Lab 6:
Phytoplankton and Bacteria Standing Stocks

Introduction

Phytoplankton, with their primary production, are the base for the pelagic food web. Estimating the amount of phytoplankton present is, therefore, a central task in all planktonic field studies. The technique to estimate the amount of phytoplankton present, i.e. the phytoplankton biomass, will depend on the level of information needed for a particular study. Generally, there are two major ways to estimate phytoplankton biomass: cell counts and chlorophyll analyses.

All phytoplankton, prokaryotic and eukaryotic, possess chlorophyll *a* as the central pigment of photosynthesis. Therefore, chlorophyll *a* lends itself as a measure of phytoplankton biomass. The amount of chlorophyll *a* in a volume of water is proportional to the present phytoplankton biomass, and due to the simplicity of performing this measurement, chlorophyll analyses are the most common measure of phytoplankton abundance. Chlorophyll measurements will not, however, reveal the species composition of the phytoplankton community. Another drawback of the chlorophyll technique is that the currency of biomass in ecology is carbon. Phytoplankton biomasses expressed in chlorophyll *a* concentrations (µg Chl.*a* l^{-1}) can be compared to one another, but to compare the phytoplankton biomass to that of bacteria or zooplankton, chlorophyll concentrations have to be converted into units of phytoplankton carbon biomass (µg C l^{-1}). This is usually achieved by a C:Chl.*a* ratio of 40 (i.e. x µg Chl.*a* l^{-1} = 40x µg C l^{-1}). However, the C:Chl.*a* ratio in phytoplankton can vary, depending on species composition and physiological state, between 20 and 100. There is, thus, a great deal of uncertainty in the conversion of chlorophyll biomass to carbon biomass.

Measurements of chlorophyll *a* are easily performed. Plankton samples are filtered onto glassfiber filters. Usually Whatman GF/F filters with a nominal pore size of 0.7 µm are used for chlorophyll analyses in warm-water systems containing a high contribution of small phytoplankton, whereas GF/C filters with a nominal pore size of 1.0 µm can be used in areas of large phytoplankton dominance (upwelling regions). The filters do not, however, possess real pores but are interwoven networks of glass fibers; the "nominal pore size" is determined by how fast waters flows through a filter under a given pressure. Field tests have revealed that GF/F filters retain >85% of picophytoplankton and are appropriate for most marine chlorophyll measurements. The filters containing the phytoplankton are then homogenized in 90% acetone, which will extract the chlorophyll and other plant pigments (carotenoids). The chlorophyll extract in acetone can be measured in a spectrophotometer or a chlorophyll fluorometer to assess the chlorophyll concentration (see specific instructions in the Lab Work section).

If taxonomic composition of phytoplankton communities is of interest, algal pigment analyses can be performed by High Performance Liquid Chromatography (HPLC). Pigments are extracted in organic solvents as in the analysis of chlorophyll *a*, but extracts are pushed through a HPLC chromatography column under high pressure. The flow rate (speed) of the different pigments through the column depends on the charge of the pigment molecules, which differs among the pigments. The different pigments are thus separated by the chromatography column and can be measured separately behind the column. Different pigments such as fucoxanthin (diatoms), zeaxanthin (cyanobacteria), diadinoxanthin (dinoflagellates) or 19'-hexanoyl-fucoxanthin (prymnesiophytes) are specific for certain algal groups, and the relative abundance of different phytoplankton groups can be established. Information on single species cannot be obtained, though.

The only way to obtain information on species composition is to count the single phytoplankton cells and to sort them according to species/genera upon counting. Doing so requires a good deal of information on algal taxonomy and is quite laborious. Nevertheless, cell counts still provide the most precise estimate of phytoplankton biomass. Each species can be measured under the microscope, and from cell dimensions, the species-specific cell volume can be calculated. Conversions from cell volume to carbon (pg C $cell^{-1}$) are well established for most plankton groups.

