CalCOFI Trainee Handbook

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What Is CalCOFI?	2
Station Map	3
Suggestions on what to bring	4
Primary Duties	5
General Guidelines	6
Line Handling	7
Drawing Salts	8
Drawing Nutrients	9
Drawing & Filtering Chlorophylls & HPLCs	10-11
Net Tow Plankton Sample Preservation	12
Oxygens	13
Fluorometry – Basic Concepts from Turner Designs	14
Glossary – definitions for common terms	20

The Organization

The *California Oceanic Cooperative Fisheries Investigation* (CalCOFI) is a unique partnership of the *California Department of Fish and Game*, the *NOAA Fisheries Service* and the *Scripps Institution of Oceanography*. The organization was formed in 1949 to study the ecological aspects of the collapse of the sardine populations off California. Today its focus has shifted to the study of the marine environment off the coast of California and the management of its living resources. The organization hosts an annual conference and publishes annually the *CalCOFI Reports*.

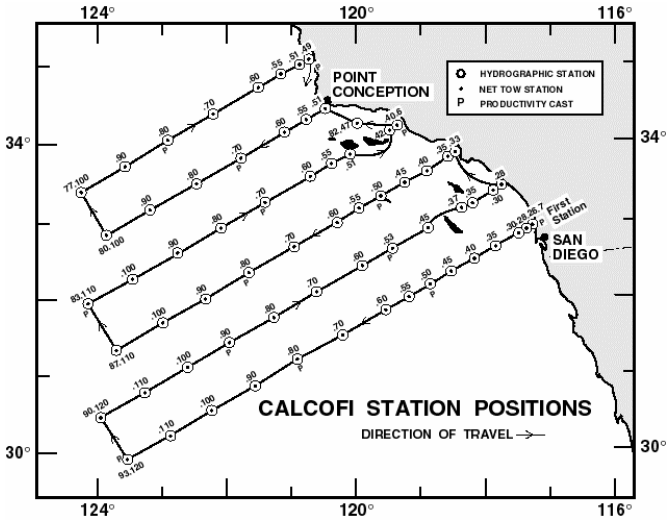
The Field Program

Since 1949, CalCOFI has organized cruises to measure the physical and chemical properties of the California Current System and census populations of organisms from phytoplankton to marine birds (see below). These cruises are the foremost observational oceanography program in the U.S. Currently, two to three week cruises are conducted quarterly. Scripps and NOAA provide equally in terms of ship time, personnel, and other cruise-related costs. On each cruise a grid of 66 stations off Southern California is occupied. At each station a whole suite of physical and chemical measurements are made to characterize the environment and census important populations of the ecosystem:

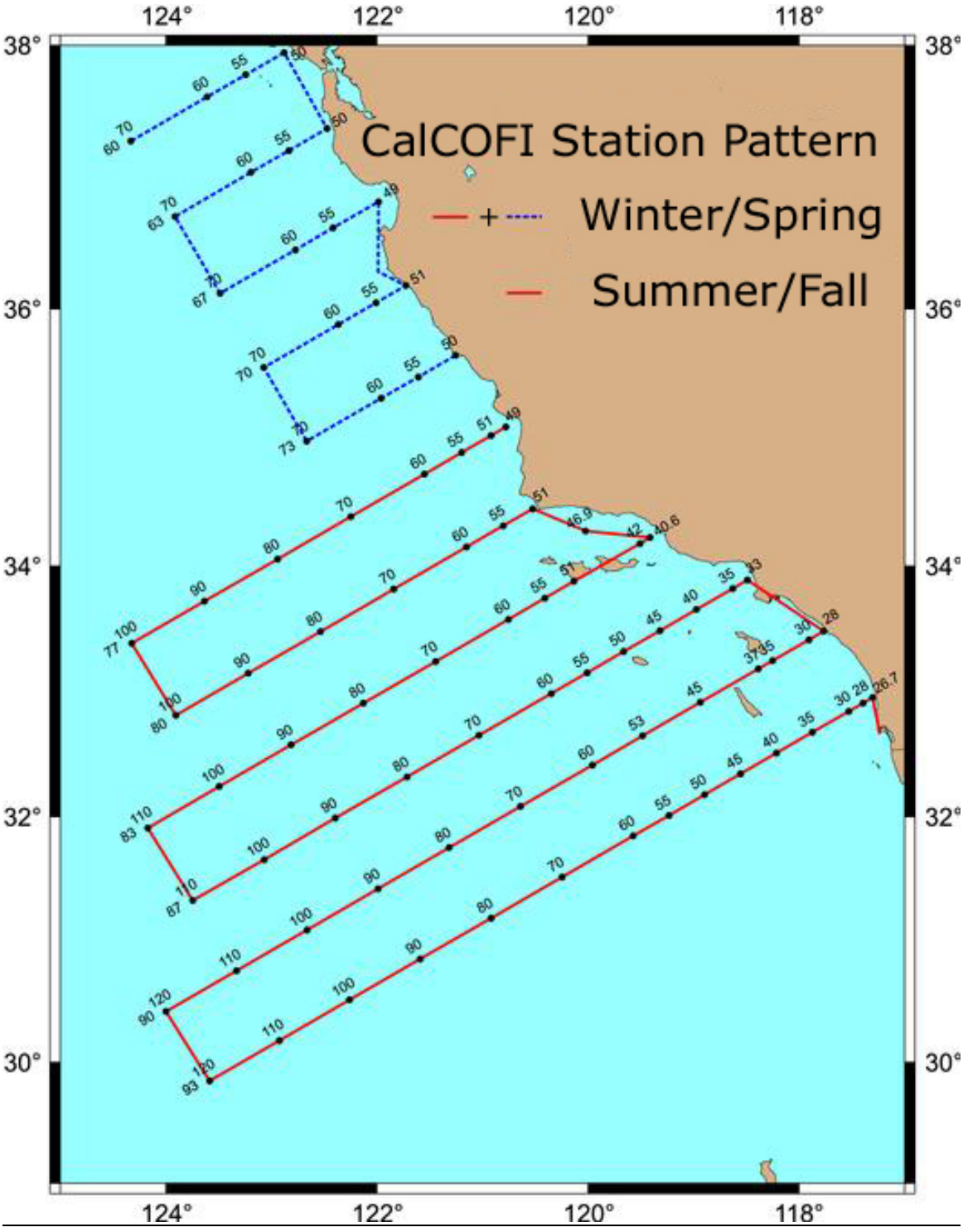
Observations

- temperature, salinity, oxygen
- water masses and currents
- concentrations of plant nutrients
- primary production
- biomass of phyto- and zooplankton
- phyto- and zooplankton biodiversity
- meteorological observations
- distribution and abundance of fish eggs, larvae, marine birds and mammals

The California Current System



At a time when there is continued call for basic research to serve societal needs, CalCOFI is a leader in furnishing the scientific underpinnings for the management of living marine resources directly to scientists within federal and state agencies.


