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## Forward Teaching by Doing

Turning a Biology Curriculum Upside Down by Clark Lindgren Ph.D.

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## **Teaching by Doing Turning a Biology Curriculum Upside Down**

by Clark Lindgren, Ph.D.

Tell me and I will forget, show me  
and I will remember,  
involve me and  
I will understand!  
— Ancient Chinese Proverb

Try to imagine the following scenario. Tommy always wanted to be a professional tuba player. He didn't have many opportunities to do serious tuba playing in high school so was thrilled when a college with a "world renowned" tuba program accepted him. He couldn't wait to begin. Unfortunately, tuba school wasn't quite what Tommy had imagined. His first class, Tuba 101, was held in a large lecture room with over 100 students and dutifully reviewed the history of the tuba. Tuba 102 the following semester focused on the theory of the tuba. Although these large Intro classes had weekly "labs" that were much more practical than the lectures, Tommy didn't actually play the tuba at all during his first year. He got to experiment with the mouthpiece once and had a few great labs exploring the valves (he even learned how to make one); however, that was as close as he got to the real thing. His second and third years were better. The classes were smaller and the lab exercises more realistic, but it wasn't until his senior year that he finally had the necessary prerequisites to sign up for Tuba 395: Independent Study. This is what he had come to college for. He would be given a tuba for the entire semester and allowed to play some music that his instructor was working on.

At this point there are several directions this scenario might go. Tommy might have a great time in his independent study, go off to Tuba graduate school and become the professional tuba player he had long dreamed of becoming. Alternatively, Tommy might have discovered in Tuba 395 that he really wasn't very good at playing the tuba or, worse yet, he didn't enjoy playing it as much as he thought he would. Regardless of the direction Tommy's tuba career takes, all of the scenarios are absurd. Who would defend a curriculum that asks students to wait until their senior year in college to actually do what they came to do? How many college students have the patience or insight to put up with this? How many potentially great tuba players would we lose using this strategy?

Yes, the scenario is absurd and fortunately for the world of tuba players, this is not how most people become professional tuba players. However, anyone who has been a science major in college will recognize the "tuba curriculum." We pack students into large lecture halls and teach them *about* science. Yes, we talk about how science is done, especially in the second and third years of our curricula. We teach labs in which students are taught parts of the process. They learn techniques and are asked to make careful measurements and/or observations, but only rarely are students involved in an actual scientific study — that is, an inquiry in which the answer is not known by anyone (including the instructor). The opportunity for students to actually do real science is reserved for much later in the curriculum and, many times, this is reserved for a subset of students who demonstrate exceptional promise for science (i.e. they passed their courses with high marks).

Several years ago my colleagues and I in the Biology Department at Grinnell College began worrying about our own "tuba curriculum." Even though we were a small private liberal arts college, we were still introducing our students to biology the way it had been done at most colleges and universities for decades. Students were first paraded through a multi-course introductory sequence in which we passed on to them the biology canon. Only then could they take courses that more closely approximated real science. Much to our dismay, this delay was becoming longer and longer. Over the previous ten years, our introduction to biology had grown from two, to three, and then to a four-course sequence as we, along with every other Biology Department in the world, had been trying to accommodate the dizzying growth in biological knowledge. Yet, even with four courses that extended through their second year of

college, we were still feeling hard pressed to give our students a complete introduction to biology. Somehow, five “Intro” courses just seemed too much. In the context of this deep curricular soul-searching, we began to entertain a very novel idea. What if we turned our curriculum upside down? What if we just bypassed the traditional Intro courses and had students do research, real research, first and then filled them in on the details/big picture later?

Although it seemed strange at first, the more we thought about an upside down curriculum, the more sense it made. First, it would prevent the bad Tommy Tuba scenario where Tommy didn’t discover until his senior year that with regard to real tuba playing he was either inept or apathetic. Students would discover right up front whether their love for biology was genuine and worth pursuing further. Second, some students might discover a love for science they didn’t know they had. These are the students who do not thrive in a traditional curriculum, but possess the skills and mettle to be first-class scientists. (How many biographies of famous scientists begin that way?) Finally, this upside down curriculum would be better for the many students who take biology as part of their general education. These are the future lawyers, business leaders and artists who have no intention of becoming scientists but are taking biology to round out their liberal arts experience. We couldn’t imagine an introduction to biology would be more useful to these future leaders than the one we were contemplating.

With fear and trepidation we began planning a one-semester course called Bio 150: Introduction to Biological Inquiry. Each of us in the department would design a section of Bio 150 that focused on a specific research area. Each section would teach students the bare minimum needed to get started on a real scientific question. The students would be shown how to perform a few techniques, how to search for and read scientific articles, and how to distinguish a good scientific question from a not-so-good question. Finally, working in groups of three (we had previously discovered that three was the magic number for group work), the students would choose a question, design and carry-out experiments to answer the question, and then present their results and an interpretation of their results in formats appropriate for the discipline.

Our first set of Bio 150 sections were announced in the fall of 2000. Students could choose one (and only one) of the following seven sections: “Building an Animal,” “Prairie restoration,” “The Language of Neurons,” “Biological Responses to Stress,” “Emerging and Re-emerging Pathogens,” “The Effects of Climate Change on Organisms,” and “What Does it Mean to be a Plant?” Since then, we have added a few more sections to our repertoire, including “Sex Life of Plants,” “Plant Genetics and the Environment,” “Survivor,” “Cell Fate: Calvin or Hobbes,” “Genes, Drugs and Toxins,” and “Animal Locomotion.”

This year will be the tenth time we have offered Bio 150. Despite some initial reticence to try such a bold curricular experiment, none of us would choose to return to the “old ways.” Why? Because Bio 150 is challenging, interesting and fun — all of the reasons we became biologists in the first place. And, it is working. It is accomplishing just what we had hoped and then some. Students who arrive in Bio 150 gung-ho about biology (i.e. the Tommy Tubas) generally love it. It is just what they needed to confirm their interest and get an early start developing their skills as young scientists. After taking Bio 150, students who want to go on in biology take a more traditional two-course sequence to round out their background and fill in the gaps in their biological knowledge. However, unlike in the past, our students are more sophisticated now. They understand why a Molecular Biologist needs to know something about ecology or why an Evolutionary Biologist must understand some physiology. It is impossible to answer even the narrowest question without help from other subfields in biology, not to mention chemistry, physics and math. Our students now appreciate, if not enjoy, the broader exposure to biology because they understand why it is necessary.

But this isn’t the experience of all of our students. Some simply do not like Bio 150. Although teachers do not usually like it when students do not enjoy their course, the reasons students offer for their displeasure with Bio 150 suggest that even these are “successes.” A few years after we began teaching Bio 150 one of my advisees announced that she didn’t want to continue in biology. She was a good student and had not done poorly in Bio 150; she just didn’t think she liked biology. Had a student reported this to me in the pre Bio 150 era I would have encouraged her to stick it out a little longer. “Maybe you will enjoy the subject matter that comes later in the sequence.”

This was common advice from me since the course content that related most directly to my specialty came at the end of our four-course Intro sequence. However, in this case my response was “tell me why you didn’t like Bio 150.” Her

answer was stunning. “I hate the ambiguity in biology! Even when you design the perfect experiment and perform it perfectly, there is still uncertainty. I understand why this is, I just don’t like it.” When I asked her what subjects she liked, her response was immediate. “I love Math! It is precise, defined and unambiguous.” I was speechless. This first year college student with only one course in biology could articulate the epistemological distinction between an experimental science like biology and a field such as mathematics. (I suspect some professional biologists do not understand this as well as she did!) My response to the student was to congratulate her on her insight and wish her well in her mathematics courses. Not surprisingly, she graduated three years later with a math major.

Our success at dissuading certain students from pursuing biology might be a unique benefit of our new curriculum; however, has Bio 150 persuaded others to embrace the field of study we all love? We think the numbers answer that question. Enrollment in Bio 150 and our other biology courses has seen steady growth over the past ten years. However, an outcome that we interpret as even more significant is the large growth in the number of students who want to do research with us. We have a long history of working with students on our research, mostly in the summer, but in the past few years student interest has grown far beyond our capacity to accommodate. We regularly have four times the number of applicants for our summer research program than we have positions available and these applicants are all our own students! The increased interest in our courses and opportunities for research suggest we have sparked some authentic enthusiasm for biology. At least we have more than compensated for those who have been “enlightened” to leave biology for another calling.

But how authentic is Bio 150? Are we succeeding in giving our students a genuine scientific experience? Are the English and Sociology majors who only take one course in biology getting what they need to be knowledgeable participants in 21st century life? The projects students carry out in Bio 150 are seldom complete, as in having been sufficiently replicated to stand alone as a scientific finding, but some have been incorporated into larger studies and published in the scientific literature. However, most of the projects have not seen the light of day. Some of the student research has contradicted previous research, both published research and research carried out in a previous year’s Bio 150. None of the student research has been ground-breaking or earth-shattering. In comparison to what we experienced as graduate students, post-docs and now faculty, well, er, uh ... yes, Bio 150 research looks exactly like real research. What students discover is that the scientific method is demanding, frustrating, and quite often tedious. Biology textbooks often give the mistaken impression that science progresses in a logical manner. In hindsight, science always appears to move in the “forward” direction. What our students experience is much more like real science. They learn that science often moves in more than one path at a time and sometimes even reverses direction. And, when it moves forward, it almost always moves at an excruciatingly slow pace. Yes, Bio 150 is painfully authentic. In the words of one student “Anyone who takes Bio 150 and still wants more is either crazy, born to be a biologist, or both.” I think Tommy would be very happy.

The goal of this handbook is to communicate to you the opportunities you have in the Departments of Biology and Chemistry, as well as the expectations we will have of your work in our courses. Because of the close interaction between courses required for the Biology, Chemistry and Biological Chemistry majors, we recognize the need to speak in one voice about our approaches to teaching science. While you will find many differences between courses in our departments, we hope you will recognize and learn the common skills that are fundamental to good scientific understanding and practice.

You should keep this manual as a reference whenever you are doing work for a biology or chemistry class. Your instructors will refer to parts of the manual for review in both introductory and advanced courses. We encourage you to make notes in your copy and give us feedback on the parts you find useful or confusing. If you lose your copy, you may consult a section directly on the web (from either department's web page), or even download another copy.

We wish you success in your learning!

*The Faculty and Staff of the Departments of Chemistry and Biology*

## I. Navigating courses in Biology and Chemistry

This section of the manual will help you plan your courses in the two departments (including a major if you so desire) and will help you be successful in your learning. Please consider this advice seriously.

### Curriculum

Listed below are sample schedules for students majoring in biology, chemistry, and biological chemistry.

Fall	Spring
<u><i>Biology</i></u>	
1 <sup>st</sup> Bio 150 or Chm 129 Math 131 (123)	Bio 150 or Chm 129 ** Math 133 (124)
2 <sup>nd</sup> Bio 251 Chm 221 <sup>**</sup>	Bio 252 ** Chm 222 **Math 209
3 <sup>rd</sup> and 4 <sup>th</sup> 5 Electives <sup>*</sup> (can include summer research or classes)	

### *Chemistry*

1 <sup>st</sup> Chm 129 Math 131 (Math 123)	Chm 130 Math 133 (Math 124)
2 <sup>nd</sup> Chm 221 <sup>**</sup> Phy 131 (Math 133)	Chm 222 Phy 132
3 <sup>rd</sup> Chem 363	Elective
4 <sup>th</sup> Elective	Elective

### *Biological Chemistry*

1 <sup>st</sup> Bio 150 or Chm 129 Math 131 (or 123)	Bio 150 or Chm 129 Math 133 (124)
2 <sup>nd</sup> Bio 251 Chm 221 <sup>**</sup>	BCM 262 Chm 222
3 <sup>rd</sup> Phys 131	Phys 132
4 <sup>th</sup> Chem 363	Elective <sup>*</sup>

\*Note that one advanced elective must be taken in the junior or senior year.

\*\* These courses are not required, but are suggested

<sup>\*\*</sup> Note that students with Chemistry AP/IB credit must take Chem 130 as a prerequisite to Chem 221.

### What choices do I have and when do I have to make them?

If you're unsure about where you're headed (Biology, Biological Chemistry, or Chemistry) you should take Bio 150, Chm 129, Math 131, and Math 133 in your first year. This will leave all options open to you. In the Fall of your second year you should enroll in Bio 251 and Chm 221. At this point (prior to the spring semester of your second year) you will need to decide if you intend to pursue the Biology, Biological Chemistry, or Chemistry major. You must declare a major, and prepare a 4-year plan, prior to registration for your 5<sup>th</sup> semester.

### **Planning for Off-campus Study**

Planning for off-campus study requires careful consideration. It is to your advantage to begin the planning process in your first year. If you are planning an off-campus experience you should plan to be away during your 5<sup>th</sup>, 6<sup>th</sup>, or 7<sup>th</sup> semester. Biological chemistry and chemistry majors should talk with their advisors about when physics and physical chemistry should be taken in relation to an off-campus experience.

### **The role of independent research**

Independent research is a central part of all three majors. It is required for chemistry major, and strongly recommended for biology and biological chemistry majors.

### **Graduating with honors**

Graduation with honors requires a minimum GPA of 3.4 overall, and 3.5 within the major. Majors are also required to conduct an independent research project, the results of which are presented at a departmental seminar.

**The award of honors is not based solely on grades and achievement in the classroom or lab. It signifies, in addition, an underlying commitment to the discipline as evidenced by participation in departmental affairs and activities, including attendance at departmental seminars.**

### **What if I'm considering going to medical school after college?**

You can major in any of the three areas above (or any other at the college), but will want to take the following science courses before you take the MCATs (Medical College Admissions Tests):

Biology 150, 251 and 252  
Chemistry 129, 130, 221 and 222  
Physics 131 and 132  
Math 131 and 133 (or 123 and 124)

Students who wish to go on to medical school right after graduation should plan to take the MCATs in April of their 3rd year. Since this limits their options in non-science courses, most students take the MCATs at the end of their senior year and spend a year following graduation gaining work experience in a medical or research setting. Grinnell's Health Professions Advisory committee (<http://www.grinnell.edu/committees/hpac/>) can help you with these decisions.

### **What if I'm considering teaching as a career?**

If you are interested in becoming a teacher, you must contact the education department prior to preregistration in the first semester of your second year. To be certified to teach in a scientific discipline at the secondary level, you'll have to take 5 education courses in addition to major requirements. See the catalogue for more specifics.

### **What if I'm not sure what I want to do?**

That's OK. You're here to explore your interests. Regardless of your potential career plans, it is to your advantage to discuss your interests with your advisor as soon as possible. Planning ahead isn't like signing a contract -- it will help you to keep your options open.

## **Everyday Keys to Success**

Science courses will vary in how much time you will spend in lecture, discussion and lab activities, and even in how these activities are structured during the week. For example, in "workshop" courses there will be no distinction between lecture or lab sessions. Despite these variations, there are two basic principles that will help you succeed in *all* your courses: **preparation** and **review**. Time spent in class will be much more valuable if you have prepared for it and much more memorable if you review your readings, notes and activities.

If you find yourself struggling in a course, consider the very specific advice found in the boxes below. Your instructor is always your best source of information and explanation, and should be your first line of defense against confusion. In addition, you are fortunate that Grinnell College makes many additional resources available to you. These include the Science Learning Center, the Math Lab, Reading and Writing labs, and the Student Affairs office. These offices provide tutors, mentors (group tutoring), and workshops on study skills and time management, among others.

### **How much time will I spend studying?**

Remember, college is a full-time job! For every hour you're spending in class you should be spending three additional hours studying, completing assignments, and preparing for upcoming classes. A typical course load of 16 credits would thus commit you to as much as 48 hours of work a week outside of class. Face it, you're going to be very busy, and you're normally going to be working 7 days a week.

### **Getting ready: tips on reading science textbooks and scientific papers**

Read your book more than once – it's not a novel. It's a valuable resource – use it as such. Keep referring back to it. Study the figures – they're there for a purpose. Remember that reading a science text takes time. Completing assigned readings before coming to class will allow you to ask questions if you are confused about any aspects of the reading.

A useful approach for text assignments is to begin with a first reading. This may be fairly quick, but your purpose is to understand the basic concepts of the text. Study the figures and consider their relation to the text. Remember, the figures are there for a reason. The authors are trying to show you something that they're discussing in the text. It should be clear to you what that is. As you read, take notes and write down any questions you have. Keep a list of terms or sections that you may not have understood.

Note that the above suggestions apply to reading a scientific paper as well. Remember that reading scientific papers typically takes more time than reading a section from your textbook.

### **Week by week success in a science course**

Always begin with your syllabus. What are the week's assignments? On Saturday you should be planning for the week ahead. What reading do you need to complete in preparation for the coming week? Some of it must be completed by Sunday evening in preparation for your Monday classes. Some of it can be completed later during the course of the week prior to specific classes. Let's consider a week and how you might approach it.

On Saturday you've examined your syllabus and you know what your assignments are. Begin with a quick overview of the reading. Look at the figures as you skim the text. What are the main concepts that the reading is addressing? Your goal here is to get the big picture. On Sunday you should read the chapter again. Your goal is to catch the details. You should be taking notes at this point. The relationship of the figures with the text should be clear to you at this stage. If not, start your list of questions that you will be asking your instructor, mentor, or teaching assistant. Keep a list of key terms and list any that you don't fully understand. You'll want to ask about these. Once you're done, get a good night's sleep.

On Monday, go to class (on time)! Take notes as you listen to your instructor. You should make marks in the margins if you're unclear about the content of some part of the lecture. These will be questions you may ask later. At the same time, if something is unclear to you, don't hesitate to ask a question in class. If you're confused, chances are good that your peers are too. Be sure to participate in discussions. Share your thoughts, your questions, and your opinions. Remember, you're going to learn from both your instructor and your fellow students. Contribute to making the classroom an interactive environment. Be sure to pick up any assignments or handouts before leaving class.

So, class is over. Is that it? Not quite yet. After class you should review your notes. Fill in any gaps in your notes. You may need to ask your instructor if you've missed something. Identify any questions you may have. Examine the notes to be certain the major concepts are clear to you. Be sure you can rework examples (i.e., problems) without the aid of your notes. If homework problems have been assigned, be certain to review the problems immediately so that you can ask any questions you may have. Try to do each problem as soon as possible. Work with a friend in the class. Working together with a peer is a good way to review.

So, you've done the reading and you've attempted the problem sets. Do you have any questions? If you do, make use of your instructor's office hours. Be sure to attend mentor sessions. These sessions are a great opportunity to review and ask questions. If you're concerned that you need more help, visit the Science Learning Center. Don't make the mistake of not using all the resources that are available to you. They're there for you, so make full use of them.



And the week continues... Be sure to keep up with the reading, problem sets, and other assignments. Don't fall behind. Once you do, it can be very hard to catch up.

### **So what to do with that first problem set?**

Well, for starters, do it! Don't use the approach that if it's worth doing, it's worth doing at the last minute. Review all the problems the day you receive them so that you can ask the instructor about anything that's unclear. Start the problem sets within a day of receiving them. Remember, most faculty start class by saying, "so, any questions"? Take advantage of this. Attempting to complete the problems early allows you to identify specific problems that you may need assistance with. Again, the later you begin, the less likely you will be to get the help you need.

### **How do I prepare for lab?**

Key to a successful lab is advance preparation. Read the lab manual and any assigned readings or problems. Your instructor may ask you to prepare your laboratory notebook in advance of the lab. Be sure to do this so that you will be prepared to work efficiently. Be certain to come prepared to ask any questions you have regarding the procedures, the conceptual content of the lab, or its relation to the overall content of the course. Most of your lab work will be done with other students – you owe it to them to come to lab prepared and on time. While conducting the lab, try to look beyond the details of the technical work and keep the main point of the exercise in mind.

### **Preparing for and responding to exams**

If you are completing course assignments and getting timely answers to your questions, you should arrive at the exam with a firm foundation to successfully answer the questions presented to you. Cramming is rarely a good strategy, and a good night's sleep is also key to your performance.

Remember that the exam is an important tool that will help you chart your progress in developing an understanding of the material. Use your exams to identify concepts that you have not yet mastered. As the old adage goes, "learn from your mistakes." Be certain to review your exam, consulting any keys that may be posted, and ask your instructor about any unresolved questions you may have.

## Orientation to the Biology Laboratory

Reviewed April 14, 2011

Any laboratory contains many hazards. An important part of your instruction is development of an awareness of these hazards and an ability to deal with them. Some of the dangers of particular concern are:

1. Chemical burns to the eyes.
2. Fire hazards.
3. Chemical toxicity.
4. Cuts and related injuries.

A discussion of safety considerations and safety equipment follows.

### [1] Selection of Chemicals

*The faculty member in charge of the lab must approve the selection of any chemical substance for a reaction or process not already specified by standard procedures.*

### [2] Obtaining Chemicals

[a] *Chemicals will only be obtained from the store room by permission of the supervising faculty member. No student will enter the store room or remove chemicals without prior consent.*

[b] *Material Safety Data Sheets (MSDS) will be posted in the lab for any hazardous chemical that will be used in the course of the lab.*

### [3] Personal Protective Equipment

[a] *When running reactions, pouring, or mixing chemicals with significant eye toxicity, goggles will be used. The degree of eye hazard presented in the lab will be left to the lab instructor's discretion. The safety goggles must be on and covering your eyes (not your forehead or throat) regardless of what you are doing.*

*Special note to contact lens wearers: Many studies within the past few years have highlighted the hazards of contact lenses in laboratories. If a corrosive liquid gets behind a lens, the process of washing out the eye is not effective unless the lens is removed. However, the natural reflex to close the affected eye makes removal of the lens nearly impossible. In addition, the newer "soft" lenses can actually absorb and concentrate vapors from the lab atmosphere, leading to eye irritation and, in some case, to damage the lenses themselves. Therefore, the wearing of contact lenses in lab is strongly discouraged.*

[b] *Gloves will be worn for handling of all chemicals that may cause irritation, allergic sensitization or skin absorption of toxic chemicals. The employee must check the MSDS for the chemical to ensure that the type of glove material used is protective for the substance.*

[c] *Listed below are several safety and emergency items generally found in each lab. Familiarize yourself with their location before beginning any lab work.*

#### Fire Extinguisher

*Fire is an ever-present hazard. Before lighting a Bunsen burner, make sure that no one is using a flammable solvent nearby. If you are uncertain, ask everyone around you. Also check that all flammable materials such as books, notebooks, paper towels and so forth are moved a safe distance away. Fire extinguishers are located in each lab and the hallways. Before beginning work be sure to note the location of the nearest fire extinguisher. To operate a fire extinguisher remove the safety pin, aim the nozzle at the base of the fire, and squeeze the handle to discharge the chemical flame retardant.*

#### Fire Blanket

*Intended to smother flames if clothing catches fire. There are fire blankets in every lab.*

#### Eye Wash Fountain

*These fountains are very useful for extended irrigation of eyes that have received a chemical burn. Eye wash fountains are available at the sinks of every lab. If you get a chemical in your eyes, it is imperative that you wash your eyes immediately.*

Safety Shower

*In the event of a serious chemical spill, where much of the body and/or face is involved safety showers are available. The safety showers in the biology department are located in the corridor outside Rooms 0610, 1003, 1010, 1011, 1809, 1814, 1819, 1823. To operate the shower, pull the lever and water will flow from the showerhead until the lever is returned to the off position. To ensure that all traces of the chemical have been removed it will be necessary to remove all contaminated clothing.*

First-Aid Kit

*First-aid kits are located in each lab. Even minor injuries should be reported to the lab instructor.*

[4] Location and Equipment to be Used for Specific Procedures

- [a] *Fume hoods will be used for all procedures involving concentrated acids, alkalis, and toxic chemicals with PEL's less than 50 ppm.*
- [b] *When using the fume hood, keep chemicals away from the face. Report any problems with hood operation.*

[5] Personal Hygiene Measures

- [a] *No eating, drinking, or smoking is permitted in the lab.*
- [b] *No mouth pipetting is allowed.*
- [c] *Your clothing should be comfortable older clothes that you can tolerate losing. The lab is not the place for three piece suits and tweed skirts. Clothing should essentially cover all of your body and should not have loose floppy sleeves or other appendages that can get in the way, catch fire easily, or catch on apparatus. Shorts are discouraged and will not be allowed on days during which strong acids and bases or other corrosive chemicals are used.*
- [d] *Shoes must be worn in the lab. The shoe must provide complete coverage of the foot. (Sandals do not provide adequate protection.)*
- [e] *Students must wash hands after handling any chemicals before leaving lab.*

[6] Spill and Leak Procedures

- [a] *Very small spills of low-toxicity chemicals can be absorbed with paper towels and allowed to evaporate under the hood after which the towel may be disposed of.*
- [b] *Larger spills: Evacuate lab, notify lab instructor.*
- [c] *For biological spills, notify the lab director and follow the spill clean-up procedure posted by the lab sink.*

[7] Waste Disposal

- [a] *The environmental officer must evaluate disposal procedures prior to the disposal of any waste at any time. No employee will dispose of any chemicals down the sink or in waste containers without approval.*
- [b] *Biohazardous materials (broth cultures, petri plates, plastic pipette tips, etc.) will be disposed of in labeled receptacles marked biohazardous waste.*
- [c] *Receptacles for broken glass and "sharps" will be provided in each lab. Broken glass and "sharps" will not be disposed of in the general trash.*

[8] Procedures for Lab Operations

- [a] *Pouring, mixing or reacting significant volumes (greater than 10 ml) of severely irritating or corrosive chemicals:*
  - {1} *Goggles or face shield, gloves and lab coat must be worn*
  - {2} *The operation should be carried out under the hood or in the sink*
  - {3} *At least one other employee should be in the lab.*

## Orientation to the Biology Laboratory

### [b] Use of volatile toxic chemicals:

- {1} All such chemicals will be handled under the hood
- {2} Gloves and eye protection will be worn
- {3} Students will wash hands after handling
- {4} Any symptoms or accidental over-exposure must be reported to the lab instructor..

