



Phycotech, Inc.

Raising the Standard in Aquatic Analyses

www.phycotech.com

Business & Technical Information

2012

620 Broad Street, Suite 100, St. Joseph, MI 49085

Phone 269-983-3654 • Fax 269-983-3653

E-mail: info@phycotech.com • Web: <http://www.phycotech.com>

PhycoTech, Inc.

Table of Contents

Mission.....	3
Executive Summary.....	4
Introducing ASA System.....	5
Question & Answer.....	6-7
Map – Processed Samples.....	8
General Technical Approach.....	9-24
09-10.....Introduction	
11-14.....Algal HPMA Mounts Protocol	
15-17.....Zooplankton HPMA Mounts Protocol	
18-23.....Quality Assurance Plan	
18.....Taxonomic Accuracy	
18.....Sample Custody	
19-23....Counting	
24.....References	
Shipment Recommendations.....	25
PhycoTech, Inc. Services Price List.....	26-27
Policies and Disclaimers.....	28
PhycoTech, Inc. Product Price List.....	29
Product Specifications.....	30
Using the Nannoplankton Chamber.....	31
Résumé – Ann St. Amand, Ph.D.....	32-38

620 Broad Street, Suite 100, St. Joseph, MI 49085

Phone 269-983-3654 • Fax 866-728-5579

Email: info@phycotech.com • Web Address: <http://www.phycotech.com>

PhycoTech, Inc.

Mission

PhycoTech, as a Christian based company, is devoted to improving environmental water quality worldwide, protecting our water resources for future generations. Included in this vision is a strong commitment to fostering environmental stewardship among our local communities' youth. We are committed to providing our staff with an environment conducive to personal growth, while providing our clients with extremely accurate and useable data, delivered in a timely manner.

PhycoTech, Inc.

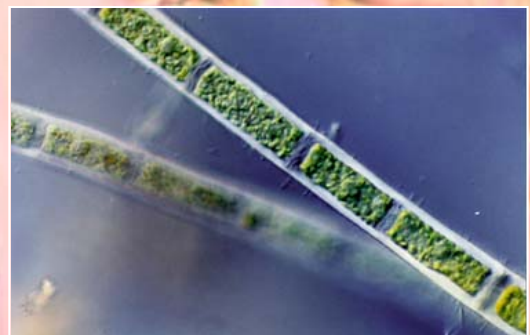
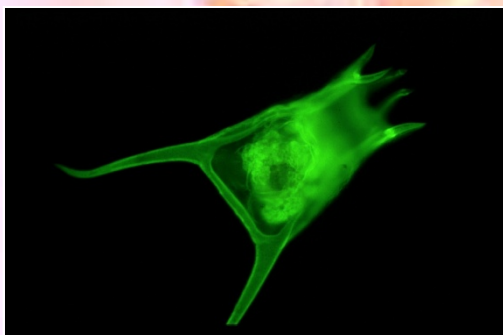
EXECUTIVE SUMMARY

PhycoTech is an environmental consulting company specializing in the analysis of freshwater and marine algae, zooplankton, macro invertebrates, and bacteria. We are a leader in our industry, providing our customers with a wide variety of related services based on the most current technology, including permanently mounted, archival slides of both algae and zooplankton. We are committed to improving water quality, while providing our employees and associates with a flexible work schedule in an atmosphere that fosters creativity, initiative, and continuous skills development.



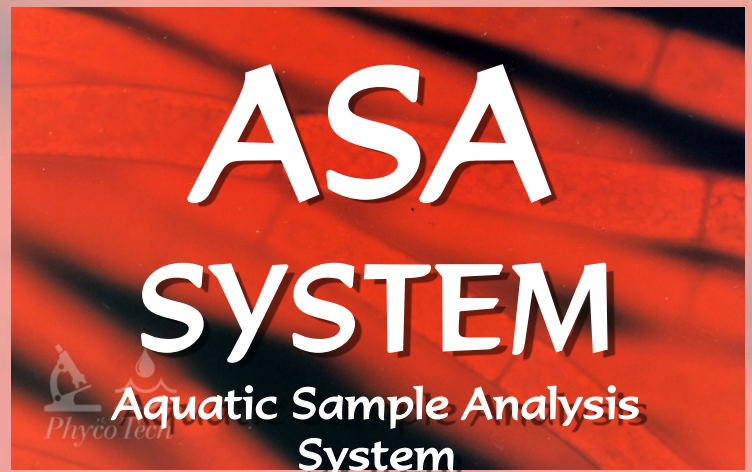
Our qualified management team is directed by Ann St. Amand, Ph.D., President and Buck Smith, Laboratory Manager. We are experienced in the direction of administration, marketing, technical services, research and development.

At present, we analyze lake, reservoir, stream, well, and marine samples (including gut analysis on fish and invertebrates) for suspended and attached algae, zooplankton, macroinvertebrates and bacteria. We also process core samples for diatoms and blue green akinetes. PhycoTech has three research grade Olympus Microscopes (two with Nomarski optics and fluorescence), and we also have access to Scanning Electron Microscopy facilities through the University of Notre Dame. We provide our customers with a range of associated services, which include permanently mounted slides, data analysis available in multiple computer formats, photographic services (2x2 slides, photographs, scanned images on CD), sales of microscopic accessories and archival services.



Introducing:

**PhycoTech's
revolutionary
proprietary data
management system!**



- 🕷 **Over 34,000 currently defined taxa including algae, zooplankton and macroinvertebrates**
- 🕷 **Over 40 Sample Calculation types, unlimited capabilities**
 - 🕸 **Including tow, attached, grab, fish gut and core samples**
- 🕷 **Over 60 Enhanced shape formulas, unlimited capabilities**
- 🕷 **Over 89 Diversity indices, calculated on abundance and biomass**
- 🕷 **Biovolume, biomass, volume and surface area on algae**
- 🕷 **Biomass on zooplankton**
- 🕷 **Standard algal data set provides both natural units and cells per volume or area**
- 🕷 **Units completely customizable**
- 🕷 **Enhanced Data File and Report capabilities**



- 🕷 **More accurate calculations**
- 🕷 **More measurement capabilities**
- 🕷 **Lower error rates**
- 🕷 **No data transcription**

PhycoTech, Inc.

Questions and Answers

Question #1 **Why is counting algae so important?**

Answer: Algal growth directly affects water quality, both functionally and aesthetically. Algae respond very quickly to environmental change, and excessive algal growth is often the first noticeable sign of degrading water quality. At the microscopic level, changes in algal species indicate increasing or decreasing water quality over time, and often give valuable information about what has or is affecting the system. Knowing the composition of the algal community, then, is extremely important for making sound management decisions.

Question #2 **Why choose PhycoTech, Inc.?**

Answer: Ann St. Amand, Ph.D. is the founder and President of PhycoTech, Inc. Dr. St. Amand personally counts all algal and bacterial samples, in fact, Dr. St. Amand has counted over 27,000 samples in her 24 years of experience. She is also qualified to analyze zooplankton and macroinvertebrate samples. This high level of expertise guarantees clients the consistency and accuracy needed in sample analysis, especially in cases where multi-year and historical data comparisons are important. In addition, PhycoTech has developed a worldwide network of taxonomic experts to assist in identification confirmation. Whereby, PhycoTech finds it is repeatedly called upon to provide QA/QC services for other laboratories. We also routinely reach 95%+ precision for our own in-house QA/QC.