CalCOFI Station Pattern
Winter/Spring
Summer/Fall
124°
122°
120°
118°
38°
36°
34°
32°
30°

Suggestions on what to bring on a CalCOFI cruise

1. **Seasick medication**. If you know you are prone to seasickness, bring any medication you know works for you. Marezine, Bonine or Scopolamine (prescription) anti-seasick medications are some examples – some work for some people; some have side effects. The ship can provide over-the-counter remedies but we suggest bringing your own. Summer and fall cruises are usually calm but the weather can get rough any time of year
2. **Clothes you can layer** – it is better to wear layers instead of heavy jackets etc since you will be moving in and out of the lab. Even summer cruises can be cool (even cold) so always bring a windbreaker and sweater or sweatshirt.
3. **Boots**: comfortable, waterproof boots are great to have since you will be sampling from the CTD and nets so your feet will be wet constantly. The more comfortable the better but even cheap ones are better than nothing.
4. **Closed-toed shoes** (or boots) are mandatory while working on deck. Steel-toed shoes/boots are great if you have them.
5. **Rain gear**: foul-weather jacket and pants are worth taking along. We can provide a few pair but sizes vary so if you have something waterproof, bring it.
6. **Sun protection** – sunglasses, a hat, sunscreen and Chap Stick – you can minimize your sun exposure but these are worth bringing.
7. **Toiletries and medications** (decongestant, Advil, etc) – the ship provides the necessities (linens, soap, etc) but bring your own personal hygiene products. The ship is relatively small so consider bathing regularly a courtesy to other people.
8. **Bathrobe & shower shoes** – makes getting to and from the head more convenient.
9. **Workout clothes/gear** – a stationary bike, stair-stepper, mat, and bench/free weights are available.
10. **Books, magazines & music** – the ship has a magazine & paperback library plus movies but the selection can vary.
11. **Binoculars & camera** – sightings of birds and marine mammals such as seals, whales or porpoises are common on CalCOFI.
12. **Snacks and goodies** – the ship provides lots of these but if you are particular, bring it. Midnight watch-standers often bring soups; tuna and other food items since meals are not serve from 6pm to 730am. There are sandwich fixings, cereals, misc leftovers and you can request the cooks set aside a plate of food from dinner.
13. **Money** – Bringing your own alcohol is NOT permitted. But the New Horizon does permit participants to buy ($1.50) one beer per day and serves wine with dinner on Sundays. The David Starr Jordan and all NOAA ships are completely dry – no alcohol permitted at all.
14. **One week's worth of clothes** - laundry facilities are available but you should bring enough clothes since the machines will be unavailable during rough weather.
15. **Fishing pole** – trolling for albacore is common, especially summer and fall, so if you like fishing and have a heavy pole with some jigs, bring them.
16. **Soft luggage** - we recommend using soft luggage to stow your clothes and other personal gear ie duffel bags etc. because large hard suitcases may not fit into your stateroom closet/locker.

Primary Duties

Tasks normally performed by trainees after training:

1. **CTD prep** – draining and rigging of CTD for deployment 20mins before station. Rosette bottles are drained, bottom lanyards unclipped; once drained, all valves are closed and rotated 90°; breathers are closed (lightly finger-tight).
2. **Sample Drawing** – trainees will learn how to sample the items in bold from the CTD.

- Refer to the sample log sheet for the bottle numbers to sample. The number of samples and the bottle they start on can vary - shallow stations have fewer bottles; others may have extra bottles with additional samples to draw. Certain sample types may not be drawn from all the bottles.
- Standard station samples drawn are:
    - Oxygens – must be drawn first to minimize contamination, usually by the CTD operator
    - **Salts** – very sensitive to fresh water contamination (ie rain) and evaporation
    - **Nutrients** – very sensitive to phosphates (soap residue on hands)
    - **Chl** – volumetric, no bubbles
    - HPLC – volumetric, filled completely; volume varies with chl conc
    - Phyto – "Pooh" sample; formalin preserved, no rinses; usually by the CTD operator
    - Prodo – Primary productivity C14 uptake experiment, done at the noon station; samples drawn by the prodo person
- All sample containers (except the Phyto-Pooh sample) require **3 rinses**.
- **Chl filtration** – ~14 chlorophyll samples are taken to the Chl van and filtered asap (usually during the Bongo net deployment)
- HPLC filtration –
- usually done along with chl filtration but may take much longer to complete because of larger volumes.

4. **Net deployment & washdown** – launch, recover and washdown Pairovet, Manta, and Bongo nets. Refer to the Fisheries Net Handbook for information on net types.
5. Zooplankton sample "pickling" – formalin-preserve net cod-ends. Let the Chief Scientist know if you are sensitive to formalin or any other chemical.
6. Secchi disc – deploy, detect secchi depth, recover
7. **Chl sample analysis** – after 24+ hours of extraction, the chlorophylls can be measured on the fluorometer during transits between stations.

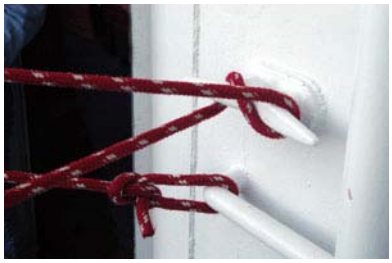
General Guidelines

- The sample bottle number **always** matches the rosette bottle number.
- The sample bottles are **rinsed three times** before the final fill.
- If you have any doubt about the sample's integrity, redraw it.
- More than one person can draw a sample type but be sure to keep the order straight, returning your bottle or tube to the right spot.
- Notify your watch leader if you have to "disappear" for more than a few minutes.
- Be available to help with CTD preps 20 mins before the station ETA. Station ETA is displayed on the navigation monitor in the ship's main lab. Occasionally, when navigating around islands or coastline, the ETA displayed may be a "way-point". These are turning points, not the actual station arrival time. Check with the watch leader if uncertain.
- If you will be unavailable to help because of other work or illness, let the watch leader know. You are expected to be available to help when you are on watch and the watch leader should not have to go looking for you.
- Check with the watch leader before leaving for meals; the CTD cast usually takes 45-50mins but shallow stations can be much shorter so don't leave until checking with him/her.
- Watches are 12 hours: 0000-1200 and 1200-0000
- Be sure to get enough sleep especially if you are on midnight (0000-1200) watch – it may take a few days to adjust to this watch.