### [c] Use of biohazardous agents (Biosafety Level 1 and 2)

- {1} Access to the lab is permitted only to students enrolled in the course.
- {2} Decontaminate lab benches with Lysol before leaving the laboratory.
- {3} Liquids are decontaminated with 10% bleach before disposal. Rinse all contaminated glassware with 10% bleach.
- {4} Mouth pipetting is not allowed.
- {5} Eating, drinking, smoking, and applying cosmetics are not permitted in the lab.
- {6} Wash hands after handling biohazard agents and also before leaving the laboratory.
- {7} Place contaminated disposable plastics and petri dishes in biohazard trashcans.
- {8} Wear laboratory coats while in the laboratory. The use of gloves is optional. However all skin abrasions and cuts should be covered. Remove protective clothing before exiting the lab.
- {9} Use extreme caution when handling hypodermic needles and sharps to avoid autoinoculation and the generation of aerosols. Needles and syringes should be disposed of in a puncture proof container for sharps.
- {10} Do not handle broken glassware directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
- {11} Spills and accidents should be immediately reported to the lab director. Refer to the spill clean-up procedure posted by the lab sink.

Before you begin any actual lab work, find each of the safety devices listed above in Section 3. Note their location and be certain that you know how to use them. If you do not, ask your instructor or an assistant to show you.

When you have done this, please sign the following statement that indicates that you have familiarized yourself with the safety equipment and that you agree to abide by the safety policies of the laboratory. (Additional copies of this statement will be provided so that you do not need to remove this page from your laboratory manual)

---

I have found and examined the safety devices mentioned and understand how to use them. Furthermore, I agree to follow all the safety rules and any other rules that my instructor delineates. I understand that failure to follow the rules will result in being asked to leave the lab.

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Instructor: \_\_\_\_\_

Course: \_\_\_\_\_

Lab Section: \_\_\_\_\_

## II. Undertaking Investigations

All scientists design investigations to address questions about natural phenomena. However, investigations can vary in approach, depending on their immediate goals:

- To practice a technique or analysis, or assess its reproducibility.
- To measure or describe something from nature.
- To create something not found in nature.
- To find a qualitative "yes or no" answer.
- To compare groups, in an experimental or observational setting.
- To determine relationships between quantitative variables.

Interesting investigations are motivated by good questions, i.e., ones that are relevant to larger issues in chemistry and biology and are answerable. Developing good questions takes time and practice, and it certainly doesn't take place in a vacuum. Your instructors will encourage you to use your prior knowledge and the scientific literature to develop good questions. Using the scientific literature BEFORE you start your investigations can help you:

- identify your area of interest and previous work relevant to it,
- define the significance of your question,
- find methodologies or techniques to carry out your investigation, and
- understand the relevance of your products/results to the discipline.

### Using the Scientific Literature

As you explore the scientific literature, it is important to appreciate the distinction between its different forms. The *primary literature* consists of journal articles, in which scientists describe and interpret the results of their investigations for the benefit of others in their field. You probably have little experience with the primary literature, and one of our goals is to help you learn to use it -- it is a wonderful resource. The *secondary literature* is variable in form and quality, ranging from scientists synthesizing a body of primary literature for other scientists, to scientists writing for the general public, to journalists writing about science for the general public. Ask your professor if it is appropriate to use secondary literature to inform your investigations. The Kistler Science Library has many resources (including librarian Kevin Engel) to help you to learn to use the scientific literature (see [www.lib.grin.edu/places/scilib](http://www.lib.grin.edu/places/scilib)).

#### Some Tips for Reading and Learning from the Primary Literature

The editors of scientific journals are scientists themselves and hence strive to make papers easily accessible to other scientists. With that in mind, here are some guidelines, which may help you read and learn from primary literature in the biological sciences.

1. Read the title. What does it mean? Write down all the questions that it raises.
2. Now read the summary/abstract. The abstract will usually provide complete clarification of the title, and describe the essence of this study. You should be able to answer all of the questions you had from the title (if the abstract is well-written and you are sufficiently well informed on the subject matter). In turn, the abstract may raise further questions. The abstract is only intended to summarize the important aspects of the study, so you will need to read the body of the paper to assess the quality of the data and the interpretation of the results upon which these conclusions are based. Similarly, reading the body of the paper will allow you to assess whether the methods and statistical analyses performed were appropriate for the question being examined.
3. Read the introduction. This is an important section because it tells you something about what is already known regarding this subject, provides any background you might need to understand the work, and it should clearly set forth the experimental question(s) the authors were addressing in their work. At this point you should have a good idea where the paper is headed. When you are finished reading the abstract and introduction you

## Choosing and Planning an Investigative Approach

should ask yourself, what are the authors doing in this paper and what is the overall importance of their work. As you read, if the authors use terms or introduce concepts that you are not familiar with, you should consult your textbook to see if it can provide any clarification.

4. Next look at the figures, graphs and tables. Read the legends and try to ascertain what's being presented visually. Is their relevance obvious to you? How do YOU interpret the data? It's worthwhile taking a moment to jot down any results that seem significant to you, prior to reading the authors' interpretation of the data.
5. Prior to reading the content of the paper, check the references and notes section for information-rich notes. Note the numbers of these references and highlight these numbers in the text. When you are reading the paper, you will now know which references are worth checking for further information that may be of interest to you.
6. Now it's time to read the content. Read through the body of the paper in its entirety and then try to answer the questions that arose from reading the abstract and title. If all questions are answered you should have superb notes that have helped determine the essence of the study and why you were interested in it in the first place.

If you have not been able to answer your questions, try to sort out why. This type of analysis is essential if you are to learn from the primary literature, rather than simply being overwhelmed by it. There are three reasons why papers might be difficult to understand:

### **Your knowledge of the subject matter is inadequate.**

This may be a problem when you are conducting your literature searches. This might involve inadequate knowledge of biological concepts, study techniques and methods of analysis. Given that it is impossible for all of us to know everything about biology, you should strive to at least develop a list of questions regarding aspects of the paper that you do not understand. You can consult your biology textbook, the Internet, your peers, and your professor for clarification. These may be good questions with which to initiate a class discussion concerning the paper.

### **The data presented are not adequate for drawing the conclusions made.**

Again, seeking clarification from peers and other sources may help you find details that you may have missed while reading the paper. You may find that you disagree with the authors' interpretation of their data. Is YOUR logic sound? This is something worth discussing in class. Don't forget that while the purpose of peer-review is to try to minimize these types of errors, reviewers are human too.

### **The paper is poorly written or just plain wrong!**

Another important aspect of peer review is to ascertain that gross errors are minimized and that the paper is readable. In most journals, this is generally well done. It is thus unusual to read papers where the statistical analyses performed are inappropriate, graphs are inappropriately labeled, or hypotheses are not rigorously tested. Such problems would usually result in a paper being rejected by a journal. That is not to say it doesn't happen, however. If you think a paper is extremely poor, you should make a concerted effort to solicit opinion from your peers about your interpretation of the work. Try to pinpoint your problems with the paper in order to facilitate an effective discussion that addresses your concerns.

## Choosing and Planning an Investigative Approach

Sometimes an investigation entails a clearly defined experiment, while in other cases a careful set of observations may be called for. Often the term "experimental" is used to describe the entire scientific process of testing ideas through careful observation and analysis; *we will use the term 'experimental' in a more restricted sense here, to refer to investigations in which the scientist manipulates some feature and observes the result.* In contrast observational studies look for predictions that arise from a theory or measure outcomes of manipulations not intentionally created by the investigator. Table 1 lists six approaches to designing a scientific investigation, as expressed by its immediate goal. Beside each approach appears (1) examples of matching activities (2) the types of analyses used, and (3) ways in which analyses may be represented. One of our common teaching goals is to help you understand how the process of science involves a mix of these approaches.

## Choosing and Planning an Investigative Approach

Table 1. Choosing an Investigative approach

Immediate Goal	Examples	Analysis	Possible Products
To learn a technique or assess its reproducibility	Dissection of a nerve	Summary statistics	Graph
	Measurement of soil carbon content	Summary statistics	Table (if necessary)
	Distillation, titration	Comparison to physical law	Graphs, outcome with error
To find a qualitative "yes or no" answer	Restriction enzyme digest	Image analysis	Pictures
	Determination of reactivity or solubility	Yield, summary statistics	Spectroscopic figures,
To compare groups, in an experimental or observational setting	Comparison of plant productivity across sites or experimental treatments	Summary statistics, t-test, or analysis of variance (if data quantitative); frequencies, chi-square analysis (if qualitative)	Bar graph, box plot, table
	Determination of structure-activity relationship	Relationship to physical principles	Bar graph
To determine relationships between two quantitative variables	Test of Beer's law	Estimate correlation coefficients or perform regression analysis.	Scatter-plot (for correlation), linear or curvilinear regression plot, table
	Test of organismal character correlations		
	Standard curve development		
To describe something in nature	Spectroscopy	Comparison to physical principles	Spectroscopic picture Picture
	Gene mapping	Recombination frequencies, database searches	Tables, pictures
	Species or community description	Summary statistics, if applicable.	
To create something not found in nature	Organic synthesis, protein purification	Characterization, yield	Figures
	Mutagenesis	Screening	Table/description
	Genetic crosses	Summary statistics, chi-squared	Bar graph/Table

You will find in your biology, chemistry, and other natural science courses that designing an investigation is often an iterative process. The types of analyses that are available and appropriate will inform, and sometimes change, the type of investigation you decide to undertake. One example of an "investigation planning form" is shown below. As you design scientific studies, you should follow a process that allows you to answer the questions on the form. Importantly, many of your investigations will be designed to address a specific hypothesis, which should be carefully justified.

As the form suggests, you should never initiate a study without first considering the data analysis that will be required, as well as the appropriate presentation products -- these issues are covered in the next section and the appendix. Developing good questions, refining testable hypotheses, and designing informative investigations are the skills we are asking you to master, because we believe they will allow you to understand both process of science *and* its results. In your biology and chemistry courses you will participate to varying degrees in the planning process of scientific investigations. For any investigation, you should be able to address these points. An example plan is given in italics but your answers will vary depending on the kind of experiment you are planning.

- What is your central scientific question?

*Does the concentration of sea urchin sperm in sea water have an effect on the number of sea urchin eggs that get fertilized?*

- If an hypothesis is appropriate for this study, state and justify your hypothesis.

*As the concentration of sperm increases, the percentage of fertilized eggs will increase.*

- What results are possible and what results do you predict?

*We predict that increasing sperm concentration will increase the percentage of eggs fertilized. It is also possible that there will be no effect on the number of eggs fertilized or that the reverse of our prediction will be true: increasing sperm concentration will decrease the percentage of eggs fertilized.*

- Describe briefly the study design and methods you will use, including experimental and/or observational features, sample sizes, number of replicates, etc.

*Our experiment will test six different concentrations of sperm: 1:40; 1:200; 1:400; 1:2000; 1:4000; and 1:10,000. For each replicate we will add 25 µl of the designated sperm dilution to a small culture dish containing 3 ml of seawater and 3 drops of concentrated eggs. Each concentration will be tested in four dishes (4 replicates). Five minutes after adding the sperm we will examine 30 eggs from each dish and observe whether or not a fertilization envelope has developed, then calculate the percent fertilization for that dish. For each sperm concentration we will calculate the average percent fertilization of the four replicates.*

- What data will you record?

*We will record the percent fertilization of four dishes of eggs for each sperm concentration.*

- When and how often will you record data?

*We plan to count the percent fertilization five minutes after the sperm dilution is added to the eggs. Another timing factor may be the amount of time the sperm have been diluted so we will make each sperm dilution right before adding it to the dish of eggs.*

- Who will record data, and when?

*We have two people in our group so to prevent bias we will each score two replicates from each of our sperm concentrations. We will do the entire experiment during class and will not need to come in at other times or set up a data collection schedule.*

- How will the data be analyzed? What statistical test (if any) will you use?

*We will calculate an average and a standard error for each sperm concentration. T-tests will be run on the entire data set to test for statistical differences in fertilization percentages between the concentrations of sperm.*

- How will you display the results of your study? If you plan to use one or more graphs, draw prototypes of them, including a sample figure caption and labeled axes. Be sure the graph presents analyzed data – not raw data. Include error bars if necessary.

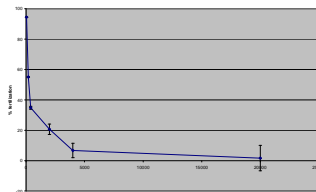


Figure 1. Average % fertilization of sea urchin eggs at six different sperm dilutions. Error bars represent  $\pm 1$  s.e.

- If you plan to use one or more tables, sketch prototypes of them, indicating what data the table will report.

*We do not plan to use a table.*

- Do the results obtained from your data and reported in the tables and graphs relate directly to your question and, if applicable, your hypothesis? Explain

*Yes, the graph of our data shows % fertilization as the dependent variable on the y-axis and the sperm dilution as the independent variable on the x-axis. These are the two variables stated in our hypothesis.*

**Materials list:**

Number and Kind of Organisms needed:

*We will need to use the sea urchin eggs and sperm supplied for the class.*

Chemicals / Solutions: 500 ml sea water, distilled water

Media: Type # of plates: Date needed:

*no media needed for this experiment*

Other:

Equipment:

*In addition to the regular equipment used for sea urchin fertilization we will need :*

*graduated cylinders 1000ml 500ml 250 ml, 100 ml 50 ml centrifuge tube, 15 ml centrifuge tube, 5 ml centrifuge tube, 4 or 5 micro-fuge tubes, Micropipettors :1000, 200, 20 µl*



## Data analysis

Planning and carrying out proper data analysis is a fundamental part of the process of scientific investigation. In this section, we mention some general techniques of data analysis that will apply across many classes. In addition to the synopsis of information in this section, Appendix A contains detailed information about the statistical tools and tests mentioned in this section. Instructions for using computer software to calculate them are also contained in Appendix A. In some cases you may have to learn analyses specific to various instruments or sub disciplines that are not covered in this handbook.

### Summary statistics

Summary statistics, which serve to describe individual data sets, are a practical tool for distilling useful information from large masses of data. While they cannot determine whether or not one data set is statistically different from another, they are valuable in their own right. Some of the summary statistics we commonly use in scientific investigations are: *the mean, the sample variance, standard deviation, and the standard error of the mean*. By using these basic statistical calculations you can transform your raw data into three or four numbers, which speak volumes about what is contained within it. Definitions and explanations of these statistics, as well as instructions for calculating them can be found in Appendix A.

### An introduction to statistical hypothesis testing

Scientists often want to compare groups of observations to see if they differ. Groups can be defined based on a categorical variable or factor created in nature (e.g., males and females), or a variable manipulated by the scientist (e.g., the amount of fertilizer given to a plant). The goal of such comparisons is to determine whether the two groups differ in a continuous variable (for example, height), which in turn can be used as support for a claim of causation, e.g., that an increase in fertilizer causes increases in plant growth. If you find a difference in mean (average) values for the continuous variable between the members of each group you sampled, you might be tempted to conclude that your grouped observations or experimental treatments have revealed a meaningful effect on the variable you measured. *Unfortunately, conclusions drawn from mean values can be misleading.* Measurement error and uncontrolled environmental variation can cause two means to differ somewhat, but it would be wrong to interpret differences caused by these factors alone to represent differences caused by the factor that you are investigating. To determine whether a difference between two means is scientifically meaningful, we need to partition out the variation in our data that is caused by a given variable or experimental manipulation from that caused by measurement error and environmental variation. In other words, we need to analyze our data using statistical hypothesis tests.

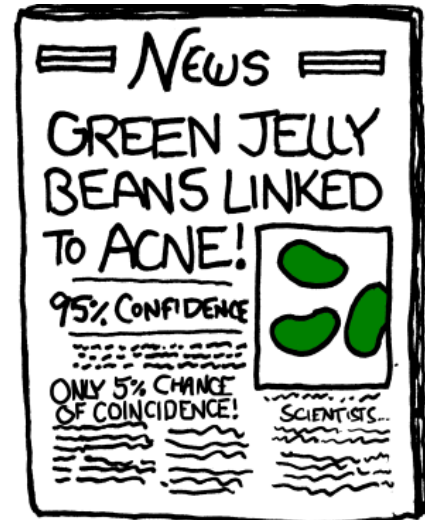
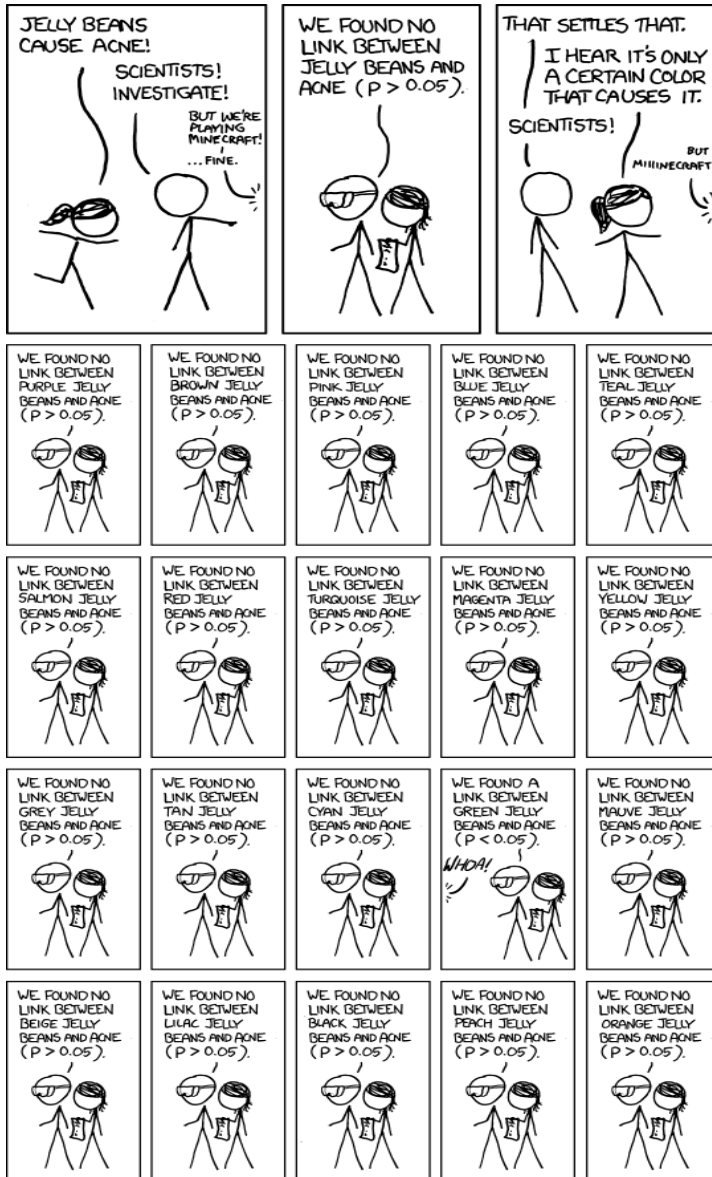
#### What is a hypothesis?

A hypothesis is an educated guess about what explains a natural phenomenon under investigation, formulated in terms of the particular data you have collected. A statistical hypothesis test allows you to discriminate between an *alternative hypothesis*, which is your estimation of the effect of a given variable or experimental manipulation on the data you have collected, and a null hypothesis, which is the idea that the variable or manipulation you're studying will have *no effect* on your data.

#### What is a statistical hypothesis test?

Statistical hypothesis testing requires you to calculate new statistics, called *test statistics*. Generally, as the absolute value of a test statistic increases, so does your confidence that you can reject the null hypothesis. How large the test statistic must be for you to reject the null hypothesis declines with sample size. You will never be certain that the alternative hypothesis is true; all you can have is some defined level of confidence that the null hypothesis is false. By convention, when the absolute value of a test statistic is so large that there's less than a 5% chance that the null hypothesis is true, (a so-called alpha or P-value of 0.05 out of 1), scientists reject the null hypothesis and tentatively accept the alternative.

When using a P-value of 0.05 it is possible that one out of twenty times the outcome is due to chance alone. These two hardworking scientists failed to keep this in mind before announcing their exciting discovery.