Question #3 **What kind of “permanent” slide does PhycoTech, Inc. provide?**

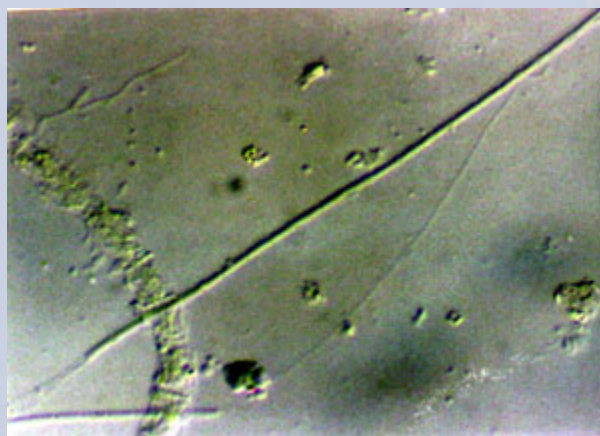
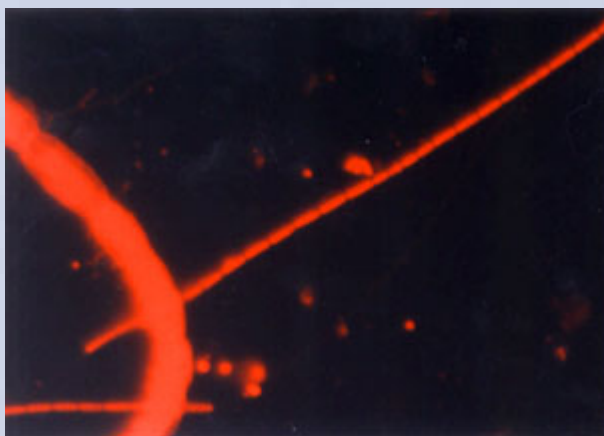
Answer: PhycoTech, Inc. produces three kinds of permanent slides: 1) HPMA mounts, 2) NAPHRAX mounts, and 3) Zooplankton mounts. Three HPMA mounts are produced per sample for all quantitative algal samples and three Naphrax mounts are produced per sample for those samples requiring species confirmation on diatoms and some Chrysophytes. All mounts have special, chemical resistant labels affixed to the left side of the slide, leaving the right side free for a voucher label, if required. A tracking code number is assigned to each sample, which is embedded under the cover slip of the HPMA slides for additional security. PhycoTech is the only laboratory in the country that produces truly permanent zooplankton mounts. One (1) mount is made per sample and a special staining/cleaning agent is used to highlight taxonomic features. Our proprietary technique for making HPMA mounts of whole samples allows you to get additional data, decades after the initial analysis, as well as maintain a permanent archive of your samples that won't degrade with time. HPMA permanent mounts can be processed on a compound microscope, allowing higher resolution and more accurate data. If glutaraldehyde is used as a preservative on algal samples, HPMA slides will maintain auto fluorescence for the lifetime of the slides. Sample slides are available upon request.

PhycoTech, Inc.

Question and Answers

Question #4 Fluorescence? Why count with it?

Answer: Epifluorescent technology is used to increase the consistency and accuracy of the sample data. PhycoTech, Inc. uses this method on samples to identify many diverse kinds of algae, including small blue-green algae. Smaller blue-green algae are extremely important ecologically and can dominate a system numerically. To be able to accurately identify and count them is absolutely vital. Fluorescence is also used as an aid in quantifying samples with high particulate levels. Without it, many taxa could not be identified properly or would be indistinguishable from bacteria. Sample preservation plays a key role in the ability to effectively use epifluorescence. Samples preserved in Lugols Iodine can fluoresce to some extent, sample must be preserved in glutaraldehyde (best) or formalin.



Fluorescent and Nomarski micrograph at 1000x showing single celled blue-green picoplankton.

A map of North America, including the United States, Canada, and Mexico, is shown against a dark blue background. The United States is highlighted in a teal color, while Canada and Mexico are in a light gray color. Numerous small, light blue teardrop-shaped markers are scattered across the United States, representing sample locations. A white rectangular box with a black border is positioned at the top center of the map, containing the text "North America" in a bold, black, sans-serif font.

North America

Over 29,000 North American samples have been processed in our lab here at PhycoTech, Inc. Not limited to the U.S., PhycoTech has processed samples from as far as Antarctica! Our President, Ann St. Amand, Ph.D., is an expert taxonomist with over 26 years of experience identifying algae and zooplankton. All algal analyses are exclusively rendered by Dr. St. Amand. Eliminating inconsistencies often found with student and/or inexperienced technician analysis, PhycoTech, Inc. is then able to guarantee accuracy and consistency for our customers' multiple year projects, and most importantly, giving them peace of mind concerning data integrity.

GENERAL TECHNICAL APPROACH

PhycoTech is the only commercial lab in North America to utilize a unique proprietary permanent mounting technique for archiving and preparing samples for enumeration. These mounts allow you to get further data at a later date, as well as maintain a permanent archive of the sample that is easily stored, maintains fluorescence, and does not degrade with time (100+ years). Permanent algal mounts allow archiving of diatoms AND soft algae. All periphyton samples to species include both HPMA mounts for the whole sample and Naphrax, acid cleaned mounts, for diatom identification to species level. Zooplankton samples are also permanently mounted using a slightly different process. Our algal taxonomist, Dr. Ann St. Amand, has over 23 years of experience and has processed over 25,000 periphyton and phytoplankton samples from both freshwater and marine systems. Dr. St. Amand is the only person who enumerates algal and zooplankton samples at PhycoTech, ensuring data integrity and consistency. Our In-house key and publication library numbers in the thousands, including the most current references. We have processed several state wide surveys in the Mid-West and Florida for phytoplankton and periphyton, each comprised of several hundreds samples. PhycoTech also consults with Federal and State Agencies, including the Corps of Engineers, on experimental design and QA/QC issues. We process samples for general water quality, as well as the determination of exotic, toxic or taste and odor producing blue-green and chrysophyte algae.

There are three state of the art microscopes used to process algal and zooplankton samples: an Olympus BX51, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (200x, 400x, 1000x), Polarized light for zebra mussel velliger counts, and Reflective Incident Light, (1000x) a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a SpotFlex digital camera attached; an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Microfire digital camera attached, and an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Ricoh Camera Back attached using traditional slide and print film. For larger material PhycoTech also has a dissecting microscope. We have access to Notre Dame's SEM facility as well. Our computer network is a newly installed Novel Network connected to 6 new 2.8+ Gig workstations, with special adaptations for graphics and extra memory for efficiently handling our new data management system, Aquatic Sample Analysis (ASA) System. All count related data is backed up daily to different media, with on-site and off-site copies weekly.

GENERAL TECHNICAL APPROACH

PhycoTech is now utilizing its proprietary data management software, ASA. This unique, powerful program not only tracks samples from receipt to data delivery within the same software program (every processing step is documented with initials and date, from Login to Analysis), but also provides significantly more information for each sample. With ASA, we are able to provide not only biovolume estimates, but volume and surface area estimates as well. Our biovolume, volume and area formulas are the most complete set available commercially, drawn from a variety of sources including current primary literature (See our Protocol), custom calculations designed in-house for complicated morphologies (e.g. Ceratium) and independently derived calculations from an Outside Engineering Firm that specializes in volumetric studies (e.g. area of a prolate, oblate ellipsoid). We also now provide data summaries on phyla, division, class down to taxa level automatically, depending on the analysis requested. In addition, our new program has the capability to calculate over 89 different diversity indices and summary statistics, including Shannon, Margalef, Alpha and Berger_Parker Diversity measures, Species Richness and Evenness, Pollution Tolerance for diatoms, Environmental Tolerance for algae, Siltation Index for diatoms, Pollution Tolerance for diatoms, Palmer Index, ACC:CMN for diatoms, in addition to others. All taxonomic information from organism down to coloniality and structure will be provided in the data set. All indices are calculated on an abundance (both Natural units/mL and Cells/mL) and total biovolume, volume and area basis, if biovolume is measured. QA/QC reports are generated from within the program, comparing dominant taxa, reporting distribution checks and doing similarity calculations between the original sample and QA/QC sample.

Reports are provided in PDF format with summary graphics by division for each sample. Data files are provided in Excel format or other spreadsheet or database formats requested by the customer.

GENERAL TECHNICAL APPROACH

Algae

The HPMA method for producing algal sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes (See references). Mounting distortion is minimal and the method provides the advantage of being able to go 100x to 1000x on the same specimen. Wet sample is always maintained in case clarification of identification is necessary. We strongly encourage our customers to use glutaraldehyde (final concentration of 0.25-0.50 %) for preservation of algal samples. It offers minimal distortion and allows the use of epifluorescence on algal samples while counting, which can dramatically improve the final results.

GENERAL PROTOCOL FOR MAKING PERMANENT ALGAL MOUNTS USING HPMA

EQUIPMENT:

Bunsen burner
Beaker tongs
Ice bath
Pyrex beakers (150 ML)
2 Dropper bottles
Mixed ester nitrocellulose filters (0.45 μm , 25 mm, plain)
Glass slides (25 mm x 75 mm)
Avery Laser Labels: #2181
Glass coverslips (25 mm x 25 mm, #1 or #1.5)
Full view series support/drying racks (102 pin)
Graduate cylinders
Dumont forceps
Glass filter towers (25 mL)
Rubber stoppers (#2, #10)
Filtration Manifold (6 station)
Vacuum pump (plus appropriate plumbing, 25-50 mm Hg)
Drying oven (60°C, not forced air)
Hood

REAGENTS:

HPMA (2-hydroxypropyl methacrylate)
Catalyst (azo-bis-iso-butyronitrile)
Iodine
Glutaraldehyde (25%, general grade)
Distilled water

CAUTION: Store HPMA and catalyst in refrigerator. Keep glutaraldehyde under hood.