Line Handling

One of the first things taught is line handling – lines are attached to the CTD to stabilize it during deployment and recovery. A rolling ship can cause it to swing dangerously when the winch lifts the 800lbs (empty)/1500lbs (full) CTD package.

Important things to remember:



1. Wrap the line around the cleat, careful not to bind on itself when the CTD moves in or outboard.

2. Keep your hands at least 12 inches (30cm) from the cleat as the line slips out.

<Wrong way Right way>

3. Always keep your line tight by pulling out the slack but do not inhibit the CTD's movement on or off the ship. A good indicator is the winch wire – if it is angled towards your cleat then it may be too tight. When the ship rolls, quickly take up the slack to prevent the CTD from swinging but let line out as the tension increases.

4. The CTD must always be secured to the deck or cleat. If the CTD is untied from the deck-eyes then the taglines must be secured by you or the cleat. Loop the line around the cleat then under itself when waiting to deploy or while securing the CTD to the deck eyes.

Deck eye

5. Be sure to watch for line tangles and do not stand inside a loop.

6. Never put yourself between the CTD and an immovable object such as the bulkhead. Watch your toes when the CTD lands – 1500lbs will crush them.

Drawing Salts

- The sample bottle number should ALWAYS match the rosette bottle number – check and double-check this during the sample drawing process. If you ever have any doubt about the sample, dump it and start over.
- The sample bottles are stored inverted – they should not be turned over until the sample has been taken. If you need to step away from sampling for any reason and have not filled the sample bottle, return it to the case **inverted**.
- The bottles should never be stored empty so as the old sample is dumped, use it to rinse any salt that may have crystallized on the threads, thimble, and cap.
- Salt samples are usually drawn from the bottom valve. Fill the bottle with ~40mls of seawater, cap loosely, shake then dump, rinsing the threads and thimble. Repeat – you should rinse the bottle 3 times; the dumping of old sample does not count as a rinse.
- The last fill should be done without interruption until overflowing, filling the bottle completely; pour ~10ml out over the thimble, place it firmly in the bottle and cap. The caps are brittle and should be tightened gently. If they crack, retrieve a replacement from the spares Ziploc, keeping the thimble in place. See photo for optimal fill height.

- Salts are taken from all closed rosette bottles unless directed otherwise.
- Sample bottles are fragile – carefully place them back in the correct slot. If you drop one on deck or into the case and crack the glass, replace the cracked bottle from the spares case, adding the bottle number. Redraw the sample.
- Once all the salts are taken:
    - fill out a sample label (on the sample log clipboard) with the time, your initials, total number of samples. Place the label in a plastic sleeve and into the box of samples

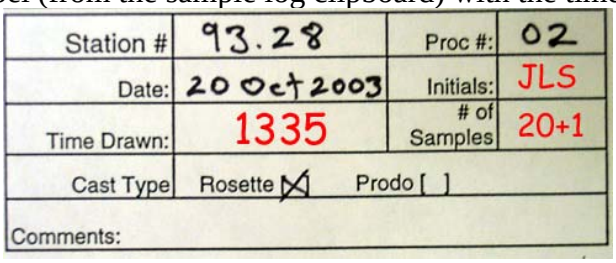
Station #	93.28	Proc #:	02
Date:	20 Oct 2003	Initials:	JRW
Time Drawn:	1330	# of Samples	20
Cast Type	Rosette [X]	Prodo [ ]	
Comments:			

    - take into the lab, adding it to the **end** of the "salt box queue"; bring out an empty case for next station.
    - add your initials to the bottom of the sample log's salt column

Common mistakes are duplicate draws; samples returned to the case out of sequence (common when more than one person is drawing salts); missing thimble insert; not enough air (can crack the bottle as it warms and not allow the analyst to load the sample); too much air (can shift the salinity value or not give the analyst enough sample to measure); and cracked or broken bottles (drops).

Drawing Nutrients

1. Retrieve an empty nutrient rack from the lab fridge if needed and record the color key on the sample log sheet.
2. Tubes are inverted when empty and should not be turned over until filled
3. Never touch the inside of the cap or tube since residue from your fingers can contaminate the sample – soap contain phosphates. If unavoidable – rinse several times.
4. Nutrients are normally drawn from the middle valve – fill the tube with ~25mls, cap loosely, shake then dump. Repeat three times.
5. Double check the tube number matches the rosette bottle number.
6. Fill the tube completely then flick out several mls so the sample reaches the base of the neck/threads. Cap tightly.
7. Draw one sample per rosette bottle (except on the surface bottle). If tube 24 is not needed, draw a duplicate surface sample.
8. Once all the nutrient samples are drawn:
    a. Carefully tip the nutrient rack until you can see the sample through the cap of each tube – if a tube is empty and not inverted, double-check the sample log and fill if necessary.
    b. fill out a sample label (from the sample log clipboard) with the time, your initials, total number of samples



    Wrap the label around tube #1 and carefully re-insert into rack – be sure to include the extra surface sample to the sample count by listing it as "+1"
    c. return the filled rack to the nutrient fridge right away.
    d. add your initials to the bottom of the sample log's nutrient column

Common mistakes are empty tubes that shouldn't be; missing duplicate surface sample; contaminated samples; and duplicate draws – two sample tubes filled from the same bottle.

Drawing and Filtering Chlorophylls & HPLCs

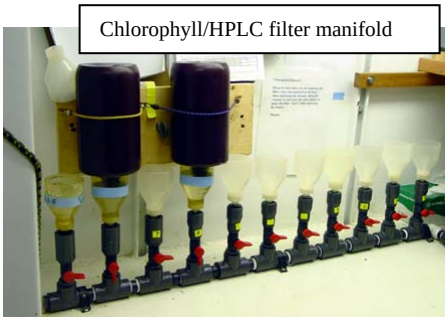
Sample Drawing:

Chlorophyll sample

1. Chl samples are drawn on all rosette bottles tripped at 200m and less so sampling on a standard 20-bottle cast usually starts at #7 (sample vol is ~140mls). For shallow stations, all the bottles may be sampled. Noontime prodo casts may have extra bottles to sample. Duplicate bottles are usually skipped. Refer to the sample log sheet to verify sampling or ask the CTD operator or watchleader.
2. Drawing from the middle valve, add ~20mls, cap loosely, shake then dump; three rinses. Double-check the sample bottle number matches the rosette bottle number.
3. Chl samples are volumetric so after rinsing, fill it completely, cap loosely, tap the bottle gently against the rosette to dislodge any bubbles then top-off, cap tightly, invert the bottle – if you see a bubble, top-off and check again. Squeezing the sides of the bottle can change the sample volume and create a persistent bubble; cup the bottle in your palm during the final fill to minimize this problem.
4. Once all the chlorophylls have been drawn, draw the HPLCs – two volumetric samples drawn into dark bottles from the second depth (usually – check the sample log). The sample volume will vary with chlorophyll concentration so check the sample log or with the watchleader for bottle size.
5. Again, three rinses then fill completely.
6. Once both chl & HPLC samples are drawn, initial the sample log.
7. Take the chlorophyll sample log from the clipboard (under the sample log) along with the chlorophyll samples and HPLCs (use a milk-crate to carry it all) to the chl van to filter ASAP. The prodo experiment filtrations in the evening may delay the filtrations so ask to be notified when the chl van is available.

