Botany 330 - Algae - Syllabus 2017

Week	Date	Reading	Lab
1	Th, Sep. 7	Chapter 1 Introduction to the Algae	Care and use of microscopes; Project descriptions
2	Tu, 12	Chapter 2 Roles of Algae in Biogeochemistry	Use of Prescott Keys (exercise 3)
	Th, 14	Chapter 3 Algae in Biotic Associations	Quantification of phytoplankton: Use of Sedgewick/ Rafter cells and inverted microscope (exercise 1)
3	Tu, 19	Chapter 4 Technological Applications of Algae	Quantification of cultured algae: Use of hemacytometer and electronic particle counter (exercise 2)
	*W, 20		*Midterm Exam question 1
	Th, 21	Chapter 5 Algal Diversity and Relationships	Field trip
4	Tu, 26	Chapter 6 Cyanobacteria	Culture methods (exercise 5) *Project decision due
	Th, 28	Chapter 7 Endosymbiosis and the Diversification of Eukaryotic Algae	*Oral presentations
5	Tu,Oct. 3	Chapter 8 Euglenoids	Culture methods, continued
	Th, 5	Chapter 9 Cryptomonads	Cyanobacterial diversity
6	*M,9		*Midterm Exam drafts due
	Tu, 10	Chapter 10 Haptophytes	Cyanobacterial diversity
	Th, 12	Chapter 11 Dinoflagellates	*Oral presentations
7	Tu, 17	Chapter 12 Stramenopiles I	Flagellate diversity
	Th, 19	Chapter 13 Stramenopiles II	Flagellate diversity
8	*M, 23		*Midterm Exam due
	Tu, 24	Chapter 14 Stramenopiles III	Diatoms of Lake Wingra (exercise 4)
	Th, 26	Chapter 15 Red Algae	DNA extraction and review, project time
9	Tu, 31	Chapter 16 Green Algae I	Xanthophytes; freshwater red algae; prasinophycean green algae
	Th, Nov. 2	Chapter 17 Green Algae II	Brown, red, & green seaweeds
10	Tu, 7	Chapter 18 Green Algae III	Midterm Lab Exam: covers specimens from Oct 4 - Nov 2
	Th, 9	Chapter 19 Green Algae IV	Green algal diversity, cont. (Trebouxiophycean and ulvophycean cultures)
11	Tu, 14	Chapter 20 Green Algae V	Green algal diversity, continued (Chlorophyceans I)
	Th, 16	Chapter 21 Phytoplankton Ecology	Green algal diversity, continued (Chlorophyceans II)
12	Tu, 21		Green algal diversity, continued (Chlorophyceans III)
	Th, 23		(Thanksgiving)
13	Tu, 28	Chapter 22 Marcoalgal and Periphyton Ecology	Green algal diversity, continued (Streptophyte Algae)
	Th, 30		Green algal diversity, continued (Streptophyte Algae)
14	Tu, Dec. 5		Using the logistic equations
	Th, 7		Lab Exam; covers specimens from Nov 9 - 29
15	Tu, 12		*Oral Presentations, Project and Report due
	*F, 15		*Final Exam drafts due
	*Th, 21		*Final Exam due

BOTANY 330 — ALGAE
See course page in Learn@UW
Lab and Staff Schedule Information
Fall 2017

Instructors	Office	Phone/email	Office Hours
Dr. Linda Graham	211 Birge	262-2640 lkgraham@wisc.edu	during lab & by appointment
Marie Trest	137 Birge	262-7475 mttrest@wisc.edu	by appointment

Course Schedule

	MON	TUES	WED	THURS	FRI
8:50					
9:55					
11:00					
12:05					
1:20		Lab-210 Birge		Lab-210 Birge	
2:25		Lab		Lab	
3:30					
4:35					

The lab will be available for independent work outside of regular class time.
Birge Hall hours are M-F 7 AM – 5 PM.

Botany 330

Course Information--Books and Grading

We aim to provide a basic background of information and training that should serve the needs of course clients in general. In addition, we will provide opportunities for specialized learning in accordance with individual student interest. Please let instructors know if accommodation to individual learning style is needed.

Textbooks:

1) Algae 3rd edition 2016—Graham, Graham, Wilcox, and Cook. The digital text is essential for completing Comm. B writing assignments, and is also meant to be used in the lab. You will purchase the digital text at http://www.ljlmpress.com/algae.html, and bring the text to class on your mobile device. Laptops and tablets work better than phones for enlarging images.

2) How to Know the Freshwater Algae - Prescott

This is the taxonomic key that we will use in the lab for algal identifications to genus. We have enough copies for each two persons to use, but these books cannot be removed from the classroom. You may want to purchase your own copy.

Exams and other Course Requirements:

To substitute for lectures, students will each individually complete 12 worksheets from 13 offered (we will delete the lowest score, or one can be missed without penalty). Worksheets must be completed by the deadlines specified. Each completed worksheet will earn up to 50 points, for a total of 600 points. Because these worksheets will be peer-graded (though instructor-checked), there can be no make-ups (much as lectures would not be made-up). Your digital textbook is the only source of information for completing worksheets; other sources are not considered suitable in terms of currency or accuracy.

Lecture exams--There will be two exams on lecture material--a mid-term and a final exam. Both exams will consist of 5 or 6, 2-4 page essays, and will be take-home exams to be completed without collaboration.

Undergraduates will answer 5 questions from a number of choices. Graduate students will be required to answer one additional question. Please consult the course document “Botany 330 – Editorial Issues” before you begin writing. Pre-grading of exams is required. For pre-grading, receipt of a preliminary draft will be required at specified times prior to final submission deadline. Preliminary drafts will be examined by Dr. Graham, who will provide suggestions for improvement of both scientific content and English expression, if needed, then returned for revision a week prior to the exam due date. Each lecture exam will be worth 100 points. Additional information is available on the exams posted at the course website.

Lab exams--Two 50 point mid-term lab exams will evaluate ability to identify on sight algal genera that were presented in lab, without the use of an identification key. Twenty or so stations will be set up in the lab; these will most often consist of a microscope with specimen on a slide, but could also consist of fossils, macroalgae or herbarium sheets. Stations will not be timed. In addition, students will be asked to use the Prescott key to identify two "unknown" microalgae. Plenty of time is available for completion of both parts of the lab exams.

Lab exercises--Five brief written reports related to class lab or field exercises will be worth 40 points each for a total of 200 points. These will be evaluated for both scientific content and English expression. An additional week beyond the first due date will be given to revise reports, if this is recommended.

Lab notebooks—Each student will keep a lab notebook of a form specified in class. Notebook content will be evaluated early in the semester, with recommendation for improvement. Notebooks will be graded at the end of the semester: 50 points total.

Project reports--A written report describing the results of individual or team field, lab, or library projects will be due at the final exam. Projects will be chosen in consultation with instructors. It is highly desirable for projects to coordinate with other work that students are doing (in research or other coursework). It is fine to submit the same report in Botany 330 and in a concurrent course, if there is substantial algal content, and with instructor approval. Examples of previous reports will be available as models. During the final lab period students will be expected to present a brief oral report (10 min or so) on their project (to

be considered as part of the project grade). Pre-grading of final project reports is available and we encourage you to take advantage of this option. Drafts for pre-grading are due at least one week prior to the final exam date, and will be returned for revision no less than three days prior to the final exam date. The project report is due at the time and date of the final exam.

Other oral presentations--At least two additional oral presentations will be required and written feedback will be provided (though not graded). Please refer to the course document “Oral presentations in Botany 330”.

Grading:

Lecture exams (2) 200 points (100 each)

Midterm lab exams (2) 100 points

Worksheets (13, drop lowest score) 600 points (50 each)

5 exercise reports 200 points (40 points each)

Lab notebook 50 points

Project report 100 points

_____

1250 points

There is no limit to the number of A, B, etc. grades given. 92-100% =A; 88-91% = AB; 80-87% = B

Regardless of point totals, because Botany 330 is a Comm. B course, all Comm. B-required components (essay exams, lab reports, and individual project presentations) must be submitted in order to achieve a passing grade.

Botany 330 essays—Editorial issues

The assigned essays were designed to mirror the characteristics of written reports that you might provide to peers in a future professional situation. So the comments I've made on your drafts are related to writing for a professional reader. For each essay, I've commented on content, length, English usage, and organization. The following numbers refer to common issues.

1. Avoid the imprecise pronouns "it" or "they," especially at the beginning of a sentence, unless the previous sentence is quite clear about the identity of the subject.

2. Aim for concise language. You will often be required to express your thoughts in a limited number of words; writing the abstract for an NSF grant proposal would be an example. So don't use superfluous words when you could use the space to instead propose even more great ideas. Evaluate the need for each and every word. Try to construct the most concise language possible.

3. Aim for precise language. Avoid imprecise words like "quite," "very," "huge." Use biological terms (your peers will likely have taken a college bio course), for precision and economy of expression. One precise term can take the place of a long string of words (see #2 above).

4. Closely focus your writing on the question or issue at hand. Don't add unnecessary material even if you think it will make you appear knowledgeable. In a professional context, your reader will not appreciate having to read through a lot of introductory material that he/she already knows, or being led away from the main topic.