### Which statistical hypothesis test do I use?

Many kinds of statistical tests exist, but some are much more commonly applied than others. Described below are a few of the simplest and most common tests used in biology and chemistry. *More detailed descriptions of the methods and uses of each test, along with examples are located in Appendix A.*

1. The *t*-test. (Appendix A pg. 42) A t-test is used when you want to compare the means of two groups. A t-test addresses the alternative hypothesis that an observed or manipulated variable (the *independent* variable) that falls into two categories affects a second variable (the *dependent* or *response* variable) that is measured on a continuous scale.
2. The *analysis of variance* (often abbreviated as ANOVA). (Appendix A pg. 45) An ANOVA is used when you want to compare more than two groups (e.g., an independent variable that falls into more than two categories).
3. *Regression analysis and correlation analysis*. (Appendix A pg. 54) Regression and correlation analyses are related techniques that are used to look at the *relationship* between two variables. These analyses differ from the t-test and ANOVA because they are used when your data do *not* fall into discrete groups, but are instead continuously distributed. Correlation analysis allows you to examine the association between two variables without making any assumption about the causal relationship between them. In other words, you can use correlation analysis when you do not know if variable 1 is influencing variable 2, or vice versa. In contrast, regression analysis is used when you believe that one variable (the independent or predictor variable) influences the other (the dependent or response variable). One result of a regression analysis is a line or curve showing the best prediction of the true relationship between variables, given the data at hand; for this reason, it is sometimes referred to as "curve-fitting."
4.  $\chi^2$  tests.  $\chi^2$  (pronounced "k-eye-square") analysis and related techniques (Appendix A pg. 61) are used when both the independent and dependent variables are categorical (i.e., fall into discrete groups), so what needs to be *analyzed* is the pattern of *frequencies* of different outcomes.

### Checking data for errors

Once you have chosen an appropriate study design and statistical approach, and after you have collected your data, it is important to examine your data for errors *before* proceeding to data analysis. Check the data for accuracy in your notebook before you transcribe them, and check them for accuracy again after you transfer them to spreadsheets or statistics programs. Look for missed entries, decimal-place errors, and misspellings. A good habit to develop is to make scatterplots or histograms of your data, which will give you a feel for the overall distribution of the data and help you spot errant entries your earlier checks missed. To be clear, data-entry errors that happen while *recording* data are quite different from outliers in the data. Correct all data-entry errors, but don't reject an unusual (but correctly recorded) data point without a valid reason. Check with your instructor before removing an outlying data point or using the q-test (below).

### Precision and relative error -- the q-test

One frequently encounters data sets in which three values are close together and one or two values are not. Does one discard the bad value and report the three good ones? Usually not. What is gained in precision will usually be lost in accuracy. The *Q test* can be helpful in making this decision. The Q test allows you to discard a data point with a certain level of certainty (usually 90% or greater). Details on the test can be found in the Appendix A pg. 64.

## Data presentation: principles of figure and table design

After analyzing your data, your next step is to summarize your data in graphs or tables. One of the goals of this activity is for you to understand what the data mean; that is why statisticians call this phase *data visualization*. Of course, graphs and tables ultimately have the function of helping you communicate your investigative results to an audience through a paper, poster or oral presentation (see Section III). You should expect this process to be iterative -- in other words, you'll have to refine your figures and tables several times before they effectively convey the important features of your results. Don't consider this a failure; it's the same concept as revising a paper before handing it in.

## Designing figures and tables for a paper or poster

Graphs and tables frequently will help the reader to understand complicated data more easily than a written description. Note, however, that if the data can be easily summarized in the text (e.g., they consist of 2-3 numbers), a figure or table is not necessary. The text should tell the reader the important points to be noted on the graphs or tables, or call out specific examples from the figure or table to illustrate a point. Obviously the same data should not be presented in two different forms (e.g., a table should not contain raw data that is summarized in a graph), so decide which form helps you tell your reader what you want him/her to know. Graphs of any kind, as well as other pictorial materials, are referred to as "Figures" in the text. Tables are called "Tables" in the text, and are numbered separately from figures. Both are numbered in the order in which they are referred to in the text (so you shouldn't refer to Figure 3 before Figure 1 is mentioned -- if you need to, renumber your figures).

**Tables**

- Use tables to present matrices of data. If it is important to show a pattern or trend, use a figure instead of a table.
- Give each table a number, but number tables separately from figures. In Chemistry, the convention is to number tables with Roman numerals (I, II, III etc.).
- Make sure that all columns of a table are clearly labeled. Each table needs a title or a legend so that it can be understood on its own. It is often appropriate to have footnotes for a table.
- Try to avoid large tables, as no one will read through them. Perhaps you can present the information better in several smaller tables.
- All tables must have legends (above the table) which contain a single phrase or sentence serving as a title for the table. It is sometimes appropriate to have footnotes for a table.
- **Do not present raw data and expect your reader to do the arithmetic before she can understand the contents of a table! While an EXCEL spreadsheet has rows and columns, it is NOT a table.**

*Example of a table:*

The table shown below presents data that are qualitative rather than quantitative. The legend clearly tells the reader what those data are about. An explanation of the nomenclature used for the colors is presented in a footnote.

Table 1: Effect of medium and temperature on color development in *Serratia marcescens* colonies.

<sup>1</sup>Numbers refer to paint color chip designations.

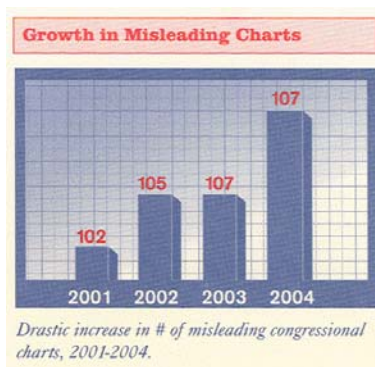
Medium	Temperature		
	4° C	22° C	37° C
Minimal Salts + glucose	no growth	pink 6 <sup>1</sup>	colorless
Minimal salts + succinate	no growth	pink 2	colorless
Peptone-glycerol	no growth	pink 7	colorless
L-agar	no growth	red 2	colorless
Trypticase-soy	no growth	brown 2	colorless

**Figures**

Data presentation: principles of figure and table design

**See Directions for Making Figures in Minitab (Appendix B, page 68) for detailed instructions on making figures using Minitab.**

Use a figure to illustrate a relationship or pattern in your data. Produce them on a computer (unless otherwise advised), using a spreadsheet, graphical or statistical package. This will allow you to easily revise figures. Be aware, however, that the computer programs do what you tell them to do, whether it makes sense of the data or not! It's your responsibility to make sure the graph accurately represents the data. As seen in the example below, you must look critically at a figure to be sure it is telling the right story!



- By convention, the independent variable (the one you manipulated or the one that you think causes variation in the other variable) is on the x-axis and the dependent variable (the result of the manipulation) is on the y-axis. Be economical of space. Do not extend the axes unnecessarily. The axes of a graph must have clear, concise labels that are large enough to read easily. Don't forget to include units, if applicable!
- If there is more than one line or bar on the figure, identify each of them.
- Relationships between variables are usually presented in a *scatterplot*. If you are fitting a curve to the data (e.g., using a regression analysis), you should include the line, the regression formula,  $r^2$  value, and p-value for the statistical technique used. In some cases, it may be appropriate to "connect the dots" to produce a *line graph*. This should never be done unless the x-axis represents a continuous variable (i.e., one in which gradations between units are meaningful), and you should ask your instructor whether a curve-fitting technique might be more appropriate.
- When the x-axis represents categories, rather than quantitative data, a *bar graph* is often preferred. If the bars represent averages of the value of the y variable, you **must** include some measure of the precision of that estimate of the mean. In most disciplines, the convention is to place an error bar on each bar that represents  $\pm 1$  S.E. (standard error of the mean).
- For papers, a clear and specific legend should be placed below each figure. A legend begins with "Figure 1." followed by a description of the figure, usually written as an incomplete sentence (!) with only the first word capitalized. If the figure includes error bars the figure legend must say what the error bars represent. You may also want to include the test statistics and p-values for statistical analyses you did. Include the sample size ( $n=$ \_\_) for each treatment. If required for clarity, you may include several more sentences, although the acceptability of this varies among disciplines (check with your instructor).

Type of Analysis	Statistical Tool	Suggested figure
Showing the distribution of data		Histogram
Presenting values of means	Summary statistics Appendix A pg. 39	Bar graph or box plot with error bars indicating standard error of the mean. Appendix B pg. 70
Comparing the means of 2 groups	t-test Appendix A pg. 42	Bar graph or box plot with error bars indicating standard error of the mean Appendix B pg. 70
Comparing the means of more than 2 groups	ANOVA Appendix A pg. 45	Bar graph or box plot with error bars indicating standard error of the mean Appendix B pg. 70
Association of 2 variables	Correlation Appendix A pg. 54	X-Y Scatter plot with a fitted line Appendix B pg. 68
Causal relationship of 2 variables	Regression Appendix A pg. 54	X-Y Scatter plot with a fitted line Appendix B pg. 68.
Comparison of frequency patterns	$\chi^2$ Appendix A pg. 61	Table or histogram Appendix B pg 69

### Checklist for Figures

All figures should be checked for the following:

- Appropriateness of figure type. Bar graphs are used when the independent variable (x-axis) is categorical. Line graphs are used for continuous relationships.
- Error bars (usually +/- s.e.) must be shown whenever means are given.
- x and y- axis must be labeled and units given.
- Axis labels should be large and fit along the entire axis. The **top graph** has appropriate sized axis labels while **the lower graph** has axis labels that are too small.
- Symbols may be used to call out significant results.
- Gridlines and background borders should be deleted from the chart.
- A figure legend must be included at the bottom or the top of the figure (below for graphs and above for tables) and it should include:
  - a figure number and a description (title) of the figure
  - the sample size (n)
  - the p-value(s) and/or test statistic(s) from the statistical analysis
  - a definition of what the error bars represent
- Figures/graphs may have titles, but *titles should be incorporated into the figure legend, not presented as a banner at the top of the figure*. Delete the titles made automatically by Excel and Minitab.
- A category legend is only included when necessary for different lines or categories. Delete if not necessary.
- Line graphs are used only when there is a continuous relationship between points on the x-axis. Check with your instructor about when curve fitting is appropriate.
- If a regression is done, include the line, regression formula, and  $r^2$  value. (none in this example).

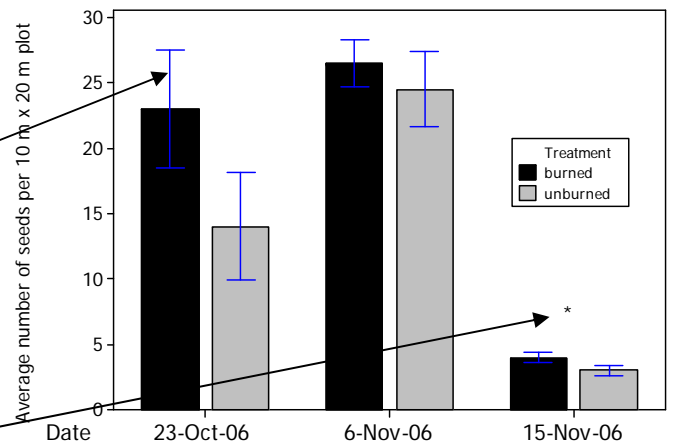


Figure 1. The effects of burning on mean number of seeds per 10m x 20m plot over three collection dates at Conard Environmental Research Area. Error bars represent 1 S.E. \* designates  $p < 0.05$ .

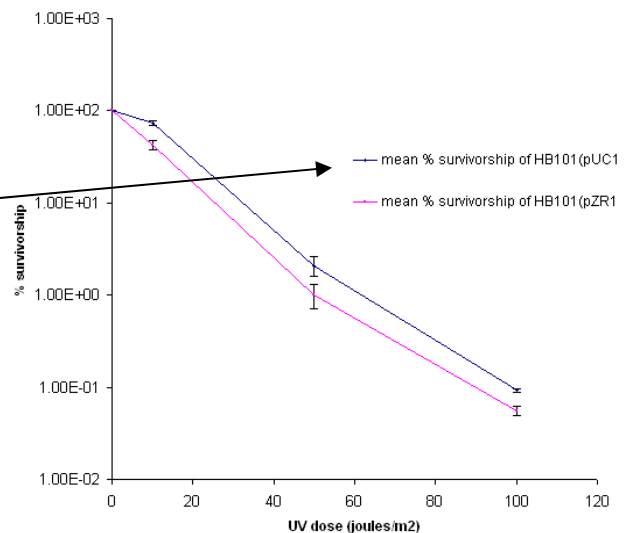


Figure1. Percent survivorship of HB101(pUC19) and HB101(pZR100) cells at varying UV doses. Data represent means (+/- 1 s.e.) of at least 3 independent trials.

## Maintaining a record: keeping a laboratory and field notebook

It is important to keep an up-to-date record of your work *every time* you are in the lab and/or field. Your lab notebook is the official place to keep a record of all the methods and results of your experiments. It serves as a source of your logic concerning your study as well as a place to include notes and observations. To guide how much information and detail should be included, use “the two year rule”. That means you should be able to understand your entries and be able to navigate in your notebook at least two years after making the report. If something looks suspicious, write it down; if something looks interesting, write it down; if you have a problem, write it down...you get the idea. A lab notebook should *always* be written in pen so that it can serve as a permanent record; a field notebook may be written in pencil, if the notebook is in danger of getting wet.

Individual professors have specific preferences for lab notebooks so check with them, but here are some general guidelines regarding the format of a lab notebook:

- At the front of the notebook, leave room for a 3-page table of contents. As you make entries, record titles and page #'s in this index. Titles should be based upon the specific experiment/activity and sometimes the technique(s) used.
- Number each page.
- After the index, you may want to only write the bulk of your entries the right hand side, reserving the left page for scratch paper that can be used to make unorganized notes or record insignificant calculations.
- For each project, record an outline or flowchart depicting the general scheme of your work. Text accompanying this should briefly describe the rationale, significance, and expected products of your study.
- For each entry (usually daily) include and clearly label the sections described below:

*Date.*

Include the date for each entry.

*General and specific objectives.*

The general objective should include information described in step 3 above or a brief summary of information described in step 3 with a reference to the page number of the complete project outline. The specific objective should briefly describe exactly what you are doing and why you are doing it. If you are conducting an isolated, simple experiment, you may only need to include a specific objective.

*Methods and Materials.*

Reference procedures in as much detail as possible. Often it is OK to include complete reference to a published procedure with notes on modifications or other information specific to your work.

*Results.*

Often these are depicted in figures and/or tables.

*Discussion.*

Usually your discussion summarizes your results, relates results to the objectives described, and indicates how your results inform future work.

**To view pages from a good example of a laboratory notebook see Appendix C pg. 79.**

### III. Communicating the Results of Scientific Investigations

While many people investigate the world for the pure love of discovery, science is only made complete when discoveries are communicated. Many scientists love to do this as well. In this section of the manual, we describe three ways scientists convey ideas to their colleagues: scientific papers, posters, and oral presentations. Each has distinct forms and conventions, although the similarities among them should also become clear. We describe how to approach each type of communication, their conventions, and our methods of evaluating them. As in all courses, it is important that you check your instructor's expectations.

#### The Scientific Paper

##### ***What is a scientific paper and why do we write them?***

A scientific paper is a formal way for working scientists to report the results of original investigations in a public and permanent fashion. Papers appear as articles in scientific journals published in print and online. Due to the importance of this "primary literature" in informing new investigations, articles published in journals undergo rigorous peer review for clarity, accuracy and importance. We want you, as a working scientist, to understand this process and contribute to the primary literature through your own investigations.

##### ***General advice***

Like papers in non-science courses, your scientific papers should be well written, creative, and thoughtful. The purpose of a scientific paper is similar to other academic writing. It is a narrative of your investigations and an argument about their meaning. In many ways, the principles of scientific writing are the same as academic writing in other disciplines:

**Audience** -- Knowledge of audience will help you decide what terms or ideas you need to define and how formal to make your language. Assume the audience consists of peers, i.e., unknown readers with a similar background in the subject matter as your classmates.

**Brevity** -- Scientific writing is often described as concise and non-ornamental. This does not mean it has to be boring.

Vigorous writing is concise. A sentence should contain no unnecessary words, a paragraph no unnecessary sentences, for the same reason that a drawing should have no unnecessary lines and a machine no unnecessary parts. This requires not that the writer make all his sentences short, or that he avoid all detail and treat his subjects only in outline, but that every word tell. (Strunk and White 1979)

**Structure** -- The structure of writing helps convey your narrative and arguments clearly. The *Advice to Authors* (below) discusses the purpose of specific sections of a scientific paper. However, the careful construction of your writing should be apparent at all levels: sections, paragraphs and sentences.

**Conventions** -- As in most disciplines, scientific papers need to conform to particular stylistic conventions; in journals these rules are given in the editors' *Advice to Authors* (see below). It is important to understand the similarities and differences in the conventions of biology and chemistry and to inquire about specific expectations and requirements of individual professors.

There are three aspects of scientific writing conventions that bear special mention:

**Voice** -- . Discuss the following passages with your instructor to make sure you understand the conventions and expectations for use of passive and active voice for papers for a particular class.



From Alley's (1996) *The Craft of Scientific Writing*:

Many scientists and engineers hold the misconception that scientific documents should be written *in the passive voice*. Not true. Because the purpose of scientific writing is to communicate (inform or persuade) as efficiently as possible, and because the most efficient way to communicate is through straightforward writing, you should use the most straightforward verbs available. Needless passive verbs slow your writing; they reduce your writing's efficiency. . . . Is passive voice wrong? No. Although the *active voice* ("The oscilloscope displayed the voltage") is stronger than the passive voice ("The voltage was displayed on the oscilloscope"), there are occasions when the passive voice is more natural. For instance,

On the second day of our wildebeest study, one of the calves wandered just a few yards from the herd and was attacked by wild dogs.

In this example, there is nothing wrong with the passive verb "was attacked" because the passive voice allows the emphasis to remain on the wildebeest calf, which is the focus of the paragraph. The key to choosing between an active and passive verb is to ask which form is more natural. . . .

Some passive voice arises in scientific writing because scientists cling to the misconception that they can never use the first person ("I" or "we"). . . . As long as the emphasis remains on your work and not you, there is nothing wrong with judicious use of the first person. . . . First, you should reserve the use of the first person for those occasional situations in which your role in the work is important – for instance, when you make an assumption. Second, you should avoid placing the first person (either "I" or "we") as the beginning word of a sentence, because that position receives heavy emphasis. Instead, have the first person follow an introductory adverb, infinitive phrase, or dependent clause.

From Day and Gastel's (2006) *How to Write and Publish a Scientific Paper*:

Let us now talk about *voice*. In any type of writing, the active voice is usually more precise and less wordy than is the passive voice. (This is not always true; if it were, we would have an Eleventh Commandment: "The passive voice should never be used.")

As noted in Chapter 11, the passive voice sometimes functions well in the Methods section. Elsewhere in the scientific paper, however, it rarely should be used.

Why, then, do scientists use so much passive voice? Perhaps this bad habit results from the erroneous idea that it is somehow impolite to use first-person pronouns. Because of this idea, the scientist commonly uses verbose (and imprecise) statements such as "It was found that" in preference to the short, unambiguous "I found."

Young scientists should renounce the false modesty of their predecessors. Do not be afraid to name the agent of the action in a sentence, even when it is "I" or "we." Once you get into the habit of saying "I found" you will also find that you tend to write "*S. aureus* produced lactate" rather than "Lactate was produced by *S. aureus*."

An example from the first paragraphs of Watson and Crick (1953):

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other.

(2) Some of the van der Waals distances appear to be too small. Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it. We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid.

**Quotation -- Never use quotation without consulting your instructor.** Quotation is extremely rare in scientific primary literature articles and used only when the author is trying highlight the *exact words* used by another

## The Scientific Paper

investigator. Compare articles in biology and chemistry journals with those in the social sciences or humanities to appreciate how different these practices are between disciplines. Paraphrase your sources carefully, and cite your sources according to the conventions of the particular journal (see below). **Please remember, however, that this rule does not grant permission to use verbatim language from sources without quotation marks!** Your College Student Handbook contains excellent advice on how to paraphrase while maintaining academic honesty.

### Honesty In Academic Work

A section of the Grinnell College Student Handbook \*\* is this the current language?

Paraphrase carefully: When you paraphrase—that is, when you put what a source says into your own words—you must not merely rearrange a few words from the source, but must recast the passage or sentence completely. In addition, you must specifically acknowledge any material that you have paraphrased or summarized, even when you have substantially reworded or rearranged it. It is not acceptable to explain similarities between your work and that of others by claiming that you read the source or sources long ago and have confused the phrases and ideas of the other author or authors with your own. Rule of thumb: when in doubt, cite.

Cite ideas and data: You are also obliged to acknowledge, whether in an in-text citation or a footnote, any idea you have borrowed from another person or source. Scholars, researchers and writers often engage in intense discussions, with each speaker confirming or modifying some aspect of another's thought. Given these circumstances, it's often difficult to credit the source for any given idea. However, such acknowledgment is part of how we honor each other's words and work. Even though, at times, you may feel as if the distinction between your ideas and the ideas of others is unclear, you must make that distinction as clear as possible. This requirement to acknowledge the ideas of others applies whether the source is a faculty member, another student, a guest lecturer, or an off-campus friend or relative.

*Authorship* -- Instructors expect students to work together to discuss the results of laboratory work and other group assignments. However, all work handed in to the instructor (quizzes, exams, problem sets, lab data analysis, papers, peer reviews, etc.) should be the work of the individual student *unless the instructor gives written permission for submission of group work.*

### **Specific Advice -- Investigations "Advice to Authors"**

identify the author(s) on the first page, use double-spacing with 1 inch margins, and number each page. If your instructor asks for an electronic submission, name the file with your name and an indication of the subject of the paper (e.g., RWasley\_Chemotaxisassay.docx).

Scientific papers usually contain sections in order: Title, Abstract, Introduction, Methods, Results, Discussion, Acknowledgments, and References.

#### **Title**

The title tells *what* the paper is about, so the best time to determine it is after you have completed your paper. A title should be informative, specific and concise. Since you are not writing a murder mystery, it is all right to tell the "ending" in the title. It is often this information that helps a reader decide if the paper is something s/he wants to read.

Under the title, place your name and "professional address," which here is your specific course and laboratory section. This information should either be placed alone on a *title page*, or at the top of the first page in order to save paper (ask your instructor).

Below are examples of titles that (1) tell the reader very little about the investigation (**Bad**), (2) give specific information about the type of investigation, though they fail to inform the reader about the nature of the results (**Better**), and (3) indicate the objective and primary conclusions of the investigation in a concise manner (**Very Good**).

**Bad:** "Lab 2 - Plant phenotypes "

**Better:** "Growth and phenotypic variation in *Solidago gigantea*"

**Very Good:** "Heritability in *Solidago gigantea* is lower for growth than for leaf size"

**Bad:** "Lab 1: Heart Lab"

**Better:** "The function of frog hearts"

**Very Good:** "Epinephrine Increases the Strength but not the Rate of Contraction of Frog Hearts"

**Bad:** "Lab 1: Growth curves"

**Better:** "Growing fibroblast cells in culture"

**Very Good:** "Lower serum concentration inhibits fibroblast cell growth *in vitro*"

**Bad:** Lab 3: Recrystallization"

**Better:** "Purification of organic compounds"

**Very Good:** "Purification of organic compound using hexane mixtures recrystallization"

**Bad:** "Laboratory exercise: Water analysis"

**Better:** "Drinking water quality in four samples"

**Very Good:** Source influences drinking water calcium content

**Bad:** "Laboratory Exercise: Greenhouse Gases"

**Better:** "Infrared Spectroscopy of Greenhouse Gases"

**Very Good:** "Molecular motion influences greenhouse gas properties"

### **Abstract**

The abstract is a summary of each major part of the paper; it includes a brief introduction to the problem being studied, a brief statement of how the study was conducted, a brief summary of the major results, and a brief statement of the significance of those results. An abstract is usually between 100-200 words. Make every word count, so you can convey the most information in these few words. Clearly, it is best to write this section after you have written each of the four sections summarized in the Abstract.

