to distinguish live and dead bacteria (BacLite) and highlight DNA and RNA of protists, bacteria and viruses (SYBR Green 1).

Electron microscopy

Electron microscopy is vital for the identification of small cells as well as examining details of larger cells. Much of the taxonomy of protists is based on fine structural details such as scales, flagella, surface patterning etc.

Scanning electron microscopy (SEM) shows the surface detail of cells.

Transmission electron microscopy (TEM) is used to examine either thin slices of material to show internal details of cells, or shadow-cast material to reveal fine surface structures of scales, flagella and other external cell components.

Flow cytometry

A Flow Cytometer analyses particles by passing them in single file through a laser beam. It can count up to 1000 cells per second, measuring for each one the light scattering properties (indicating size and complexity), as well as yellow-green, orange, and red fluorescence. Autofluorescence (from chlorophylls and other pigments) as well as the use of fluorescent stains help distinguish cell types.

The capacity to rapidly count large numbers of particles greatly increases the reliability and precision of cell concentration estimates, however the cells are not directly identified and must be examined by microscopy.

Pigment analysis

Phytoplankton, like all photosynthetic plants, use chlorophylls and carotenoids to absorb light for photosynthesis. Chlorophyll *a*, or a derivative, is present in all types of phytoplankton and is commonly used as an indicator of the phytoplankton biomass. It enables living phytoplankton to be distinguished from zooplankton, detritus and dead phytoplankton. It is highly fluorescent and can be measured in unconcentrated seawater samples. Other chlorophylls and carotenoids are present in phytoplankton, many of which have restricted taxonomic distribution. These pigments can be used as quantitative markers for particular taxonomic groups.

Fluorometry

The concentration of chlorophyll *a* in surface waters is continuously monitored on cruises of the Aurora Australis by measuring the fluorescence of water pumped from an intake at 7m depth. The temperature and salinity of this water is measured simultaneously.

Vertical profiles of chlorophyll *a* are measured with a fluorometer attached to the CTD. This shows the vertical distribution of phytoplankton much better than discrete samples from the rosette sampler (typically collected at 15m intervals).

Note however that fluorescence is reduced by sunlight and the response per unit chlorophyll varies during the day.

HPLC

HPLC (high performance liquid chromatography) is used to separate, identify and quantify the various chlorophylls and carotenoids in phytoplankton. Many of these are markers for particular taxa and can be used to estimate their contributions to the phytoplankton community.

HPLC is an excellent technique for mapping populations, since it is feasible to analyse more than 1000 samples per cruise. However it does not identify taxa directly and must be combined with microscopy to determine the key species present.

Special software (CHEMTAX) was developed collaboratively with CSIRO Marine Research to calculate the relative contributions of different groups of phytoplankton from the pigment content of field samples.

Feeding experiments

Studies of feeding by protists that are heterotrophic (dependent on external food) or mixotrophic (can use photosynthesis or external food) use two approaches:

First approach

Uptake of fluorescently labelled particles (dextrans, bacteria, latex microspheres, algae) is detected using epifluorescent microscopy or flow cytometry. Results show the size spectrum and uptake rate of food available to protists. Cultures of the choanoflagellate *Acanthocorbis unguiculata* have been incubated with fluorescent microspheres as a surrogate food source. Smaller microspheres (0.25 μm diameter) are ingested faster, and by a larger percentage of cells, than larger FM (0.5 and 1.0 μm diameter).

Second approach

The growth rate of phytoplankton and the grazing rate of protozoa and zooplankton upon them can be measured simultaneously by incubating a series of water samples with various dilutions with filtered seawater. In brief, the phytoplankton grow at the same rate irrespective of dilution, but the grazing rate declines at higher dilutions because the food particles are further apart and harder to find. Extrapolation to infinite dilution estimates the phytoplankton growth rate in the absence of grazing. Knowing the growth rate, the grazing rate can be determined.

Response to UV irradiation

Field and laboratory studies have been undertaken to determine the tolerance of microbial species and communities to UV exposure. Ozone depletion over Antarctica enhances the UV-B (280 - 320 nm wavelength) irradiation, which penetrates near-surface waters to 50 m depth. UV radiation has been shown to reduce growth, production and survival in the top 10 -15 m of the water column.

UV light can impact all levels of the microbial community. Microbes differ greatly in their susceptibility to UV-induced damage and significant changes to community structure and function have been observed in natural assemblages exposed to antarctic sunlight.

Cultures

Controlled laboratory studies of the physiology of key protist species are performed using cultures maintained at the Australian Antarctic Division. Cultures are isolated by selecting single cells from a field sample and maintained in various nutrient-enriched culture media based on seawater supplemented with various nutrients.

Marine microbes - Agents of death

Marine microbes are subject to attack by a range of organisms with voracious appetites and a variety of feeding strategies, but many are also consumers.

Viruses - inject their DNA or RNA and take over the cell metabolism of the host resulting in viral multiplication and eventual cell rupture.

Protozoa -

1. Puncturers - Many dinoflagellates and ciliates pierce the prey cell with a peduncle, digesting and absorbing its contents.

1. Engulfers - cover prey cells with their cytoplasm and digest them. This is the prime strategy for amoeboid cells but some other cells, notably dinoflagellates, can project a veil of cytoplasm (pallium) externally through their cell wall to enclose prey. They can sometimes successfully engulf cells larger than themselves.

1. Ingesters - feeding currents created by cilia or flagella draw prey cells to the predator where they are captured and ingested e.g. ciliates and choanoflagellates.

Mesoplankton -

1. Biters - copepods
2. Swallowers - salps
3. Crushers - krill

Microbes have evolved various strategies for avoiding or resisting these modes of attack, giving them a competitive advantage.