Larger phytoplankton (>10 µm) is counted on an inverted microscope by the Utermöhl technique. The inverted microscope technique was introduced in 1931 by the German limnologist Utermöhl. He designed an assortment of sedimentation tubes of known volume (typically between 10 and 100 ml), which are placed on top of a special microscope slide with a shallow sample chamber (Fig. 1). The bottom of the sample chamber is made of a thin glass plate, not thicker than a normal cover slip. The water sample is filled into the sedimentation chamber (tube) and a cover glass is positioned without any air bubble at the

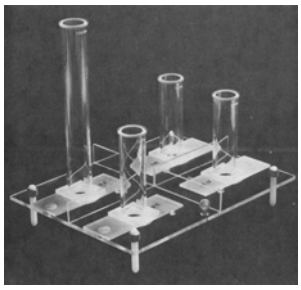

Fig. 1: Utermöhl settling chambers for inverted microscope counts of phytoplankton, settling chambers (tubes) mounted

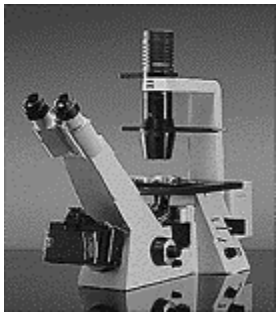

Fig. 2: Carl Zeiss Axiovert inverted microscope; note that the light comes from the top and the objectives are below the stage

top of the tube. The assembly is allowed to stay for 24 hrs on a leveled surface. During this time, all plankton cells sediment to the bottom of the assembly. Fixation by Lugol's iodine solution rather than formaldehyde helps plankton sedimentation because the iodine increases the density (specific weight) of the plankton cells. Prior to analysis, the settling chamber (tube) is slit aside carefully by another, rectangular glass plate that will cover the bottom chamber. No air bubble shall arise in the bottom chamber. The water in the settling tube is discarded. The bottom plate is placed on an inverted microscope, on which the objectives are below the microscope stage rather than above (Fig. 2). The Utermöhl technique is restricted to cells >10 µm. Smaller cells will not settle quantitatively in the settling chambers because their specific weight, even after Lugol's iodine addition, is not sufficient.

Small (<10 µm) phytoplankton is counted by epifluorescence microscopy. Water samples are filtered onto 0.2 µm pore-size polycarbonate filters. In contrast to the glassfiber filters, polycarbonate filters possess defined pores and a very smooth surface so that all cells lie on the filter surface and in the same optical plane. The filters are placed on an epifluorescence microscope and are illuminated by dark blue light, which causes the chlorophyll in the phytoplankton cells to fluoresce red. Small phytoplankton can then be counted as red fluorescent cells. In picocyanobacteria, i.e. *Synechococcus* spp., the blue light will also excite a yellow fluorescence of the accessory pigment phycoerythrin. The yellow phycoerythrin and the red chlorophyll fluorescence from these cells will mix to an orange fluorescence, by which *Synechococcus* spp. can be differentiated from other small phytoplankton.

Recent technical development made a technique for automated cell counting of pico- and nanophytoplankton available to marine studies: flow cytometry. A flow cytometer is basically an electronic particle counter. A sample stream is led through a capillary (mostly 120 µm diameter) system illuminated by an argon laser emitting blue (488 nm) light. The blue light excites the chlorophyll and phycoerythrin fluorescence as the epifluorescence microscope does, and in addition all cells passing the laser beam will scatter light. For each cell, the amount of fluorescence and of light is measured and recorded. Flow cytometry will not only give the total number of cells but will also provide information on cell size (by light scatter) and cellular chlorophyll content (by red fluorescence). The introduction of flow cytometry into marine science facilitated a burst in studies on the distribution and importance of marine picophytoplankton in the last decade.