HPLC sample bottles

Filtrations:


Chlorophyll/HPLC filter manifold

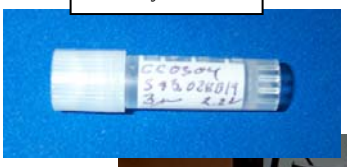
1. The chl van filter manifold should have filters installed but double-check. Turn on the vacuum pump and **carefully** pour each sample into the filter funnel. These are volumetric samples so it is important not to lose any by spilling or ill-fitted funnels. You may want to start the HPLCs first since they can take a long time and can be filtering while the chl samples are processed.
2. If you spill **any** sample, discard the remainder, replace the filter, redraw the sample from the rosette. You can wait to do this after all the other samples are done filtering but do it before removing the filters so the vial/sample sequence remain intact. If you wait too long the rosette bottle may be drained or dumped. Be sure to pour the redrawn sample into the correct funnel.
3. Monitor the sample filtration and close the valve immediately after the sample is filtered.

4. Once all the samples are filtered, turn off the vacuum, and remove an empty tube rack from the fridge. Open the sample tube storage bin and fill the rack with the next set of tubes; the tubes are consecutively numbered and should continue the sequence from last station – verify this by looking at the chl sample log (clipboard) from the previous station. Also check the tube volume contains 8 mls (level should be above label area).

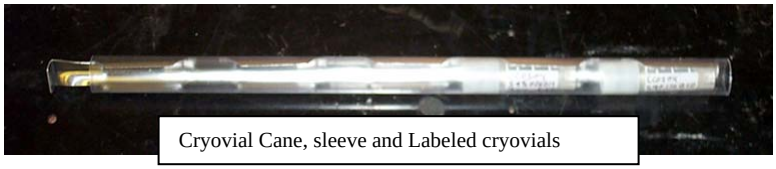
Acetone-filled Tube

5. Remove the filter funnel and the tube cap and carefully tweeze the filter into the tube - rolling it on the filter base then sliding it carefully into the tube, 1cm below the surface; if the filter tears, be sure all the pieces are retrieved and submerged. If the filter drops onto the counter or floor you will have to **redo the sample** (you can protect the counter with a fresh paper towel or bench protector sheet). Repeat for all the samples. Be sure all the tubes are capped **tightly**. Keep track of the tube, sample & funnel order
6. Fill out the chlorophyll sample log with filtration time, your initials, the tube numbers.
7. Place the tube rack in the fridge; bungie the door closed. Samples are light-sensitive so be sure they are in the dark (in the fridge, box or foil-covered) until they are analyzed.
8. Turn on the vacuum and open the valves slightly. Tweeze new GFF filters onto each filter base, centering them carefully on the frit then reattach the funnel, being sure the filter stays centered (vacuum helps) and funnel seats properly by wriggling it slightly. (If this step is not done correctly then next sample will spill out the sides and require a sample redraw).
9. Check the HPLCs and if they are not done filtering you will have to come back every 15-20mins to check them.
10. Return the empty chl sample bottles to the wet lab and check-in with the watchleader (unless we have already left station).
11. Once the HPLCs are filtered, label two cryovials with the station info (follow the example) and record the time, volume filtered and initial the HPLC log.
12. Tweeze each filter into a cryovial and cap tightly. Take these vials and the empty sample bottles to the wet lab.
13. The filters are frozen in the liquid nitrogen (~196°C) dewar, use eye protection and gloves; clip the vials into a labeled cane and load into the labeled canister to freeze.

Cryovial



Liquid Nitrogen Dewar



Cryovial Cane, sleeve and Labeled cryovials

14. Return the two dark bottles to the HPLC milk-crate.

Common mistakes: spillage - sample loss from pouring or squeezing the sides of the bottle when uncapping; filter bypass - filter was not centered on the frit; funnel was loose or skewed; filter falls onto counter or floor when funnel is removed or during transfer to vial; vial skipping and/or disorder; pour a sample into a funnel that already has a sample; new filters are not installed for next station.

Plankton Sample Preservation

1) After completion of the net tow, concentrate the plankton sample into the cod end by washing down the net with the deck seawater hose (**DO NOT use fresh water**). Be sure to spray the net from the outside to minimize damage of the delicate plankton in the sample.

2) Once the sample is concentrated into the cod end, use a screwdriver to loosen the hose clamp. Give the cod end a tug to detach it from the PVC coupler. If the sample will overflow the cod-end when removing it from the coupler, grab a bucket and remove the sample over the bucket. Make sure to wash down any sample remaining in the net into the bucket as all our plankton tows are quantitative (no sample can be lost).

3) Take the cod end into the preservation sink area. Remove the appropriate jar from the sample box (pint jar for Pairovet and Manta, quart jar for Bongo; all boxes are labeled accordingly) and set it into the sink rack. Be sure the begin-tow time is written on both inside & outside labels, using pencil for inside and Sharpie for outside. Peel off the outside label backing and stick to jar lid. Put the inside label into jar.

4) Using the filtered seawater hose, concentrate the plankton into the bottom of the cod end. It is ok to gently spray the inside of the cod end at this point but not so hard you will damage the organisms. Once the plankton are sufficiently concentrated towards the bottom, invert the cod end into the draining sock (a wooden dowel helps). There are 2 different mesh sizes of draining sock, **always** make sure you use the appropriate size mesh for the sample you are preserving (.150 for Pairovet samples, .333 for Manta or Bongo). The draining sock is helpful because it allows you to use as much seawater as necessary to thoroughly rinse the cod end. Gently spray the cod end with enough seawater to remove all the plankton clinging to it. It is very important not to lose any part of the sample or it will no longer be quantitative. Large pieces of kelp or grass or anything obviously not plankton can be rinsed off thoroughly and discarded at this point. Using a spoon, transfer the plankton into the jar. Rinse the spoon into the draining sock and then invert the draining sock into the jar. Wash down any of the remaining plankton into the jar with the rest of the sample, filling the jar with seawater to just below the shoulder.