The following is a good example of a Biology abstract.

Fluoxetine (Prozac) is a frequently prescribed antidepressant, identified as a selective serotonin reuptake inhibitor. Prozac's function as an SSRI leads to the popular belief that its antidepressant mechanism is related simply to an increased serotonin level in the synapse, due to the blockage of the serotonin reuptake pump. However, while some previous research has suggested that Prozac acts as an agonist, other research has suggested that Prozac also acts as an antagonist of 5HT<sub>2C</sub> receptors, a function apparently contradictory to its role as an SSRI. We sought to further elucidate Prozac's effect on 5HT<sub>2C</sub> receptors in the crayfish neuromuscular junction. To determine if Prozac acts as an antagonist of 5HT<sub>2C</sub> receptors in crayfish *Procambrius clarkii* neuromuscular junctions, we compared excitatory postsynaptic potential (EPSP) amplitudes of control/5HT treatments, and control/Prozac and 5HT. Our results suggest that Fluoxetine does indeed act as an antagonist of 5HT<sub>2C</sub> receptors in the crayfish neuromuscular junction.

### **Introduction**

The introduction should briefly describe the background information for the reader to understand *why* the investigation was done. This should include the reasons for choosing the question being asked (*why* is it interesting?), some background on the system under investigation, and, if applicable, justification for hypotheses. A good introduction will mention the major issues that will be considered in the *Discussion* section, and that is why it may be helpful to write it, and particularly revise it, after finishing the other sections.

There are many ways to organize an introduction depending on its length and audience, but one principle is to begin by developing the general importance of your question, then move to the ideas leading to your specific study and question. Assume that the reader is at least moderately familiar with the general subject of the paper. In Biology, unless you are studying a model organism (e.g., *Drosophila*, *Arabidopsis*, *E. coli* etc.), it is important to describe enough aspects of its natural history that the reader can appreciate why it was chosen for the study. (If lengthy, this is sometimes placed in the Methods section). If you are using a particular experimental method, provide a rationale for having selected it. If your study tests a particular hypothesis, you might end your

introduction by stating your hypothesis (with justification, of course) and describing in the most general terms how your investigation addressed it.

The following are two examples of good Biology introductions.

### *Biology Introduction Example 1*

As urbanization pushes human settlements into natural ecosystems, the scientific community must develop novel paradigms to understand the ecological dynamics of these heterogeneous habitats and to quantify the impact of human activities on biological processes. Urban landscapes can be complex matrixes of disparate factors that both contribute to disturbance, such as chemical pollution, and nurture biodiversity, such as well-designed parks. This complexity makes it difficult to tease out the ecologically relevant impact of urban disturbances (McDonnell & Pickett 1990) and evaluate the quality of ecosystem dynamics at local sites (Sadler et al 2006). To simplify these assessments, research in urban ecology employs the “gradient paradigm” which examines the variation in factors related to human disturbance with distance from a city center to less densely populated suburban and rural areas (Austin 1987).

Researchers then seek to relate this gradient of anthropogenic disturbance to easily measured indicators. These indicators can include populations or communities that are especially sensitive to various manifestations of human activity, such as soil compaction or the amount of paved area. Noss (1990) suggests that the organisms be wide spread, manageable to sample, and responsive to environmental changes (Figure 1).

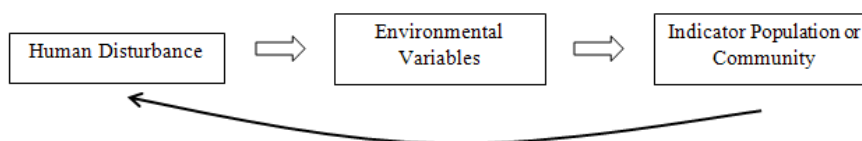


Figure 1. Biological indicators reflect the influence of human disturbance on environmental variables. In turn, the magnitude and spread of human disturbance can be extrapolated by assessing the indicator.

Previous research has identified bird and butterfly populations as reliable biological indicators that are remarkably sensitive to gradients of urbanization (Blair 1999). These models, however, respond to large-scale changes in the landscape, not small-scale changes that could impact micro-sites in vegetation or soil characteristics. Soil arthropods may fill this need by serving as ubiquitous, minimally motile, and easy-to-collect indicators of local site variables.

Additionally, the abundance and community structure of these arthropods has important ecological implications; soil organisms influence the rate of litter decomposition (Seastedt 1983) and balance of soil nutrients (Blair et al 1992). Some soil arthropod species graze on bacteria and fungus, impacting microbial communities (Dress & Boerner 2003).

Based on comparisons between sites with distinct natural histories, soil organisms do respond to dramatic disturbances, such as intense grazing (Mikola et al 2001; Clapperton et al 2002) and fire (Dress & Boerner 2004). Clapperton et al (2002) also concluded that Acarina, a group that dominates most samples across a wide range of environments (Dress & Boerner 2003; Cedpeda-Pizarro & Whitford 1989 and others), are especially sensitive to soil disturbance. Less research, however, has investigated the soil community's responses to finer-scale variation like that associated with urban landscapes.

To determine if soil microarthropods can be used as an accurate biological indicator, this study examined their abundance and group diversity along a transect from the urban middle to the rural edge of a college campus in Grinnell, Iowa. We collected soil arthropod samples and assessed relevant site and landscape variables along the Prairie Walk, a kilometer long strip of prairie plantings.

The study investigated the following questions:

- 1) Does the Prairie Walk accurately represent an urban-rural gradient? Do site variables, such as soil compaction, organic matter, moisture, and temperature, and landscape variables, such as impervious surfaces, correlate with distance from the campus?
- 2) Do site and landscape variables—including soil compaction, soil moisture, soil temperature, soil organic matter, and proportion of landscape covered by impervious surfaces—reflect the abundance and diversity of soil microarthropods?
- 3) Does the abundance and diversity of biotic communities below ground, at the surface, and in the air correspond to an urban-rural gradient?

We anticipated that the sites furthest from campus would be subjected to less intense human disturbance and would exhibit greater percent soil organic matter and moisture and lower soil compaction, soil temperature, and surrounding impervious surfaces. Vegetation height may correlate with higher soil moisture and lower soil temperature because the leaves would shade the soil surface. Most likely, the most abundant and diverse soil

microarthropod communities would inhabit these sites on the rural end of the gradient (Figure 2). We expected the arthropod communities both below and above ground to respond similarly to the urban-rural gradient.

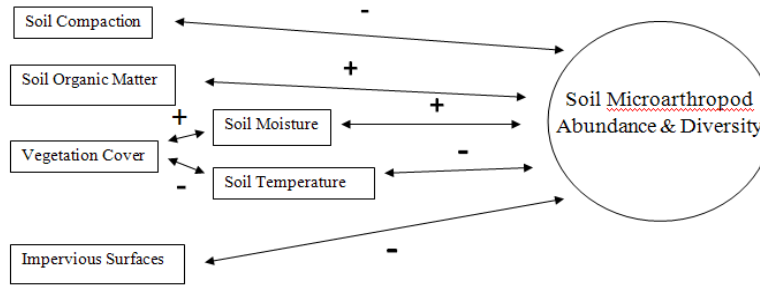


Figure 2. Schematic depicting predicted correlations between localized, environmental variables and soil arthropod abundance and diversity.

### Biology Introduction Example 2

The knowledge of how nerves function is imperative to understand bodily functions in any animal. Many chemicals are involved in the nervous system and most of them have multiple effects. For example, 5-HT, one of the most abundant neurotransmitters in the nervous system, has been shown to increase excitatory postsynaptic potential (EPSP) amplitudes in the crayfish neuromuscular junction (Dropic et al. 2005 and Etkorn et al. 2006). The exact mechanism behind this is unknown, but the increase in EPSP amplitudes could be due to one of two intracellular calcium release receptors—that of either IP<sub>3</sub> or ryanodine (Mattson et al. 2000). Past research has also suggested a link between the effects of IP<sub>3</sub> inhibitors and 5-HT on EPSP amplitudes (Dropic et al. 2005). 2-APB is known to be an IP<sub>3</sub> inhibitor but may affect EPSP amplitudes through mechanisms other than an IP<sub>3</sub>-induced Ca<sup>2+</sup> release (Dropic et al. 2005). Through application of the IP<sub>3</sub> inhibitor 2-APB, we aim to determine whether 5-HT affects EPSP amplitudes through IP<sub>3</sub>-induced Ca<sup>2+</sup> release and if 2-APB and 5-HT have an unknown combined effect on EPSPs. This research is important because understanding the effects of 5-HT and the mechanisms through which it affects EPSPs will lead to a greater understanding of many neurological functions and how they occur.

We hypothesized that the application of an IP<sub>3</sub> inhibitor, 2-APB, will negate the effects of 5-HT on EPSP amplitudes. We already know that 5-HT causes an increase in EPSP amplitude (Etkorn et al. 2006), however, we wanted to know the combined effects of 5-HT and 2-APB. Our data supported our hypotheses that 5-HT works through an IP<sub>3</sub>-induced Ca<sup>2+</sup> release and that 2-APB decreases EPSP amplitudes when 5-HT has previously been applied to the preparation.

### Methods

This section should carefully explain *how* the research was done. The level of detail should allow the reader to know exactly what you did and be able to repeat your study. Organize the sections logically, and not necessarily chronologically; the Methods section is not a diary of what you did every day. Use subheadings if there are more than a few paragraphs. Include all materials used, *but do not make lists*. Describe the exact conditions employed, how you gathered the data, and how you analyzed it precisely enough that someone else could repeat it. You may cite the lab manual or other sources for common techniques. If you develop your own technique, explain it in sufficient detail that another person could replicate your work. If you are doing a field study, indicate the location of the study and the dates on which it was carried out (since this may be important to the results). Do NOT do this for a laboratory study.

Fatal flaws: Do not present your Methods as a diary or the materials as a list. Write in complete sentences and organized paragraphs.

Below are some examples of good and bad Methods section practices:

#### Bad:

- “We measured growth rates and wrote down the data.” How did you measure growth rates? You don’t need to tell the reader that you recorded the data or entered it into the computer.
- “The data were entered into Excel for manipulation.” Likewise, graphical or spreadsheet programs do not need to be mentioned.

## The Scientific Paper

- “The cells were washed in saline and resuspended in medium” What solution did you use to wash the cells?
- "On the first lab day, we extracted the DNA and froze it. Then, the next week, we ran the DNA on an agarose gel." The methods section is not a diary of your lab work. Describe what you did concisely and in a logical order. It needn't be the exact order you did it in, unless that is critical.
- “Student t-tests are based on the principle...” Commonly used statistical tests generally need no explanation or citation. You should mention, however, what techniques were used to test which predictions.

### Good:

- "We used a t-test to determine whether mean photosynthetic rates differed between the two light environments."
- “We prepared 5 solutions ranging in concentration from .0050M to .010M, by serially diluting a solution of .050M silver nitrate.”
- “We pelleted cells at 5000 x g for 5 minutes and then washed them in 0.05 M NaCl. Cells were pelleted again and resuspended at a concentration of 108 cells per ml in peptone-glycerol broth.”

The following are two examples of good biology Methods sections.

### *Biology Methods Example 1*

To establish populations of *Ceratopteris richardii* of different densities, we obtained 10 mg of pre-sterilized wild type spores from Carolina Biological Supply (Burlington, NC). We added 0.4 ml sterile water to suspend the spores and spread one drop of the suspension on a 60 mm petri dish containing a culture medium described in Klekowski (1969). This plate was labeled A. Five additional plates labeled B through F were each sown with one drop of a spore suspension that was two-fold more dilute than the previous one. These cultures were maintained in a culture dome under continuous light from four 34 Watt cool white, fluorescent bulbs ( $40 \mu\text{mol}/\text{m}^2/\text{sec}$ ) and with the temperature at  $28 \pm 2^\circ \text{C}$ .

Determinations of the percent spore germination and gametophyte composition (as % males) were made at 7 and 14 days after inoculation (DAI) respectively. Determinations for plates A-C were made using a sampling method that counted between 5 and  $10 \text{ cm}^2$  on each plate while plates D-F were counted in their entirety.

### *Biology Methods Example 2*

For both the quantitative cell count and the turbidometric mass determination, the lab instructor supplied samples of the same 24-hour old stock culture of *Serratia marcescens*.

**Quantitative Cell Count of *S. marcescens*** An aliquot of the stock culture of *S. marcescens* (approximately  $10^9$  cells/mL) was diluted serially in sterile saline solution to generate three solutions with dilution factors ( $F_d$ ) of  $10^5$ ,  $10^6$  and  $10^7$ . Utilizing a flame sterilized glass spreader,  $100 \mu\text{L}$  of each solution was spread onto sterilized peptone/glycerol agar plates, and the plates were then incubated for a 40 h period at  $30^\circ \text{C}$ , at which point visible colonies were counted. Plates yielding colony forming unit (CFU) counts between 25 and 250 were used to determine viable cell counts of the original undiluted culture ( $\text{CFU mL}^{-1}$ ).

**Turbidometric Mass Determination of *S. marcescens*** An aliquot of the stock culture of *S. marcescens* (approximately  $10^9$  cells/mL) was diluted serially in sterile saline solution to generate four solutions with dilution factors ( $F_d$ ) of 1.25, 2.5, 5, and 10. The absorption of these four solutions and an aliquot of the original culture were then measured at 550 nm using a Cary50 spectrophotometer utilizing a 1 cm pathlength plastic cuvette.

## Results

Raw data you have collected should not appear in your paper. Rather, the *Results* section should summarize your findings and present your data for the reader to evaluate. One good way to approach the text of the Results

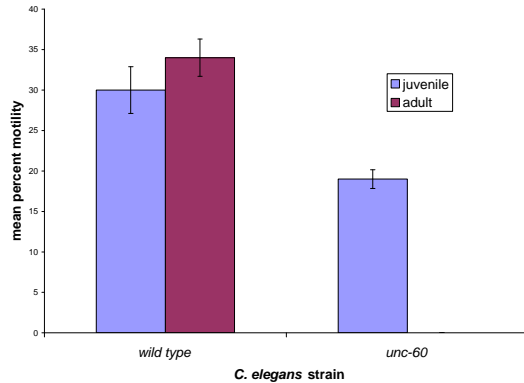
section is to develop a set of questions about the data you gathered. Do not use questions that begin with “Why” - these necessarily involve interpretation and should be addressed in the *Discussion* section. Write your Results section by answering each of these questions in a logical order. Refer to Figures and Tables as you describe the results.

Graphs and tables help the reader understand complicated data more easily than a written description. Note, however, that if the data can be easily summarized in the text, a figure or table is not necessary. The text should tell the reader the important points trends shown on the graphs or tables. Obviously the same data should not be presented in two different forms (e.g., a table should not contain data also summarized in a graph), so decide which format best informs your reader. Refer to graphs of any kind, as well as other pictorial materials, as “Figures” in the text. Call rows and columns of numbers and text “Tables” and number them separately from figures. Number tables and figures in the order in which you refer to them in the text. Call out each figure and table in the text. Ask your instructor whether tables and figures should be imbedded in the text, or placed in order at the end of the paper.

The following are two examples of good biology Results sections.

### Biology Results Example 1

Our chemotaxis assay quantified the difference in motility between juvenile and adult *unc-60* mutants by measuring the ability to detect and move towards an *E. coli* food source. Both wild type juveniles and wild type adults were motile and exhibited a similar rate of chemotaxis (Fig.1  $t = -1.1$ ,  $p > 0.05$ ). The *unc-60* juvenile worms possessed some motility, and the adults did not move at all. Our study shows that the *unc-60* mutation becomes more debilitating with age, as 19% of the juvenile mutants were able to move to the *E. coli* ring, while 100% of the adult mutants remained completely paralyzed (Fig.1  $t = -16.45$ ,  $p < 0.05$ ). We also found that the *unc-60* juveniles exhibit less motility (19%) than the wild type juveniles (30%) (Fig.1  $t = -3.5$ ,  $p < 0.05$ ).



**Figure 1.** *Unc-60* mutants vs. wild type, adult vs. juvenile positive chemotaxis toward *E. coli* results. *Unc 60* juveniles are shown to be motile and can chemotax (19%), while adults were completely immotile ( $t = -16.45$ ,  $p < 0.05$ ). Wild type juveniles and adults are fully motile and maintain similar levels of motility (30% and 34% respectively,  $t = -1.1$ ,  $p > 0.05$ ). Motility was determined as presence in the *E. coli* perimeter. Error bars represent S.E.  $n = 3$  in all cases.

**Biology Results Example 2** -- Adapted from Pasachnik, S. and G.R. Ruthig. 2004. Versatility of Habitat Use in Three Sympatric Species of Plethodontid Salamanders. *Journal of Herpetology* 38: 434-437.

After accounting for the block effect, the species- treatment interaction was not significant (Table 1). However, both species and treatment effects were significant (Table 1). A posthoc Scheffe test determined that *E. cirrigera* had a significantly higher gain in mass than *P. cinereus* when all three habitat treatments were combined ( $p < 0.05$ ) (Fig. 1). An additional Scheffe test determined that all individuals (regardless of species) within the stream treatment gained significantly less mass than individuals in either the bank or forest treatments ( $p < 0.05$ ) (Fig. 1)

TABLE 1. ANOVA results for the change in mass/initial mass data. Change in mass is the difference in mass from the beginning of the experiment to the end of the experiment. Species include *Desmognathus fuscus*, *Eurycea cirrigera*, and *Plethodon cinereus*. Treatments include stream, stream bank, and forest.

	df	Mean	F	P
MODEL	17	0.027	2.87	0.001
BLOCK	9	0.024	2.16	0.016
SPECIES	2	0.063	6.72	0.002
TREATMENT	2	0.059	6.32	0.003
SPECIES × TREATMENT	4	0.008	0.86	0.492

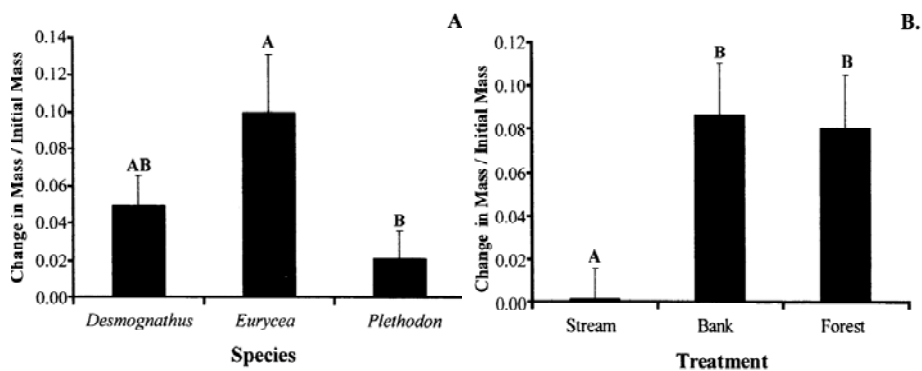


FIG. 1. (A) Species performance, independent of habitat treatment for *Desmognathus fuscus*, *Eurycea cirrigera*, and *Plethodon cinereus*. (B) Treatment performance, independent of species, in the stream, bank, and forest habitat simulations. Differing letters depict significant differences. Error bars represent standard error of the mean. N ranges from 6-10.

## Discussion

The role of the *Discussion* section is to interpret the meaning of your results. Proceed in this section from the specifics of your study to the general question that motivated the study (just the opposite from the Introduction!). Consider addressing the following points in order:

1. Remind the reader of important trends in your data and how those results relate to your hypothesis or goals.
2. Provide an explanation for the most interesting or relevant results. This might include references to other studies that showed similar or different results. Include references that help support your explanations.
3. Discuss the relevance of your results and their interpretation to the larger questions that motivated the study.
4. End the discussion with a summary, the “take home lesson” that you want your reader to remember about your work. Indicate interesting future directions for study, rather than simply summarize (again) your results. In other words, build on the interpretations you have just provided, so that those arguments in the previous paragraphs matter. Some papers have this summary as a *Conclusion* section.

The length of the Discussion section depends on the scope of your study. A good way to approach the writing of this section is to consider each of the above points as the subject of a short paragraph. You can later expand or combine these after laying out your ideas.

Here are some tips on common errors in Discussion sections:

- Do not infer that because your hypothesis wasn't supported, you made a mistake. “Negative” results can be important too, since they may suggest that your hypothesis was incorrect. What would be the benefit of



testing hypotheses, if you could never reject them? If you did make an error somewhere in your investigation, acknowledge it and move on.

- Do not omit or minimize discussion of findings that you did not expect. Such results are often the most interesting.
- Do not center your discussion around a proposal to repeat your experiment with a larger sample size! This is often true and not as interesting as suggestions for new investigations that arise from your findings.
- Do not end the paper with the phrase, “. . . but of course more work needs to be done.” Describe what *kind* of work would be the most interesting extensions of the study and why.

### **Acknowledgments**

In this section, thank any persons who contributed any significant help during the study. Such contributions include help in experimental design, collection of data, preparation of graphs, drawings or the manuscript, critiquing a draft of the manuscript, and financial or physical support of the work. Always acknowledge your partners in group projects!

### **References**

Standards for citation vary somewhat among journals. The *Investigations* formats are similar to those in many (but not all) journals in biology or chemistry. **It is critical that you do not use MLA format or footnotes to cite your references.** Since the forms differ between biology and chemistry journals (and even within the disciplines), it is also critical to ask your instructor which of the standards below apply to your class. When scientists submit a paper for publication, they read and adhere to the guidelines set for each particular journal. **In the same manner, you should think of each paper you submit as needing to adhere to the guidelines set by each particular professor.**

**List only the papers or other publications that were *directly cited* in your paper. A References section is not a bibliography. Citing a paper means you read it -- reading the abstract is NOT sufficient, unless specifically allowed by your instructor.**

#### *Investigations* Biology References Convention

Cite references in one of two ways in the text of your paper:

1. Mention the authors' names as part of your sentence followed by the year of publication in parentheses. When there are three or more authors, give the first author's last name, followed by "et al." (Latin for "and others"):

**Sullivan et al. (1998) described the use of delta-crystallin as a marker of lens induction during differentiation.**

2. Place authors' names and the year of publication in parentheses following ideas or results from the article:

**Experimental studies of several species indicate that tradeoffs between growth and male function may not be predicted by resource allocation models alone, unless meristem availability is also considered as a resource (Eckhart and Seger 1999).**

List references alphabetically according to the first author. Standards for the reference sections vary widely among journals, primarily in details of punctuation. Please use the appropriate form for each type of reference below:

#### **Journal article:**

Author(s). Year. Title. *Journal* Volume:pages.

Sullivan, C.H., P.C. Marker, J.M. Thorn, and J.D. Brown. 1998. Reliability of delta-crystallin as a marker for studies of chick lens induction. *Differentiation* 64: 1-9.

If you cite more than one paper by the same author(s), the papers should be listed chronologically (earliest first).

If a paper has more than five authors, use "et al" after listing the first five.

**Book chapter of edited volumes**

Author(s). Year. Title of chapter. *In* Editors names (eds.). Title of book. Publisher, Place of Publication, pages.

Eckhart, V.M., Seger, J. 1999. Phenological and developmental costs of male sex function in hermaphroditic plants. *In* Vuorisalo, T.O. and Mutikainen, P.K. (eds.). Life history evolution in plants. Kluwer, Dordrecht, pp. 195-213.

**Book**

Author(s). Year. Title. Publisher, Place of Publication.

Voyles, B.A. 1993. The biology of viruses. Moseby, St. Louis.