In any ecosystem, the importance of nutrient availability to the primary producers is well established. The release of these nutrients, locked in the tissue of dead organisms and the waste products of living organisms, is the role assumed by decomposers. **Bacteria** are the major group of marine (or aquatic in general) decomposers. Bacteria are quite abundant in most marine systems. Their abundance averages usually about 5×10^6 cells ml^{-1} but can range from 10^3 to 10^8 ml^{-1}. Bacterial abundance and production mostly increases with increasing phytoplankton abundance and production. Of the estimated 1500 bacteria species, only 12% are considered marine; some of them are found only as resistant spores in marine sediments, and it is unknown if they are truly marine or simply transported from terrestrial systems into the marine sediments. The number of bacteria species, both in marine systems and in general, increases, however, by the month as new molecular techniques and gene sequencing provides new insight into the diversity of prokaryotes. Another severe factor limiting the knowledge on the diversity of marine bacteria is that only 10% of marine bacteria are cultivable; roughly 90% of marine bacteria escape culture attempts, and little is know about their taxonomic position and physiology.

Bacteria can be grouped into three major clusters: Archaebacteria, Eubacteria, and phototrophic bacteria (cyanobacteria). The cyanobacteria, although prokaryotes, are usually included in the treatment of phytoplankton. Archaebacteria and Eubacteria are mostly heterotrophic (there are some chemoautotrophic Eubacteria in specific environments).

Archaebacteria lack murein (ester lipids) in the cell walls, which is characteristic of eubacteria; instead, they have ether lipids, as well as a number of different cell-wall constituents. Archaebacteria also differ from eubacteria in the structure of their ribosomal RNAs, which are used in genetic testing to assess the degree of genetic relatedness among different species. Archaebacteria were first discovered from extreme

environments (hot vents, hot springs) and long considered to be restricted to such extreme environments. A study published in 2001 revealed, however, that up to half the pelagic bacteria off Hawaii can belong to the Archaea.

Eubacteria are the most common and important bacteria in all environments. Three basic shapes are evident among these bacteria: **cocci,** round, spherical forms; **bacilli** or **rods**, rod-shaped, cylindrical forms of differing length; **spirillae**, helical or bent forms (Fig. 3). Bacteria can further occur as single cells or in cell aggregates of various sizes. A substantial fraction of marine pelagic bacteria also occur attached to organic particles or on the surface of other organisms. Cocci are generally non-motile, but rods and spirillae can possess flagella for locomotion.

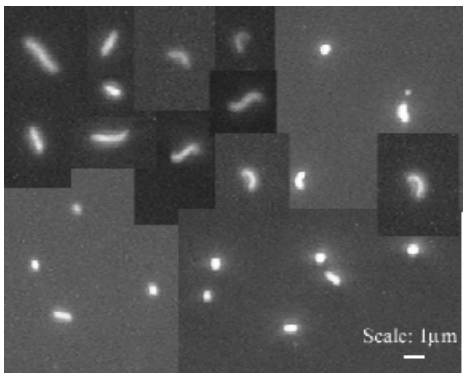

Fig. 3: Planktonic bacteria as seen under the epifluorescence microscope after DAPI staining, 100× objective. Long rods (upper left), spirillae (upper center), small rods and cocci. Scale bar 1 µm. Sample from the Gulf of Mexico.

Pelagic bacteria are usually also counted by epifluorescence microscopy. Water samples are filtered onto black 0.2 µm pore-size polycarbonate filters. Bacteria cells are then stained by the fluorescent DNA dyes acridine orange or DAPI. Although generally specific to DNA, these dyes will stain more or less the whole bacterium cell under the high concentrations applied. Recently, a flow cytometric technique to quantify pelagic bacteria was developed, which will facilitate and speed bacteria counting in the future (a digital reprint on this work can be downloaded from the internet at the URL http://www.jochemnet.de/papers/AME_BactGulf.pdf).