5) **Wear eye protection**. Add a squirt of supersaturated sodium borate to the sample for buffering purposes. Formalin is slightly acidic so the borate raises the pH of the sample to neutral. Add 10 cc. to pint jars, 20 cc. to quart jars. The syringe is usually marked with a "P" for Pint and a "Q" for Quart.

6) To add full strength formalin, unclip the plastic binder attached to the tygon tubing of the formalin rig to allow for flow. Hold the 60 cc. syringe firmly and pull out the stopper to the desired amount (25 cc. for Pint and 50 cc. for Quart). **Re-clip the plastic binder** and dispense the formalin into the jar. Be careful not to spill any or push so hard the formalin squirts out the back of the syringe. Formalin is very caustic and extra care should be taken when dispensing it. If you have any allergies to formalin then you should not help with this step of the preservation process.

7) After the formalin is added, secure the lid tightly and invert the jar a few times to ensure proper mixing of sample & chemicals. Return the sample jar back to the correctly labeled box.

8). Rinse the cod end with the deck seawater hose and invert it back to the original position. Reattach the cod end to the coupler and tighten the hose clamp securely.
Thank you for all your help, it's greatly appreciated !!

OXYGENS

Pickling Oxygen Samples using Carpenter's Modified Winkler Titration Method:

Seawater samples are drawn into a calibrated volumetric flasks using tygon tubing. The flask is rinsed three times then overflowed with twice the sample volume. Carefully removing the sampling tube to prevent the influx of bubbles, the sample is then fixed with manganous chloride (MnCl2) and sodium iodate/sodium hydroxide (NaI/NaOH). A stable precipitate forms. The flask is stoppered and shaken vigorously to homogenize. A sample label is filled out and the case is covered. After settling for several minutes, a second shake is performed to insure all the oxygen is fixed. The case is added to the O2 sample queue. Autotitrator oxygen samples are drawn and fixed in the same manner but the temperature of the sample is measured and recorded.

The relevant chemical reactions occurring throughout the procedure are outlined below:

$Mn^{2+} + 2OH^- + 1/2\ O_2 \longrightarrow$ oxygen-manganese complex + H_2O (1)

oxygen-manganese complex + $4H^+ + 2I^- \longrightarrow I_2 + Mn^{2+} + 2H_2O$ (2)

$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$ (3)

Addition of the manganous chloride and the alkaline-iodide results in the formation of an insoluble oxygen-manganese complex (1), the precipitate in step 2. The oxygen is stable in this form for several days. Both the manganous chloride and the alkaline-iodide are added in excess to ensure reaction with all of the oxygen. Treatment with the sulfuric acid dissolves the complex and liberates free iodine (2), imparting the distinctive yellow-gold color. The amount of free iodine is proportional to the amount of oxygen dissolved in the original sample. By titrating a measured portion of the sample against a standardized sodium thiosulfate solution (3), the amount of free iodine—and the corresponding amount of oxygen—is determined. The starch "indicator" (which forms a distinctly colored complex with the free iodine) is used to provide an unmistakable visual endpoint for the titration.

The concentration of dissolved oxygen (DO) is one of the most important indicators of the overall health of a body of water. Waters with consistently high levels of DO (> 6 mg/L) typically support the most diverse biological communities. Waters with consistently low DO levels (< 3 mg/L) may be virtually devoid of aquatic life or may harbor only a few species adapted to such conditions.

Fluorometery

Frequently Asked Questions About Fluorometric Chlorophyll Analysis

Q: Why measure chlorophyll?

A: All plant life contains the primary photosynthetic pigment chlorophyll *a*. Microscopic, planktonic plants, or phytoplankton, occupy the lit zone of all water bodies. With over 70% of the surface of the earth covered in water, phytoplankton and photosynthetic bacteria are responsible for almost ½ of the planets primary production while their total biomass comprises less then 1% of the total plant biomass. These extraordinarily efficient plants also act as the single largest CO_2 sink on earth. For these reasons alone it should be clear that there is an interest in measuring concentrations of phytoplankton. Chlorophyll *a* fluorescence is the most versatile, sensitive and easy way to measure the concentrations of phytoplankton in water.

The quantitation, through extracted analysis, or estimation, through *in vivo* analysis, of chlorophyll *a* concentration supplies information on the abundance of phytoplankton present in all aquatic environments. Since chlorophyll-containing organisms are the first step in most food chains, the health and /or abundance of these primary producers will have cascading effects to all higher organisms. Therefore, the determination of chlorophyll concentration is one of the key indices in monitoring the health of any natural system.

Chlorophyll measurements are also used to directly monitor phytoplankton populations. Examples include, but are not limited to, the monitoring of chlorophyll in natural marine and freshwater environments, reservoirs, water and sewage treatment plants, and aquacultural systems.

Q: How do fluorometers detect and quantify chlorophyll *a* in water?

A: Fluorescence is the phenomena of some compounds to absorb specific wavelengths of light and almost instantaneously emit longer wavelengths of light. Chlorophyll *a* naturally absorbs blue light and emits, or fluoresces, red light. Fluorometers detect chlorophyll *a* by transmitting an excitation beam of light in the blue range (440nm for extracted analysis and 460nm for *in vivo* analysis) and by detecting the light fluoresced by cells or chlorophyll in a sample at 685nm (red). Generally, this fluorescence is directly proportional to the concentration of the material in question.

Q: What is the difference between *in vivo*, *in vitro* , and extracted chlorophyll analysis?

A: *In vitro* (meaning 'in glass' and referring to 'in an artificial environment or outside the living organism') chlorophyll analysis is another term for extracted analysis. It entails the concentration of chlorophyll containing cells onto a filter followed by the extraction of the chlorophyll *a* from the cells. *In vivo* (meaning 'within a living organism') chlorophyll analysis simply refers to the analysis of chlorophyll in the natural environment or, in our case, in the living algal cells.

Q: What is in vivo chlorophyll analysis?