**Web pages (use sparingly!)**

Author. publication date. Page title. Site title. URL. date you accessed it.

Harr, J. 2002. Plants of Cedar Creek – Asteraceae. Cedar Creek LTER.  
<http://cedarcreek.umn.edu/plants/narratives/asteraceae.html> . 7 January 2004.

*Investigations* Chemistry References Convention

Citations appear either as footnotes or endnotes. They are numbered in the text in the order referred. Numbers are either in superscript or enclosed in brackets, as in the following example:

**"(Name) and co-workers [1,2] have previously studied the effect of molecular weight on vapor pressure for chlorinated compounds."**

You may also cite a source at the end of a paraphrased sentence:

**"Mass Spectrometry has previously been shown to be a rapid, sensitive technique for determination of molecular weights of organic compounds. [3]"**

The standard format for full citations is:

1. Lastname, I. N.; Author2, I.N. "Title", *Journal Name* ,  
Year, Volume, PageNumber.

Examples are below:

Levandoski, M.M., Lin, Y., Moise, L., McLaughlin, J., Cooper, E., and Hawrot, E. "Chimeric analysis of a nicotinic acetylcholine receptor reveals amino acids conferring sensitivity to a-bungarotoxin", *J. Biol. Chem.*, **1999**, 274, 26113.

Marzluff, E.M.; Campbell, S.; Rodgers, M.T.; Beauchamp, J.L. "Collisional Activation of Large Molecules is an Efficient Process", *J. Am. Chem. Soc.*, **1994**, 116 , 6947-6948.

**Are you interested in streamlining the processes of doing your references section? Read the following!**

RefWorks is a bibliographic software package available at no cost to Grinnell College students through the Web that enables you to:

- build a bibliography in a wide variety of citation styles (including styles recommended in "Investigations")
- import references from many data sources
- organize your research
- include citations while you write your paper

## The Scientific Poster

Since RefWorks is available through the Web, there is no software to download and update and you can access your personal account from any computer connected to the Internet.

RefWorks can be accessed at: <https://www.refworks.com/Refworks/>

If you are logging in for the first time, you will be asked to register. You will receive an e-mail confirming your registration. You can also access RefWorks from off-campus using a Group Code available from any Librarian. Questions? Contact Kevin Engel ([engelk]; Science 2105; x4234) or another Librarian (x3353; [query]).

### Evaluation of Scientific Papers

Evaluation and revision is the key to better writing. This means you must start writing early, finish a complete draft well before the due date, seek out reviewers, and revise. Scientists never send manuscripts to journals without having several people not associated with the study read and evaluate it. A good reviewer reads critically, i.e., s/he lets the author know both the strengths and weaknesses of the writing. People who just like to tear things (and people) apart, or who are afraid or unwilling to point out problems, are equally useless as reviewers. So who should review your manuscript? The candidates:

**The author** -- Take some time away from your paper and then come back to it as a reviewer. Read it aloud. How does it sound? Does one sentence flow to the next? Is there needless repetition? Do your explanations make sense? This may sound foolish, but it is a tested method. It may also help you learn to recognize your own bad writing habits.

**Other students** -- Since other students are your intended audience, why not test out the paper on one of them? You may want to pick someone NOT in your class, particularly if all members of the class are writing a paper on a similar topic. Your reviewer should be thanked in your *Acknowledgments* section.

**The Writing Lab** -- The folks in the Writing Lab are a wonderful resource. Going to the Writing Lab is not a punishment, but an opportunity. Many of your professors have used the writing lab for their own work, including this very document. Make an appointment and start writing well in advance.

**The professor** -- Your instructor may be willing work with you on your paper. Make an appointment well ahead of time (professors have busy schedules) and come in well prepared to discuss *specific* aspects of the paper. Don't expect your instructor to read a full draft at a moment's notice to screen for problems.

***Do you get the sense that all this can't be done the night before the due date?  
You got it.***

Methods of evaluations of papers will vary among your professors. In **Appendix C on page 73** we show two examples of evaluation rubrics that have been used in Biology classes for evaluating scientific papers. Ask your instructor how s/he will evaluate your paper.

## The Scientific Poster

### What is a poster and why do you do it?

Professional scientists regularly present the results of their work at local, national, and international meetings. At most scientific meetings, posters are the primary means by which scientists exchange information about their work. The poster, although a smaller unit than the published journal article, is thus a fully professional entity, and almost always the first form in which your story is made public. It is also the most egalitarian form of presentation in that tenured researchers and students alike use it. Its principal advantage is that it promotes extensive two-way communication between the presenter and the audience. Not only are results and conclusions presented to the

audience, but also the presenting scientist usually receives ideas and suggestions that help in planning future experiments.

What is a poster? A poster is a visual way of presenting scientific results. A good poster is virtually self-explanatory; it will contain the elements of a paper (Title, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, and References), but it is a distinct form in which different elements are emphasized. There are several examples of research posters distributed around the science building. Look them over. If you still have questions or are unclear about the elements and structure of posters, talk to your instructor.

The poster audience may be divided into three main groups. At professional meetings, Group 1 comprises those colleagues, collaborators, and students who follow work in your area of biology very closely. In Bio 150, that means the other students who have chosen to focus on topics very similar to yours. This group is familiar enough with the methods and background of your work not to find detail intimidating. At professional meetings, Group 2 includes those scientists who work in the same general area as you, but not on your particular specialty. This group is much larger than Group 1; in this course, it includes the other members of your course. At professional meetings, Group 3 would include those researchers whose work is largely unrelated to yours. In this course, it includes other students within the Science Division who may come to view your poster, as well as the very general audience likely to be present at Parents' Weekend. Keep in mind that your poster must address the needs and abilities of all three groups in order to be successful!

### Contents of a Scientific Poster

#### *Title and Author Panel*

The title should be descriptive but short, in **boldface** letters 1.5 inches high. The authors' names may be somewhat smaller.

#### *Abstract*

This is a short (50-100 word) summary of your research. It should be completely self-contained (that is, independent of the rest of the poster). This is the one portion of a poster that is commonly published.

#### *Introduction*

Here you introduce the topic of the work, briefly summarize any relevant background information, including a short review of the work of other investigators, and succinctly state the objectives or hypothesis.

#### *Methods*

Unless the primary focus of the poster is the novelty of its experimental methods, this section should be kept to a minimum. There must, however, be sufficient detail to permit the reader to understand what was done and evaluate the appropriateness of the experimental design and technique.

There is some disagreement, both within biology and between biologists and chemists, over how long this section should be and what it should contain. Some alternatives:

- Include relevant methods within the text associated with figures in the Results section, and don't include a separate Materials and Methods section at all.
- Include a Materials and Methods section, but make it extremely brief and heavily larded with citations (including the lab manual); additional methodological details may be included in figure legends when necessary
- Use bulleted lists of procedures; sketches, figures, diagrams, or photos of equipment; and a listing of conditions. If detailed materials and methods are required, they may be appended in smaller type.

Check with your instructor to find out what s/he prefers.

### *Results*

This is a **summarized** report of your observations, not your interpretation of the results. Present your results in a logical sequence, not the sequence in which they were obtained. Remember that this is primarily a visual, rather than verbal, presentation. Graphical representation of data is almost always more effective than tables or text. Use text only to explicate the figures and, if necessary, to make transitions between figures. Number all figures and tables consecutively (e.g., Fig. 1, Fig. 2, Table 1, Table 2, etc.). Raw data should be included only when absolutely necessary; if in doubt, ask your instructor.

### *Discussion*

Here you analyze and discuss your findings, though less expansively than in a paper. Summaries such as numbered or bulleted items may be used. You should point out the general meaning and importance of your results, and relate them to those of other investigators (be sure to cite their work appropriately!). You should also include a description of further work that could be done in this area.

### *Conclusions*

This includes a few brief and concise statements summarizing your work.

### *References*

Here you should list sources that were cited in your poster.

### *Acknowledgments*

You can acknowledge funding sources, individuals, facilities, and personal conversations that aided you in your research.

### **Presenting a poster**

Be ready with a short oral summary of the main points of your poster. A concise synopsis of the purpose of your experiments, the results you achieved, and the conclusions you draw is very useful. Also, prepare brief explanations of the important features of each panel, particularly for those including tables or figures. This preparation will allow you to “walk through” the poster with anyone who expresses interest.

### **Criteria for evaluating posters.**

The assessment of your posters will be based upon criteria that will vary somewhat among different courses. There are two poster evaluation forms on **page 76 of Appendix C** that give a feel for what poster evaluators are looking for. The first form, a tripartite scheme, has been used for many biology courses in the past. Parts one and two, which address the "science" of the experiment, carry more weight than part three, which addresses the aesthetics of the poster.

**Appendix C contains detailed instructions for making posters in Power Point (pg 82) and important information about the Biology 150 Poster Session (pg 83).**

## The Scientific Presentation

### What is the role of a 15-minute oral presentation?

Scientific presentations, like posters, are ephemeral. You have only a brief interval in which to convince others of the significance of your data and leave a lasting impression. Every effort must be made to make it easy for the audience to comprehend and remember the main points of your talk.

### How should a scientific presentation be organized?

As with a paper or poster, one way to ensure that people will remember your main result is to include it in the title. Repetition is also important, both within the structure of the talk and in reinforcing the main points on a figure or slide with what you say. In the words of an old adage, first you should tell people what you are about to tell them, then you should tell them, and in conclusion you should repeat what you told them.

Many students prefer to use PowerPoint, a computer program that permits electronic versions of slides to be projected onto a screen, while others are more comfortable with overheads. Ask your instructor what s/he prefers.

Here are a few tips for talks:

- A talk should include an Introduction, Materials and Methods, Results, Discussion, and Conclusions. A talk is therefore structured much like a paper or poster. A talk is unlike a paper in that it is advisable for you to offer a brief interpretation of your results immediately after describing the results to the audience, while the relevant figure is still being shown.
- A common problem is running over the time allotted for a talk (at professional meetings, as well as biology courses here at Grinnell!). A good rule of thumb is to allocate about a minute per slide. If you have 30 slides or overheads to squeeze into a 15-minute talk, chances are you won't make it. Keep in mind that you should allocate 2-3 minutes for questions at the end of your talk, which means that only 12-13 minutes of a 15-minute talk should be taken up by you.
- Try to maintain a high information:ink ratio. That is, don't include any information that is not relevant to your point. It is often especially difficult to cleave to this rule in PowerPoint presentations, where it's easy to get caught up in the glitz and gimmicks! Focus on content and clarity, rather than flash. Often, flash not only is irrelevant, but also actively prevents the viewer from perceiving content. For example, while it is fun for you to play with presentations that move around on the screen, an audience will be sufficiently distracted that it will be unable to hear anything you say while the movement is being visually tracked.
- Limit the amount of information on each slide. A good slide will include no more than 5 points. If slides include figure or data tables, they should also include a line of text identifying the main point. If you feel that you need to make more than 5 points, make another slide.
- You cannot include more points by talking faster! The audience will not hear what you have to say unless you are speaking at a rate of about 100 words per minute or slower. There is a document on PowerPoint presentations available on the Biology Department website (<http://www.grinnell.edu/academic/biology/links/>), or through Stephanie Peterson in the Science Office. It contains points that may also be useful to those who choose to use overheads, and we recommend that all students peruse a copy.

### Giving group presentations

Usually the easiest way to give a group presentation is to assign responsibility for particular sections of the talk to particular members of the group. While this may streamline production, it results in a poor talk unless the entire group gets together to discuss everything before and after working on each section. You need to know what will

happen in the other sections of the talk in order to do a good job on your section. This is especially important because poor transitions between segments can lose an audience.

### Handling questions from the audience

- Wait for the questioner to complete his or her question before you begin to answer, and then repeat the question so that everyone in the room can hear.
- Never interrupt unless the question is extremely vague and rambling. If you must interrupt, do so tactfully, and attempt to rephrase the question.
- Take a moment to think before answering, and don't be afraid to ask for clarification if you aren't sure what the question means. Questions that arise during the course of the talk should be answered immediately if they resolve ambiguities in the presentation, and postponed if they will interrupt the flow of the talk and distract the audience.
- If you don't know the answer to a question, you should just say so. You can use this as an opportunity to query the audience, suggest sources where the questioner might find the answer, or offer to find out the answer and get back to the questioner at a later time.

An excellent website where you can get more tips on preparing talks:

<http://www.kumc.edu/SAH/OTEd/jradel/effective.html>

### Evaluation

In **Appendix C on page 77** there are two oral presentation evaluation forms that provide a feel for what instructors are looking for in a good oral presentation.

## Appendix A - A Primer of Statistical Analysis

### Summary statistics

Whenever you gather data, you need to calculate some statistics to describe them. We call these summary statistics. All quantitative sciences apply summary statistics, the most commonly used of which are described below; the Appendix includes several examples to illustrate these concepts.

#### The mean

The *mean* is the average of a set of observations. The mean is also a measure of *central tendency*, as it often tells you where most of the values occur. Calculate the mean by summing ( $\Sigma$ ) the individual observations ( $x_i$ ) and dividing by the total number of observations ( $n$ )

$$\bar{x} = \frac{\sum x_i}{n}$$

#### The sample variance

The sample variance is a measure of dispersion; it tells you how much your data vary around the measure of central tendency. Estimating dispersion is very important because variability is an inherent part of collecting data. Some variability arises from real differences among individual values, while some arises from measurement error. Calculate the sample variance using the following formula:

$$S^2 = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

The sample variance has a minimum of zero (if all your observations are the same) and increases with the variation among **observations**.

#### The standard deviation

The standard deviation is another measure of dispersion. If you can calculate the sample variance, you can easily calculate the standard deviation; the standard deviation is simply the square root of the variance:

$$S = \sqrt{S^2}$$

Like the sample variance, the standard deviation has a minimum of zero (if all your observations are the same) and increases with the variation among **observations**.

#### The standard error of the mean

A quantity that is related to the sample variance and standard deviation is the *standard error* of the mean. It can be calculated by dividing the standard deviation of your sample ( $S$ ) by the square root of the sample size:

$$SE = \frac{S}{\sqrt{n}}$$

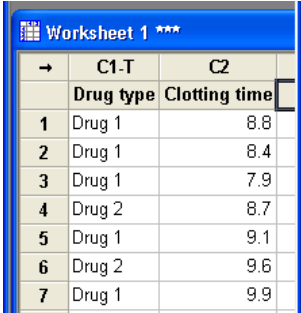
One useful property of the standard error is that, all things being equal, it decreases as sample size ( $n$ ) increases. This property makes sense. An estimate of a mean will become more precise as you collect more data, simply because you have more information. This precision is reflected in your standard error, which will also become smaller with sample size. Put another way, your confidence in your estimate of the mean will increase as sample size increases. Because the standard error reflects the precision of, and therefore your confidence in, the estimate of the mean, **the mean and the standard error should always be presented together**. In contrast to the standard error, neither the variance nor the standard deviation decrease as sample size ( $n$ ) increases. As such, the variance and standard deviation are estimates of how *variable* your data are, while the standard error is an estimate of how *precise* your estimate of the mean is.



## Summary Statistics

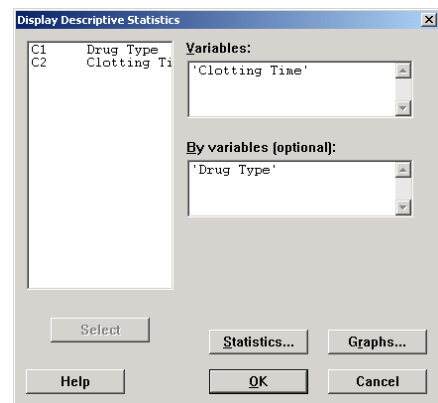
### How to calculate summary statistics in Minitab

1. Enter your data so that one or more labeled columns contain a grouping variable (e.g., your experimental treatments/independent variables) and one or more additional columns contain the dependent variables with numerical data you wish to analyze.

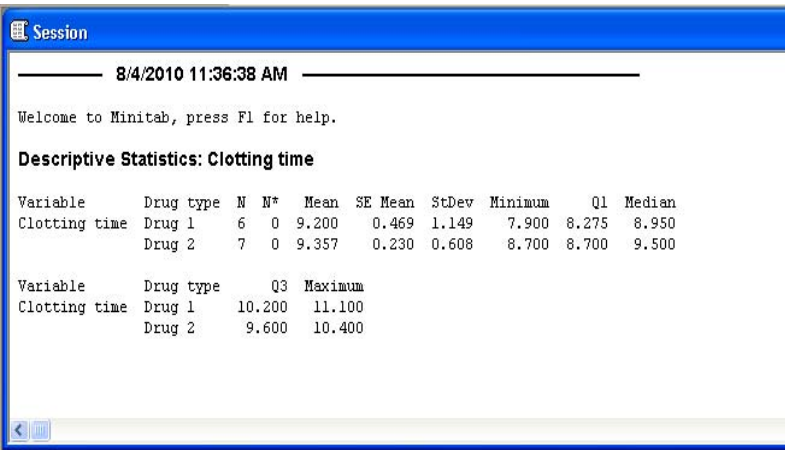


	C1-T	C2
	Drug type	Clotting time
1	Drug 1	8.8
2	Drug 1	8.4
3	Drug 1	7.9
4	Drug 2	8.7
5	Drug 1	9.1
6	Drug 2	9.6
7	Drug 1	9.9

2. Go to the menu **Stats> Basic Statistics > Display Descriptive Statistics**. Click in the **Variables** box to make it active and choose the data you wish to analyze (dependent variable) by double clicking on it in the box on the left side of the window. If you wish to analyze the data by groups or categories (independent variable) click in the **By Variables** box and then double click the columns at the left which contain the group or category information. The **Statistics** button lets you choose which statistics will appear as output in the session window.



3. Click OK to view the descriptive statistics output in the session window.



8/4/2010 11:36:38 AM

Welcome to Minitab, press F1 for help.

**Descriptive Statistics: Clotting time**

Variable	Drug type	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Clotting time	Drug 1	6	0	9.200	0.469	1.149	7.900	8.275	8.950
	Drug 2	7	0	9.357	0.230	0.608	8.700	8.700	9.500

Variable	Drug type	Q3	Maximum
Clotting time	Drug 1	10.200	11.100
	Drug 2	9.600	10.400

## Summary Statistics

### Example problems

For each of the samples below, calculate the mean, variance, standard deviation, and standard error of the mean.

- Human blood-clotting times (in minutes) of individuals that received one of two different drugs. (Data from JH Zar's *Biostatistical Analysis*, 4<sup>th</sup> ed.)

Treatment	
Drug 1	Drug 2
8.8	9.9
8.4	9.0
7.9	11.1
8.7	9.6
9.1	8.7
9.6	10.4
	9.5

- Mean serum cholesterol levels (mg/100ml) of male and female turtles. (Data from JH Zar's *Biostatistical Analysis*, 4<sup>th</sup> ed.)

Females	Males
220.1	223.4
218.6	221.5
229.6	230.2
228.8	224.3
222.0	223.8
224.1	230.8
226.5	

- Total biomass (g) of prairie plants growing in each of 20, 0.5 m<sup>2</sup> plots. Half of the plots were burned in the fall, while the others were unmanipulated. All plots were sampled the following spring, with biomass used as an estimate of growth.

Treatment	
Burned	Unburned
10.56	8.85
11.97	8.01
9.01	7.13
10.33	7.50
9.53	9.10
12.10	7.87
8.88	6.80
8.50	9.50
10.20	8.88
11.55	6.56

## Statistical hypothesis testing

### The t-test

The formula for the t-test is as follows:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S_{\bar{X}_1 - \bar{X}_2}}$$

where

$$\bar{X}_1 \text{ and } \bar{X}_2$$

are the means of each your two groups and

$$S_{\bar{X}_1 - \bar{X}_2}$$

is the standard error of the difference between groups 1 and 2. Thus the t-value is the ratio of the difference between your groups to the precision of your estimate of that difference.

The standard error of the difference between groups is mathematically equivalent to the standard error of the combined (or pooled) groups. Two steps are needed to calculate the pooled standard error. First, you have to calculate the pooled variance, using the following formula:

$$S_p^2 = \frac{\sum(X_i - \bar{X}_1)^2 + \sum(X_j - \bar{X}_2)^2}{n_1 + n_2 - 2}$$

where

$$S_p^2$$

is the pooled variance,  $n_1$  and  $n_2$  are the number of observations in groups 1 and 2, respectively, and

$$\sum(X_i - \bar{X}_1)^2 \text{ and } \sum(X_j - \bar{X}_2)^2$$

are the sum of squares for groups 1 and 2, respectively. The formula for sum of squares should look familiar to you, as it is also used to calculate the normal sample variance.

After you have calculated the pooled variance, you can calculate the pooled standard error using the following equation:

$$S_{\bar{X}_1 - \bar{X}_2} = \sqrt{\frac{S_p^2}{n_1} + \frac{S_p^2}{n_2}}$$

Although you will probably calculate your t-tests in Minitab or other software, you should have a conceptual understanding of what the computer is doing to your data. As such, you might want to do a few t-tests by hand, using the above formula. As noted earlier, the t-value is the ratio of the difference *between* your groups to the precision of that estimate. The latter quantity will be larger when the variability *within* your groups is larger, and smaller when sample size increases.

## The t-test

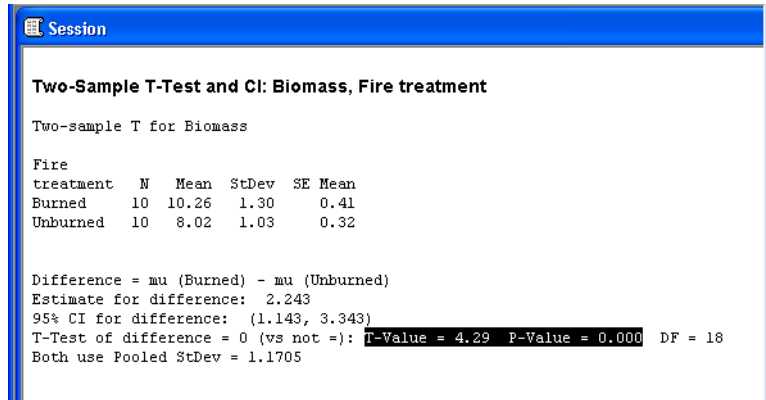
If the difference between your groups is relatively large compared to the precision of your estimate, then your t-value will be relatively large. A relatively *large* t-value suggests that the difference between your groups is caused by a given variable or experimental manipulation, rather than by chance natural variation. Statistical tables or computer packages can tell you the probability of getting that t-value by chance, which is the P-value for your comparison. By convention, if the P-value is less than 0.05 (a 1 out of 20 chance), one rejects the null hypothesis of no difference in means, and accepts the alternative hypothesis that the two groups differ.

If the difference between your groups is similar to the precision of your estimate, your t-value will be relatively small. A relatively *small* t-value suggests that the difference between your treatment groups is largely due to chance natural variation and measurement error, rather than to a given variable or manipulation. By convention, we do not reject a null hypothesis when the chance of getting a t-value is greater than 0.05.

### Application of the t-test: an example

Let's go back to our fire-prairie plant study (sample problem 3 in section A.1). Because we have two groups (burned vs. unburned), we can analyze our data using a t-test. When we do this test, we obtain a test statistic of  $t = 4.29$ . This value was calculated using Minitab; you should calculate the t-value by hand and see if it matches. The t-test output from Minitab is inserted below, with the t-statistic and p-value in bold.