GENERAL TECHNICAL APPROACH

METHOD:

SAMPLE:

1. Add enough glutaraldehyde to bring the final concentration to approximately 0.25% to 0.5% (for periphyton samples or "bloom tows", increase the final concentration to approximately 0.5%-1%). Keep the sample dark and refrigerate if possible.
2. Remove the sample from the refrigerator and let it warm to room temperature before mounting.

RESIN:

1. Prepare ice bath in plastic tub.
2. Measure 25 mL of HPMA and 0.025 g of catalyst into a 150 mL beaker.
3. Deal with HPMA under hood and use gloves for both HPMA and catalyst.
4. Under hood, light Bunsen burner and set to high flame.
5. Heat HPMA (with catalyst added) until you see density currents starting to form. Cool mixture by swirling in ice bath, and return to flame.

DO NOT LET MIXTURE BOIL!!*

6. Keep heating and cooling, alternately, until the mixture is approximately the thickness of Karo syrup. Make sure the mixture is cool when it reaches this point or it will polymerize further.
7. Transfer to a clean, glass jar for storage until usage.

The entire procedure takes 1 to 2 hours, depending on how brave you are.

***CAUTION!! THIS REACTION IS EXOTHERMIC ONCE IT REACHES A CERTAIN TEMPERATURE AND WILL TAKE PLACE ALMOST EXPLOSIVELY IF YOU LET IT GET TOO HOT. THE FUMES ARE TOXIC. KEEP WATER OUT OF THE PRE-POLYMER.**

NOTE: Wash beakers in ethanol by letting them soak for 24 to 48 hours twice; wash with soap and rinse with distilled water. Be careful to keep dust out of the beakers when making the resin.

Fill 2 amber dropper bottles with resin. Add crystalline iodine to one of the bottles until the resin is nearly opaque. The iodine-resin will be slightly thicker than normal resin. (Resin is light sensitive -- be sure to cover the extra resin with foil.)

GENERAL TECHNICAL APPROACH

SLIDES:

MAKE THREE SLIDES FOR EACH SAMPLE

SHAKE SAMPLE WELL (100 TIMES-phyt. or 200 TIMES-peri)

Use Millipore 6-place stainless steel manifold and Millipore Filtration Towers.

1. Put membrane filters onto filtration bases and wet with distilled water. Drain excess water through filter. If filter has any opaque areas (very white when wet), replace with another filter.
2. Assemble filter towers.
3. Measure out phytoplankton sample using micro-pipetor or macropipetor (use graduate cylinder for very dilute samples, e.g. 30+ mL). For periphyton samples, remove sample with micropipetor (usually from 0.05-0.5 mL) and dilute to 10 mL in a graduated cylinder with distilled water. Agitate to mix. Choose sample volume so that each field at 200x contains approximately 20-30 cells.
4. Add sample to the tower and open valve. For periphyton samples or large phytoplankton samples using cylinders, rinse graduate cylinder into tower. Filter sample until water just clears the filter surface. Close valve and remove filtration tower just after the water disappears from the inner edge of the tower.
5. Place filter, FACE down, on a cover slip (# 1.5). Be careful to avoid bubbles under the filter.
6. Samples:
 - A. Samples preserved in glutaraldehyde:
3 slides: Add 1-2 drops of clear resin to the back of the filter, and rotate the cover slip until the resin covers the back of the filter.
 - B. Samples preserved in lugols:
3 slides: Add 1-2 drops of the iodine-resin to the back of the filter, and rotate the cover slip until the resin covers the back of the filter.
7. Place cover slips on the drying rack and place in drying oven for 12 to 24 hours.
8. Remove cover slips from oven. Add 1 drop of resin to the filter side of the cover slip and attach to a labeled slide. Add as little resin as possible to cover the filter surface!!!! The less resin, the faster it will polymerize and the better the prep.

GENERAL TECHNICAL APPROACH

SLIDES continued:

9. Put slides in the oven and let polymerize for approximately 24 hours. If the resin is not completely polymerized, replace and heat for as long as 2-3 days. Make sure that the slides are completely polymerized before you store them or they will run and/or evaporate!!!! And believe me, its a mess!!!!

10. Label slides with ASA generated labels. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

GENERAL TECHNICAL APPROACH

Zooplankton

The HPMA method for producing zooplankton sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes (See references). Mounting distortion is minimal. Wet sample is always maintained in case clarification of identification is necessary. Lignin Pink Double Stain allows for better visualization of animals and highlights critical morphological structures necessary for identification. Preferred preservative for zooplankton is 70% EtOH at a optimal 70:30 ratio. Lugol's Iodine can also be used, but sometimes interferes with staining and obscures structures. Please call if the EtOH preservation method is not a viable option.

GENERAL PROTOCOL FOR MAKING PERMANENT ZOOPLANKTON MOUNTS USING HPMA

EQUIPMENT:

Bunsen burner
Beaker tongs
Ice bath
Pyrex beakers (150 ML)
2 Dropper bottles
Mixed ester nitrocellulose filters (5.0 μ m, 47 mm, plain)
Analyslide (47 mm)
Laser Labels: 1½ x ¾ inch
Full view series support/drying racks (102 pin)
Graduate cylinders
Dumont forceps
Lignin Pink Double Stain
Glass filter tower (250 mL)
Filter Flask
Rubber stopper (#8)
Glass Microanalysis Filter Holder 47 mm disc
Vacuum hand pump
Drying oven (43°C, not forced air)
Hood

REAGENTS:

HPMA (2-hydroxypropyl methacrylate)
Catalyst (azo-bis-iso-butyronitrile)
Iodine
Alcohol (70% ETOH)
Distilled water

CAUTION: Store HPMA and catalyst in refrigerator.

GENERAL TECHNICAL APPROACH

METHOD:

SAMPLE:

1. Add enough alcohol to bring the final concentration to approximately 70%, or Lugols until a dark tea color.

RESIN:

1. Prepare ice bath in plastic tub.
2. Measure 25 mL of HPMA and 0.025 g of catalyst into a 150 mL beaker.
3. Deal with HPMA under hood and use gloves for both HPMA and catalyst.
4. Under hood, light Bunsen burner and set to high flame.
5. Heat HPMA (with catalyst added) until you see density currents starting to form. Cool mixture by swirling in ice bath, and return to flame.

DO NOT LET MIXTURE BOIL!!*

6. Keep heating and cooling, alternately, until the mixture is approximately the thickness of Karo syrup. Make sure the mixture is cool when it reaches this point or it will polymerize further.
7. Transfer to a clean, glass jar for storage until usage.

The entire procedure takes 1 to 2 hours, depending on how brave you are.

***CAUTION!! THIS REACTION IS EXOTHERMIC ONCE IT REACHES A CERTAIN TEMPERATURE AND WILL TAKE PLACE ALMOST EXPLOSIVELY IF YOU LET IT GET TOO HOT. THE FUMES ARE TOXIC. KEEP WATER OUT OF THE PRE-POLYMER.**

NOTE: Wash beakers in ethanol by letting them soak for 24 to 48 hours twice; wash with soap and rinse with distilled water. Be careful to keep dust out of the beakers when making the resin.

Fill 2 amber dropper bottles with resin. Add crystalline iodine to one of the bottles until the resin is nearly opaque. The iodine-resin will be slightly thicker than normal resin. (Resin is light sensitive -- be sure to cover the extra resin with foil.)

GENERAL TECHNICAL APPROACH

SLIDES:

**MAKE ONE SLIDE FOR EACH SAMPLE
SHAKE SAMPLE GENTLY 50 TIMES**

If necessary, split sample with Folsom plankton splitter.