A: *In vivo* chlorophyll analysis is the fluorescent detection of chlorophyll *a* in living algal and cyanobacterial cells in water. In this technique, the excitation light from the fluorometer passes through the untreated sample water and excites chlorophyll within the living cells of the algae present. There are several factors that make *in vivo* analysis a semi-quantitative measure at best. Environmental parameters, physiology, morphology, light history and the presence of interfering compounds all play a role in altering the relationship between fluorescence and the concentrations of chlorophyll *a*. Examples of interfering materials include other plant pigments, degradation

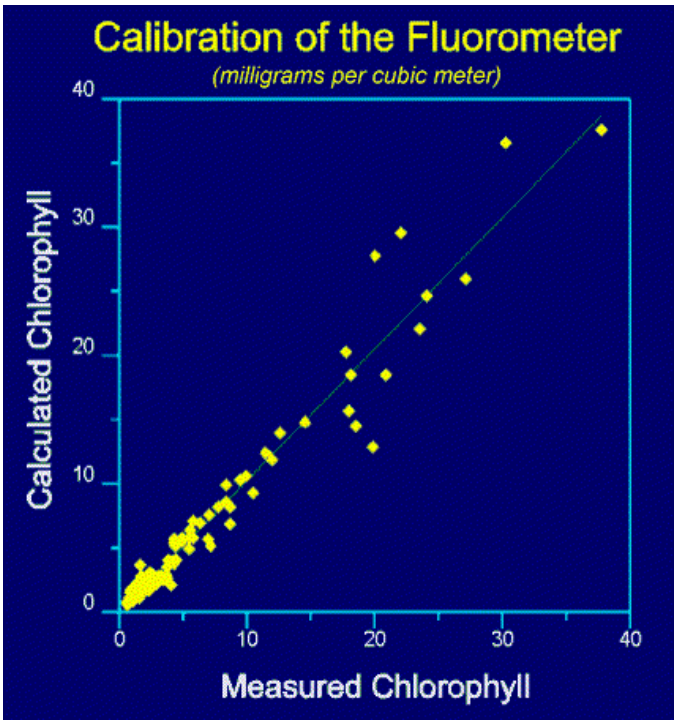
products, dissolved organic matter, and turbidity. *In vivo* fluorescence data supplies information on the relative distribution of chlorophyll concentrations and usually correlate well with extracted chlorophyll *a* samples.

In vivo detection has several very useful applications. An example is the monitoring of general trends in chlorophyll concentrations in real time. It is very easy to obtain large amounts of data using in vivo instrumentation and is an excellent means of following trends and estimating chlorophyll concentration. With the introduction of the SCUFA® submersible fluorometer, verticals profiling and mooring applications are now possible. Other examples of *in vivo* applications include continuous monitoring along a ship's track using the 10-AU configured with a flowcell and discrete sampling used to monitor algal concentrations in natural or laboratory phytoplankton populations. Examples of discrete sampling applications include aquaculture and hatchery systems, water treatment facilities, reservoir monitoring, and aquatic research (see the *Aqua*fluor™).

If water samples are taken, the *in vivo* data can be correlated to extracted chlorophyll *a* data to estimate actual concentrations. Otherwise, the *in vivo* data can be used as a relative measurement to identify trends and patterns.

Q: How do you calibrate a fluorometer for extracted chlorophyll *a* analysis?

A: Allow the fluorometer to warm-up for the time specified in the User's Manual. Measure the fluorescence of each standard at sensitivity settings that provide mid-scale readings (refer to your User's Manual for proper calibration procedures). Follow directions under section 10.0 from E.P.A. Method 445.0 (Revision 1.2) for the calibration and standardization procedure using the traditional acidification technique or the non-acidification method. E.P.A. Method 445.0 calls for filtering onto glass fiber filters (GFF) filters and grinding of the filters. This step may not be necessary in some systems and tests should be run to compare extractions with and without grinding. Non-grinding techniques can use either GFF or membrane filters that will dissolve in the solvent.



Q: What environmental factors interfere with *in vivo* chlorophyll analysis?

A: Light, temperature, water quality, and dissolved components can all have significant effects on fluorescent readings independent of the chlorophyll concentration. However, all of these factors can be controlled and/or corrected to a degree if the user is aware of their effects.

Temperature has an inverse relationship with fluorescence. For example, in a vertical profile, as the temperature decreases, the fluorescence will increase independent of chlorophyll concentration. The *in vivo* chlorophyll fluorescence response changes at a rate of 1.4% per °C. A temperature drop of 10 °C in a vertical profile would result in a 14% overestimation of chlorophyll

at the coldest point. Turner Designs' field and submersible instruments have the capability to automatically compensate for temperature effects.

Light history can have significant affects on the fluorescence in algal cells. For example, at low light levels, algal cells can optimize the light uptake by pushing chloroplasts to the outer edge of the cell or by producing more chlorophyll per cell. Both of these responses can result in data increases the fluorescence signal while the algal biomass may be unchanged. To lessen the effects, opaque hose should always be used when sampling natural waters with a field fluorometer. The transport time of the water in the hose will dark-adapt cells to an extent, significantly reducing fluorescence error caused by variations in the light history of the cells.

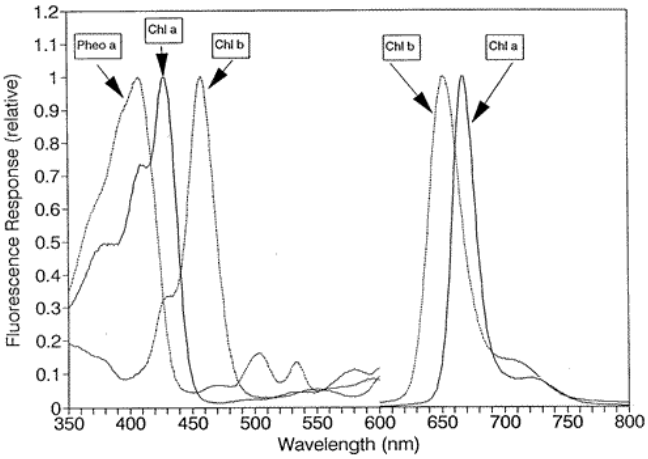
Dissolved organic matter (DOM), chlorophyll degradation products (pheophytins), chlorophyll *b* & *c* and turbidity can also falsely increase the chlorophyll *a* fluorescence signal. If the dissolved interfering compounds are suspected to be significant, it is worth conducting a quick study to look at the effects by comparing the fluorescence from filtered and non-filtered water samples or from noting the fluorescence signal below the photic zone and using this value as a blank.

Q: What is the effect of varying species on fluorometric chlorophyll analysis?