5. Avoid constructing complex sentences having more than one major point.

6. Don't mix multiple topics in the same paragraph. Start each paragraph with a topic sentence that alerts the reader to the paragraph's subject and stick to that subject.

7. Define critical terms–those forming the main topic of a paragraph–especially if the terms will not be widely understood by your readers.

8. Construct transitional sentences at the ends of paragraphs to link to the next.

9. Use proper singular and plural forms of the words important to our field: bacterium, bacteria (there is no such word as bacterias!); alga, algae (no such word as algaes!); genus, genera (no such word as generas!); mitochondrion, mitochondria. Use the adjectival form of these words when appropriate: bacterial decomposition, algal ecology, generic name, mitochondrial genome.

10. Match singular subjects with singular verbs, and match plural subjects with plural verbs.

11. “Phosphorus” is an unusual word. As the result of its origin, the most appropriate use for the spelling “phosphorus” is as a noun, and the most appropriate use of the spelling “phosphorous” is as an adjective. Examples: Photosynthetic organisms require phosphorus for producing NADP, as well as ATP and DNA. Phosphorous concentrations can be determined by colorimetric methods.

12. Cite references within the text of your writing.

13. Avoid overly colloquial, informal language. In writing for a professional audience, use professional expressions, with few exceptions.

14. Use your word processor’s spell-checker and grammar checker!

15. Try to use active voice more often than passive voice. Passive voice is more acceptable in professional scientific writing than in general writing, however.

16. “It’s” is a contraction and means only “it is.” “Its” (without an apostrophe) is a possessive; do not use “it’s” as a possessive. Examples of correct use: The heterokont cell uses its longer, hairy flagellum for propulsion. It’s not possible to see the hairs on this flagellum without using an electron microscope.

17. “Affect” is a verb (not used as a noun). In contrast, “effect” is mostly used as a noun, though sometimes also as a verb meaning “cause or produce” as in “to effect a change.” Example of appropriate uses: The concentration of carbon dioxide in the water will affect phytoplankton photosynthetic rate. The hurricane had a devastating effect on coastal wetlands.

18. The appropriate use of “which” and “that” is a common problem. I recommend checking a reliable usage reference. “Which” typically follows a comma, while “that” does not.

19. Despite the fact that many scientists conflate the terms “theory” and “hypothesis,” these terms are not synonyms. An hypothesis is a tentative explanation (an educated guess) that requires testing before it can be accepted. A scientific theory is a broad explanation that is widely accepted among qualified scientists because it is strongly supported by experiment and/or observation. For example, the Cell Theory (the cell is the basic unit of living things and arises from preexisting cells) is not a hypothesis. It is important not to mistakenly give your reader the impression that an explanation is widely accepted when it is not by describing an untested hypothesis as a “theory.”

20. The expression ‘et al.’ means “and others;” al. is short for “alia.” It is therefore important to place a period after ‘al.’(but not after ‘et’).

21. When forming a sentence with several clauses, ensure that they are of the same form. For example: Algae are defined as organisms that are generally photosynthetic, primarily live in aquatic habitats, and are structurally and reproductively simpler than land plants. Test such complex sentences by considering each part independently to see if it makes sense: algae are organisms that are generally photosynthetic; algae are organisms that primarily live in aquatic habitats; algae are organisms that are structurally and reproductively simpler than land plants.

22. Be sure to use the terms "population" and "community" appropriately; these terms are not synonyms. Check the definitions in your general biology/botany texts if necessary.

23. In evolutionary explanations, use the language of selection and adaptation. For example, the sentence "Diatoms produce chitin fibrils *in order to* stay afloat in well-lit surface waters" is not quite accurate because individual diatoms can't consciously choose to produce the fibrils or not. "Chitin fibrils are adaptations that allow diatoms to remain afloat in well-lit surface waters" is more accurate because this expression uses the language of selection and adaptation. An ancestral diatom acquired a mutation that allowed it to produce chitin fibrils, which aided photosynthesis, thus fostering reproduction and persistence of the mutation in the population.

Oral Presentations in Botany 330.

Because this is a Comm. B course, oral presentations are part of the requirements. Here are some recommendations based on previous Botany 330 class presentations.

Standing vs sitting presentation position. In some professional settings it may be appropriate to remain seated during your presentation. One example would be if everyone were sitting around a table; in this case each speaker could be easily seen. However, in our classroom some seated speakers would be hidden from view by others.

In the past, I have noticed that everyone watched speakers who stood at the front of the room, perhaps because we were conscious that the speaker would notice if we didn't seem to be paying attention. In contrast, when speakers remained seated, we didn't all watch the speaker. This could give the speaker the impression that the audience isn't paying attention.

Even in situations where everyone is sitting around a table, you might want to consider standing for a presentation. This gives you more professional authority and may be especially helpful for people who don't have commanding voices. As a listener, when you can't see a speaker, make an effort to shift positions a bit so that you can make eye contact.

Visual aids. Presenting technical material in a professional setting often involves using unfamiliar terminology, as is the case for our presentations. If terms are unfamiliar to you, you can assume that at least some of your professional colleagues having similar backgrounds also will be unfamiliar with the terms. For this reason, it is always useful to write key terms or concepts in some way that everyone can see them. Further, some key terms may need to be defined in written form. This will aid listeners who are taking notes from your presentation. Even those who don't take notes will understand and remember what you said better if you write down key terms and ideas. (If you don't know how to pronounce a term or a person's name, find out how ahead of time.)

In our case, the chalkboard, signs, handouts, or overhead projector transparency are useful low-tech visual aid options. These methods have the advantage that they are useful even when high technology presentation is not possible or fails. Though you may use Powerpoint and the digital projector for your final oral presentation, the major advantage of projection is that it allows people sitting far away from a speaker in a large room to see the visual aids. (I use it mainly because the TA usually needs to fill the chalkboard with lab instructions prior to lecture time, and because the powerpoints are useful to students who've had to miss class.)

I usually have backup low-tech visual aids in case of technology failure. At scientific conferences and other settings, I have noticed that engineers and NASA employees tend to favor overhead transparencies (which can be printed in color from powerpoint files). (Do they know more about frequency of technology failure?) Overhead projectors are

low-cost, versatile, and widely available. For these and other reasons, overhead transparencies are my choice of backup visual aid. I once had to give an after-dinner presentation to hundreds of people in a hotel ballroom that turned out to lack facilities for digital projection. Fortunately, I had brought along overhead transparencies of my Powerpoint slides, which saved the day (for me, at least).

Capturing and keeping listener attention. People will pay closer attention to your presentation if you make eye contact with your audience and show enthusiasm for your subject. (Humans seem preadapted—maybe from watching for predators—to pay attention to moving objects.) But try not to go overboard with the body motion, unless you have designed a theatrical presentation, our next topic.

Under some professional circumstances, and our class is included, it is appropriate to use skits, songs, comedy or other theatrical means to transmit information in an entertaining way. You can be sure that your audience will remember your message! However, prior to designing a theatrical presentation obtain your supervisor's approval! Inappropriate language or dress will not be welcome in most science professional settings.

Opening and final words. I recommend starting your presentations with a powerful opening and/or closing statement. Short, succinct opening and closing statements can really attract listener attention and aid memory.

ALGAE - BOTANY 330

GENERAL LABORATORY PROCEDURES

As you come to lab, check the board for the day's agenda, and instructions. There will generally be a list of samples to study, or class exercises and demonstrations. Sometimes there may be short field expeditions. When these are finished, the time remaining is available for individual projects. Instructors will be present during scheduled lab time to assist you with projects. You are welcome to work in the lab outside of regularly scheduled classtime, but except for scheduled appointments, your instructors may not be available to help you during unscheduled times.

Studying microalgal samples: Algal samples may be provided as unialgal cultures (no other algae present), or as mixtures. In the latter case, we will put images of the target alga on the TV screen so that you know which form you are supposed to find and study. Unialgal cultures may occur in liquid media or on the surface of agar in tubes (instructions for sampling these are given below). First, make a slide of one of the specimens; organisms can be observed in any order. Then look it up in the text to read about salient features. Observe the features mentioned in the text and draw the algae for inclusion in lab notebooks. Drawing helps to focus attention upon distinguishing features, and can be a useful aid in studying for the mid-term lab exam. Feel free to ask for instructor help in adjusting microscopes, finding algae, or finding distinguishing characteristics of algal taxa.

Second, pretend that you do not know the name of the organism at hand, then try to key it out using the Prescott key, and see if you get the right answer! If not, backtrack from the correct genus in the key to see where you made the wrong choice(s). Feel free to request instructor assistance after you have given it a good try, but before frustration sets in. In this way you will gradually learn to use the identification key for unknown forms. For each organism presented in lab, indicate the list of key choices made in your lab notebook. The lab exams will test your ability to identify unknowns.

In order to avoid mass confusion, make special efforts to avoid mixing algae from separate cultures. Do not use the same pipette for more than one sample.