1. Put membrane filter onto filtration base and wet with distilled water. Drain excess water through filter. If filter has any opaque areas (very white when wet), replace with another filter.
2. Assemble filter tower.
3. Measure out zooplankton sample using graduate cylinder. Choose sample volume so that each field at 100x contains approximately 5-10 animals.
4. Add one drop of Lignin Pink to graduated cylinder for every 5mL of sample. Let sample sit for 15 minutes.
5. Place entire contents of graduated cylinder into filter tower. Rinse graduate cylinder into tower twice. Filter sample (using vacuum hand pump) until water just clears the filter surface. Remove filtration tower just after the water disappears from the inner edge of the tower.
6. Place filter, FACE up, on analyslide (47 mm). Be careful to avoid bubbles under the filter.
7. Add 8-10 drops of clear resin to the filter, and rotate the analyslide until the resin covers the whole filter.
8. Place analyslide on the drying rack and place in drying oven for 12 to 24 hours.
9. Remove analyslide from oven. Add just enough resin to the filter to cover the filter surface!!!! The less resin, the faster it will polymerize and the better the prep.
10. Put slides in the oven and let polymerize for approximately 24 hours. If the resin is not completely polymerized, replace and heat for as long as 2-3 days. Make sure that the slides are completely polymerized before you store them or they will run and/or evaporate!!!! And believe me, it's a mess!!!!
11. Label slides with ASA generated labels. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

GENERAL TECHNICAL APPROACH

Quality Assurance Plan

TAXONOMIC ACCURACY

Dr. Ann St. Amand, a senior level phycologist and taxonomic expert, will perform all phytoplankton, periphyton, and zooplankton identifications, enumerations, and biovolume/biomass measurements. Dr. St. Amand has published extensively in the area of algal ecology and has processed over 27,000 algal and bacterial samples, and is qualified to analyze zooplankton and macroinvertebrates. Outside taxonomists will be utilized for taxonomic verifications when necessary.

All samples are initially test mounted for counting density before final mounting. Any major questionable IDs are noted in the database during counting, and indicated on the report as uncertain for taxonomic clarity. If enough sample is present, samples are sent out to other taxonomists for taxonomic confirmation. Distribution is checked on approximately every tenth sample, during the counting process. All biovolume calculations have been verified by comparing with current literature, and by comparing calculations using outside mathematical consultations.

SAMPLE CUSTODY

The chain-of-custody requirements for all laboratory operations for each sample (broadly interpreted to include procedures for the preparation of reagents or supplies which become an integral part of the sample, record keeping associated with sample acquisition, documentation of sample preservation, sample labeling, sample tracking to establish chain-of-custody, and shipping and packing) and laboratory analysis (i.e., laboratory coding, storage, check-out, and documentation of sample movement) will be fully documented in our data management software. Each sample received will be assigned an individual tracking number. The sample bottle, chain-of-custody, and sample log sheet, which accompanies each sample sent, are then used in conjunction with one another, to enter the samples individual tracking number and all available sample information, into our sample database, ASA. The database allows for quick and accurate tracking of each sample received by PhycoTech. Dated and initialed entries by appropriate personnel on all worksheets and in the log database are required for data validation. All information entered into ASA is fully QA/QC'd for content and accuracy. Sample receipt is confirmed with each customer. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

GENERAL TECHNICAL APPROACH

COUNTING

Microscope: There are two microscopes used to process algal samples: Our primary microscope, an Olympus BX51, research-grade compound microscope equipped with Brightfield optics (40x, 100x, 200x, 400x, 1000x), Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (200x, 400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a SpotFlex digital camera attached. For larger material PhycoTech also has a dissecting microscope. The BX60 is a secondary microscope with similar optics to the BX51, used for algal and zooplankton identification as a back-up microscope. There is also an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Ricoh Camera Back attached using traditional slide and print film.

Data Entry: Samples are enumerated within ASA directly. ASA is a database driven program with an integrated virtual TallyMeter module, containing over 130 databases. Up to 400 taxa can be enumerated within any one sample, and the entire database currently contains over 33,000 taxa, including algae, zooplankton, macroinvertebrates and bacteria. All calculations are completed within ASA, including concentrations, biovolumes, biomasses and diversity indices. Data files are also generated by ASA and saved in Excel format, while reports are formatted and saved to pdf format utilizing Microsoft Access, including summary graphics on a per sample basis. PhycoTech can then format data files in any format required by the customer, either horizontally or vertically oriented. QA/QC on counting is a recount done on approximately every 10th sample. ASA produces a QA/QC report comparing the original sample and the recount sample (quantitatively and qualitatively), including the distribution check. Samples pass that are within 10% of the QA/QC recount, quantitatively. Percent similarity may vary up to 20% on exceptionally diverse or sparse samples.

Phytoplankton: The magnification used will depend on the size of the dominant taxa and the size and number of particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size. If the sample is dominated by cells below 10-20 μm or the cells are fragile and difficult to identify, the majority of counting will be completed at 400x-1000x. Measuring for biovolume includes measuring GALD and additional measurements including length, width and depth of different aspects of the colony or cell. ASA allows up to 28 separate measurements per taxa. Cell and colony shapes are approximated to a geometric figure and or figures and the appropriate calculations made. Currently, ASA has over 44 different shapes defined. From 10 up to a total of 30 natural units (sometimes higher on exceptionally variable taxa) are measured for each taxa depending on variability and number encountered.

GENERAL TECHNICAL APPROACH

Phytoplankton continued:

Use ONE of the following methods depending on sample composition:

A. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae greater than 10-20 μm in GALD, count a minimum of 300 natural units and 15 fields at 200x (when possible, maximum of 100 fields). In addition, count taxa below 10 μm or fragile, difficult to identify taxa at 400x (minimum of 100 natural units and 10 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ μm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

B. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae less than 10-20 μm in GALD or is dominated by fragile, difficult to identify taxa, count a minimum of 400 natural units and 15 fields at 400x (when possible, maximum of 100 fields). In addition, count taxa above 20-30 μm in GALD at 200x (minimum of 15 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ μm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

C. DOMINATED BY DIATOMS: If the sample is dominated by diatoms other than large, easily identified taxa (e.g. Asterionella), count a minimum of 15 fields at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, count soft algae according to size distribution (see A or B above) for a minimum of 15 fields at either 200x or 400x. Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ μm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

NOTE: The goal, regardless of magnification, is to enumerate and identify a minimum of 400 natural units per sample exclusive of misc. microflagellates.

GENERAL TECHNICAL APPROACH

Periphyton: The magnification used will depend on the dominant taxa. If the sample is dominated by diatoms, the majority of counting will be completed at 1000x. If the sample is dominated by soft algae, the majority of counting will be completed at 200-400x, whichever is appropriate considering cell size and particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size.

Use ONE of the following methods depending on sample composition:

A. **DOMINATED BY SOFT ALGAE:** If the sample is dominated by soft algae greater than 10-20 μm in GALD, count a minimum of 300 natural units and 15 fields at 200x (when possible, maximum of 100 fields). In addition, count taxa below 10 μm or fragile, difficult to identify taxa at 400x (minimum of 100 natural units and 10 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ μm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

B. **DOMINATED BY SOFT ALGAE:** If the sample is dominated by soft algae less than 10-20 μm in GALD or is dominated by fragile, difficult to identify taxa, count a minimum of 400 natural units and 15 fields at 400x (when possible, maximum of 100 fields). In addition, count taxa above 10-20 μm GALD at 200x (minimum of 15 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ μm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

C. **DOMINATED BY DIATOMS:** If the sample is dominated by diatoms, count a minimum of 15 fields at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, count soft algae according to size distribution (see A or B above) for a minimum of 15 fields at either 200x or 400x. Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide).

GENERAL TECHNICAL APPROACH

Periphyton continued:

C. DOMINATED BY DIATOMS continued: Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ μm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

NOTE: The goal, regardless of magnification, is to enumerate and identify a minimum of 400 natural units per sample exclusive of misc. microflagellates.

Acid Cleaning: If species identifications for diatoms are required or unknown diatom taxa are present for Phytoplankton or Periphyton samples, acid cleaned mounts in Naphrax.

Naphrax mounts are prepared according to the following procedure:

1. Take 5-20mL of sample and transfer to a clean, 250mL Pyrex beaker in the hood. Add room-temperature nitric acid to a total volume of 40-60mL.
2. Cover with a watch glass.
3. After at least 24 hours has elapsed, carefully siphon off acid using glass siphon. Dilute acid and discard down drain with lots of extra water (Let water run for a minimum of 30 minutes after discarding acid).
4. a) Transfer remaining sample to a centrifuge tube and bring volume up to 14mL with distilled water. Cap tube, mix well, and centrifuge at 3000 RPM for 5 minutes. Remove tube and carefully remove supernatant to the 2mL volume marker with a micropipetor. Bring volume back up to 14mL with distilled water, mix well, and repeat process. Complete a minimum of 6 centrifuge cycles. Check pH. If pH is lower than 7, repeat centrifuging process until the pH reaches 7.

b) On the final cycle, remove supernatant to the 1 mL volume marker and bring volume back to 5 mL. Mix well to suspend pellet and decant into the storage bottle. Rinse the centrifuge tube 2 more times with 5 mL of distilled water and decant into the storage bottle. The total volume of the cleaned sample should be 15 mL. If the sample is very sparse, lower final volume.
5. Using a pasture pipette, transfer enough sample to a cover slip (#1, 22mm square) to cover the entire area and place in a vibration-free area until dry.