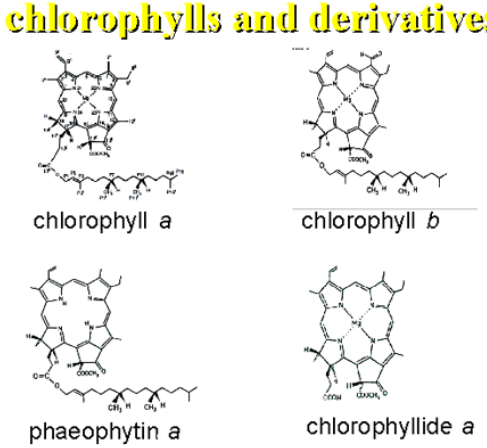
A: Different species of phytoplankton have varied morphologies (cell packaging), physiological states, size, and chlorophyll *a* : carbon ratios. All of these factors can affect the fluorescence emitted from a cell under a specific excitation light intensity. Luckily, in natural environments, the phytoplankton assemblage is diverse enough that much of the variation in fluorescence resulting from the factors above balance eachother out so the net effect on the fluorescence reading is reduced. Nevertheless, the researcher needs to be aware of the potential for error in readings caused by these factors to better interpret *in vivo* data.

Q: How does other chlorophylls and degradation products affect extracted chlorophyll analysis?

A: All chlorophyll pigments and their degradation products (pheophytins) have their own unique excitation and emission spectra. Unfortunately, these spectra overlap significantly with the fluorescence spectra of chlorophyll *a* due to the similar chemical structure (see Figure 2). Due to the overlap in fluorescence spectra, the presence of one can result in an interference in the measurement of another, resulting in an under or overestimation of the pigment in question.



In the case of chlorophyll *a*, interfering pigments to be aware of are chlorophyll b, chlorophyll *c*, and pheophytin *a* (see figure 3). Chlorophyll *b* causes the most interference in freshwater systems with high concentrations of chlorophytes and/or prasinophytes and in marine systems with high concentrations of prochlorophytes. In chlorophyll extraction, the interference results during the acidification step of the traditional extraction technique. Chlorophyll *b* undergoes a wavelength shift when acidified,



resulting in an underestimation of chlorophyll *a* and an overestimation of pheophytin. In environments with high chlorophyll *b* concentrations, we strongly recommend using the Welschmeyer (non-acidification) method.

High concentrations of chlorophyll *c* can result in a slight overestimation of chlorophyll *a* and an underestimation of pheophytin a sometimes even resulting in negative pheophytin readings. It has been reported that a chl *a* : chl *b* ratio of 1 : 1 , which is the highest ratio which could occur in nature, would result in a chl *a* overestimation of 10%.

High concentrations of any of the interfering compounds will result in an increase to *in vivo* chlorophyll readings. The magnitude of the increase will depend on the instrumentation used. Instruments using filters with a wider bandpass will be more effected by interfering compounds than instrument with narrower bandpass filters.

Q: What is a secondary standard?

A: A secondary standard is used as an alternative to a primary calibration standard. It is often used when primary standards are expensive, difficult to obtain, or unstable. In the case of chlorophyll *a*, a secondary standard can be used the majority of time for calibration because liquid chlorophyll *a* standards are expensive, time-consuming, and photosensitive. To properly use a secondary standard, you must first calibrate with a primary liquid standard of the fluorophore of interest. You can then obtain the equivalent value of the secondary standard. Henceforth, you may calibrate using the secondary standard using the value you obtained for it initially. An occasional calibration using a primary standard to recheck the stability is recommended.

In the past, secondary standards have been more stable than the primary standards they mimic, but have still required special storage and handling conditions with relatively short lifetimes. Examples of these include coproporphrin and fluorescent dyes such as Rhodamine WT.

Turner Designs has developed a solid secondary standard that is stable under ambient light and temperatures with no special treatment or storage required. This new secondary standard will greatly reduce time, cost, and trouble in fluorescent chlorophyll analysis procedure.

Q: What is the best solvent and procedure for the extraction of chlorophyll *a*?

A: The most commonly used extraction solvent is a 90% acetone 10% DI water solution. Other solvents, such as methanol, ethanol and acetone/DMSO mixtures are also commonly used and can improve extraction efficiency with specific phytoplankton or may be found useful for the extraction of sediment samples.

There is no 'best' solvent or procedure for chlorophyll extraction. Several work well and have their own pros and cons. The E.P.A. Method 445.0 describes the recommended step-by-step process for analysis using 90% acetone. There are many factors in the extraction process that can lead to different results. Several examples of these factors will be discussed below in hopes reducing some of the variability.

An excellent resource for sample collection, storage and extraction methods is the UNESCO publication, Phytoplankton Pigments in Oceanography.

Water collection, storage, and filtration:
Because pigment is being extracted from living cells, it is critical to use consistent techniques of obtaining water, filtering and storing filters. The living cell is sensitive to changes in the environment such as temperature and light. Conditions leading to cell death or damage will affect chlorophyll concentrations.

In the collection of water samples, it is important to make certain that the collection containers are clean of all chemicals. They should be rinsed several times in the sample water. Once collected, if samples cannot be filtered immediately, they should be stored quickly on ice in the dark. The time between collection and filtration should be as brief as possible and should not exceed 4 hrs.

Specifics on the recommended filtration and storage procedure can be found in E.P.A. Method 445.0. An excellent resource for sample collection, storage and extraction methods is the UNESCO publication, Phytoplankton Pigments in Oceanography.

Q: How do I take and store discrete water samples in the field?

A: For discrete *in vivo* analysis, water samples should be measured as soon as possible after collection. The same time constraints should be placed on the filtering of water samples that are to be used for extracted analysis. From the time of collection to measurement, the samples should be stored in the dark on ice. Remember that the cells are living and significant time in a container will alter the physiological state of the algal cells, resulting in misrepresented chlorophyll data compared to the natural situation.

Discrete samples need to be kept at the same temperature. This is most easily accomplished through the use of a water bath. The bath should be covered from direct light. When the samples are being measured in the fluorometer, a 'time in the instrument' must be established. Use the discrete sample averaging function on the 10-AU or TD-700 Fluorometer or wait for the reading to stabilize (~10 seconds) and record the fluorescence. If this time is not monitored the heat and light in the instrument will cause fluorescence to change.

When developing you own sample collection and storage procedures, it is recommended to run your own experiment to test change in fluorescence over a given transport time by analyzing samples from a given sample at hour or half-hour intervals.

Q: How do I calculate actual chlorophyll *a* concentrations from my fluorometric data?