The development of molecular probes, especially oligonucleotide probes for ribosomal RNA sequences, now allows quantifying different bacteria groups in the field. Based on molecular results, eubacteria are distinguished in α-proteobacteria, β-proteobacteria, γ-proteobacteria, and the Cytophaga/Flavobacter group. These groups are established by similarity in the sequence of ribosomal RNA and DNA. New techniques such as in-situ hybridization allow labeling the different bacteria types with fluorescent molecular probes and quantifying them under the epifluorescence microscope. From these experiments it appears that α-proteobacteria are more important in marine waters and β-proteobacteria are more important in freshwater systems.

In coastal areas, especially near densely populated area, human and other terrestrial bacteria can be encountered in seawater. Of particular interest among them are those that live in the digestive tract of humans and mammals. These *coliform* bacteria, named after the most common human digestive tract bacterium *Escherichia coli*, do not ordinarily live for long outside the mammal body and do not form resistant spores. The coliform bacteria are not pathogenic (disease-causing), but their abundance may be indicative of the presence of pathogenic bacteria, such as those that cause salmonella, hepatitis, and a variety of staphylococcus and streptococcus infections. The abundance of coliform bacteria is therefore utilized to monitor the water quality, especially where of public interest (public beaches, pools, etc.). Fecal coliforms counts are also used to establish the safety of water for shellfish harvesting. Filter feeders such as oysters and mussels concentrate bacteria from the water with other suspended organic material. The safety level of coliform counts for shellfish harvesting is, therefore, relatively stringent: 700 cells l^{-1}. Public beaches and recreational waters must be closed at counts above 10,000 l^{-1}. In 1991, the EPA eliminated the requirement for coliform counts for drinking water, instituting regulations on the absence or presence of coliform bacteria only. This was done in response to studies that demonstrated that the level of coliform bacteria was not quantitatively related to the potential for an outbreak of waterborne disease, and that the presence or absence of coliforms provided adequate water quality information. The number of coliforms is established by plating water samples onto agar plates that contain specific nutrients for the growth of these bacteria (for details see Lab Work section). Faster and more precise estimates can be achieved by filtering water samples onto membrane filters that contain the same specific growth factors. In both cases, color reactions facilitate the detection of coliform bacteria on the agar plates or filters.

Lab Work

1. Phytoplankton abundance by chlorophyll measurement

We will determine the chlorophyll *a* concentration of the phytoplankton communities of the four field stations sampled during the first field trip. We already measured the nutrient concentrations at the sampled field stations, so we do have information at hand that can lead us to a first **hypothesis** on the expected phytoplankton biomass distribution among the sampled stations. Upon your knowledge on the role of inorganic nutrients for phytoplankton production and the nutrient concentrations measured for the field stations (refer to your lab journals), develop a hypothesis for the distribution of chlorophyll phytoplankton biomass among the sampled stations. Note this hypothesis in your lab journal for later discussion in your lab report.

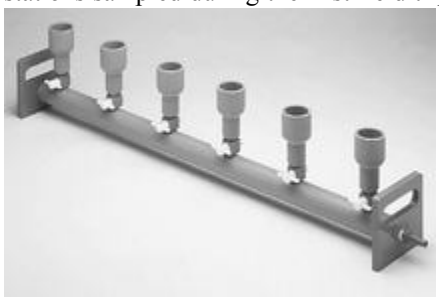

Fig. 4: Filtration manifold

After the field trip, your instructor has filtered a known volume of water sample (0.5 or 1 L) onto 25 mm Whatman GF/F glassfiber filters using a filtration manifold (Fig. 4). The filtration manifold holds six filter funnels, which allows filtration of multiple samples at a time. The filtered water is gathered in a 20 L filtration flask, and a vacuum pump or a lab vacuum line provides the vacuum. For all plankton filtrations, vacuum should never exceed –300 mbar to avoid destruction of cells.

Ask your instructor for the filtered volume. You will need it to calculate the chlorophyll concentration in the water sample after your measurement. After filtration, the filters were placed in 20 ml scintillation vials and stored frozen (-20°C) until this lab class.