GENERAL TECHNICAL APPROACH

Acid Cleaning continued:

6. Add 1 small drop of Naphrax to the cover slip and invert onto a slide. Compress the coverslip with a clean object and place in an oven (60oC) for 1-3 hours, or finish on a hot plate.
7. Ring cover slip with fingernail polish and store.
8. Identify taxa at 1000x under oil immersion. Reference taxa are identified using a diamond scribing objective and permanent ink labels.

Zooplankton: Zooplankton are enumerated at 100x to 200x, depending on the average size of animal present (structures can be viewed at 400x, if necessary). Counting procedure is consistent with Standard Methods, with the target being 200 animals. Studies requiring greater precision or focusing on diversity require a higher counting threshold. Generally, when the sample is sparse, the entire slide is counted. Measurements for biomass include length, width and depth. ASA calculates biomass on crustaceans using published length/weight regressions, and on rotifers using biovolume formulae where biovolume is then converted to biomass. ASA can also use constant weights. If requested, customers may provide custom biomass calculations for ASA to use as well.

GENERAL TECHNICAL APPROACH

REFERENCES

Bergquist, A.M. 1985. Effects of herbivory on phytoplankton community composition, size structure and primary production. Ph. D. Dissertation. University of Notre Dame, Notre Dame, Indiana, USA.

Crumpton, W.G. 1987. A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. *Limnol. Oceanogr.* 32: 1154-1159.

St. Amand, A. 1990. Mechanisms controlling metalimnetic communities and the importance of metalimnetic phytoplankton to whole lake primary productivity. Ph.D. Dissertation. University of Notre Dame, Notre Dame, Indiana, USA.

Olrik, K., et. al. 1998. Methods for Quantitative Assessment of Phytoplankton in Freshwaters, part I. Naturvårdsverket, Stockholm.

Hillebrand, H., et. al. 1999. Biovolume Calculation for Pelagic and Benthic Microalgae. *Journal of Phycology.* 35: 403-424.

Standard Methods for Examination of Water and Wastewater, 18th ed., 2005. American Public Health Association, 1015 18th Street, N.W., Washington, DC 20036.

SHIPMENT RECOMMENDATIONS & ASSOCIATED COSTS

Due to the steady increase in shipping and handling expenses, PhycoTech, Inc. requires that you send a pre-paid shipping label with each cooler sent to us that you want returned to you. If you do not want your cooler(s) to be returned to you, please write "disposable cooler" on the exterior surface of the cooler or enclose a memo with each cooler stating that the cooler is disposable.

Shipment Information for Samples:

1. Samples should still be shipped to us via overnight service
2. All coolers that you wish to have returned to you **MUST** contain a pre-paid shipping label!
3. PhycoTech cannot accept ANY weekend deliveries.

Alternate methods of packaging samples:

(either method will be more economical than shipping an actual cooler)

1. Securely tape each container lid closed.
2. Place each sample container into a sealed bag for either shipping method.
Dark, plastic sample containers are highly recommended.
3. All containers (plastic and glass) should be firmly packed with Styrofoam, paper, or vermiculite (to avoid breakage)
4. All containers should be shipped with freezer packs or ice, sealed in airtight bags

Proceed to either

- A. Place bagged samples into a disposable Styrofoam cooler and put the cooler into a corrugated box.

OR

- B. Place bagged samples into a corrugated box lined with Styrofoam sheeting.

ANALYSIS SERVICES

Identifications, Data & Graphical Analysis \$294.00 per hour

(Minimum charge for services is \$294.00)

Phytoplankton Analysis

Prices for phytoplankton analysis (3 HPMA slides/sample). Species counts do not include acid cleaning. Please specify if you want acid cleaning for diatom identifications to the species level for an additional \$109.00 per sample. *Many common, planktonic diatoms are easily speciated without acid cleaning.

Calculation Level	With Biovolume Calculations	Without Biovolume Calculations
Species Count	\$329.00	\$239.00
Genus Count	\$218.00	\$155.00
Division Count	N/A	\$149.00

Additional Phytoplankton Services

Targeted for Algal Blooms - Faster Turn Around
Please call for process times.

Please NOTE: Prices are for Freshwater Samples ONLY (no marine) and Bulk Pricing DOES NOT APPLY!

Calculation Level	With 3 HPMA Slides	Without HPMA Slides
Rapid Assay: Qualitative Species (when possible) Assay weighted for Biovolume	\$168.00	\$112.00
Toxic/Bloom Scan, One Species	\$118.00	N/A

Periphyton Analysis

Prices for periphyton analysis (3 HPMA slides/sample). All periphyton samples received by PhycoTech, Inc. that require the removal of periphyton from substrate (i.e. rocks, sticks, etc.) are subject to an additional charge of \$32.00 per sample.

Calculation Level	With Biovolume Calculations	Without Biovolume Calculations
Species Count (All periphyton counts to the species level for diatoms include acid cleaning and at least three Naphrax mounts.)	\$596.00	\$508.00
Genus Count	\$299.00	\$239.00
Division Count	N/A	\$180.00
Sediment Core Diatoms	N/A	\$717.00
Sediment Core Akinetes	N/A	\$149.00
Diatom count to species or below for diversity purposes	Please Inquire - Based on time or taxa encountered, not counting threshold.	

Additional Algal Services

Bacteria

Total Counts (DAPI Method) Per Sample	\$150.00
---------------------------------------	----------

Biomass

(Reported in mg or g)

Dry Weight Per Sample	\$42.00
-----------------------	---------

Dry Weight/Ash-Free Dry Weight Per Sample	\$62.00
---	---------

Chlorophyll-A

Per Sample	\$90.00
------------	---------

PhycoTech, Inc. reserves the right to refuse improperly collected or leaking samples. There is a \$50.00/sample expedite fee for reporting within 4 weeks and a \$100.00/sample expedite fee for 48 hour business day turn around IF feasible (subject to prepayment). Algal Counts include report and raw data file (multiple formats available) and optically clear permanent slides (3 per sample). Units available as cells/mL (cm2) or natural units/mL (cm2). Analysis prices include report and raw data file ONLY. Interpretations, extensive explanations, and modifications to the initial report will be billed at \$294.00 per hour. Prices are per sample unless otherwise noted. One Sample = One Bottle

Zooplankton Analysis

Zooplankton counts include report and raw data file (multiple formats available) and optically clear permanent slides (one per sample). Counts reported as animals/L. Please contact us before sending Marine Samples. Charges for the analysis remain the same as what is requested even in the event no zooplankton or adult male copepods are found in the sample.

Zooplankton prices are based on one (1) slide per sample, if the samples are Daphnid dominated, we suggest going to three (3) slides at an additional cost of \$54/sample. Please specify 1 or 3 slides when requesting a quote, unless 3 slides are specified, we will base zooplankton counts and pricing on 1 slide.

Calculation Level	With Biomass Estimates	Without Biomass Estimates
Species Count (Copepods require Adult Males)	\$232.00	\$186.00
Genus Count (Copepods require Adult Males)	\$193.00	\$159.00
Zebra Mussel Veligers (no permanent slides, SR counts only, 10 Chambers)	\$165.00	\$159.00

Macroinvertebrate Analysis

Calculation Level	Price Per Sample
Family Count	\$126.00

[For additional identification levels, please contact us info@phycotech.com](mailto:info@phycotech.com)

RELATED SERVICES

Archiving

Permanent Microscope Slide Mounts	Price Per Sample
Slide mount only, (HPMA, 3 slides per sample)	\$118.00
Naphrax diatom mount (3 slides per sample)	\$155.00
Zooplankton permanent slide	\$108.00

Digital Photography

Per Image	\$31.00
-----------	---------

Miscellaneous Services

Culturing	\$428.00
-----------	----------

PhycoTech, Inc. reserves the right to refuse improperly collected or leaking samples.

There is a \$50.00/sample expedite fee for reporting within 4 weeks and a \$100.00/sample expedite fee for 48 hour business day turn around IF feasible (subject to prepayment). Algal Counts include report and raw data file (multiple formats available) and optically clear permanent slides (3 per sample). Units available as cells/mL (cm2) or natural units/mL (cm2). Analysis prices include report and raw data file ONLY. Interpretations, extensive explanations, and modifications to the initial report will be billed at \$294.00 per hour.