If samples are in liquid, use a pipette to remove just a little algae to a slide, and cover with a cover slip. Remove excess liquid with a tissue, so that the cover slip is not floating. If cultures are on agar, heat a loop to glowing cherry red in the flame of an alcohol lamp. Let the loop cool, then retrieve a little algal material and place in a drop of water on a slide, adding cover slip. You may need to tease masses of algae apart with dissecting needles or forceps before adding the cover slip to avoid making preparations that are too thick to see through. Reagents such as ink, nickel sulfate or iodine are usually added before putting the cover slip on, though they may be drawn through a preparation by using a tissue applied to the side of the cover slip opposite to the point at which reagent was added.

Reuse slides--wash with water and dry with a tissue before storing in your drawer. But don't reuse cover slips--they are difficult to clean sufficiently, and may break, causing injury. Dispose of cover slips, pipettes, and other disposable glass in the "glass trash box," not the regular trash.

Preparing for lab exams: A list of genera for which you will be responsible will be provided at least one week prior to the lab exams. In the meantime, assume that you are responsible for all of the genera presented in lab. It is not advisable to rely upon web images to study for the lab exam because these are often different species and sometimes misidentified. You are welcome to come to lab outside of regularly scheduled class hours (no other class uses the room). You will be informed of the location of a key to the lab (but be careful to return the key, and to lock up the room before you leave the lab).

Things to remember:

1) Bring your copies of Graham, Graham, Wilcox and Cook, and Prescott to lab each time, or leave them in your cabinet.

2) Treat microscopes well. Rotate microscope objectives to low power before removing slides!!! When changing objectives, turn the turret, not the lens barrels!! Use fine adjustment with high power objective lenses!!! Learn to adjust microscopes for best images at differing magnifications--it makes a lot of difference! Clean lenses with lens tissue (not lab tissues) at frequent intervals.

3) Make an effort to avoid mixing samples. Fresh pipettes are cheaper than isolated cultures!

SUMMARY OF WRITTEN LAB EXERCISE REPORTS

These exercises were designed to provide 1) instruction in some important techniques useful in studying algae (and other organisms), 2) experience in technical writing, 3) and numeracy practice. You might encounter similar assignments in your future occupation if you are asked to prepare technical reports or grant proposals. Due dates to be announced.

Exercise 1. Use of the Sedgewick-Rafter Cell and Inverted Microscope/Settling Chamber (Utermöhl) Techniques for Counting Algae in Mixed Samples.

Follow the instructions in I A and I B of "Quantitative Determination of Algal Density and Growth" (with modifications and additional advice supplied in class) to count the algae in the mixed assemblage provided, by two methods. You will have to take turns using the two inverted microscopes available. You can work in pairs, sharing the counting effort, and reporting results as a team.

Turn in a written statement of at least a page in length, with counts, and a statement of your perception of the pros/cons of using the Sedgewick-Rafter vs the inverted microscope method for counting algae in mixed assemblages. 40 points.

Exercise 2. Comparison of the Use of the Hemacytometer vs Coulter Counter Method for Counting Algal Cells in Unialgal Cultures.

Use the instructions in II B in "Quantitative Determination of Algal Density and Growth" to make a hemacytometer count of the algae in a cultured sample that we will provide. You will have to take turns, because we do not have enough chambers for everyone to do this exercise at the same time. Be sure to mix the sample well before removing an aliquot! Do at least 5 replicate counts.

Now, with the aid of the instructor, count the same sample, on the same day, with the Coulter Counter--the whole class can use the same 5 replicate counts. Pairs of students may share the counting efforts and preparation of the report. Turn in a statement at least one page in length of the comparative counts (are they significantly different?), and your perception of the pros/cons and potential sources of error for the two methods. 40 points.

Exercise 3. Algal Collections from Lake Mendota and another WI source

Turn in 5 separate sheets of drawing paper for each collection, each with a single large (i.e. fill the whole page) drawing of an identified algal genus from samples provided from Lake Mendota and another body of water. Instructors will help with identifications, on request. Label distinguishing characteristics such as chloroplast(s), flagella, trichocysts, mucilaginous sheaths, or heterocytes. The point of this exercise is to foster observational powers and an appreciation of diversity differences in eutrophic and oligotrophic waterbodies. Put your name on each sheet and staple the sheets together. Compare the two samples. Write at least half a page on your impressions of diversity in the two bodies of water. Were any of the genera you found in other collections also present in L. Mendota and vice versa? 40 points.

Exercise 4. Diatoms of Lake Wingra.

Read "Sample Preparation, Methods, and Literature for Diatoms." The most effective method for assaying many diatoms from natural collections requires cleaning, because distinctive frustule markings are obscured by cell contents (chloroplast, etc.). The most effective methods for making permanent mounts of diatoms require use of strong acids, which we prefer not to attempt in this course, because of safety considerations. Therefore, we have assembled a collection of permanent slides made from L. Wingra collection for your use.

First, examine a fresh collection of L. Wingra diatoms (mostly members of the periphyton associated with water milfoil). Make a list of the genera that you can confidently identify on the basis of cell or colony shape, or presence of stalks (as with *Cymbella*.). Use the Prescott key to start. Consult other references as needed.

Now, examine the prepared slides, and make a list of all the species that you can confidently identify on the basis of frustule shape or ornamentation. You will want to use the notebooks of photos of identified L. Wingra diatoms. The photos were made from the same cleaned preparations that you are using.

This exercise should be done individually. Turn in the two lists, together with a brief statement of a page or so comparing the lists and explaining why they might be different. 40 points.

Exercise 5. Algal Isolation & Culture Techniques.

Read "Isolation and Culture of Algae," and watch demonstrations for: 1) pulling micropipettes, 2) spraying plates, 3) streaking plates, 4) single cell/colony/filament isolation, 5) making algal culture media & use of the autoclave, & 6) function of the algal growth room/chambers. Make sure that you have heard all 6 demonstrations.

Now, from a field collection recommended by an instructor, use the media provided to make several single-alga isolations. You can choose to isolate desmids, chrysophytes, cryptomonads, diatoms, or blue-greens. You will need to make a supply of micropipettes. These don't need to be autoclaved because the heat of pulling them sterilizes them, but use a separate pipette for each organism. Also try the spraying technique. You will need to check your isolates for growth and contamination at various points in the semester; you may need to subculture isolates.

Turn in at least one unialgal culture, identified to genus, with information on origin, date isolated, and isolator (you) written on the tube/dish with Sharpie. Each individual should turn in at least one culture. The culture should be accompanied by a single page description of the isolation process and interesting aspects of the organism. 40 points.

PRESERVATION OF ALGAL SAMPLES

For the purposes of this course, we recommend that you store algal collections in the glass-door refrigerator in the teaching lab as soon as possible after sampling, in lieu of adding chemical preservatives. We have found that most algal forms will survive long enough to complete projects within the scope of a semester, and you will not be exposed to toxic preservatives. If large zooplankton are present, it is a good idea to filter them out so they won't eat all the algae before you get a chance to see them. Cheesecloth works well to filter zooplankton. The rest of this document provides preservation information that you may find useful outside this class.

Storage periods require addition of chemicals that reduce bacterial activity and autolysis of algal cells. The major preservatives in use today (formalin, Lugol's solution, and glutaraldehyde) are discussed in Wetzel & Likens (1990), Limnological Methods, Springer-Verlag, on page 140. The discussion below is based on our personal experience in use of these preservatives.

The single best preservative, in terms of cell structure retention, is buffered glutaraldehyde. Wetzel & Likens recommend use of 3% glutaraldehyde, neutralized to pH 7 with NaOH, and suggest that the glutaraldehyde should be filtered to remove particles. Glutaraldehyde may be purchased in various grades--if you use high grade 70% glutaraldehyde in ampules, which is designed for use in ultrastructure studies, there will be little problem with particles. It is not desirable to filter glutaraldehyde because it is highly irritating to mucous membranes. Filtration (if done) should occur in a chemical hood. Also, 3% glutaraldehyde is too high in concentration for retention of normal cell shape of wall-less flagellates, which may shrivel or disintegrate completely (0.5% or less is better for these forms). We recommend that you try to match the pH and osmolality of the system sampled by use of appropriate buffer. When you are ready to count/identify algae that have been preserved in glutaraldehyde, you should wash the glutaraldehyde off with use of a micropore filter apparatus and buffer wash, if you are using a filter-based method for counting/identification. If you are using a settling chamber, merely cover the chamber with glass so that you are not exposed to glutaraldehyde fumes.

Many published sampling protocols make use of Lugol's solution. You add this preservative in amounts to achieve a 1% final concentration (1 part per 100). To make Lugol's, dissolve 10g I2 (pure iodine, which is toxic) and 20g KI in 200 ml distilled water along with 20 ml concentrated glacial acetic acid. Solution should be stored in the dark, preferably in a glass bottle with ground glass stopper. The iodine in Lugol's is effectively bacteriostatic, but it causes a number of changes in algal cells. For example, iodine will bind with starch to form a blue-black complex. This reaction is useful in identifying starch, which is present in some algal groups, but not in others. On the other hand, green algal cells often accumulate so much starch that treatment with Lugol's renders the whole chloroplast or whole cell so dark in color that identification is difficult or impossible. Moreover, in our experience, Lugol's solution does not preserve cell structure well in many cases, making identification difficult. We prefer to preserve in glutaraldehyde, and use Lugol's as a reagent to identify starch when necessary. Though formalin is often used to preserve certain marine algae, we do not recommend the use of formalin to preserve freshwater algae because cellular features required for identification are often not retained.