Place your GF/F filter from the glass vial stored at –20°C into a homogenizer (filtered surface up) and add 10 ml of 90% acetone by bottle dispenser. Carefully grind filter in acetone until fully homogenized. Take care not to spill any acetone and wear lab coat and goggles. Fill all acetone with filter debris into a labeled glass centrifuge tube and store on ice in bench cooler.

Warning: Label all vials and tubes with label tape and ball pen. Do not use marker, because acetone might dissolve your labeling if drops are running along the vial or tube surface.

When all groups have extracted their chlorophyll filters, all centrifuge tubes are placed in a desktop centrifuge. Make sure to balance the centrifuge by placing tubes into opposite holders of the centrifuge head. Spin tubes at 70% setting for 10 min. Remove one tube at a time carefully and decant supernatant into your glass scintillation vial. Take care to leave pellet in tube.

Chlorophyll concentrations will be determined on a Genesys UV10 spectrophotometer. Fill your chlorophyll extract into the 50 mm quartz cuvette so that the cuvette is ¾ full. One cuvette will always serve as blank. Adjust photometer wavelength to 750 nm, set blank cuvette to zero, turn cuvette carousel to position 2 and read absorption at 750 nm. Then tune photometer wavelength to 663 nm, zero blank cuvette, and take your reading for 663 nm. Repeat these steps for wavelengths of 645 nm and 630 nm. After you have completed your measurements, discard your acetone extract into the provided acetone waste bottle. *Do not discard your sample into the sink!* The acetone will damage the plastic plumbing and presents a hazardous waste that is prohibited from draining down the sink.

750 nm is used as a correction for any particulate material in your extract; 663, 645, and 630 nm are the absorption maxima for chlorophyll *a*, *b*, and *c*, respectively. Chlorophyll *a* in units of µg l^{-1} is calculated as:

$$\text{Chl.a} = \frac{11.87 \times E_{663\text{-}750} - 1.54 \times E_{645\text{-}750} - 0.08 \times E_{630\text{-}750} \times v(ac)}{V \times L}$$

with $E_{663\text{-}750}$ = absorption at 663 nm corrected for absorption at 750 nm (E_{663}-E_{750}), etc; V = filtered sample volume [l], L = cuvet length [cm], v(ac) = volume of acetone [ml]. Note that 5 cm cuvettes are used.

Report your phytoplankton chlorophyll concentration to the spreadsheet on the central lab computer. In your final lab report, discuss the chlorophyll concentrations at the different stations in relation to the ambient nutrient concentration we measured in an earlier lab. Does the distribution of phytoplankton correspond to your expectations formulated in your hypothesis?

2. Phytoplankton abundance by the Utermöhl counting technique

Phytoplankton net samples provided us with sufficient material of the larger phytoplankton forms to study the taxonomic composition and to get acquainted with the different forms of phytoplankton in our field samples. Net samples cannot provide, however, quantitative information on phytoplankton abundance. The net concentrates the phytoplankton, but the factor of concentration is not known unless specific devices are used to measure the water flow through the net mouth during sampling (a technique commonly used for zooplankton quantification from zooplankton net samples). The net samples also cannot provide us with the phytoplankton smaller than the mesh size, usually 20 µm.

After we have studied the present taxa at our field stations using the net samples, we will now quantify the abundance and species composition of larger phytoplankton by the Utermöhl technique. FIU can only provide one inverted microscope at this time, so sample analyses have to done in shifts among the four study groups. Each group assigns one member to perform the Utermöhl counts for their station.

The instructor placed 50 ml of Lugol's iodine preserved water sample into the settling chambers 24 hrs prior to the class. During class, the settling chambers (cylinders, see Fig. 1) will be removed from the counting chambers and the counting chambers covered with a cover glass. The counting chambers can now be placed on the inverted microscope. At low (20× objective) magnification, scan the sample and note what forms of phytoplankton are present, and which forms are dominant. Try to identify the abundant forms and include sketch drawings in your lab journal. Decide on which forms are abundant enough for quantitative counting. Due to time constraints in the lab class, we will have to neglect the smaller forms at this time.