Prices are per sample unless otherwise noted. One Sample = One Bottle

PhycoTech, Inc.

STORE POLICIES & DISCLAIMERS

Charges and Fees

All prices shown are US dollars Prices are subject to change at any time and without notice.

Michigan residents are subject to a 6% state sales tax, unless they have tax-exempt status. PhycoTech, Inc. requires a tax-exempt number and a signature to verify this status.

Pre-payment is **required** with each order. Personal and Corporate checks are accepted.

International Orders

All international orders must also be pre-paid. International shipments are subject to individual country requirements and processing these orders may take longer. International customers are responsible for conversion fees, shipping costs, customs charges, and all taxes and duty charges if applicable.

Return/Refund Policy

Every item we sell is carefully inspected before it is shipped. If merchandise is defective or damaged upon receipt please contact us **immediately** after your package arrives for a return authorization claim number. All claims **MUST** be made within 3 days of receipt of order. Shipping charges will not be refunded. Returned products must be in absolutely new and unused condition for refund. All items are subject to a 20% restocking fee.

Warranty

We offer a one year warranty on all products under normal usage conditions. Please contact us if you experience any problems with our products.

Shipping Information

We ship via FedEx. Shipping charges are estimated at the time of order and are subject to change. Shipping via overnight does not guarantee you will receive your product the next day, but is dependant on product stock and availability. We ship your product as soon possible, however, please allow 3 to 6 weeks for delivery.



620 Broad Street, Suite 100, St. Joseph, MI 49085

Phone (269) 983-3654 • Fax (269) 983-3653

info@phycotech.com

www.phycotech.com

PhycoTech, Inc.

PRODUCTS PRICE LIST

25mm x 75mm, used up to 400x-600x on a compound scope. Significantly thinner than either a Palmer-Maloney or a hemocytometer. Each chamber is individually calibrated to contain a volume between 60-85µL. The PhycoTech nanoplankton counting chamber is fashioned after the Palmer-Maloney counting cell, and is used to hold a known volume of sample for quantitative counting of cells with a microscope. The PhycoTech nanoplankton counting chamber has several advantages over other cells and hemocytometer: 1) It is thinner, which allows examination and counting of microalgae at higher magnification, 2) larger cells will not be crushed by the weight of the coverslip, as this chamber uses a #1, 22 mm² coverslip, 3) it is less expensive and easier to use, and 4) it is individually calibrated. It is suitable for counting organisms up to approximately 300 µm in length. It can be used up to 600x using a high, dry objective. Each PhycoTech nanoplankton counting chamber has been individually calibrated.

Item Number	Product Description	Price
P-0015	Nanoplankton Chamber	\$79.00

Sedgwick Rafter Cells

The PhycoTech Sedgwick-Rafter Counting Cell is a standard counting cell designed for counting larger plankton on a compound microscope (up to 200x) or on an inverted microscope (higher magnifications possible). The cell volume is exactly 1.0 mL and consists of a ceramic rectangular frame mounted on a 1" x 3" glass slide. Cell dimensions are 50 x 20 x 1 mm (1.97" x 0.7870" x 0.04"). Includes two cover glasses in a plastic Snap-Lock case.

Item Number	Product Description	Price
P-0042	Sedgwick Rafter Cell	\$101.00

The PhycoTech Sedgwick-Rafter Counting Cell is improved with a bottom grid! Description is same as above with a 5 mm x 5 mm etched grid. Also includes two cover glasses in a plastic Snap-Lock case.

Item Number	Product Description	Price
P-0042G	*NEW* Sedgwick Rafter Cell with Bottom Grid	\$170.00

Micro-Organism Reference DVD

Micro-Organism Reference Photographic Image Set (over 2100 images), represents 12 freshwater divisions and classes, both algae and zooplankton. The set also includes multiple microscopic enhancements including Bright field, Phase, Nomarski and Epifluorescent shots. All images in low (72 dpi) and high (300 dpi) resolution

Item Number	Product Description	Price
P-011_CDR	Micro-Organism Reference DVD	\$291.00

HPMA Mounting Medium

Pre-polymerized HPMA mounting resin (125mL will make approximately 450 slides)

Item Number	Product Description	Price
P-0059	125mL Bottle	\$185.00

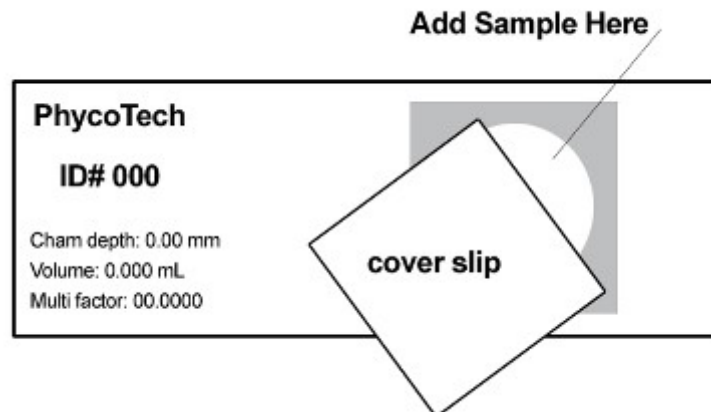


PhycoTech, Inc.

CHAMBER SPECIFICATIONS

Nannoplankton Counting Chamber:

- 25mm x 75mm
- Used up to 400x – 600x on a compound microscope
- Significantly thinner prep than either a Palmer-Maloney cell or a hemocytometer
- Uses a standard #1, 22mm cover slip
- Each chamber is individually calibrated to contain a volume between 65 – 85 μL
- Calibration information is bonded permanently to the slide



Property of PhycoTech:
Reproduction of specifications herein is strictly prohibited

Note: Consumer is responsible for user tax if applicable.
PhycoTech, Inc. is not liable for any tax penalties associated with the usage of
our products

PhycoTech, Inc.

NANNOPLANKTON CHAMBER

Information and Instructions

ID# example*

This Nannoplankton chamber is fashioned after the Palmer-Malone counting chamber, and is used to hold a known volume of sample for quantitative counting of cells with a microscope. This Nannoplankton chamber has several advantages over other chambers and hemocytometers: 1) It is thinner, which allows examination and counting of microalgae at higher magnification, 2) larger cells will not be crushed by the weight of the coverslip, as this chamber uses a #1, 22mm² coverslip, 3) it is less expensive and easy to use, and 4) it is individually calibrated. It is suitable for counting organisms up to approximately 300 µm in length. It can be used up to 600x using a high, dry objective.

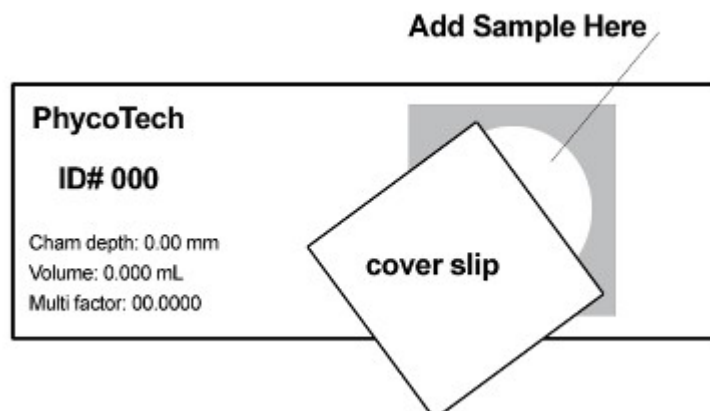
Each chamber has been individually calibrated and this particular chamber holds exactly 0.08* milliliters (mL). Retain underlined data for future use with this chamber. After counting all of the algae in the entire chamber, simply multiply by 12.5* to calculate the number of cells/mL.

A less tedious counting method involves extrapolating from counts on several individual microscope fields. To do this, measure the diameter of the field at a given power with a stage micrometer. Then calculate the area of the field using the formula 3.1459 (π) times the radius squared (radius = ½ of the diameter): area = π x r². Use millimeters for all length measurements. To convert this value into volume, multiply by the depth of the chamber, which is calibrated to 0.35*mm for this chamber. The formula for calculating volume is (area) x (depth). Keep in mind that 1000 mm³ is equal to 1 mL.

To use this chamber, place a #1, 22mm² coverslip on the side and leave a small space to add sample as illustrated below. As the sample fills the chamber, it will draw the coverslip over the chamber. Then position the coverslip over the entire opening to seal in the sample. If air bubbles are present, repeat the filling process. Live samples can be examined immediately and preserved samples after settling.