Because the p-value associated with our t-statistic is  $<0.05$ , we can accept the alternative hypothesis that burning influences prairie plant growth. If the p-value for our t-statistic had been  $>0.05$ , then we would have accepted the null hypothesis that there is no relationship between fire and prairie plant growth.



```
Session

Two-Sample T-Test and CI: Biomass, Fire treatment

Two-sample T for Biomass

Fire
treatment  N   Mean  StDev  SE Mean
Burned     10  10.26  1.30   0.41
Unburned   10   8.02  1.03   0.32

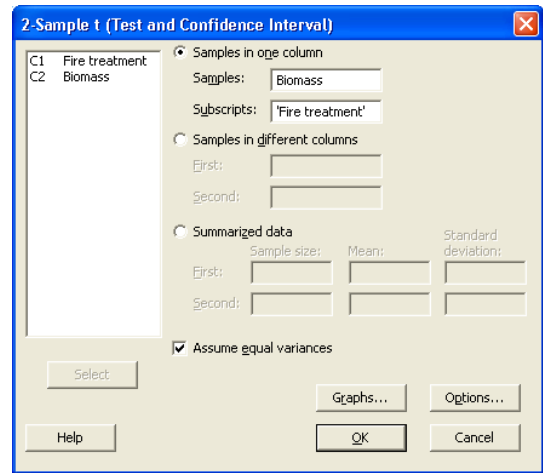
Difference = mu (Burned) - mu (Unburned)
Estimate for difference:  2.243
95% CI for difference:  (1.143, 3.343)
T-Test of difference = 0 (vs not =):  T-Value = 4.29  P-Value = 0.000  DF = 18
Both use Pooled StDev = 1.1705
```

**How to do a t-test in Minitab**

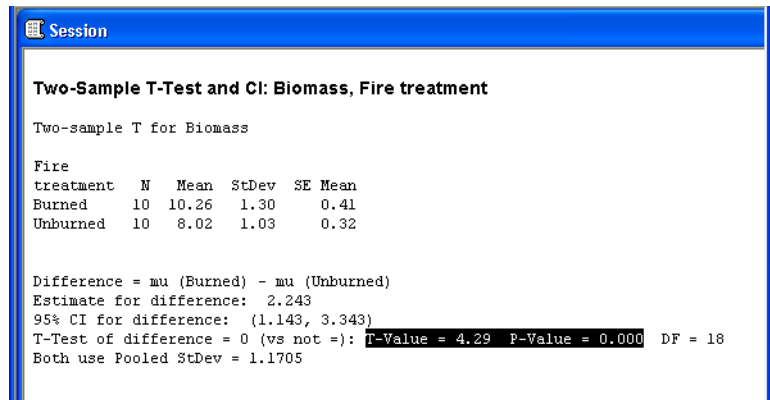
1. Enter your data so that one labeled column contains the independent variable (e.g., for the prairie experiment use Fire treatment) and a second labeled column contains the dependent variable (e.g., the Biomass values).
2. From the top menu bar, select **Stat > Basic statistics > 2-Sample t**.

	C1-T	C2	C3
	<b>Fire treatment</b>	<b>Biomass</b>	
4	Burned	10.33	
5	Burned	9.53	
6	Burned	12.10	
7	Burned	8.88	
8	Burned	8.50	
9	Burned	10.20	
10	Burned	11.55	
11	Unburned	8.85	
12	Unburned	8.01	

3. In the dialog box, place your dependent variable (what you measured) in the **Sample** box, place the independent variable (what you manipulated) in the **Subscripts** box, and click the **Assume Equal Variances** box.



4. Click **OK** to view the t-test output in the session window. The T- value and p-value are highlighted at right.



**Example Problems:**

Carry out t-tests of the differences between the means in sample problems 1 and 2 in section the section on summary statistics.

## The analysis of variance (ANOVA)

### One-way analysis of variance

Though the t-test is one of the most commonly used statistical hypothesis tests, it has limitations. The major limitation is that the t-test can be used to compare the means of only two groups at a time. Scientists often find that they need to compare the means of three or more groups. The statistical hypothesis test used to compare the means of three or more groups is the analysis of variance (ANOVA).

#### ANOVA: an example

ANOVA calculations are tedious enough that they are rarely done by hand, but there is no better way to really understand this particular statistical test. As an example, consider again the issue of the role of controlled fire in prairie restorations. One particularly contentious issue among restoration ecologists is the timing of prairie burns. Although natural fires may primarily have been sparked by late-summer lightening strikes, most controlled burns are done during the spring or fall. The timing of burning may strongly influence the outcome of prairie restorations because burns done at different times of year can favor dramatically different plant species. As scientists, you can collect data to help resolve such issues. For example, you could collect data to answer the following question: How does the timing of controlled burns influence the biomass of desirable prairie plant species? An example of the data you might collect is in Table 2, below.

Table 2. Total biomass (g) of *Rudbeckia hirta* (Black-eyed Susan) growing in each of 15, 0.5 m<sup>2</sup> plots. One third of the plots were burned in the spring, late summer, and fall of 1998, respectively. All plots were sampled during summer 1999.

Treatment		
Spring	Late Summer	Fall
0.10	5.56	3.85
0.61	6.97	3.01
1.91	3.01	2.13
2.99	5.33	2.50
1.06	3.53	6.10
Mean = 1.33	Mean = 4.88	Mean = 3.52

Before we go any further, we need to state null and alternative hypotheses. For example, the null hypothesis could be

$H_0$ : The timing of controlled burning does not influence biomass of *Rudbeckia hirta*.

While the alternative hypothesis could read as follows:

$H_A$ : The timing of controlled burns influences the biomass of *Rudbeckia hirta*.

#### ANOVA: calculations

To analyze these data using an ANOVA, you first need to calculate the *grand mean*. The grand mean is simply a fancy term for the mean of all of your observations, regardless of group. The grand mean can be calculated using the following formula:

$$\bar{Y} = \frac{1}{an} \sum_a \sum_n Y$$

Where  $a$  is the number of groups,  $n$  is the number of observations within each group, and  $Y$  is a single observation. Applying this formula to our prairie burn data, where  $a = 3$  and  $n = 5$ , gives the following grand mean:

## The analysis of variance

$$\bar{Y} = \frac{1}{15}(0.10 + 0.61 + 1.91 + 2.99 + 1.06 + 5.56 + \dots + 3.53 + 3.85 + 3.01 + 2.13 + 2.50 + 6.10) = 3.24$$

Once you have calculated the grand mean, you need to calculate the *sum of squares among groups* ( $SS_{\text{among}}$ ). The  $SS_{\text{among}}$  is an estimate of the variation *among* your groups or, more precisely, an estimate of the deviation of the group means from the grand mean. The  $SS_{\text{among}}$  can be calculated using the following formula:

$$SS_{\text{among}} = n \sum_a (\bar{Y} - \bar{Y})^2$$

Where  $a$  is the number of groups,  $n$  is the number of observations within each group, and

$$\bar{Y}$$

is the mean of each group. Applying this formula to our prairie burn data, where  $a = 3$  and  $n = 5$ , gives the following  $SS_{\text{among}}$ :

$$SS_{\text{among}} = 5((3.52 - 3.24)^2 + (1.33 - 3.24)^2 + (4.88 - 3.24)^2) = 32.08$$

The last thing you need to calculate is the *sum of squares within groups* ( $SS_{\text{within}}$ ). The  $SS_{\text{within}}$  is an estimate of the variation among observations *within* groups, or, more precisely, an estimate of the deviation of the observations within each group from the group mean. The  $SS_{\text{within}}$  can be calculated using the following formula:

$$SS_{\text{within}} = \sum_a \sum_n (Y - \bar{Y})^2$$

Where  $a$  is the number of groups,  $n$  is the number of observations within each group,  $Y$  is an individual observation, and

$$\bar{Y}$$

is the mean of each group. Applying this formula to our prairie burn data, where  $a = 3$  and  $n = 5$ , gives the following  $SS_{\text{within}}$ :

$$SS_{\text{within}} = (0.10 - 1.33)^2 + (0.61 - 1.33)^2 + \dots + (2.50 - 3.52)^2 + (6.10 - 3.52)^2 = 25.55$$

### ANOVA: the *F*-value

The test statistic for an ANOVA is called an **F-value**. The *F*-value for an ANOVA is calculated using the following formula:

$$F = \frac{\left( \frac{SS_{\text{among}}}{a-1} \right)}{\left( \frac{SS_{\text{within}}}{a(n-1)} \right)}$$

Where  $a$  is the number of groups and  $n$  is the number of observations within each group. Applying this formula to our prairie burn data, where  $a = 3$  and  $n = 5$ , gives the following *F*-value:

$$F = \frac{(32.08/2)}{(25.55/12)} = 7.57$$

If you look at the formula for the  $F$ -value, you can see that it is essentially the ratio of the variation *among* groups to the variation *within* groups. If the variation among groups is relatively large compared to the variation within groups, then the  $F$ -value will be relatively large. A relatively *large*  $F$ -value suggests that the variation among groups is largely caused by a given variable or experimental manipulation (in our example, the timing of burning), rather than chance variation. If the variation among groups is similar to the variation within groups, the  $F$ -value will be relatively small. A relatively *small*  $F$ -value suggests that the difference among groups is largely due to chance natural variation and measurement error, rather than to a given variable or manipulation. This interpretation of an  $F$ -value should sound familiar to you, because it is very similar to the interpretation of a  $t$ -statistic discussed above.

#### ANOVA: Minitab output

For a data set of any size, an ANOVA is extremely tedious to calculate by hand. As such, you will be using Minitab to do ANOVAs (see below). The Minitab ANOVA output for our prairie burn study is shown below.

```

Session

General Linear Model: Total Biomass versus Season

Factor Type Levels Values
Season fixed 3 Fall, Late Summer, Spring

Analysis of Variance for Total Biomass, using Adjusted SS for Tests

Source DF Seq SS Adj SS Adj MS F P
Season 2 31.998 31.998 15.999 7.52 0.008
Error 12 25.546 25.546 2.129
Total 14 57.545

S = 1.45906 R-Sq = 55.61% R-Sq(adj) = 48.21%

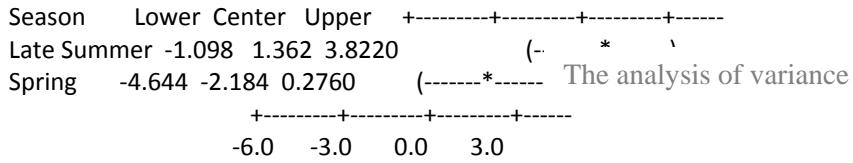
```

By custom, the results of an ANOVA are expressed in the above format, which not surprisingly is called an *ANOVA table*. In this table, estimates of  $SS_{\text{among}}$  and  $SS_{\text{within}}$  appear in the “Seq SS” column, and these same values divided by their degrees of freedom appear in the “Adj MS” (for “adjusted mean square”) column. The  $F$ -value (i.e., the ratio of adjusted Mean Squares) and other values in this table are not identical to the values calculated earlier in this handout because of rounding error in the hand calculations. The table also contains the  **$P$ -value** associated with the  $F$ -value. To review, a  $P$ -value ranges from 0 to 1, and is the probability of calculating a given test statistic assuming that the means of your groups are identical. The larger the test statistic is, the lower the chance ( $P$ ) that that an observed difference among groups is due to chance environmental variation, and the greater the chance that a difference has biological causation. With a sample size of 15 observations, the probability that the differences we see among spring, late summer, and fall burns are due to chance alone is 0.008. This value is bolded in the Minitab output. That is, the  $p$ -value equals 0.008. We can therefore reject the null hypothesis that burning season has no effect on *R. hirta* biomass, and accept the alternative hypothesis that it burning season does affect *R. hirta* biomass. If the  $P$ -value for our  $F$ -value had been  $>0.05$ , then we would have accepted that null hypothesis.

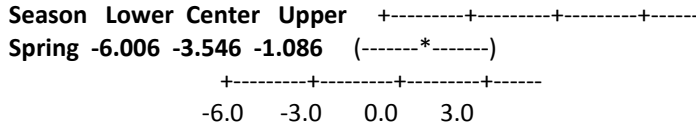
Note that the ANOVA will *not* tell you anything about pairwise differences between groups unless you run a pairwise comparison test. In this case we ran a Tukey’s pairwise comparison. The details of how this test works are beyond the scope of this handbook. Briefly, two means are considered significantly different if their true difference is very unlikely to be zero. The “Tukey 95.0% Simultaneous Confidence Intervals” below estimate the uncertainty around the true differences between each pair of means. Notice (in the bold-face output) that only in the case of the Late Summer mean subtracted from the Spring mean does the confidence interval fail to include zero and does the corresponding  $P$ -value fall below 0.05 (0.0061, to be exact).



Tukey 95.0% Simultaneous Confidence Intervals  
 Response Variable Total Biomass  
 All Pairwise Comparisons among Levels of Season  
 Season = Fall subtracted from:



Season = Late Summer subtracted from:



Tukey Simultaneous Tests  
 Response Variable Total Biomass  
 All Pairwise Comparisons among Levels of Season  
 Season = Fall subtracted from:

Season	Difference of Means	SE of Difference	Adjusted T-Value	P-Value
Late Summer	1.362	0.9228	1.476	<b>0.3361</b>
Spring	-2.184	0.9228	-2.367	<b>0.0843</b>

Season = Late Summer subtracted from:

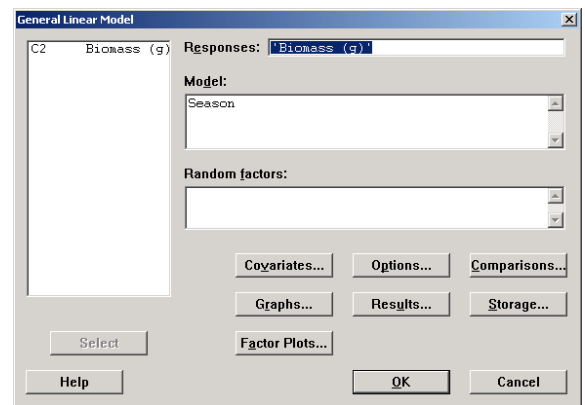
Season	Difference of Means	SE of Difference	Adjusted T-Value	P-Value
Spring	-3.546	0.9228	-3.843	<b>0.0061</b>

**How to do an ANOVA with pair-wise comparisons in Minitab**

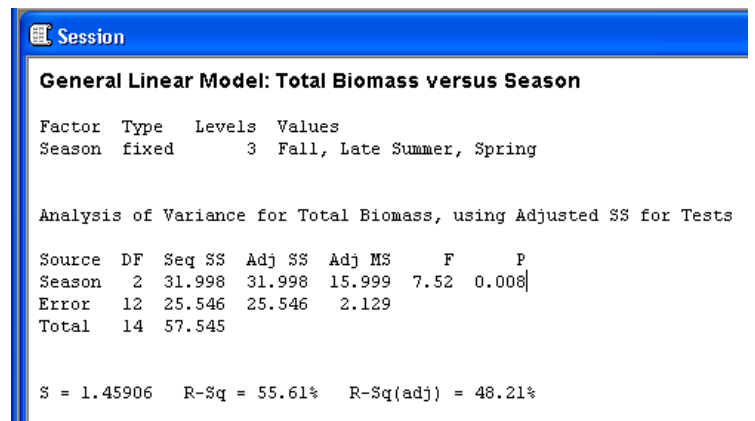
1. Enter your data so that one labeled column contains the experimental treatment information (independent variable). In the prairie burn example the entries are “spring”, “summer”, and “fall”. A second labeled column contains the dependent variable data (what you measured). It is biomass in this example.
2. From the menu bar select **Stat > ANOVA > general linear model**.

↓	C1-T	C2
	Season	Biomass (g)
1	spring	0.10
2	spring	0.61
3	late summer	5.56
4	late summer	6.97
5	fall	3.85
6	fall	3.01
7		

3. In the dialog window, place the dependent variable (what you measured) in the **Response** box. Place the independent variable (what you manipulated) in the **Model** box.



4. The output that appears in the session window is shown at right and also shown and explained previously in the One Way Analysis of Variance.



5. To make pair-wise comparisons between groups click the **Comparisons** button and choose **Pair-wise comparisons**. In the **Terms** box enter the column with the independent variable (use the same one you used for the Model box in the ANOVA window) and choose a **Method**. **Tukeys** is a common method to run. Click **OK** for the Comparisons box and **OK** again to run the ANOVA. The ANOVA and the Tukey’s comparison output is shown in the ANOVA output section.
6. An **Interval Plot** is useful for illustrating the results of an ANOVA. See the graphing section for instructions (Appendix B) or use the Graph options button in the ANOVA window.

**Example problems**

Carry out One-way ANOVAs for the following data sets:

1. Three different methods were used to measure dissolved oxygen (mg/kg) content of lake water. (Data from JH Zar's *Biostatistical Analysis*, 4<sup>th</sup> ed.)

Method		
1	2	3
10.96	10.88	10.73
10.77	10.75	10.79
10.90	10.80	10.78
10.69	10.81	10.82
10.87	10.70	10.88
10.60	10.82	10.81

2. Number of eggs laid per female per day for females from each of three lines of *Drosophila melanogaster*. The RS and SS lines were selected for resistance and susceptibility to DDT. The NS line is the nonselected control. (Data from RR Sokal and FJ Rohlf's *Biometry*, 3<sup>rd</sup> ed.)

Line		
RS	SS	NS
12.8	38.4	35.4
21.6	32.9	27.4
14.8	48.5	19.3
23.1	20.9	41.8
34.6	11.6	20.3
19.7	22.3	37.6
22.6	30.2	36.9
29.6	33.4	37.3

3. Strontium concentrations (mg/ml) in three different bodies of water. (Data from JH Zar's *Biostatistical Analysis*, 4<sup>th</sup> ed.)

Grayson's Pond	Beaver Lake	Angler's Cove
28.2	39.6	46.3
33.2	40.8	42.1
36.4	37.9	43.5
34.6	37.1	48.8
29.1	43.6	43.7
31.0	42.4	40.1

**Factorial Analysis of Variance**

**A two-way ANOVA**

A one-way analysis of variance (ANOVA), is appropriate for evaluating variation in a dependent variable among two or more levels of a single categorical independent variable.

Suppose, however, that you've designed an experiment that involves *two or more levels* of each of *two or more independent variables*, in *all possible combinations*. This is called a *factorial* design. The simplest example of this situation would be an experiment that applied two levels of each of two treatment variables, giving four combinations. Consider a concrete example.

Amy Lindahl (class of 1999) investigated the response to interspecific competition of two subspecies of the California native plant *Clarkia xantiana*, by cultivating individuals of each subspecies both alone and in competition with four individuals of a non-native grass called *Bromus tectorum* (Table. 1).

Table 1. Design of a two-by-two factorial interspecific competition experiment involving *C. xantiana* subspecies with contrasting life histories.

Subspecies of <i>C. xantiana</i>	Interspecific competition treatment	
	No competition	With <i>B. tectorum</i>
	<i>xantiana</i>	20 replicates
<i>parviflora</i>	20 replicates	20 replicates

Amy wanted to learn three things in this experiment. First, she wanted to find out whether competition reduced individual performance (estimated by shoot biomass, node number, and other measures of adult plant size). Second, she wanted to learn whether an individual's subspecies affected its overall size. Third, and most importantly, she wanted to learn whether individuals the two subspecies responded differently to competition. That is, she wanted to assess whether the reduction in performance associated with competition with grass differed between the subspecies -- whether one subspecies had superior competitive ability to the other. In a statistical sense, this third goal required her to test for the significance of the *interaction* between competition and subspecies.

Amy analyzed her data with a two-way ANOVA. The appropriate model for this experiment is: Response variable = competition + subspecies + competition\*subspecies + error.

"Competition\*subspecies" should be read as "competition by subspecies." Table 2 contains a fake data set that has the same structure (but not as much replication) as Amy's.

Table 2. These fake data depict authenticated fact.

Competition treatment	Subspecies	Shoot biomass (mg)
None	<i>xantiana</i>	113
None	<i>xantiana</i>	87
None	<i>xantiana</i>	102
None	<i>xantiana</i>	98
None	<i>parviflora</i>	12
None	<i>parviflora</i>	9
None	<i>parviflora</i>	11
None	<i>parviflora</i>	12
<i>Bromus</i>	<i>xantiana</i>	88
<i>Bromus</i>	<i>xantiana</i>	92
<i>Bromus</i>	<i>xantiana</i>	85
<i>Bromus</i>	<i>xantiana</i>	95
<i>Bromus</i>	<i>parviflora</i>	6
<i>Bromus</i>	<i>parviflora</i>	8
<i>Bromus</i>	<i>parviflora</i>	7
<i>Bromus</i>	<i>parviflora</i>	7

To analyze data like these, go to "Stat" then to "ANOVA" and then to "General linear model" in Minitab. In the "response" box, place your response variable (e.g., shoot biomass, in Table 2). In the "model" box, click or type in "competition", "subspecies", and "competition\*subspecies". If you run the analysis on the above data, you'll get Table 3's output (among other things), which is edited for clarity.

## Factorial Analysis of Variance

Table 3. Two-way ANOVA of effects of competition treatment and subspecies identity (see Table 2).

Source	df	Adj MS	F	P
Competition	1	196.0	5.1	0.034
Subspecies	1	29584.0	861.67	0.000
Competition*subspecies	1	36.0	1.05	0.326
Error	12	34.3		
Total	15			

The *F*-statistics test whether each of the "sources" of variance contributes significantly to the total variance in the data. In the above case, there are significant effects of **competition** (plants grew larger without it, across both subspecies) and **subspecies** (subspecies *xantiana* individuals grew larger than subspecies *parviflora* individuals, both in and out of competition), but there is not a significant effect of **the interaction between competition and subspecies**. In this hypothetical case, the effect of competition did not differ between the subspecies. We cannot conclude that subspecies *parviflora* is the weaker interspecific competitor.

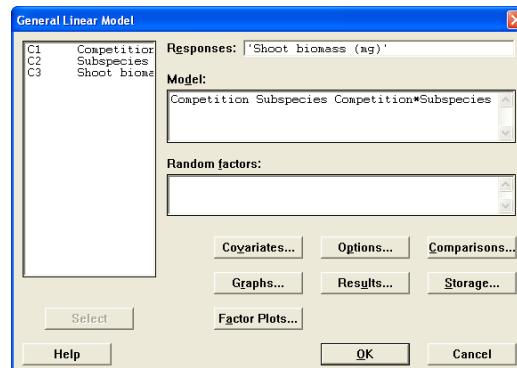
To analyze experiments that use more than two categories you'll need to scale up these directions. A three-way ANOVA, for example, requires testing for the significance of three main effects, three two-way interactions, and one three-way interaction.

### How to do a Two way ANOVA in Minitab using the General Linear Model

1. Enter your data so each data point is a row with columns for as many independent (models) and dependent (response) variables as you have applied. (e.g. for this experiment there are 2 independent variables (Competition and Subspecies) and one dependent variable (shoot biomass (mg))).
2. Go to the Stat menu and select **ANOVA>general linear model**.