Quantitative Determinations of Algal Density and Growth

There are four major techniques for counting algae. Two of these are used when counting algae in mixtures, as from field sampling or competition experiments, while the other two are used when counting unialgal samples, such as in growth or bioassay experiments. In each technique pair, one is "high-tech," requiring an expensive instrument, whereas the other is relatively "low-tech" and could be used in remote locations.

I. Counting algae in mixed assemblages.

A. "Low-tech" method--Sedgewick-Rafter chamber. This chamber is constructed from a thin brass plate from which a precise internal area has been cut; the plate is epoxied to a glass slide. Large, rectangular glass cover slips form the top of the chamber. The chamber's dimensions are 50X20X1 mm, with an area of 1000 mm^2, and a volume of 1.0 ml. We have found that the chambers may have a greater depth than 1 mm with the layer of epoxy and hold a greater volume than 1.0 ml; therefore, we will measure the sample added to the chamber.

To fill the Sedgewick-Rafter chamber, place the cover slip on top of the chamber, but at an oblique angle, so that the chamber is only partially covered. Watch the instructor's demonstration.

Use a pipette to measure 1.2 ml of well-mixed sample and fill the chamber, then gently nudge the cover slip so that it covers the chamber completely. The cover slip should not float, nor should there be any air bubbles; the former results from over-filling, the latter from under-filling the chamber. Now, let the sample settle for at least 15 minutes before beginning to count algae (the time can be used for calibrating the counting grid--see next paragraph).

Counts are done with the 4X or the 10X objectives of the compound microscope (depth of field and lens length preclude the use of higher magnification objectives). A Whipple disk is inserted into one of the ocular lenses in order to provide a sample grid. It is necessary to first determine the area (A) of the Whipple field for each set of ocular and objective lenses used. This is accomplished with a stage micrometer. At 4X the width of the grid field is _____mm; the length is _____mm. The area (A) is thus _____mm.

Now place the Sedgewick-Rafter chamber on the microscope stage. Observe 10 randomly-chosen fields, counting every alga within the boundaries of the Whipple grid field and every other alga lying on a boundary line. The average number of cells is N. The density of algae (D = cells/ml) in the original sample is calculated:

$$D = \frac{N \times A_{\text{Sedgewick-Rafter}}/A_{\text{Whipple}}}{\text{Volume}}$$

Find the average value of D for each alga in the sample provided to you.

For maximum statistical accuracy with the least time expenditure, it has been recommended to count 2 Whipple fields in each of 12 chambers (Woelkerling, et al., 1974. Hydrobiologia 48: 95-107). However, in our case we have streamlined the process due to time constraints.

Rinse out your chamber, then refill it with the mixed field sample provided. Do not attempt to count the algae, but note the difficulties involved in trying to accurately identify small cells (since you can't use the 40X objective lens). One strategy for doing this might be to do a preliminary survey of the community from a regular slide, identifying the major taxa (using the 40X or 100X lenses if necessary). Counts could then be done, since you now have a mental catalogue of the small forms.

I B. "High-tech"--Inverted microscope method. The expensive component here is the inverted microscope, whose great advantage is that settling chamber depth does not preclude use of high magnification objective lenses. Special "Utermöhl" chambers can be purchased, but they are expensive. Chambers can be constructed from large cover slips, plastic bottle caps, and plastic syringe barrels. If a long focal-length lens is available, chambers may be constructed by gluing syringe barrels to glass slides. A measured volume of sample is added to the chamber and allowed to settle for at least an hour. Time periods as long as 24 hours are preferred, especially if small algae are present in the sample (these will settle only very slowly). A total of 20 random Whipple field should be counted (follow procedures described above for determining the area (A) of the Whipple field for the magnification used, usually 400X). For counting procedures that can be used to achieve increased accuracy, see Sandgren and Robinson (1984), Br. J. Phycol. 19: 67-72.

II. Counting unialgal samples (unicells, small colonies, or relatively short filaments).

A. "Low-tech" method--hemacytometer. The hematocytometer was developed for counting cells in blood samples (now this is mostly done with electronic paticle counters). There are two delicate, mirrored surfaces that must not be scratched. There are special thick cover slips in the box with hemacytometer; do not throw these away; do not use a regular cover slip with the hemacytometer. Make sure that the mirrored surfaces and special cover slip are clean by wiping them gently with a lens tissue (NOT LAB TISSUES) moistened with water. Now, place the cover slip on top of the two mirrored surfaces, and use a pipette to introduce a drop of well-mixed algal suspension beneath the cover slip. The entire mirrored surface area should be wet, but liquid should not spill into the adjacent channels. Allow to settle at least 15 minutes.

Each mirrored surface has a grid etched upon the surface. Each grid is composed on 9 squares, each 1 mm along the sides. These 9 squares are further subdivided into small

areas. See the magnified, printed version posted on the bulletin board. The chamber is 0.1 mm deep. Each 9 mm^2 grid holds exactly 0.0009 ml of sample. You have the choice of counting the algae in the entire grid; counting algae in only one of the 9 squares, then multiplying by 9; or counting algae in an even smaller area, and multiplying accordingly. Counts of about 30 cells per unit area are desirable for accuracy. If there are more than 30 cells per unit, you will find it hard to keep track of whether you counted them or not. In this case, dilute the sample, or count algae in a smaller-sized unit and multiply. Repeat the process ten times and determine a mean. If the mean number of cells in the whole grid area were 3×10^3, then the density of cells in the original sample would be 3.3×10^5 cells/ml.

B. "High-tech" method--electronic particle counter (e.g. Coulter counter). In spite of relatively high cost, an electronic particle counter is highly recommended for doing growth or bioassay studies that require many counts and high accuracy. In addition, the instrument will provide particle size/biovolume distributions. The principle of operation is that particles, suspended in an electrolyte solution, are sized and counted by passing them though an aperture having a particular path of current flow for a given length of time. As the particles displace an equal volume of electrolyte in the aperture, they place resistance in the path of the current, resulting in current and voltage changes. The magnitude of the change is directly proportional to the volume of the particle; the number of changes per unit time is proportional to the number of particles in the sample. When opened, the stopcock introduces vacuum into the system, draws sample through the aperture, and unbalances the mercury in the manometer. The mercury flows past the "start" contact and resets the counter to zero. When the stopcock is closed, the mercury starts to return to its balanced position and draws sample through the aperture. As the column of mercury flows past the "stop" position, it initiates electronic counting and when it passes the "stop" contact, the count ceases. The distance traveled by the mercury column can be calibrated to provide a reproducible sample volume.

The electrolyte used is 1% NaCl which has been filtered to remove particulates. Samples are dispersed into electrolyte in special sample beakers. The dilution factor is determined experimentally, and is designed to yield the best distribution.

The control settings are to be done only by an expert. You should not change the threshold controls, amplification settings, aperture/current setting, bandwidth selector, separate/locked switch, gain control, vacuum control regulator, stirring motor rheostat, or manometer without seeking the assistance of an expert operator.

Place the sample to be analyzed on the platform of the sample stand. Open the control stopcock and watch the readout return to zero and the pattern appear on the oscilloscope screen. The unit will count all particles above the size determined with the threshold dial, then display the count on the numeric readout. Write the count down.

Variously-sized aperture tubes are available for use in counting variously-sized particles; the aperture size is chosen to match that of particles. Aperture tubes are quite fragile and expensive. They should be handled only by an expert operator. In using an aperture of 50 um of less to count very small particles, extreme care must be taken to reduce background counts. Electrolyte should be filtered at least 3 times. When large particles are to be counted, care must be taken to reduce error due to settling.

III. Growth rate and generation time determinations.

In comparing the results of bioassay experiments, or growth of various algae under the same or varying conditions, it is necessary to determine growth rates experimentally. Cell density data are usually obtained with a hemacytometer or electronic particle counter.

Growth curves are prepared from data obtained by sampling cultures at intervals, such as once per day, depending upon the growth rate of the alga. Plots of number of cells against time (in days) can be made, and from these curves can be calculated specific growth rate or growth constant (u) and division or generation time (tg).

A typical growth curve will show a lag phase, an exponential or log phase, and a stationary or plateau phase, where increase in density has leveled off. In the stationary phase, growth is likely limited by resources such as light or nutrients. Growth rate (u) is calculated with the following equation:

$$u = \frac{\ln X2 - \ln X1}{t2 - t1}$$

where X1 and X2 are densities at times t1 and t2.

Division time (tg) is calculated with the following equation (Guillard, 1973):

$$tg = 0.6931/u$$

Useful references:

Schoen, S. 1988. Cell counting. In, Lobban, et al. (eds), Experimental Phycology. A Laboratory Manual. Cambridge University Press, Cambridge, pp. 16-22.

Guillard, R.R.L. 1973. Division rates. In, Stein (ed), Handbook of Phycological Methods, V. 1, Cambridge University Press, Cambridge, pp. 289-312.