Move the microscope stage so that you start at the left end of the plankton chamber. The eyepiece of the microscope contains a measuring scale (Fig. 5). Imagine horizontal lines from both ends of the graticule scale; count all cells within the view field between the two imaginary lines. Then move microscope stage one view field further and repeat counting for the next view field, etc. until you reach the other side of the plankton chamber. Always keep track of your counts. If phytoplankton is very abundant in your sample, several random view fields rather than one whole strip from one end to the other of the counting chamber can be counted. Differentiate at least the different phytoplankton groups (centric diatoms, pennate diatoms, dinoflagellates) in your counts.

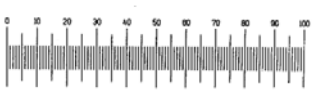

Fig. 5: Microscope eyepiece graticule with micrometer scale

For each species/form, compute the total number of cells counted. Convert these numbers to cell numbers per liter by

$$N\ [\text{cells}\ l^{-1}] = \frac{\text{area of chamber bottom} \times n \times 1000}{\text{area counted} \times 50}$$

with area of chamber bottom = 506.71 mm²; area counted = 12.7 mm² for one whole strip at 20× objective, and (0.50 × number of counted view fields) for view fields at 20× objective; n = counted events; 1000/50 = normalization of sediment chamber volume (50 ml) to 1 liter water sample. This equation reduces to

$N\ [\text{cells}\ l^{-1}] = n \times 797.96$ [for one whole strip at 20× objective]

$N\ [\text{cells}\ l^{-1}] = n \times 20161.25 \div (\textit{number of view fields counted})$ [for view fields at 20× objective]

Compute the cell abundance of centric diatoms, pennate diatoms, and dinoflagellates and report them to the spreadsheet on the central lab computer. Discuss the total abundance of phytoplankton as well as the taxonomic composition of phytoplankton (relative dominance of taxa) and the differences among the four field stations in your final lab report.

3. Abundance of heterotrophic bacteria

3.a. Total abundance of heterotrophic bacteria by flow cytometry

Direct counts of all heterotrophic bacteria can be achieved by epifluorescence microscopy of flow cytometry. The time constraints of this class do not allow, however, presentation of these techniques. Your instructor will estimate the total abundance of heterotrophic bacteria at the four sampled field station by flow cytometry outside the time of this class. In the next class, your instructor will present you a data printout of the cytometric bacteria counts.

Include this printout in your final lab report. Discuss the abundance of bacteria and the differences among the stations in relation to factor governing the distribution of bacteria: nutrient concentration and phytoplankton biomass. Does the bacteria abundance follow the abundance of phytoplankton among the four sampled stations? Does the bacteria abundance at the different stations explain the differences in dissolved oxygen (% saturation) that you measured during the field trip (refer to your lab journal for oxygen saturation values)?

3.b. Abundance of cultivable heterotrophic bacteria – plate counts

Your instructor will provide two nutrient plates for heterotrophic bacteria for each group. These plates in plastic petri dishes contain a standard bacteria growth medium solidified by agar, a polysaccharide derived from macroalgae. The nutrient plates contain 0.3% beef extract, 0.5% peptone, 1.5% agar, and are prepared in full-strength filtered seawater. You will bring out small amounts of water sample containing the marine bacteria onto the agar plates, a process called inoculation. Cultivable bacteria will start to grow on the agar plates and form small colonies visible to the naked eye (after 24-48 hrs). Each colony stems from one single bacterium cell brought onto the plate by inoculation.

Precautions: Since this medium provides a good growth for all kinds of bacteria, you have to attempt to prevent contamination of the plates by any other than your marine bacteria from your samples. Do not open the petri dishes unless necessary for inoculating. While inoculating, do not breathe on the plates and do not touch the plates with your fingers or your skin. Use only sterile equipment to inoculate.