	C1-T	C2-T	C3
	Competition	Subspecies	Shoot biomass (mg)
1	None	xantiana	113
2	None	xantiana	87
3	None	xantiana	102
4	None	xantiana	98
5	None	parviflora	12
6	None	parviflora	9
7	None	parviflora	11
8	None	parviflora	12
9	Bromus	xantiana	88
10	Bromus	xantiana	92
11	Bromus	xantiana	85
12	Bromus	xantiana	95
13	Bromus	parviflora	6
14	Bromus	parviflora	8

3. In the dialog window, place the dependent variable (the one you are measuring, shoot height) in the **Response box**. In the **Model box** enter the treatment variables by clicking on them or typing in "competition", "subspecies", and "competition\*subspecies". Delete any spaces in the last term. Check out the other buttons to see if the options available would be helpful to the analysis you are doing. The **Graph** button may be useful.

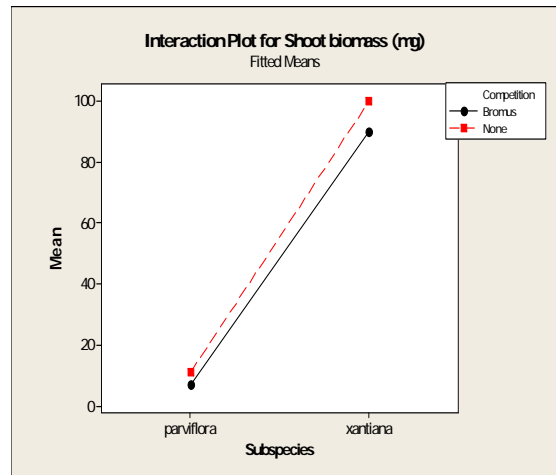
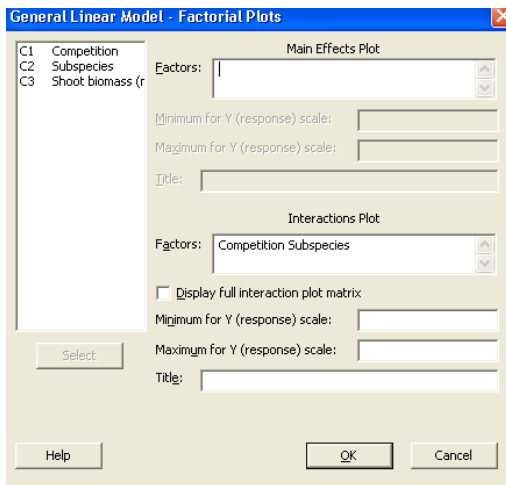


## Factorial Analysis of Variance

4. Click **OK** to view the output. It will show a p-value for each of the two treatments and for the interaction of the two treatments

Source	df	Adj MS	F	P
Competition	1	196.0	5.1	0.034
Subspecies	1	29584.0	861.67	0.000
Competition*subspecies	1	36.0	1.05	0.326
Error	12	34.3		
Total	15			

5. When you are doing a Two-way ANOVA an **Interaction Plot** is a good way to convey results. To do so, click the **Factor Plots** box in step three above. In the Factor Plots window click in the **Factors** box in the **Interactions Plot** section (not the Main Effects Plot section) and enter the factors you wish to plot, click **OK**. Minitab will make a plot similar to the one shown below on the right. You should remove the titles and make a figure legend if it is to be included in a paper.



## Correlation and Regression Analysis

The t-test and analysis of variance are used to compare group means. But not all data neatly fall into discrete groups. In many cases, scientists are interested in studying the relationship between two or more *continuously distributed* variables. Two related statistical hypothesis tests are used to examine relationships between such variables: *correlation* and *regression*.

### Correlation analysis

Correlation analysis is used to analyze the relationship between two continuously distributed variables. This analysis does not make any assumptions about the causal relationship between variables. That is, you do not have to specify if variable 1 is responsible for variation in variable 2, or vice-versa. Thus, correlation analysis is a very useful for analyzing data collected in the early, exploratory phases of a study, before you have formulated cause-and-effect hypotheses.

As an example, consider the measurements of flowers in Table 3, below.

Table 3. Length and width of *Lobelia siphilitica* (Great lobelia) flowers growing at CERA during Fall 1999.

Length	Width
17.616	4.811
16.858	4.280
17.123	5.697
18.416	5.790
19.673	5.593
17.093	6.116
18.322	5.238
16.875	5.618
18.111	6.412
17.744	5.896
Mean = 17.783	Mean = 5.545

Suppose we are interested in seeing if there is a strong relationship between the length and width of these prairie flowers. For example, do long flowers also tend to be wide? Correlation, rather than regression, would be the appropriate way to analyze these data because the causal relationship between flower length and width is unclear. We cannot discriminate between the hypotheses that (1) variation in flower length is the cause of variation in flower width or (2) variation in flower width is the cause of variation in flower length. But the advantage of correlation analysis is that you can estimate the association between two variables without knowing anything about causal relationships between them.

The *correlation coefficient* ( $r$ ) is calculated using the following formula:

$$r = \frac{C_{12}}{\sqrt{V_1 V_2}}$$

where  $C_{12}$  is the covariance between variables 1 and 2,  $V_1$  is the variance of variable 1, and  $V_2$  is the variance of variable 2. The *covariance* is calculated using the following formula:

$$C_{12} = \frac{\sum (X_1 - \bar{X}_1)(X_2 - \bar{X}_2)}{n - 1}$$

where  $n$  is the number of paired observations and  $X_1$  and  $X_2$  are your two variables. Applying this formula to our prairie flower data gives the following covariance:

$$C_{12} = \frac{(17.616 - 17.783)(4.811 - 5.545) + \dots + (17.744 - 17.783)(5.896 - 5.545)}{9} = 0.120338$$

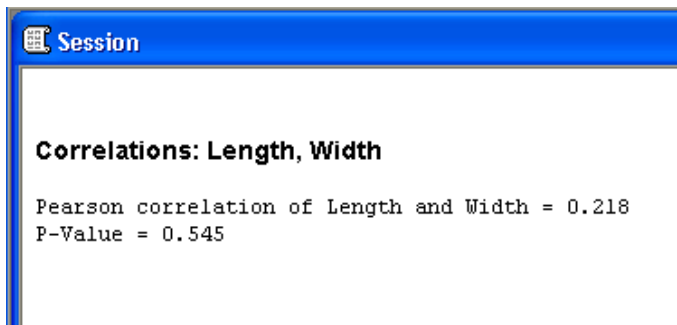
The covariance is an estimate of whether the variables' deviations from their respective means tend to be in the same direction ( $C_{12} > 0$ ), in different directions ( $C_{12} < 0$ ), or follow no strong pattern ( $C_{12}$  is approximately 0).

Now that we have calculated the covariance, we can go back and calculate the Pearson correlation coefficient:

$$r = \frac{0.120338}{\sqrt{(0.776898)(0.391631)}} = 0.218$$

The correlation coefficient varies from -1 to 1. If  $r$  is close to one, there is a strong positive relationship between the variables. If  $r$  is close to -1, there is a strong negative relationship between the variables. And if  $r$  is close to 0, there is a weak relationship between the variables. As such, the correlation coefficient that we calculated for our prairie plant data ( $r = 0.218$ ) indicates that there is a positive, but relatively weak, relationship between the length and width of flowers in our sample. Correlation coefficients can be calculated using Minitab; see below for instructions.

If you were to use Minitab to calculate the correlation coefficient between flower length and width you would get the following output:



```

Session

Correlations: Length, Width

Pearson correlation of Length and Width = 0.218
P-Value = 0.545

```

### Regression analysis

*Regression* analysis is closely related to correlation; both techniques are used to examine the relationship between continuously distributed variables. But regression and correlation differ in that only regression analysis requires you to specify a causal relationship between your variables. In regression one variable is the *independent* or *predictor variable*, whereas the other variable is the *dependent* or *response variable*. The independent variable is believed to be the *cause* of variation in the dependent variable. Thus regression is preferred to correlation when you have some pre-existing hypothesis about the relationship between your variables. Regression analysis could not have been used to analyze the prairie flower data discussed above because we had no scientific rationale to determine which variable was independent and which was dependent.

To demonstrate how regression analysis works, consider the measurements of prairie plant flowers in Table 4, below.



Table 4. Flower width and fruit set of *Lobelia siphilitica* (Great lobelia) plants growing at CERA during Fall 1999.

Width	Fruits/plant
4.811	60
4.280	14
5.697	34
5.790	35
5.593	20
5.618	12
6.412	23
5.873	69
5.346	54
4.900	18
Mean = 5.432	Mean = 33.9

Supposed we are interested in determining if plants with wide flowers produce more fruits than those with narrow flowers. Although this hypothesis may seem farfetched, flower width can potentially influence the ability of bees to deposit pollen on *L. siphilitica*. Because this pollen is necessary for *L. siphilitica* to produce fruits, flower width may influence fruit set. These data are a good candidate for analysis by regression, as there is clearly a predictor variable (flower width) and a response variable (fruits/plant). There is nothing to stop us from analyzing these data using correlation analysis. But as you'll see below, regression will tell us much more about the relationship between our variables than correlation analysis does.

When you use regression to analyze data, you are fitting your data to a straight line. This line can be represented using the following equation:

$$Y_i = \alpha + \beta X_i$$

where  $Y$  and  $X$  are, respectively, your dependent and independent variables,  $\alpha$  is the y-intercept of the line, and  $\beta$  is the slope of the line. We are usually interested in estimating  $\beta$ , which is a measure of the strength of the relationship between your two variables.  $\beta$  can be estimated using the following formula:

$$\beta = \frac{\sum (X_i - \bar{X})(Y_i - \bar{Y})}{\sum (X_i - \bar{X})^2}$$

Where  $X$  and  $Y$  are, respectively, your independent and dependent variables. Notice that the numerator of this formula is identical to the numerator of the formula for the covariance that was discussed above. Once you have calculated  $\beta$ , you can calculate  $\alpha$  simply by substituting  $\beta$ , the mean of  $X$ , and the mean of  $Y$  into the above formula for a regression line. Once you have calculated  $\alpha$  and  $\beta$ , you can plug any value of  $X$  into the formula to get the predicted value of  $Y$ . This ability to predict  $Y$  from  $X$  is unique to regression analysis; you cannot make such predictions based on correlation analysis.

Applying this formula to our prairie flower data gives the following  $\beta$ :

$$\beta = \frac{(4.811 - 5.432)(60 - 33.9) + \dots + (4.900 - 5.432)(18 - 33.9)}{(4.811 - 5.432)^2 + \dots + (4.900 - 5.432)^2} = 3.615$$

Once you have calculated  $\beta$ , you can calculate a *test statistic* to determine if  $X$  explains a significant amount of the variation in  $Y$ . The appropriate test statistic is called an *F-value*. Calculating an *F-value* is a multi-step process. First, you need to calculate the *total sum of squares*. The total sum of squares is simply a measure of the variability of the dependent variable  $Y$ , and it can be calculated using the following formula:

$$totalSS = \sum (Y_i - \bar{Y})^2$$

where  $Y$  is your dependent variable. Applying this formula to our prairie flower data gives the following total sum of squares:

$$totalSS = (60 - 33.9)^2 + \dots + (18 - 33.9)^2 = 3758.900$$

Next, you need to calculate the *regression sum of squares*. The regression sum of squares is simply the amount of variation in the dependent variable  $Y$  that is accounted for by the independent variable  $X$ , and can be calculated using the following formula:

$$regressionSS = \frac{(\sum (X_i - \bar{X})(Y_i - \bar{Y}))^2}{\sum (X_i - \bar{X})^2}$$

Where  $X$  and  $Y$  are, respectively, your independent and dependent variables. Notice that this formula is very similar to the formula for  $\beta$ . Applying this formula to our prairie flower data gives the following regression sum of squares:

$$regressionSS = \frac{((4.811 - 5.432)(60 - 33.9) + \dots + (4.900 - 5.432)(18 - 33.9))^2}{(4.811 - 5.432)^2 + \dots + (4.900 - 5.432)^2} = 44.659$$

The last value that you need to calculate before you can estimate  $F$  is the *residual sum of squares*. The residual sum of squares is an estimate of the amount of variation in the dependent variable  $Y$  that is *not* explained by the independent variable  $X$ . As you will remember, the total sum of squares is an estimate of the variation in the dependent variable  $Y$ , and the regression sum of squares is the amount of variation in  $Y$  that is explained by the independent variable  $X$ . As such, the residual sum of squares can be obtained by simple subtraction:

$$residualSS = totalSS - regressionSS$$

Applying this formula to our prairie flower data gives the following residual sum of squares:

$$residualSS = 3758.900 - 44.659 = 3714.241$$

The test statistic  $F$  can now be calculated using the following formula:

$$F = \frac{regressionSS}{residualSS / n - 2}$$

Where  $n$  is the number of paired ( $X$ - $Y$ ) observations in your data set. Applying this formula to our prairie flower data gives the following  $F$ -value:

$$F = \frac{44.659}{3714.241 / 8} = 0.096$$

The last quantity that you can calculate is  $r^2$ , the *coefficient of determination*. The  $r^2$  varies from 0 to 1 and is an estimate of the proportion of the total variation in the dependent variable  $Y$  that is explained by the independent variable  $X$ . The tighter the fit of the data to the regression line, the larger  $r^2$  is. The  $r^2$  can be calculated using the following formula:

$$r^2 = \frac{regressionSS}{totalSS}$$

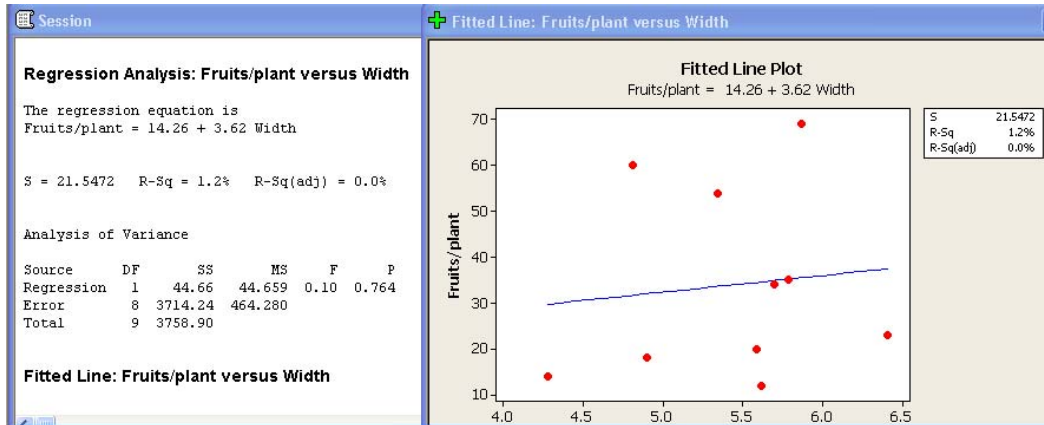
## Correlation and Regression Analysis

$$r^2 = \frac{44.659}{3758.900} = 0.012$$

Applying this formula to our prairie flower data gives the following  $r^2$ :

This  $r^2$  indicates that flower width explains ~1% of the variation in fruit set. (Note: The  $r^2$  can also be calculated by squaring the **correlation coefficient,  $r$** .)

If you were to use Minitab to analyze the relationship between flower width and fruit set you would get the following output:



In addition to the equation for the estimated regression line (with  $Y$ -intercept and slope  $[\beta]$ ), you should see many familiar quantities in this output, including  $r^2$ ,  $F$ , residual (“error”) sum of squares, regression sum of squares, total sum of squares, and the now familiar (hopefully!)  $p$ -value. To review, a  $P$ -value ranges from 0 to 1, and is the probability of calculating a given test statistic. The larger the test statistic is, the lower the chance ( $P$ ) that that the relationship between your dependent and independent variables is due to chance alone, and the greater the chance that a relationship has biological causation. With a sample size of 10  $X$ - $Y$  pairs, the probability that a relationship is due to chance alone is 0.764. That is, the  $P$ -value equals 0.764. By convention, most biologists agree that if the probability of a certain test statistic is greater than 0.05, then it is likely that the relationship between variables is due to chance variation or measurement error, rather than any biological causation. So based on our test statistic, we can accept the null hypothesis that flower width does not influence fruit set of *Lobelia siphilitica*

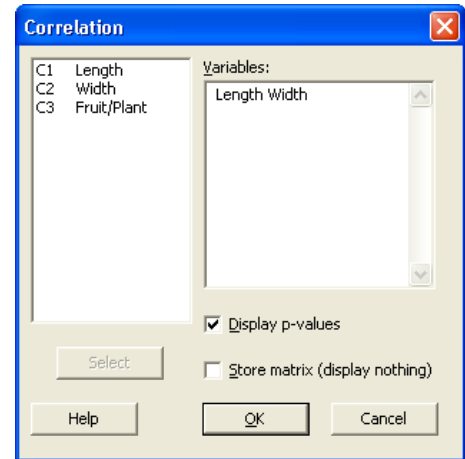
### How to do correlation analysis in Minitab

1. Enter your data so that each variable is in a separate column. Label your columns appropriately. For example, in our prairie flower example, the columns could be labeled "length" and "width".

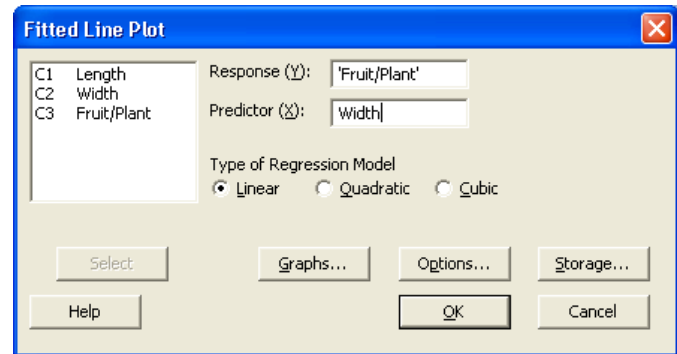
+	C1	C2	C3
	Length	Width	
1	17.616	4.811	
2	16.858	4.280	
3	17.123	5.697	
4	18.416	5.790	
5	19.673	5.593	
6	17.093	6.116	
7	18.322	5.238	

## Correlation and Regression Analysis

2. Select **Stat > Basic Statistics > Correlation**. Place your variable's names in the **Variables** box.



3. Click OK to view the correlation coefficient(s) output and associated p-value. An example output from a Minitab correlation is shown and explained in the previous section.



### How to do regression analysis with a fitted line plot in Minitab

1. Enter your data so that each variable is in a separate column. Label your columns appropriately. In our prairie flower example, the columns could be labeled "fruit set" and "width".
2. Select **Stat > Regression > Fitted line plot**.
3. Enter the appropriate data into the **Response(Y)** and the **Predictor(X)** boxes. Generally the Response(Y) data is the dependent variable (what is measured) and the Predictor(X) data is the independent variable (what you are testing).
4. Click **OK** to view the regression analysis output and the corresponding graph. An example of a Minitab regression output is shown and explained in the previous section.
5. In the graph, replace the titles with a figure legend. To delete the titles, right click on them and choose **delete**. To add a figure legend create a text box within the graph and type in the appropriate text. The **text box button** **T** is on the tool bar at the top of the window. Click on it and use the crosshairs to drag open a text box to type in. Alternatively, you can incorporate the figure legend into the text below the figure.

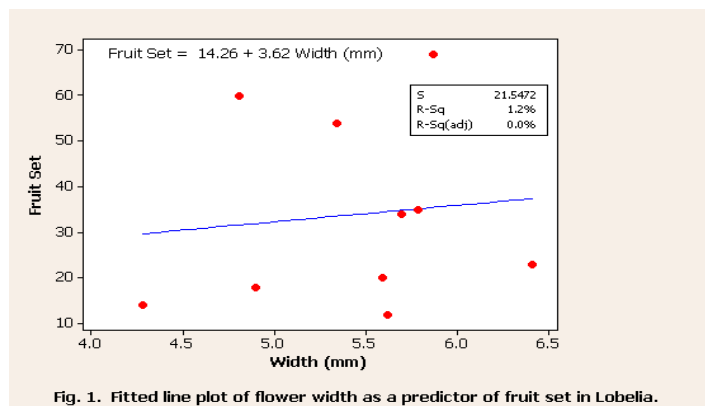


Fig. 1. Fitted line plot of flower width as a predictor of fruit set in Lobelia.

**Example Problems**

Use regression and correlation to analyze the following data sets.

1. Weight loss (in mg) of *Tribolium* beetles raised at different relative humidities. (Data from RR Sokal and FJ Rohlf's *Biometry*, 3<sup>rd</sup> ed.)

Weight loss	Percent relative humidity
8.98	0
8.14	12
6.67	29.5
6.08	43
5.90	53
5.83	62.5
4.68	75.5
4.20	85
3.72	93

2. Heart rate and oxygen uptake ( $VO_2$ ) for a single human. (Data from DS Moore and GP McCabe, *Introduction to the practice of statistics*, 3<sup>rd</sup> ed.)

Heart rate	$VO_2$
94	0.473
95	0.929
96	0.753
104	1.178
106	1.292
108	1.403
110	1.499
113	1.529
115	1.746
118	1.749
121	1.897

3. Development time (in days) of the potato leafhopper from egg to adult at various temperatures (in °F). (Data from RR Sokal and FJ Rohlf's *Biometry*, 3<sup>rd</sup> ed.)

Temperature	Development time
59.8	58.1
67.6	27.3
70.0	26.8
70.4	26.3
74.0	19.1
75.3	19.0
78.0	16.5
80.4	15.9
81.4	14.8
83.2	14.2
88.4	14.4

**Analysis of categorical data:  $\chi^2$  analysis**

Not all scientific data are quantitative and/or continuously distributed. Some are more appropriately scored as categories. For example, in most organisms, sex expression (male, female, hermaphrodite in animals and plants; mating type in some other organisms) falls into discrete categories. When you want to evaluate patterns in variables like these, it is often appropriate to use  $\chi^2$  analysis.

**1. Tests of goodness-of-fit**

Sometimes you want to know whether the frequency of data in different categories fits some expected distribution. For example, in transmission genetics, one might test whether progeny phenotype ratios in the  $F_2$  generation of a monohybrid cross fit a 3:1 expectation (under single-locus, fully dominant inheritance). Here's an example from Gregor Mendel's original inheritance experiments on garden peas (*Pisum sativum*). Mendel crossed two true-breeding varieties of peas, one with purple flowers and one with white flowers. In the first hybrid generation (the  $F_1$ ), all the plants had purple flowers. In the second hybrid generation, created by allowing the  $F_1$  to self-pollinate, he obtained 705 purple-flowered plants and 224 white-flowered plants, out of 929 total (Observed numbers in Table 5, below). Mendel had to figure out the rules of inheritance without an a priori expectation about phenotypic ratios, but we can take advantage of what he learned to test the hypothesis that these numbers are close enough to the expected 3:1 ratio that any deviations are likely to be due to chance. We can calculate the expected number of purple-flowered plants by multiplying the total number of plants (929) by the proportion expected to be purple (0.75). The expected number of white-flowered plants can be calculated by multiplying the total number of plants by the proportion expected to be white (0.25). These expected numbers are in Table 5, below.

**Table 5. Observed and expected number of white- and purple-flowered *Pisum sativum* plants**

	Color	
	Purple	White
<b>Observed number</b>	<b>705</b>	<b>224</b>
<b>Expected number</b>	<b>696.75</b>	<b>232.25</b>

You can calculate the chi-square test statistic ( $\chi^2$ ) using the following formula

$$\sum \left( \frac{(\text{observed number} - \text{expected number})^2}{\text{expected number}} \right)$$

If you plug the values in Table 5 into this formula, you get a  $\chi^2$  value of 0.391. The  $\chi^2$ , like all other test statistics, has an associated p-value. For a data set with two categories (white and purple flowers), the p-value is <0.05 if the  $\chi^2$  is greater than 3.84. Because our  $\chi^2$  was less than 3.84, we would conclude that the observed ratio of purple to white-flowered plants (705/224) and the expected ratio of purple to white-flowered plants (696.75/232.25 or 3/1) are not significantly different. In other words, the observed ratio of purple to white-flowered plants fits the 3:1 model we proposed.