Periphyton Analysis

Periphyton species diversity and numbers are usually assessed by one of two approaches: 1) scraping, peeling, or using a sticky material to remove algae from a determined area of natural substrate (rocks or plant surfaces) and examining the scrapings microscopically and for chlorophyll or other pigment content, or 2) by placing artifical substrates in the water for later retrieval, followed by taxonomic and chlorophyll analyses. Commonly used artificial substrates include unglazed clay tiles and glass slides. Slides are not thought to represent natural substrates as well as tiles, but they don't need to be scraped (which can damage the algae and disrupt community structure) in order to examine them by light microscopy. Non-transparent surfaces can be studied by SEM (which, however, is expensive).

I like to use glass slides because they can easily be mounted in slide holders and anchored at various depths in the water. One can also observe ecologically-significant internal features of the algae, such as parasites or endosymbionts, and see algae at the bottom of a stack or aggregation of cells. Upon retrieval, slides bearing algae can be transported back to the lab in 1% glutaraldehyde; this prevents decomposition or other changes. Back at the lab, slides are rinsed to remove sand and sediment, and algae removed from one side by wiping with a paper towel. Then the algae are dehydrated by submerging slides in increasing concentrations of ethanol for 15 minutes each, and stained with fast green dissolved in 95% ethanol. Rinsing with 100% ethanol removes excess stain and completes the dehydration process. Slides should then be submersed briefly in xylenes in a fume hood, whereupon they are removed and a drop of permount added to the algae-coated surface. A large cover slip is added at an angle to avoid bubble formation, then the slide is left to dry on a slide drier. Addition of a lead weight helps to flatten the preparation. When the permount is hard, excess permount is removed and slides are ready to label, store, and examine by LM.

Percent cover of species can be estimated in small quadrats marked on the cover slip, or you can use an ocular grid (Whipple disk) to define areas for analysis. Biovolume can be estimated for various species using data provided in Hillebrand, et al. (1999)-- Biovolume calculation for pelagic and benthic microalgae, Journal of Phycology 35:403-424. Algae that were dead when collected can be recognized by lack of evidence of cytoplasmic contents, which normally stain well with fast green. This stain also aids in recognition of taxonomically important features such as chloroplast shape and presence of pyrenoids.

If SEM examination is required, the slides or other substrates are treated as above except that they are not stained with fast green. Ethanol-submerged substrates are then critical-point dried and gold-coated prior to examination with the SEM.

Chlorophyll may be extracted from slides or samples scraped from substrates; Dimethyl sulfoxide (DMSO) is the best solvent for use with green algae, and is as good a solvent as 90% acetone for diatoms and cyanobacteria (Shoaf and Lium, 1976, Oceanogr. & Limnol. pp 927). Algae are filtered onto a 0.45 micrometer membrane filter that is soluble in DMSO and the filter ground in 3-4 ml of DMSO for 3 minutes at room temperature. After grinding, an equal volume of 90% acetone is added, mixed, then centrifuged for 10 minutes at about 5,000g. Absorbance of the supernatant is determined at appropriate wavelengths, 663 nm for chla and 645 nm for chl b. Equations used for acetone extractions are also appropriate for DMSO. Although toxicity of DMSO is low, it penetrates skin and thus contact with this solvent should be limited.

Botany 330

Sample Preparation Methods and Literature for Diatoms

Bob Mitchell, 1980

Cleaning

A. Hydrogen Peroxide

1. Add 75ml 30% H_2O_2 to sample in 1000 ml beaker.
2. Let stand for 24 hours. (Avoid evaporation.)
3. Add pinch of $K_2Cr_2O_7$ (potassium dichromate).
4. Let stand for 2 hours, then boil.
5. Add distilled water, let settle, decant. Repeat until liquid is colorless.

B. Potassium Permanganate

1. Add HCl to sample until no further reaction.
2. Add distilled water, let settle, decant.
3. Add 20ml H_2SO_4 and 10-15ml saturated $KMnO_4$ solution until sample turns purple.
4. Add 10ml oxalic acid until solution is colorless.
5. Add distilled water, let settle, decant. Repeat (8-10x).
6. Add 1-2ml strong ammonia to final washing to prevent clumping of cells. Wash again. (I have not tried this but it sounds a bit questionable since the frustules will dissolve in a base.)

C. Nitric Acid (other acids form precipitates with hard water)

1. Put sample and an equal volume of conc. HNO_3 in a Kjeldhal flask (or 600ml beaker). Add boiling chips.
2. Boil for about 20-30 minutes or until reaction ceases.
3. Add pinch of potassium dichromate until no effervescence. (This step seems only to be useful when you are working with a lot of organic matter e.g., sediment or epiphytes on their host).
4. Add distilled water to sample in a beaker, let settle (at least 4 hours), decant. Repeat until pH = 7 with litmus paper.

D. Plankton (lightly silicified forms)

1. Preserve and let settle, decant.
2. Add 95% EtOH or Chlorox bleach, let sit for ± 24 hours.
3. Decant, add distilled water, repeat.

Slide Preparation

1. Wipe cover slips (#1.5) with a very dilute solution of Photo-flo or alcohol to remove residues from glass. [Don't add Photo-flo to diatom suspension because it will dissolve the frustules!]

2. Dilute suspension to convenient density. If suspension is dilute spread directly on cover slip with a drop of dilute Photo-flo. If suspension is concentrated fill cover slip with Photo-flo solution and then add a few drops of the diatom suspension.

3. Let dry at room temperature overnight. A cover helps keep out dust.

4. In a fume-hood place a microscope slide on a hotplate and add ± 5 drops of mounting resin (hotplate should be set just lower than temperature where resin will boil).

5. Quickly invert cover slip onto resin and let toluene boil off until bubbling slows.

6. Remove microscope slide from hotplate and press out bubbles and excess resin. Let cool.

7. Trim excess resin from edges with razor blade.

8. This may be altered by adding resin to cover slips with a dried diatom suspension and letting the solvent evaporate overnight at room temperature, then inverting them onto the hot microscope slides. This may help with more complete embedding.

Note: The acids, oxidizers, solvents, and resins used for these methods are nasty! Some are known carcinogens and the others are probably as bad. Use a hood or very well ventilated area.

Literature

A. Methods

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Hendey, N.I. 1964. An introductory account of the smaller algae of British Coastal Waters. Part 5. Bacillariophyceae. (Diatoms) Ministry of Agriculture, Fisheries and Food. Fishery Investigation Series 4. London: Her Majesty's Stationery Office.

Isolation and Culture of Algae

Algal cultures are essential when conducting competition studies, bioassays, assessment of zooplankton food preferences, and determination of algal life histories. They are also necessary for molecular systematic work. Algal cultures may be "unialgal," which means they contain only one kind of alga, usually a clonal population (but which may contain bacteria, fungi, or protozoa), or cultures may be "axenic," meaning that they contain only one alga and no bacteria, fungi or protozoa. There are four major techniques for obtaining unialgal isolates: streaking, spraying, serial dilution, and single-cell isolations. Streaking and spraying are useful for single-celled, colonial, or filamentous algae that will grow on an agar surface; cultures of some flagellates, such as *Chlamydomonas* and *Cryptomonas* may also be obtained by these procedures. Many flagellates, however, as well as other types of algae must be isolated by single-organism isolations or serial-dilution techniques. We will practice spraying and single-organism isolations.

Spraying. In this technique, a stream of compressed air is used to disperse algal cells from a mixture onto the surface of a petri plate containing growth medium solidified with agar. Hold a petri plate about 18 inches from the touching tips of two Pasteur pipettes. One of these is attached to an airline via a hose, and mounted onto a ringstand. The other pipette is suspended tip-up into a container holding the algal mixture. The airflow from the first pipette creates a vacuum that draws a stream of algae-containing liquid up from the container through the second pipette. The airflow also sprays the suspended algae through the air, where they can be intercepted by the agar plate.

Single-cell/colony/filament isolations. The first step in this procedure is to prepare a number of "micropipettes" (very fine-tipped pipettes) from glass Pasteur pipettes. Hold a pipette in both hands; the tip end is held with a forceps so that the glass near the tip is within the flame of a bunsen burner (gas flame). The pipette is held in the flame only until the glass becomes slightly soft. This is determined by testing for flexibility by moving the tip with the forceps. Then the pipette is removed from the flame and pulled out straight. or at an angle so that there is a bend. **If you pull the pipette while it is still in the flame, it will seal up, so don't do this.** Always remove the pipette before pulling it! Use the forceps to break the tip. You can vary the diameter of the finely pulled tip by changing the speed of pulling; the diameter of a slowly-pulled tip will be greater than that of a rapidly-pulled tip. You would want a narrow diameter tip if you are trying to isolate very small algae, but a larger diameter tip is required for large cells. Try to match the diameter of the pipette tip to the size of the algal cells to be isolated.

Prepare a multiwell plate with sterilized media in each well. Place multiple drops of sterilized media or water onto the inside surface of a sterile petri plate. Attach a micropipette to a length of rubber tubing, attach a ethanol-sterilized mouthpiece to the the other end of the tubing, and put the mouthpiece in your mouth. Place a petri dish of algae on the stage of a dissecting microscope and locate the single cell/colony/filament to be isolated. Then find the tip of the micropipette and move it to the vicinity of the alga, then suck it up into the pipette tip, then stop the suction. Try to avoid sucking up any other algae. Now remove the pipette from the dish, then blow the liquid+alga into one of the drops of water on a petri plate). Break off and dispose of the portion of the micropipette tip that contained liquid; this has been contaminated. The micropipette can continue to be used until all of the pulled portion has been consumed. Now use the micropipette

to transfer the isolated alga from the first drop into a series of fresh drops. This is a washing procedure that helps remove contaminants. After transfer through 5-10 drops, transfer the alga into a well of the multiwell plate holding liquid growth medium suitable for that particular species. Repeat the procedure. Usually several attempts are made because not all isolated algae will continue to grow, or some may be contaminated with other algal cells.