**Example of numbers that are similar to those for each individually calibrated chamber.*

Note: Consumer is responsible for user tax if applicable. PhycoTech, Inc. is not liable for any tax penalties associated with the usage of our products.



Ann L. St. Amand, Ph.D., CLP

PhycoTech, Inc
620 Broad St., Ste. 100
St. Joseph, MI 49085

Voice 269.983.3654 E-Mail astamand@phycotech.com

Phycologist & Certified Lake Professional

Professional Experience

President. PhycoTech, Inc. 1990-Present. St. Joseph, Michigan. Provide identification and enumeration of suspended and attached algal, zooplankton and bacterial samples utilizing a unique, permanent mounting technique. Also provide photographic, statistical and interpretive services involving algal samples and ecological data.

Research Associate. University of Notre Dame. 1991-1995. Department of Civil Engineering/Geological Sciences. Involved in project relating composition and biomass of periphytic biolayer in artificial stream ecosystems to PCB transfer within stream sediments.

Research Associate. University of Notre Dame. 1989-1991. Department of Civil Engineering. Involved in project relating groundwater quality to surface water quality including preliminary data acquisition and grant submission. Also involved in data analysis for a collaborative project on the environmental effects of oil-field brine application for road maintenance.

Faculty. Practicum in Aquatic Ecology, University of Notre Dame Environmental Research Center. June 1990. Taught limnology section of summer field course.

Research Assistant. University of Notre Dame. 1988-1989. Identified and enumerated phytoplankton samples from three northern Wisconsin lakes.

Teaching Assistant. University of Notre Dame. 1985-1988.

Field Intern. The Nature Conservancy (Indiana Chapter). November 1984-January 1985. Habitat management and landowner responsibilities within wetland and prairie habitats.

Education

Ph.D. University of Notre Dame, Notre Dame, Indiana. Aquatic Biology Program. Defense: April 12, 1990. Dissertation: Mechanisms Controlling Metalimnetic Communities and the Importance of Metalimnetic Phytoplankton to Whole Lake Primary Productivity.

B.S. Purdue University, West Lafayette, Indiana. 1984. Ecology, Evolutionary, and Population Biology.

Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana. 1980-1982.

Certifications

Certified Lake Professional 2003/2006/2009

North American Lake Management Society

Ann L. St. Amand, Ph.D., CLP

Honors

Cornerstone Chamber of Commerce: Entrepreneur of the Year, Commercial, 2008
Environmental Business Journal: Silver Medal: Consulting and Engineering award, 2008
Secchi Disk Award. North American Lake Management Society. 2006.
Merlin Hanson Challenge Award, Cornerstone Alliance. February 2000.
Corporate Award, North American Lake Management Society. November 1999.
Best Student Paper. North American Lake Management Society. November 1988.
Graduate Student Travel Award. North American Lake Management Society. 1988.
Arthur J. Schmitt Dissertation-Year Fellowship - 1988/1989. University of Notre Dame.
Best Student Paper. North American Lake Management Society. November 1987.
IBM Travel Fund for Women. University of Notre Dame. 1987.

Grants

Department of Natural Resources and Environmental Control, Tree Planting Grant, Upton Middle School, 2010
Heart of Cook. Education Grant. UpStream Project, Upton Middle School. May, 2007/2008/2010.
Berrien County Recycling Grant, Upton Middle School, December 2007/2010.
Florida Department of Environmental Protection: Research Grant. Joint project between PhycoTech and CyanoLab. Investigations in the biology and the ecological impact of *Cylindrospermopsis raciborskii* in Florida Lakes. September 2005.
National Science Foundation: Small Business Innovation Research Grant. 1997. Computerized Algal Identification System. Phase I.
National Science Foundation: Research Planning Grant. 1991. Role of the periphytic biolayer in mediating the transfer and transformation of PCB's within stream sediments.
National Science Foundation: Doctoral Dissertation Improvement Grant. 1988.
Alternate states of metalimnetic systems resulting from cascading trophic interactions. Sigma Xi. 1988. Variation in primary productivity and biomass accumulation in metalimnetic and epilimnetic algal populations.
Indiana Academy of Science. 1987. Species-specific growth and loss rates within metalimnetic algal populations.

Ann L. St. Amand, Ph.D., CLP

Presentations

St. Amand, A. and K. Wagner (Co-Chairs). 1991-Present. Algal Identification Workshop. Annual Meeting of the North American Lake Management Society.

Graham, J.L., KA Loftin, BH Rosen, A St Amand, 2010. Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste-and-Odor Studies in Lakes and Reservoirs, National Water Quality Monitoring Conference Workshop.

St. Amand, A; J.L. Graham, J. R. Jones, 2009 Occurrence of microcystin-producing Cyanophyta along a Midwestern United States nutrient gradient, Annual Meeting of the North American Lake Management Society.

St. Amand, A. 2007. Multiple Data Analysis Techniques and Challenges for Analyzing Phytoplankton Data from the National Lakes Survey, Summer 2007. 20th Annual National Conference Enhancing the States' Lake Management Programs: Interpreting Lake Quality Data for Diverse Audiences. April 24-27, 2007 Chicago, IL.

St. Amand, A. 2006. Occurrence and Toxicity of *Cylindrospermopsis* and other toxigenic blue-greens in the Midwest. Illinois Lake Management Association Annual Meeting. March, 2006.

St. Amand, A., Graham, J. and Jones, J. 2005. Co-Chairs. Special Session: Toxic Freshwater Cyanobacteria – Global Perspectives on North American Occurrence and Regulation. International Symposium of the North American Lake Management Society. November 9, 2005.

St. Amand, A., Dyle, J., Chapman, A., and Eilers, J. 2005. Efficacy of Molecular DNA Methods for Confirming Species Identifications on Morphologically Variable Populations of Toxin Producing *Anabaena* (Nostocales). Special Session: Toxic Freshwater Cyanobacteria – Global Perspectives on North American Occurrence and Regulation. Ann St. Amand, Co-Chair. International Symposium of the North American Lake Management Society. November 9, 2005.

Eilers, J.M. and St. Amand, A. 2005. Multiple Scenarios for Fisheries to Increase Potentially Toxin Producing Cyanobacteria Populations in Selected Oregon Lakes. EPA ISOC-HAB meetings, Raleigh, NC. September 7, 2005.

St. Amand, A., Hoover, R., Jermanb, G., and Rozanov, A. 2005. Morphology and Elemental Composition of Recent and Fossil Cyanobacteria. Proceedings of the Annual Symposium of the International Society for Optical Engineering, SPIE, July 31, 2005.

St. Amand, A. and K. Wagner. 2003 Water quality factors driving blue-green algal blooms in lakes with relatively low nutrient concentrations. Annual Meeting of the North American Lake Management Society.

St. Amand, A. 2003. *Cylindrospermopsis raciborskii* and its distribution changes across the US. Annual Meeting of the North American Lake Management Society.

St. Amand, A. and K. Wagner. 2003. Ecology and Control of Nuisance Algae workshop. Annual Meeting of the North American Lake Management Society.

Ann L. St. Amand, Ph.D., CLP

St. Amand, A. and J. M. Eilers. 2002. Blue-green akinetes from sediment cores as a tool for assessing water quality changes. Annual Meeting of the North American Lake Management Society.

St. Amand, A. 2002. *Cylindrospermopsis raciborskii*: Distribution changes over the last decade across the United States and Implications for Water Quality in the Midwest. Meetings of the Midwest Aquatic Plant Management Society.

St. Amand, A. 2001. *Cylindrospermopsis raciborski*: Distribution, Ecology and Implications for Drinking Water Supplies and Recreational Use. Indiana Department of Environmental Management, meeting of the Task Force on Toxic Blue-Green Algae.

Wang, H., St. Amand, A., and Gray, K.A. 1992. Role of the periphytic biolayer in mediating the transfer and transformation of PCB's within stream sediments. Hazardous Waste Conference, Center for Bioengineering and Pollution Control, University of Notre Dame.

St. Amand, A.L., P.A. Soranno, and S.R. Carpenter. 1989. Nutrient cycling dynamics associated with manipulations in fish communities. Annual Meeting of the International Association for Great Lakes Research.

St. Amand, A.L., and S.R. Carpenter. 1989. Species-specific responses to nutrient regeneration among metalimnetic and epilimnetic algae. Annual Meeting of the Ecological Society of America.