A: ACIDIFICATION METHOD

Prior to running sample on the fluorometer, the instrument must be calibrated with a pure chlorophyll *a* standard and the maximum acid ratio must be determined by measuring the fluorescence of the standard before and after acidification. If a fluorometer other than a digital Turner Designs instrument is being used, the fluorometer sensitivity coefficient may also need to be determined. For further information please refer to EPA Method 445.0.

$$\text{chl a} = K\,(F_m / F_m - 1) \times (F_b - F_a) \times (v/V)$$

$$\text{pheo a} = K\,(F_m / F_m - 1) \times [(F_m \times F_a - F_b)] \times (v/V)$$

If necessary, the result can be multiplied by a dilution factor.

where:
K= sensitivity coefficient, equal to 1 on 10-AU
Fm = max acid ratio Fb/Fa of pure chlorophyll *a* standard
Fb = fluorescence before acidification
Fa = fluorescence after acidification
Fo = fluorescence signal of sample

v = extract volume (L)
V= volume filtered (L)

NON-ACIDIFICATION METHOD (Chlorophyll *a* concentration only)

1) Collect fluorescence data (one number/sample)
**** DO NOT ACIDIFY****
2) Plug data into following equation:

chl a = (Fo x v) / V

where:
Fo = fluorescence signal of sample
v = extract volume (L)
V= volume filtered (L)

Q: Does the E.P.A. approve fluorometric chlorophyll analysis?

A: Yes, the E.P.A. has published Method 445.0 which covers the in vitro(extraction) fluorometric analysis of chlorophyll *a*. In the most recent revision (Rev 1.2 Sept., 1997), the E.P.A. also approves the use of the non-acidification method, which is less susceptible to interfering compounds such as chlorophyll *b*. This filter kit supplies only chlorophyll *a* concentrations with no information on pheophytin concentration.

Q: How can I compare chlorophyll data obtained through different measurement techniques?

A: All detection instrumentation used in chlorophyll analysis will result in chlorophyll concentrations that are directly comparable. A side-by-side comparison between a fluorometer and a spectrophotometer is easily done but would require dilution of the chlorophyll sample to put it within the linear range of the fluorometer. A sample that is in range on a spectrophotometer will be over-range on a fluorometer.

Q: Why use a fluorometer over a spectrophotometer for extracted chlorophyll analysis?

A: Benefits of fluorescence over spectrophotometry include the capability of *in vivo* detection, sensitivity, durability, versatility (accepts a wide range of discrete sample cells and flow cells, accepts AC or DC power, and the user can choose and quickly change between many optical kits), ease of use, stability, ease of transport, and a small footprint.

For oceanographic research, the greater sensitivity of fluorescence results in less time and work in the analysis because much less water must be filtered for extracted analysis. The superior sensitivity also enables *in vivo* detection of chlorophyll concentration of <1µg/L.

Freshwater researchers now have an extremely accurate and easy way to measure chlorophyll *a* even with high chlorophyll b concentrations using the non-acidification optical kit. Fluorometers also allow for in-line monitoring to collect data in real time.

Q: What are the chlorophyll detection limits of Turner Designs Fluorometers?

A: The TD-700 Laboratory Fluorometer and the 10-AU Field Fluorometer with a red sensitive photomultiplier tube (PMT), have extracted chlorophyll detection limits of 0.02µg/L using a 13mm diameter test tube and 0.01µg/L using a 25mm test tube. The SCUFA® Submersible Fluorometer can detect *in vivo* chlorophyll concentrations to 0.02µg/L.

Glossary

CTD – stands for **C**onductivity-**T**emperature-**D**epth and refers to the electronic instrument deployed into the ocean to measure these and other parameters. Electronic sensors measure different aspects of the ocean while the CTD is lowered to a terminal depth (on CalCOFI ~500m, depth permitting). These data are transmitted up the conductive cable and display on the CTD computer screen real-time. The screen plots created during the downcast are used to determine the upcast optimal bottle closure depths/spacing. After being lowered to the terminal depth without stopping, the **rosette** (array of 24 bottles mounted on the frame that surrounds the CTD electronics) bottles are sequentially closed at specific target depths as the CTD is raised. Although the terms CTD or rosette may be used interchangeably when referring to the complete package, the CTD is the electronic portion; the rosette and **carousel** (central hub that controls the bottle closure) are the bottle array. The CTD electronics may be deployed independent of the rosette.

rosette – metal frame and bottle array that surrounds the CTD electronics. CalCOFI uses 24 ten liter bottles; other programs may use 6, 12, 24, or 36 bottles of various volumes. The **carousel** (or **pylon**) holds the bottle **lanyards** until the CTD data aquisition programs sends a command to close a specific bottle. CalCOFI's bottle closure sequence is bottle #1 at terminal depth (usually ~500m) ending with bottle #20 at surface (~2m). The 4 extra bottles are for discretionary use (except the Santa Barbara Basin station where all 24 are closed).

Carousel (or **pylon**) – electronic hub centered on the rosette that holds the bottles open until it receives the release command. Bottle closure is usually sequential but the software can be program to close the bottle in any order.

Lanyard – nautical term used to describe a short line or rope that secures or rigs something. In reference to the CTD/rosette, the nylon rigging used to keep the bottle top & bottom caps open.

HPLC - High-pressure liquid chromatography; high-performance liquid chromatography.

Fluorometer - instrument used to measure fluorescent materials; for Chl-a, it emits a excitation wavelength of light ~460nm and detects the emission return at ~685nm.

Fluorescence - the phenomena of some compounds to absorb specific wavelengths of light and instantaneously emit longer wavelengths of light energy

Chlorophyll *a* - the green photosynthetic pigment contained in all living algae that can be directly measured and used as the primary indicator of algal biomass.

Chlorophyll *b* - an accessory pigment found in some algae that can effect the accuracy of chlorophyll a determinations

Extraction - the process of using a solvent (acetone) to remove the chlorophyll from the algal cells.

Eutrophic - term used to describe a nutrient-rich body of water.

In vivo - Term used for measuring chlorophyll that is contained naturally within the algal cells

In-Situ - Term synonymous with *in vivo*

In-vitro - Term used when measuring chlorophyll that is extracted from the algal cells

Oligotrophic - term used to describe a nutrient-poor body of water.

Pheophytin - term for degraded chlorophyll that occurs naturally or as a result of acidification.

Phytoplankton - the photosynthesizing constituent of plankton, mainly unicellular algae

Phycoerythrin - the accessory pigment found primarily in marine cyanobacteria species such as Synechococcus spp

Primary Standard - a sample of the exact same material being measured, and ideally at a known concentration level.

Quenching - term refers to factors that reduce, or quench, fluorescence.

Secondary Standard - a sample material that can be used as a secondary measure for calibration, and provides a stable reference that is repeatable.