A particularly effective means of obtaining unialgal cultures is isolation of zoospores immediately after they have been released from parental cell walls, but before they stop swimming and attached to a surface. Recently-released zoospores are devoid of contaminants, unlike the surfaces of most algal cells. But catching zoospores requires a steady hand and experience.

Filaments can be grabbed with a slightly curved pipette tip and dragged through soft agar (less than 1%) to remove contaminants. It is best to begin with young branches or filament tips which have not yet been extensively epiphytized.

Antibiotics can be added to the growth medium to discourage growth of contaminating cyanobacteria and other bacteria. Addition of germanium dioxide will inhibit growth of diatoms.

Axenic cultures (beyond the scope of this course) can be obtained by treating isolated algae to an extensive washing procedure, and/or with one or more antibiotics. Resistant stages such as zygotes or akinetes can be treated with bleach to kill epiphytes, then planted on agar for germination. It is usually necessary to try several different concentrations of bleach and times of exposure to find a treatment that will kill epiphytes without harming the alga.

Place the tubes/dishes with isolated algae into the culture room and allow growth to occur for 3-4 weeks. Examine them with the dissecting scope for signs of growth or contamination.

Freshwater Growth Media used in this class:

1) BBM is Bold's basal medium, chemically defined; good for many green algae.

2) Soil-water is undefined and used for algae whose nutritional requirements are unknown, or which will not grow on simple inorganic media. The soil should be loam from a site where herbicides have not recently been used. Sometimes it is advisable to add a dried pea to the medium before autoclaving.

3) SD11 is a defined medium that is somewhat more complex than BBM; it contains a vitamin mixture. Good for many green algae.

4) DYIII is a defined medium to which vitamins are often added, used for culture of chrysophytes and cryptomonads as well as some dinoflagellates.

Reference: Stein (ed.) 1973. Handbook of Phycological Methods. Culture methods and growth measurements. Cambridge University Press.

Botany 330 Take-Home Midterm Exam 2017

I. Introduction. These essay questions are designed to foster integrative thinking, comprehend the peer-reviewed scientific literature, and express findings in a clear way to a peer audience of scientists. *The essays are meant to model the kinds of short reports that you might write in a future professional situation.* In almost any biology-related field, you will be expected to summarize important information for the rest of your work group in a concise, yet readable way, and by mandated deadlines.

In these essays, you are writing for a reader who has earned at least an undergraduate degree in biological science; you are NOT writing for the general public, or general college students. Please use good English, and employ technical language and high-level concepts from Bot. 330 and your previous biology courses!

Draft essays that have been submitted by deadlines will be edited to let you know exactly what changes you must make to achieve an excellent grade. If you comply with specified deadlines and editorial recommendations, you should be able to earn all or most of the available points for essay exams!

Past experience indicates that procrastination may be your worst enemy. All of the information you need to write the Bot. 330 mid-term (and final) exams is already available to you on the course website and in your digital textbook. *Don't wait until close to deadlines to start writing.* **Read the first half of your textbook right away!**

II. Essential components. Each of your answers is expected to combine relevant material from the **textbook** with content from a particular, very recent peer-reviewed **article**, in about equal amounts. The textbook reflects compendia of information that you might regularly rely upon in a future professional situation and the peer-reviewed articles display the latest thinking, techniques, and results related to a more focused problem.

Don't use *any* other sources of information to write your essays, unless you receive instructor permission, because other sources are likely less accurate and current.

So, don't forget to include both sources of material (text & article) in your essays. It is common for people to become so focused on the articles that they forget to incorporate essential background information from the text. Text information will come from multiple chapters; use digital searches to find all relevant material.

When discussing articles, it is very important to discuss major methods used in the study, because without that information, it would be difficult for a peer reader to evaluate the accuracy of the results. So, don't forget methods!

III. Getting started. To get everyone on the right track, please turn in a draft answer to the *first question* by 5 pm on Wed. Sept. 20, 2017. *This first draft essay is so important that 10 points will be deduced from the total for the midterm exam if this*

draft is late.

All drafts and final essays should be 2-4 double-spaced pages long, with 12-point easy-to-read typeface such as Times New Roman. *Outlines are not acceptable as drafts.* Upload draft files (whose filenames incorporate your surname: e.g. TrestmidtermdraftQ1) to the Botany 330 Learn@UW assignment marked "midterm exam essay1".

Draft essay 1 will be edited and returned via Botany 330 Learn@UW by October 2. Don't wait for this edit before proceeding with other essays, but after receiving edited midterm essay1, use the information gained to polish all drafts.

In addition to midterm essay1, undergraduates must also answer 4 of the questions posed below; graduate students must write about any additional 5. (It is a graduate school requirement that in mixed courses, graduate students must perform work not required of undergraduates.)

IV. Additional Deadlines. Drafts of the other 4-5 essays are due on or before 5 pm, October 9 in the Learn@UW assignment "midterm exam drafts". Points will be deducted if all drafts have not been turned in on time.

Please link all draft midterm essay files together and incorporate your surname in the filename to reduce odds of misplacing files. Example: Graham draft midterm. Edits will be returned via Learn@UW by Oct. 16, in time to incorporate suggested modifications into drafts of all essays. Most edits will be completed well before Oct. 16, so check Learn@UW to see if your edits are there earlier, then go right ahead with revisions.

The final version of all midterm essays is due by 5 pm on October 23 in the Learn@UW assignment "midterm exam final version". Ten points will be deducted if any portion of the final version midterm exam is late.

The purpose of the deadlines and deduction of points if deadlines are missed is to foreshadow processes that occur in workplaces. There are always deadlines in workplaces and consequences for missing them, so by imposing deadlines we are not being mean, but to foster work habits that will benefit you later.

Please link all revised midterm essay files together and incorporate your surname in the filename to reduce odds of misplaced files. Example: Trest revised midterm

V. Recommended Process.

- Start by reading the entire first half of your digital textbook, because this step will help you to know where essential material is located.
- Next, read a question, noting key words that you can use as search terms to locate all relevant textbook information.

- Take notes from relevant sections of the textbook, a step that will aid your understanding of the associated article.
- Read the associated article, taking notes on research goals, methods, and results most relevant to the exam question.
- Organize text and article notes in a logical sequence that first provides reader with essential overview background information from textbook, then a specific example (the article).
- Read the course document "Editorial Issues," which addresses common writing errors made by past Bot. 330 students. We recommend printing & posting this document for easy referral throughout this course.
- Examples of two common errors to avoid: "Algae" is a plural word for which the singular is "alga;" so, "algae are beautiful", NOT "algae ~~is~~ beautiful." The subject "None" is a contraction of "no one," hence takes a singular verb: "None of these algae **is** known to be toxic," NOT "None of these algae ~~are~~ known to be toxic."
- Write a first version and then revise at least twice before submitting as a draft essay, aiming for increased clarity and to remove excess verbiage.
- Correct spelling and grammar errors indicated by your word processor.

The articles you will write about were chosen for currency and relevancy to issues of wide concern. The journals that published these articles represent the highest-rated & most demanding publication sources. In the future, these are among the sources you can trust to provide authoritative and carefully peer-reviewed information. Beware of new for-profit biology journals that publish work that has not been adequately peer-reviewed or edited. This assignment will help you recognize high-quality publication sources for use in your future work. This is much like distinguishing real from fake news.

VI. Ethics and scholarly responsibilities. Please cite the article author(s) appropriately within your essays in this form: (Brown et al. 2016).

Notice the locations of literature citations in scientific articles (and your textbook). Follow these models. Do not credit article authors for knowledge generated by previous workers, such as information provided in article introduction sections.

At the end of each essay provide article author names, date, article title, journal name, volume, & pages. It is not necessary for you to cite the textbook.

Collaboration with your peers in the composition of these essays is specifically prohibited, though it is perfectly fine to discuss articles amongst yourselves or with instructors.

It is also prohibited for you to have your essays edited or "polished" in any way by anyone other than current Botany 330 course instructors. Don't ask friends to help or

use professional editing services. The reason for this is to ensure than you will receive the maximum practice in writing and revising, to benefit your future success.

You are not expected to necessarily agree with the conclusions of authors; a skeptical view or alternative opinion might be justified. However, essay content should be professionally unemotional. Any personal opinion must be supported by peer-reviewed literature. Personal attacks on authors (known as *ad hominem* attacks) are not acceptable.

Do not use quotations, because quotations are rarely employed in scientific writing for peers. You might have learned to use quotations in writing for a non-science course, but as you can see from reading scientific articles and your textbook, quotations are not appropriate for our purposes. Instead, paraphrase, a process that shows that you comprehend the material. *Again, no quotations!*

Q1. The general public would probably consider toxin-producing cyanobacteria to be the single most important topic in algal biology. How does complete genomic sequencing help us to better understand toxin-producing cyanobacteria?