St. Amand, A.L., P.A. Soranno, and S.R. Carpenter. 1988. Nutrient deficiency indicators: Growth bioassays vs. physiological indicators for assessing ecosystem stress. Annual Meeting of the North American Lake Management Society.

St. Amand, A.L. and S.R. Carpenter. 1988. Metalimnetic and epilimnetic algae: Differences in nutrient limitation and grazing response when exposed to varying assemblages of zooplankton. 1988 Annual Meeting of the Ecological Society of America.

St. Amand, A.L. and S.R. Carpenter. 1987. Variable responses of *Anabaena circinalis* to grazing. Annual Meeting of the North American Lake Management Society.

St. Amand, A. and S.R. Carpenter. 1987. Metalimnetic algae: Species specific growth and loss rates. Annual Meeting of the American Society of Limnology and Oceanography.

Publications

St Amand, A.L. 2010. Chapter 7: Chlorophyta. AWWA Algal Manual. pp. 147-166.

Graham, J. L.; J.M. Jacoby; A St Amand. 2009. The NALMS Blue Green Initiative. *LakeLine*. 29(2): 12-15.

St. Amand AL, Roefer P, LaBounty JF, Tietjen T, Bolt D. 2009. Response of the algal community in Boulder Basin, Lake Mead to the introduction of Quagga Mussels and reduced water levels. *Journal of Lake Reservoir. Management*. *In review*

Ann L. St. Amand, Ph.D., CLP

Eilers, J. M.; Loomis, D.; Amand, A. St.: Vogel, A.; Jackson, L.; Kann, J.: Eilers, B.; Truemper, H.; Cornett, J.; Sweets, R. 2007. Biological effects of repeated fish introductions in a formerly fishless lake: Diamond Lake, Oregon, USA. *Fundamental and Applied Limnology/Archiv fur Hydrobiologie*, Volume 169, Number 4, pp. 265-277(13).

St. Amand, A., J. Dyle, M. Aubel, A. Chapman and J. Eilers. 2007. Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing *Anabaena* (Nostocales). *Lake and Reservoir Management*. 23(2):193 - 202

St. Amand, A. and A. Chapman. 2007. Using Plankton Data. *LakeLine*. 27 (1):34 - 40.

Holland, T. A. St. Amand, and G. Good. 2006. Otter Lake '05—A Successful Response. *LakeLine*. 26(2):52 – 56.

St. Amand, A., Hoover, R., Jermanb, G.,and Rozanov, A. 2005. Morphology and Elemental Composition of Recent and Fossil Cyanobacteria. Proceedings of the Annual Symposium of the International Society for Optical Engineering, SPIE, July 31, 2005. In Press.

St. Amand, A. & Wagner, K. (2004). Stalking slime: The value of monitoring your lake. *LakeLine*, 24 (1), 14-16.

Eilers, J. M., Kann, J., Cornett, J., Moser, K., & St. Amand, A. (2004). Paleolimnological evidence of change in a shallow, hypereutrophic lake: Upper Klamath Lake, Oregon USA. *Hydrobiologia*, 520, 7-18.

Eilers, J. M., & St. Amand, A. (2004). *Recent paleolimnology of Blue Lake, OR*. Report to the Oregon Department of Fish & Wildlife. Roseburg, Oregon.

Kostel J. A., Gray K. A., & St. Amand A. L. (2003). The impact of metal and organic contaminants on the structure of periphyton in lotic sediments: Observations at various scales. *International Journal of Sediment Research*, 19 (2), 227-235.

St. Amand, A. (2002). *Cylindrospermopsis*: An invasive toxic alga. *LakeLine*, 22 (1), 36-38.

Havens, K. E., Beaver, J. R., East, T. E., Rodusky, A. J., Sharfstein, B., St. Amand, A., & Steinman, A. D. (2001). Nutrient effects on producers and consumers in the littoral plankton and periphyton of a subtropical lake. *Archiv fur Hydrobiologie*, 152, 177-201.

Eilers, J. M., Beaver, J., St. Amand, A., & Cornett, J. (2001). *Historical changes of zooplankton and cyanobacteria in Diamond Lake, Oregon, based on analysis of the sediment record*. Report to the Oregon Department of Fish & Wildlife. Roseburg, Oregon.

Frost, T. M., Descy, J. P., DeStasio, B. T., Gerrish, G., Hood, J., Hurley, J. P., & St. Amand, A. L. (2000). Evaluations of phytoplankton communities using varied techniques: A multi-media comparison of lakes in Northern Wisconsin USA. *Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie*, 27, 1023-1030.

Ann L. St. Amand, Ph.D., CLP

Wang, H., Kostel, J. A., St. Amand, A. L., & Gray, K. A. (1999). The response of a laboratory stream system to PCB exposure: Study of periphytic and sediment accumulation patterns. *Water Research*, 33 (18), 3749-3761.

Kostel, J., Wang, H., St. Amand, A. L., & Gray, K. A. (1999). Use of a novel laboratory stream system to study the ecological impact of PCB's in a periphytic biolayer. *Water Research*, 33 (18), 3765-3748.

Cottingham, K. L., Carpenter, S. R., & St. Amand, A. L. (1998). Responses of epilimnetic phytoplankton to experimental nutrient enrichment in three small seepage lakes. *Journal of Plankton Research*, 20, 1889-1914.

St. Amand, A. (1995). Algae: Nature's artwork. *LakeLine*, 15 (3), 10-11.

St. Amand, A. L. (1994). A photographic key to the algae of the University of Notre Dame Environmental Research Center. University of Notre Dame. Ongoing. St. Amand, A. (1995). Algae: Nature's artwork. *LakeLine*, 15 (3), 10-11.

St. Amand, A., & Carpenter, S. R. (1993). Plankton vertical structure. Cascading Trophic Interactions. In S. R. Carpenter & J. F. Kitchell, (Eds.), *The Trophic Cascade in Lakes*. Cambridge, UK: Cambridge University Press.

Carpenter, S. R., Morrice, J., Elser, J. J., St. Amand, A., & MacKay, N. A. (1993). Phytoplankton community dynamics. Cascading Trophic Interactions. In S. R. Carpenter & J. F. Kitchell, (Eds.), *The Trophic Cascade in Lakes*. Cambridge, UK: Cambridge University Press.

Carpenter, S. R., Morrice, J., Soranno, P. A., Elser, J. J., MacKay, N. A., & St. Amand, A. (1993). Dynamics of primary production. Cascading Trophic Interactions. In S. R. Carpenter & J. F. Kitchell, (Eds.), *The Trophic Cascade in Lakes*. Cambridge, UK: Cambridge University Press.

St. Amand, A. L. (1990). Mechanisms controlling metalimnetic communities and the importance of metalimnetic phytoplankton to whole lake primary productivity. Ph.D. dissertation, University of Notre Dame.

St. Amand, A. L., Soranno, S. A., Carpenter, S. R., & Elser, J. J. (1989). Algal nutrient deficiency: Growth bioassays versus physiological indicators. *Lake Reservoir Management*, 5, 27-35.

Elser, J. J., Goff, N. C., MacKay, N. A., St. Amand, A. L., Elser, M. M., & Carpenter, S. R. (1987). Species-specific algal responses to zooplankton: Experimental and field observations in three nutrient-limited lakes. *Journal of Plankton Research*, 9, 699-717.

Ann L. St. Amand, Ph.D., CLP

Technical Skills

General

Experimental/Monitoring Design
Biostatistics

Taxonomic Skills

Phytoplankton (marine and freshwater)
Periphyton (marine and freshwater)
Core analysis for diatoms and akinetes
Macrophytes (familiarity)
Zooplankton
Benthic Macroinvertebrates (including chironomids)
Zebra/Quagga Mussel Veligers

Professional Societies/Journals

American Water Works Association
British Phycological Society
International Association of Diatom Research
International Phycological Society
International Society for Optical Engineering
Journal for Harmful Algae
North American Lake Management Society (National & Michigan Chapter)
Phycological Society of America

Offices

NALMS (Administrative Council 1992-1994), NALMS (Chapters' Chair 1995-1998, Region 5 Director, 2001-2004, Secretary, 2005-2006), Michigan NALMS (President 1999-2002, Secretary 1993-1996, 2002-2007), Chapter Representative to NALMS 1994 to present, Steering Committee WFB (1994-1996), MLSA (Science Advisory Committee 1999-2004), Moderator, Berrien County Science Olympiad, Water Quality Section (2001 to present), JTG Chair, Plankton Section, Standard Methods for the Examination of Waste Water and Water..

PhycoTech