Inoculation: Label the *bottom* of your agar plate by name and station number with a permanent marker. Keep your labeling small so it does not interfere with counting the colonies later. You will label the *bottom* of your dish for two reasons: (1) the top part might be lost or placed on the wrong plate by accident; it is the bottom part that contains your sample, so labeling the bottom part will prevent any accidental confusion. (2) Agar plates are stored upside down, so labeling the bottom will make the label more readable when you store a high number of plates (you usually stack them). Then

1. Take a fresh yellow pipettor tip from the box by attaching it to the Gilson Pipetman without touching the tip with your fingers. Take care not to touch anything with the pipettor tip. Adjust the Pipetman to 100 µl (dial at 1-0-0).
2. Dispense 100 µl of your field sample onto the center of one agar plate. Open the top of the petri dish only half so you can slip the pipettor inside. Do not fully remove the top. Do not touch the agar with the pipettor tip.
3. Take the glass spreading rod and dip it into the beaker with 70% alcohol for sterilization.
4. Hold the alcohol-wett spreading rod over the Bunsen burner flame to burn off the alcohol
5. Let spreading rod briefly cool in the air, not touching your skin or the table.
6. Remove top part of the petri dish.
7. Place spreading rod on the agar surface without applying pressure (if rod sinks into the agar, then it was still too hot and melted the agar). Turn the petri dish under the spreading rod, thereby spreading the 100 µl of water sample homogeneously over the whole agar plate.
8. Place top on petri dish and turn petri dish upside down

The petri dishes will be collected after the class in incubated for 24 to 47 hours in an incubator at 37°C. Within this time, cultivable bacteria should form colonies. After incubation, the petri dishes will be stored in a refrigerator at 4°C for inspection in the next class.

During the next class, take your petri dishes and place them on the colony counter, bottom side up (so you look “through” the bottom. The colony counter (Fig. 6) has a magnifying glass to facilitate recognition

Fig. 6: Colony counter

of small colonies. Count the colonies on your plate. Use a permanent marker to mark all the colonies you have counted to avoid confusion as to which colonies are counted and which are not yet counted. Convert your colony counts to bacterial abundance. Remember that each colony is assumed to have originated from one single bacterial cell. Thus, the number of colonies on your plate represents the number of cultivable bacteria in 100 µl of your field sample. Multiply your colony count by 10 to convert to bacteria cells ml^{-1}.

Report your final abundance of cultivable bacteria to the spreadsheet on the central lab computer. For your final lab report, discuss the differences in cultivable bacteria among the sampled station. How do the plate counts relate to the total number of bacteria estimated by flow cytometry? Which percentage of the total bacteria is cultivable on the provided general bacteria growth medium?

3.c. Abundance of coliform bacteria – plate counts

The abundance of coliform bacteria by plate counts follows the working pattern described for the cultivable bacteria plate counts in section 3.b. Instead of agar plates with general bacteria growth medium, special agar plates for coliform bacteria will be provided. Two different media will be used, and each group will get two plates of each medium to inoculate.

One medium is Hektoen Enteric Agar. If coliforms are present in your inoculum, colonies growing on Hektoen Enteric Agar will appear bright orange. The other medium is Levine Eosin Methylene Blue Agar (EMB). Coliforms growing on EMB Agar will appear as shiny dark-green colonies.

Following the protocol given in section 3.b., inoculate each plate with 100 µl of your field sample. The plates will be collected after the class and incubated at 37°C for 24 hrs, then stored in a refrigerator at 4°C until colony counting during the next class. Count your colonies in the next class, using the colony counter, and convert your coliform bacteria numbers to cells ml^{-1} by multiplying by 10. Report your coliform bacteria abundance (average of the two plates) for each medium to the spreadsheet on the central lab computer.

Did you grow any coliform bacteria on your plates? What does the number of coliforms (cells ml^{-1}) tell about the water quality at the different field stations? Refer to the Introduction section for coliform cell numbers in relation to recreational and mussel harvesting waters. Does the distribution of coliform bacteria concur with your expectations? Discuss these questions in your final lab report.