Sub-components of answer in an appropriate order (note: bullet points are to get you started; subsequent questions will not include this level of detail):

- Explain why cyanobacteria, and particularly *Anabaena*, are important to society (Ch. 3 and 6). This discussion must include a description of toxins, particularly anatoxin-a.

- Explain *methods* used to isolate and grow cultures of cyanobacteria, assess toxin production by chemical means, assess relationships among toxin-producing cyanobacteria, and identify genes involved in the synthesis of cyanobacterial toxins (Chapter 5). Explain why algal isolate names include numbers (or letter-number combos) and why this is important.

- Provide a specific example by discussing the article Brown et al. (2016) "Structural and functional analysis of the finished genome of the recently isolated toxic *Anabaena* sp. WA102," published in *BMC Genomics*. You can obtain this article by using university library digital resources or Google Scholar.

- First, tell your reader the *goal* of the investigation reported by the article.

- Finish with major results described by the article, how they were obtained (key methods) and how the results are useful to science and/or society.

- Your answer should address the importance of genome-level characterization of cyanobacterial strains (e.g. important features you can't

learn using a microscope alone), differences between the genomes of examined strains and why those differences are ecologically important, and the benefits of long-read (vs short-read) sequencing technology.

- Briefly tie discussion of the article back to the initial question, but don't write a flowery, poetic, simplified, or detailed summary paragraph. Your professional reader will not need or want that.

2. For many biologists, the origin of oxygenic photosynthesis is one of the most important scientific questions out there, and the timing and mechanism of the origin of aerobic respiration might be a close second. When and how did oxygenic photosynthesis first arise, and when and how did aerobic respiration arise in cyanobacteria and close relatives? Textbook Chapters 1, 2, and 6 are essential. Relevant article: Soo et al. (2017) On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria, *Science* 355:1436-1440.

3. Many scientists, industrialists, and others would vote for renewable algal biofuels as a supremely important algal topic. How can genomic techniques and innovative imaging be used to aid production of renewable biofuels from algae? Begin by explaining three major ways in which algae are used to generate renewable biofuels, ending with lipid extraction. Chapter 5 will aid understanding of genomics. With this background, explain why and how Ota et al. 2016 did the work described in the article Highly efficient lipid production in the green alga *Parachlorella kessleri*: draft genome and transcriptome endorsed by whole-cell 3D ultrastructure, *Biotechnology for Biofuels* 9:13.

4. Increasing levels of greenhouse gases in Earth's atmosphere have important climatic and biotic effects. How are cyanobacteria likely to response to high CO_2? Start by summarizing the diverse environmental roles of cyanobacteria past and present (Chapter 2 & 6). Focus on nitrogen fixation and the global importance of the genus *Trichodesmium*. Describe *Trichodesmiu*m and its habitat. Finally, explain how and why Hutchins et al. (2015) did the work described in their article "Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide."

5. Changing levels of greenhouse gases in Earth's atmosphere have had significant climatic and biotic effects. How do eukaryotic algae cope with low CO_2 and how might they respond to a higher CO_2 future? Begin by discussing CO_2 acquisition processes in eukaryotic algae, explaining how these differ from those present in cyanobacteria (Chapter 2). Focus on the role of carbonic anhydrase in the carbon-concentration process, and discuss current knowledge about carbon-concentration mechanisms in photosynthetic stramenopiles. Describe *Nannochloropsis* and its ecological and societal importance (Chapters 4 and 13). Employ the article by Gee and Niyogi (2017) "The carbonic anhydrase CAH1 is an essential component of the carbon-concentrating mechanism in *Nannochloropsis oceanica*" as a specific example.

6. Algae interact with other organisms in many significant ways. Provide an overview of the spectrum of algal associations (Chapter 3) then focus on algal associations with ciliates. Discuss at least two phyla of eukaryotic algae known to occur as ciliate endosymbionts, one common in freshwaters and the other in marine systems. Explain kleptoplastidy (Chapter 9). Explain the benefits received by the partners (material exchanges). Use the Qiu et al. (2016) article "Cryptophyte farming by symbiotic ciliate host detected in situ" as a specific example. If you answer this question, please do not also answer question 7.

7. Algae interact with diverse other organisms, in sometimes complex ways. Provide an overview of the spectrum of algal associations (Chapter 3) then focus on endosymbiosis and herbivory. Define endosymbiosis and describe freshwater ciliates that harbor algal endosymbionts. List and briefly describe animals that rely upon algal food; be sure to include copepods. Employ the DeLong et al. (2016) article "Predators catalyze an increase in chloroviruses by foraging on the symbiotic hosts of zoochlorellae" as a specific example of complex biotic interactions involving algae. If you answer this question, please do not also answer question 6.

8. Understanding how algae respond to excess light is important in cultivating algae for industrial applications and ecological aspects involving algal motility and distribution in the water column. How is excess light damaging to algal cells? How do algae protect themselves from the damaging effects of excess light? Provide an overview of various algal light protection mechanisms (Chapter 1) then focus on cyanobacteria and orange carotenoid protein (Chapter 6). Describe the cyanobacterial phycobilisome in some detail. Use information provided in Harris et al. (2016) "Orange carotenoid protein burrows into the phycobilisome to provide photoprotection" to provide more-detailed information about how the structure of orange carotenoid protein is key to its function.

9. Understanding the process by which eukaryotic cells originated by endosymbiosis has diverse ecological, evolutionary, industrial, and medical implications. Use Chapter 6 to provide an overview of the impact of endosymbiosis in the origin of the eukaryotic algae. Focus on controversy surrounding the number of times primary plastids are thought to have originated. Briefly discuss the three algal phyla characterized by primary plastids, as well as alternative hypotheses about the nature of some of the so-called "primary" plastids. Finally, focus on *Paulinella*, explaining how study of this rhizarian protist has been helpful in understanding the process of primary endosymbiosis. Employ the article by Nowack et al. (2016) "Gene transfers from diverse bacteria compensate for reductive genome evolution in the chromatophore of *Paulinella chromatophora*" as an example. Chapter 5 will be helpful in understanding the concept of HGT.

10. Common misconceptions about the roles of algae in nature and algal responses in cultivation systems (e.g. for renewable biofuels) are based on a widespread lack of understanding that algae do not form a monophyletic group, and thus display a

wide range of structure and physiology. What eukaryotic supergroups include algae? For each algal group, list some distinctive features that you think are ecologically or industrially important. Base this overview of eukaryotic algal diversity on information in Chapter 5, then focus on the supergroup Rhizaria, and describe the evolutionary importance of algal representatives of the Rhizaria (Chapter 7). Use the article by Biard et al. (2016) "In situ imaging reveals the biomass of giant protists in the global ocean" to expand your discussion to include the ecological importance of Rhizaria and their associations with algae. Be sure to look at the photographic images provided in the supplementary data of this paper.

Botany 330 Final Exam 2017

I. Introduction. These essay questions are designed to foster integrative thinking, comprehend the peer-reviewed scientific literature, and express findings in a clear way to a peer audience of scientists. *The essays are meant to model the kinds of short reports that you might write in a future professional situation.* In almost any biology-related field, you will be expected to summarize important information for the rest of your work group in a concise, yet readable way, and by mandated deadlines.

In these essays, you are writing for a reader who has earned at least an undergraduate degree in biological science; you are NOT writing for the general public, or general college students. Please use good English, and employ technical language and high-level concepts from Bot. 330 and your previous biology courses!

Draft essays that have been submitted by deadlines will be edited to let you know exactly what changes you must make to achieve an excellent grade. If you comply with specified deadlines and editorial recommendations, you should be able to earn all or most of the available points for essay exams!

Past experience indicates that procrastination may be your worst enemy. All of the information you need to write the Bot. 330 final exams is available to you on the course website and in your digital textbook. *Don't wait until close to deadlines to start writing.* **Read any parts of your textbook that you have not already assimilated right away!**

II. Essential components. Each of your answers is expected to combine relevant material from the **textbook** with content from a particular, very recent peer-reviewed **article**, in about equal amounts. The textbook reflects compendia of information that you might regularly rely upon in a future professional situation and the peer-reviewed articles display the latest thinking, techniques, and results related to a more focused problem.

Don't use *any* other sources of information to write your essays, unless you receive instructor permission, because other sources are likely less accurate and current.

So, don't forget to include both sources of material (text & article) in your essays. It is common for people to become so focused on the articles that they forget to incorporate essential background information from the text. Text information will come from multiple chapters; use digital searches to find all relevant material.

When discussing articles, it is very important to discuss major methods used in the study, because without that information, it would be difficult for a peer reader to evaluate the accuracy of the results. So, don't forget methods!

III. Format. The format of final exam draft and final essays is the same as for the midterm**:** 2-4 double-spaced pages, 12-point easy-to-read typeface such as Times

New Roman. *Outlines are not acceptable as drafts.* Upload draft files (whose filenames incorporate your surname: e.g. Trestfinalexamdraft) to the Botany 330 Learn@UW assignment marked "final exam drafts".

Undergraduates must answer 5 of the questions posed below; graduate students must write 6. (It is a graduate school requirement that in mixed courses, graduate students must perform work not required of undergraduates.)

IV. Additional Deadlines. Drafts of the 5-6 essays are due on or before 5 pm, December 15. Points will be deducted if all drafts have not been turned in on time.

Please link all draft essay files together and incorporate your surname in the filename to reduce odds of misplacing files. Example: Grahamdraftfinal. Edits will be returned via Learn@UW by December 19, though most edits will be completed well before then, so check Learn@UW to see if your edits are there earlier, then go right ahead with revisions.

The final version of all final exam essays is due by 5pm December 21 in the Learn@UW assignment "final exam final version"**.** Ten points will be deducted if any part of the final exam is late.

Note: The purpose of the deadlines and deduction of points if deadlines are missed is to foreshadow processes that occur in workplaces. There are always deadlines in workplaces and consequences for missing them, so by imposing deadlines we are not being mean, but rather aim to foster work habits that will benefit you later.

V. Recommended Process.

- Start by reading the entire digital textbook if you haven't done that already, to know where essential material is located.
- Next, choose a final exam question, noting key words that you can use as search terms to locate all relevant textbook information.
- Take notes from relevant sections of the textbook, a step that will aid your understanding of the associated article.
- Read the associated article, taking notes on research goals, methods, and results most relevant to the exam question.
- Organize text and article notes in a logical sequence that first provides reader with essential overview background information from textbook, then a specific example (the article).
- Make sure you haven't made any of the errors noted in the course document "Editorial Issues," common writing/science mistakes made by past Bot. 330 students.
- Examples of common English usage errors to avoid: 1) "Algae" is a plural word for which the singular is "alga;" so, "algae are beautiful", NOT "algae ~~is~~ beautiful." (Ditto for bacterium, bacteria and fungi, fungus.) 2) The subject "None" is a contraction of "no one," hence takes a singular verb: "None of

these algae **is** known to be toxic," NOT "None of these algae ~~are~~ known to be toxic."
- Examples of common science errors to avoid: 1) hypothesis (yet to be substantiated) vs theory (in science, a body of well-established knowledge); 2) in photosynthesis, the product oxygen derives from water (NOT CO_2).
- Write a first version and then revise at least twice before submitting as a draft essay, aiming to increase clarity and remove excess verbiage.
- Correct all spelling and grammar errors indicated by your word processor.

VI. Ethics and scholarly responsibilities. Please cite the article author(s) appropriately within your essays in this form: (Brown et al. 2016).

Notice the locations of literature citations in scientific articles (and your textbook). Follow these models. Do not credit article authors for knowledge generated by previous workers, e.g. information provided in article introduction sections.

At the end of each essay provide article author names, date, article title, journal name, volume, & pages. It is not necessary for you to cite the textbook.

Collaboration with your peers in the composition of these essays is specifically prohibited, though it is perfectly fine to discuss articles amongst yourselves or with instructors.

It is also prohibited for you to have your essays edited or "polished" in any way by anyone other than current Botany 330 course instructors. Don't ask friends to help or use professional editing services. The reason for this is to ensure than you will receive the maximum practice in writing and revising, to benefit your future success.

You are not expected to necessarily agree with the conclusions of authors; a skeptical view or alternative opinion might be justified. However, essay content should be professionally unemotional. Any personal opinion must be supported by peer-reviewed literature. Personal attacks on authors (known as *ad hominem* attacks) are not acceptable.

Do not use quotations, because quotations are rarely employed in scientific writing for peers. You might have learned to use quotations in writing for a non-science course, or perhaps writing about science for the general public. By contrast, as you can see from reading scientific articles and your textbook, quotations are rarely used in writing for science peers. Instead, paraphrase, a process that shows that you comprehend the material. *Again, no quotations!*

1. The production of calcium carbonate coccoliths by coccolithophorids is ecologically-, geologically-, and industrially-important, but the cellular mechanisms involved in calcification by haptophytes have been unclear. Begin by explaining why coccolithophorids are ecologically, geologically, and

industrially significant. (Include a discussion of the value to haptophytes of producing coccoliths.) Follow with a description of what has been known about cellular development of coccoliths and organic scales. Discuss the methods and findings of Gal et al. (2016), "Macromolecular recognition directs calcium ions to coccolith mineralization sites."

2. Diatoms are widely recognized for their ecological, geological and industrial importance, but how diatoms have adapted to stressful environments remains unclear. Begin by describing the ecological, geological and industrial importance of diatoms, eventually focusing on sea ice diatoms. Follow with a description of methods and findings of Mock et al. (2017) "Evolutionary genomics of the cold-adapted diatom *Fragilariopsis cylindrus*. Be sure to discuss the importance of the comparison with genomics of temperate diatoms.

3. Kelps are known for industrial and ecological importance. Begin by describing industrial applications of kelp, and follow with a discussion of the ecological roles of kelp species. Provide at least three examples of kelp genera together with their known biotic associations and geographical distributions. Discuss the methods and findings of the Krumhansl et al. (2016) article, "Global patterns of kelp forest change over the past half-century." How is global environmental change likely to affect kelp distribution?

4. Acquisition by certain early eukaryotes of a primary plastid dramatically changed these organisms' genomes and cell biology. Begin by discussing current concepts of primary plastid origins (single or multiple) and at least three key lineages of primary plastid algae. Describe at least three cellular changes that occurred in the host or endosymbiont during the transformation of a prokaryotic endosymbiont into a primary plastid. Consider the methods and findings of the Sumiya et al. (2016) paper, "Chloroplast division checkpoint in eukaryotic algae," which describes work done with a red alga. Note that these authors uncritically accept the "single primary plastid origin hypothesis," which allows them to assume that the work also applies to the glaucophyte and green lineages. What evidence cited by these authors suggests that the observations may apply primarily to red algae?

5. Comparative analysis of chloroplast genomes are widely employed in phylogenomic analyses to comprehend the diversification of land plants, but the value of chloroplast genomes for understanding early green algal diversification has not been clear. Describe what is known about early diversification of Viridiplantae (green algae + land plants), and focus on prasinophytes, then the genus *Pyramimonas*. Explain the importance of this genus. Discuss methods and results reported by Satjarak and Graham (2017)

"Comparative DNA sequence analyses of *Pyramimonas parkeae* (Prasinophyceae) chloroplast genomes."

6. Cyanobacterial blooms have such strong societal impacts that biological phenomena that might affect such blooms are of wide interest. Some researchers have cited evidence that allelopathic repression of competing eukaryotic algae might play a role in bloom formation. Start by providing an overview of cyanobacterial blooms: which genera are common bloom-formers, what features of these genera foster bloom formation, what physical and biological conditions foster bloom formation, and what effects do blooms have on other organisms? Segue to a discussion of the evidence for possible allelopathic effects and how this might happen in nature. Discuss evidence from the Dunker et al. (2017) article "A fateful meeting of two phytoplankton species…"; an investigation that employed a trebouxiophyte green alga. Does this information aid the prevention of cyanobacterial blooms or help to comprehend their biological effects?

7. The chlorophycean green algal genus *Chlamydomonas* is widely known as a model photosynthetic eukaryote in cell biology. Begin by surveying the structure, ecology, and relationships of *Chlamydomonas*, following with comments about how widely information obtained from *Chlamydomonas* can be extrapolated. Discuss how *Chlamydomonas* has been used to understand the genetic basis of photosynthesis, flagellar function, and light receptors. Focus upon cryptochrome and then the methods and findings reported by Zou et al. (2017) "An animal-like cryptochrome controls the *Chlamydomonas* sexual cycle." Evaluate the relevance of these observations to plant biology.

8. The streptophyte green algae include species regarded as closely related to land plants, and therefore are useful sources of information about how plant traits originated. Begin with a survey of the streptophyte green algae, providing at least three examples of genera other than *Klebsormidium*. Describe features of those genera of interest to plant evolution. Then focus on the genus *Klebsormidium*; discuss what has been known about its structure, reproduction, ecology, relationships, and genomics. Focus on the Ohtaka et al. (2017) report "Primitive auxin response without TIR1 and Aux/IAA in the charophyte (meaning "streptophyte") alga *Klebsormidium nitens*." What does this investigation reveal about the evolution of plant auxin response pathways?

9. The question of how diverse, related organisms (e.g. phytoplankton algae in a pond) co-exist has puzzled ecologists for decades. Discuss what has been known about co-existence of diverse phytoplankton species based on structural features. Describe investigations and hypotheses that have attempted to explain higher than expected species diversity. Consider the methods and findings of Narwani et al. (2017) "Ecological interactions and coexistence are predicted by gene expression similarity in freshwater green

algae." How has the addition of molecular methods affected our understanding of freshwater phytoplankton community ecology? What are some caveats of this work (e.g. are lab bi-cultures adequate models of natural behavior in multispecies associations)?

10. Most people know that alien species are of global ecological concern, but few realize that humans play a major role in the spread of invasive algal species. Discuss what is known generally about invasive marine phytoplankton and macroalgal species. Provide at least 5 examples of algal species that can be invasive, and discuss their ecological impacts. Explain how humans have been involved in these species' spread. Use the Seebens et al. (2016) article "Predicting the spread of marine species introduced by global shipping" as an example of how people study the roles of human activities in the spread of invasive algal species.