Table 6. Critical values of the  $\chi^2$  statistic

d.f.      critical  $\chi^2$  ( $p < 0.05$ )

1	3.84
2	5.99
3	7.81
4	9.49
5	11.1
6	12.6
7	14.1

**2. Tests of association between categorical variables**

Sometimes you have no a priori expectation about the frequencies of different data, but you want to know whether there is some non-random association between variables. Consider the following data on late-summer prairie species (Table 6). Some plants have flowers that are radially symmetrical (like a pumpkin pie). That is, if you make *any* lengthwise section that goes through the center of such a flower, the resulting halves look alike, except that they are mirror images. Other plants have flowers with bilateral symmetry (like a human body). Here, only one *specific* section through flowers gives symmetrical halves. While it is not a hard-and-fast rule, plants with bilaterally symmetric flowers tend to have relatively specialized (exclusive) relationships with their animal pollinators, while those with radially symmetrical flowers tend to have rather diverse flower visitors. Flower color also relates to pollination biology, because of variation in the sensory capabilities of different animal pollinators. A  $\chi^2$  test can be used to determine if there is any association between flower color and symmetry.

**Table 6. Animal-pollinated angiosperm species observed at the CERA (September 1996), sorted by flower symmetry and color. Each combination of symmetry and color is referred to as a cell.**

Color	Symmetry	
	Radial	Bilateral
White	1	10
Yellow	2	13
Blue or violet	7	9

To calculate a  $\chi^2$ , you need to determine the number you would expect in each cell if there was *no* association between flower color and symmetry. To calculate the expected number, take the total for a cell's row, multiply it by the total for a cell's column, and divide that product by the grand total. For example, the expected number of yellow, radially symmetrical flowers is:

$$[(2 + 13) \times (1 + 2 + 7)] \div 42 \approx 3.6$$

If you plug the data in Table 6 into the formula in A.6.1, you get a  $\chi^2$  of 5.816. For a data set with three rows and two columns, the p-value is  $< 0.05$  if the  $\chi^2$  is greater than 5.99. Because our  $\chi^2$  was less than 5.99, we would conclude the floral symmetry and color were not significantly associated.

**How to do  $\chi^2$  analysis in Minitab**

1. Enter your data so that the table of counts you want to analyze appears in labeled columns. In the above example, just enter the radial symmetry counts in one column and the bilateral symmetry counts in an adjacent column.
2. From the menu bar select **Stat > Tables > Chi-square Test**.

3. In the dialog box, enter the column *Analysis of categorical data:  $\chi^2$  analysis*

4. Click **OK** to view the  $\chi^2$  analysis output, which includes an exact p-value.

### Example problems

Use chi-square to analyze the following data sets.

- Results of a cross between two varieties of the bean *Phaseolus vulgaris*. The expected ratio was 18:6:6:2:12:4:12:4. For a data set with 8 categories, the p-value is <0.05 if  $\chi^2$  is greater than 14.067. (Data from RR Sokal and FJ Rohlf's *Biometry*, 3<sup>rd</sup> ed.)

Phenotype	Observed numbers
Purple/buff	63
Purple/testaceous	31
Red/buff	28
Red/testaceous	12
Purple	39
Oxblood red	16
Buff	40
Testaceous	12

- Fate of mice that received either pathogenic bacteria, or pathogenic bacteria, followed by an antiserum. For a data set with two rows and two columns, the p-value is <0.05 if  $\chi^2$  is greater than 3.84. (Data from RR Sokal and FJ Rohlf's *Biometry*, 3<sup>rd</sup> ed.)

	Fate	
	Dead	Alive
Bacteria and antiserum	13	44
Bacteria only	25	29

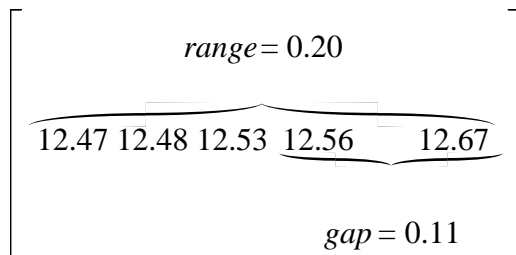
- Disease status and number of cigarettes smoked per day for 2225 males. For a data set with five rows and two columns, the p-value is <0.05 if  $\chi^2$  is greater than 9.488. (Data from RR Sokal and FJ Rohlf's *Biometry*, 3<sup>rd</sup> ed.)

	Disease status	
	Lung cancer	Healthy
Never smoked	15	822
1-10	36	136
11-20	133	328
21-40	226	311
≥41	127	91



**Precision and relative error -- the q-test**

Consider the following set of data points: 12.47, 12.48, 12.53, 12.56, and 12.67. You suspect the last data point to be too far removed from the rest. To determine a Q value, compare the *gap* between the removed value and the nearest value to the *range* of all values:



$$Q = \frac{gap}{range} = \frac{0.11}{0.20} = 0.55$$

Compare this Q value to one listed in a table for your number of data points and the level of confidence you wish to use. A table for the 90% confidence level is shown below. If your Q value exceeds the tabulated value, you may safely discard the questionable result; otherwise, it must be kept. In using the Q test, bear in mind that the test is less reliable with the fewer data points you use for comparison.

Number of Observations	3	4	5	6	7	8	9	10
Q	0.94	0.76	0.64	0.56	0.51	0.47	0.44	0.41

Of course, a first result obtained before skills have been mastered, or results in which a recognizable error was made can be discarded. Otherwise it is best and more honest to report all results.

## Appendix B - How to Use Minitab

### Running Statistical Tests in Minitab

This appendix contains valuable information about how to use Minitab software but the actual instructions for running statistical tests are located in Appendix A, A Primer of Statistical Analysis. For each statistical test you will find background on how each test works, how to interpret the statistical output, sample data sets and problems, and detailed instructions for running each test. The page numbers where sections begin are listed here for your convenience:

Summary Statistics	pg. 39
t-test.	pg.42
One way ANOVA with pair-wise comparisons	pg.45
Two way ANOVA in Minitab using the General Linear Model	pg.51
Correlation and Regression Analysis	pg.54

### Minitab Basics:

#### A quick look at what you see when you open the software

More students have learned statistics with Minitab software than with any other statistical software package. Even though it has advanced capabilities, it is quite straightforward and useful for performing basic statistical functions. To get you started we will cover a few basic items that should help you become familiar with Minitab and how it “thinks” so you will be comfortable with it right from the start.

Minitab has three main windows: a Session window where numerical results of statistical tests and calculations are displayed, a data window for data worksheets with columns and rows of data, and a graph window for displaying graphs. Simply click in the window you want to become active. Multiple data and graph windows may be open for each Session window. A Project Manager window is also available in the bottom left corner. It becomes useful when you have multiple windows open.

The screenshot shows the Minitab software interface with several windows open. Labels on the left side point to specific parts of the interface:

- Title Bar**: Points to the top blue bar of the main window.
- Menu bar**: Points to the menu items (File, Edit, Data, Calc, Stat, Graph, Editor, Tools, Window, Help).
- Tool bars**: Points to the icons below the menu bar.
- Graph Window**: Points to the 'Interval Plot of Total Biomass' window.
- Session Window**: Points to the 'General Linear Model: Total Biomass versus Season' window.
- Data Window**: Points to the 'Worksheet 1' window.
- Generic column names**: Points to the column headers C1, C2, C3, C4, C5, C6.
- Column names**: Points to the specific column headers 'Season' and 'Total Biomass'.
- Row numbers**: Points to the row numbers 1 through 14.
- Project Manager Icon**: Points to the 'Project' icon in the bottom left corner.

The 'Interval Plot of Total Biomass' window displays a 95% CI for the Mean. The y-axis is 'Total Biomass' (0 to 7) and the x-axis is 'Season' (Fall, Late Summer, Spring). The plot shows three data points with vertical error bars representing the 95% confidence intervals.

The 'General Linear Model: Total Biomass versus Season' window displays the following ANOVA table:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Season	2	31.998	31.998	15.999	7.52	0.008
Error	12	25.546	25.546	2.129		
Total	14	57.544				

The 'Worksheet 1' window displays the following data:

Season	Total Biomass	
1	Spring	0.10
2	Spring	0.61
3	Spring	1.91
4	Spring	2.99
5	Spring	1.06
6	Late Summer	5.56
7	Late Summer	6.97
8	Late Summer	3.01
9	Late Summer	6.33
10	Late Summer	3.63
11	Fall	3.86
12	Fall	3.01
13	Fall	2.13
14	Fall	7.60

**Important information about the data window**

*Formatting data*

It is best to format the data columns before adding any data to the data window. You can format Minitab columns as Numeric, Text, or Date/Time by right clicking on the generic column name (C1, C2, etc.) and choosing Format Data. Minitab automatically formats columns that have not already had formats assigned to them as whatever type of character you first enter into the column. For example, if you type a word, that column becomes a text column, and a T appears next to the generic column name (see Fig. 2 below). It is not possible to do any calculations or statistical tests on Text formatted columns. The next section shows you how to change from one data format to another, so don't worry if you inadvertently assigned the wrong format to a column.

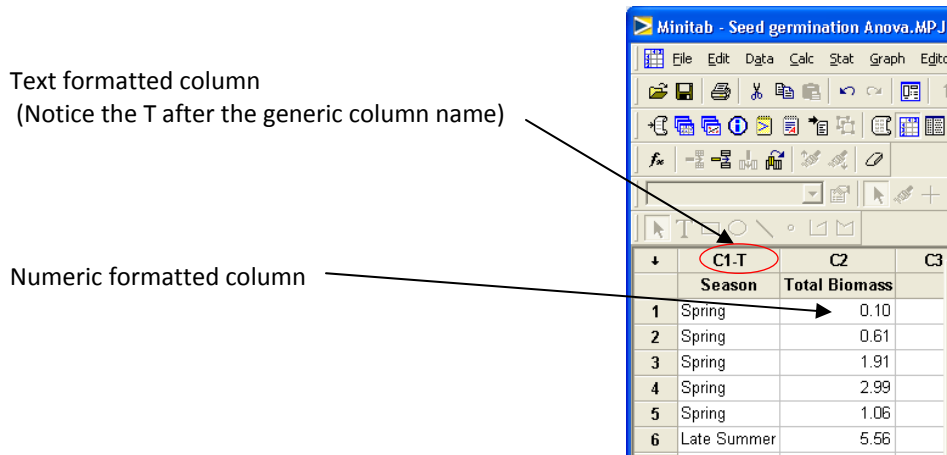
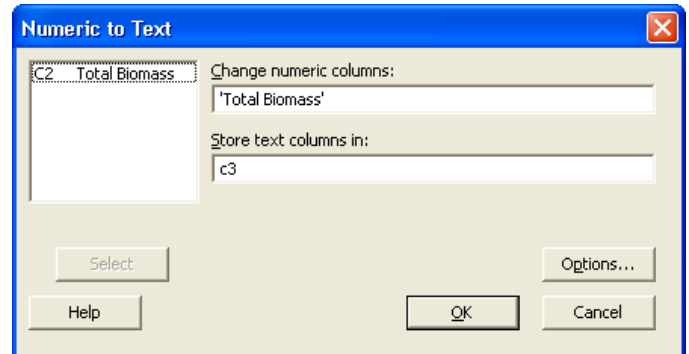


Figure 2. Minitab data formatting.

*Changing data type*

To change text data to numeric (or numeric data to text) go to the **Data** menu and pull down to **Change Data Type**. There you can choose the appropriate changes to make. A dialog box will open up asking you to choose which column of data to change. Choose the appropriate column by double clicking on it at the left side of the window. It should appear in the **Change numeric (text) columns** area. Next click in the **Store text (numeric) columns** area and, if you want your data to be in the same location, choose the same column. If you want a new location, type in a new column location. Click OK.



*Stacking and Unstacking Data*

There are two main ways of entering data into Minitab; stacked and unstacked. It is most useful to enter data in the stacked form but at times a different arrangement is required (i.e. for a paired T-test ). Stacked data are where each column is a separate variable, meaning that the independent variable (manipulation) would have one column and the dependent variable (measurements) would all be in another column. Unstacked data have the independent variables as the column titles and the corresponding measurements entered under the column titles. See Figures 3 and 4.

	C1-T	C2	C3	C4	C5	C6
	Season	Total Biomass				
2	Spring	0.61				
3	Spring	1.91				
4	Spring	2.99				
5	Spring	1.06				
6	Late Summer	5.56				
7	Late Summer	6.97				
8	Late Summer	3.01				
9	Late Summer	6.33				
10	Late Summer	3.53				
11	Fall	3.05				
12	Fall	3.01				
13	Fall	2.13				
14	Fall	2.50				
15	Fall	6.10				

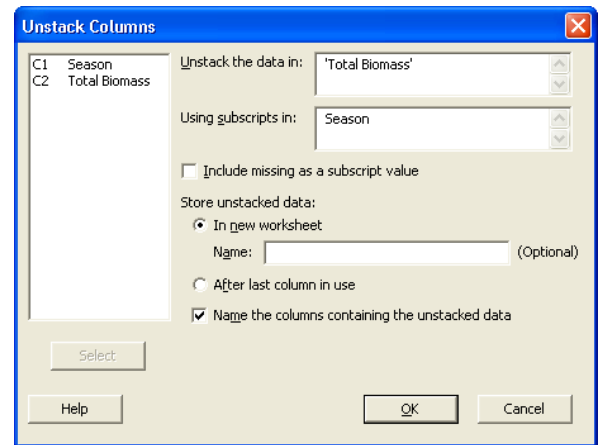
Figure 3. Stacked Data.

	C1	C2	C3
	Total Biomass_Fall	Total Biomass_Late Summer	Total Biomass_Spring
1	3.85	5.56	0.10
2	3.01	6.97	0.61
3	2.13	3.01	1.91
4	2.50	5.33	2.99
5	6.10	3.53	1.06
6			

Figure 4. The same data after unstacking

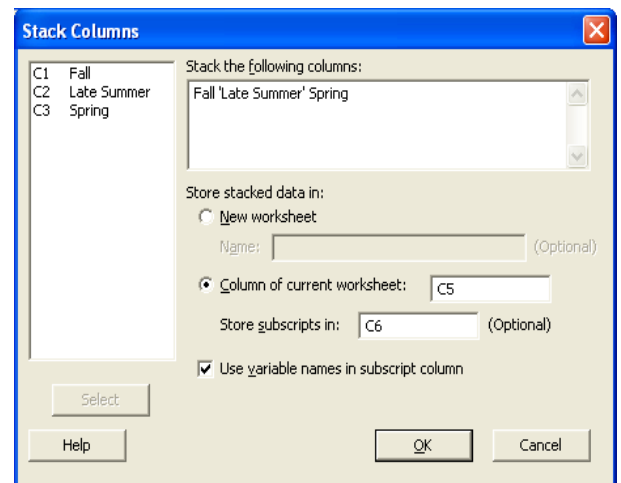
### Unstacking Data

To unstack data go to the **Data** menu and pull down to **Unstack columns**. While the cursor is in the **Unstack the data in** box double click on the dependent variable data (what you measured-total biomass). While the cursor is in the **Using subscripts in** box double click on the independent variable (Season). Next choose where you would like to put the unstacked data (a new worksheet works well) and check **name the columns containing unstacked data**. Click **OK**. The data should now look similar to Figure 4.



### Stacking Data

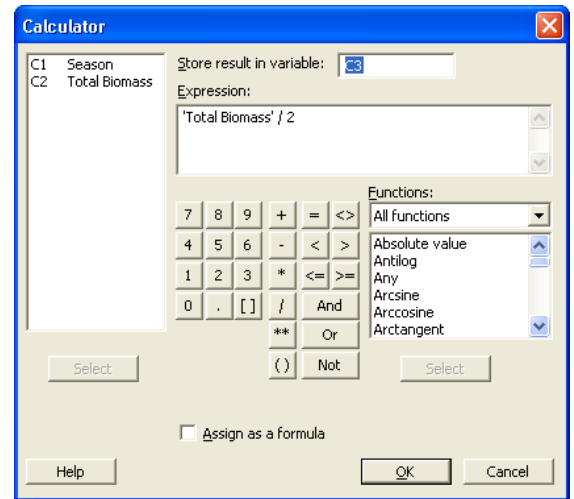
To stack data from several different columns into one column, go to the **Data** menu, pull down to **Stack**, and then choose **Columns**. With the cursor in the **Stack the Following Columns** box, double click on any of the columns at the left, designating the numerical data you want to stack. Next, choose where to store the stacked data, either in **New Worksheet** or **Column of Current Worksheet** by checking the appropriate box. Type in a column number to designate where you want the numerical data to be and the subscripts to be stored. The subscripts come from the titles or column numbers at the top of the columns you are stacking and subscripts are generally the independent variables of the experiment. Click **O.K.**



## Directions For Making Figures Using Minitab

### Running Calculations in Minitab

At times it is convenient to perform the same calculation on an entire column of data. Minitab is equipped to do this. Say that in our seed germination example you wanted to divide the number of seeds germinated by 2. Simply choose **Calc** from the menu bar and pull down to **Calculator**. When the dialog box opens choose a location for the calculations to be stored by typing in a generic column title into the **Store result in variable** box. Click in the **Expression** box and then double click on the column of data you wish to run the calculations on. Once the column appears, it is simply a matter of writing a formula (like you would for a calculator) and then clicking **OK**. The calculated data will appear in the assigned column. Notice that there is also a **Functions** area to the right of the calculator where you can choose functions to perform on the data.



### Directions For Making Figures Using Minitab

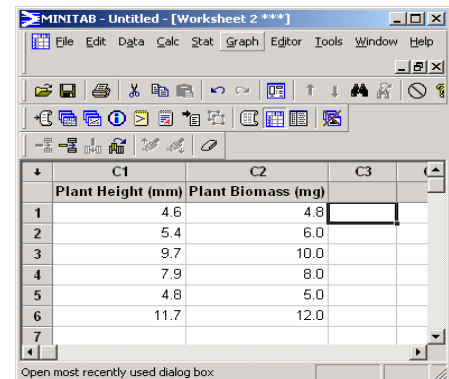
#### X-Y or Scatter plot

Use this sort of plot to illustrate the relationship between two variables measured for the same sample or individual (see Statistics sections *Correlation* and *Regression* in the Appendix). If you do a correlation analysis, place the r-value (Pearson's correlation coefficient) as text within the figure.

If you propose that X values cause or predict Y values, you will want to present the result as a *regression* analysis (see Statistic section for how this analysis is done and what it means).

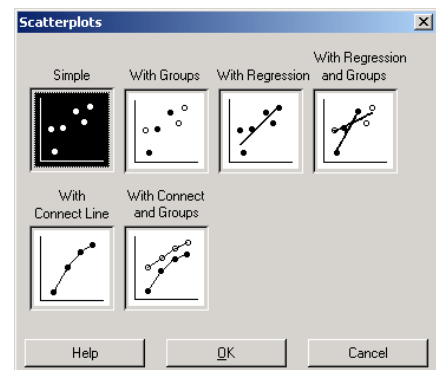
#### Creating a scatter plot in Minitab

Label two columns with the names and units of the two variables and then enter the paired data in each of the columns. Go to the **Graph** menu at the top of the window and drag down to **scatter plot**.



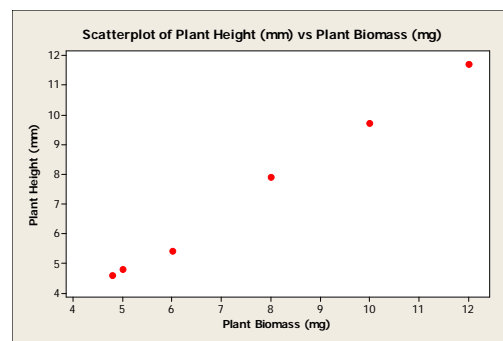
	C1	C2	C3
	Plant Height (mm)	Plant Biomass (mg)	
1	4.6	4.8	
2	5.4	6.0	
3	9.7	10.0	
4	7.9	8.0	
5	4.8	5.0	
6	11.7	12.0	


In the scatter plot window select a **scatter plot** (in this example we used a simple scatter plot) and click **OK**. *If you are doing a regression analysis, you should see the Statistics section (pg. 61) and do a regression analysis with a fitted line plot. Do not use the Graph>scatter plot menu for your regression plots because you will not get all the information you need.*



## Directions For Making Figures Using Minitab

A scatter plot will appear. Remove the background colors and lines by double clicking on the background of the figure and choosing white under the custom color options.



Finally, remove the title and subtitle by right clicking and choosing **delete**. Add a figure legend either by incorporating it into the text of the document, directly below the figure, or by dragging open a text box within the graph and typing in the appropriate text. In Minitab the text box button  is located on the tool bar.

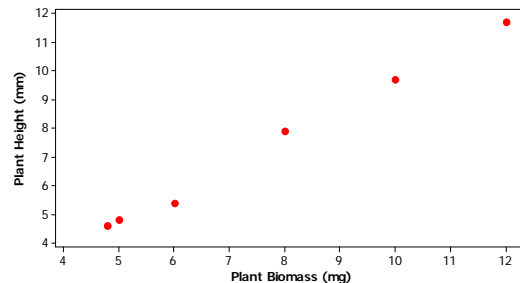


Figure 1. Scatterplot showing plant height (mm) v.s plant biomass (mg) 14 days after seeds were sown.

### Histogram

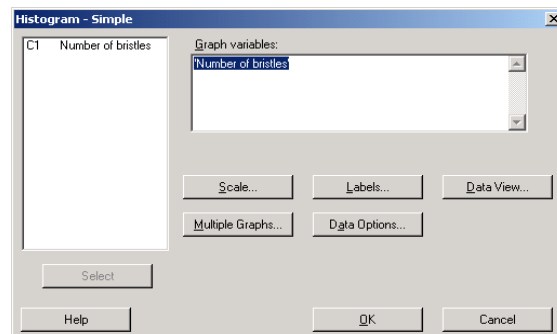
Use a histogram to show the character of the *distribution* of values measured. Minitab makes histograms quite easily.

### Creating a Histogram chart in Minitab


Label a column (in the gray shaded square) and enter your data in the cells below.

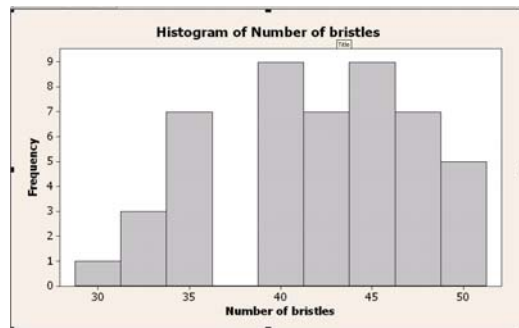
	C1	C2	C3	C4
	Number of bristles			
1	30			
2	33			
3	33			
4	33			
5	36			
6	36			
7	36			
8	36			
9	36			
10	36			
11	36			
12	39			

Go to the **Graph** menu and choose **histogram**. In the histogram dialog box, double click on the data in the left window to add it to the graph variables on the right side of the box. If you have multiple columns of data, you can click on several data columns to get either combined or separate histograms. Click **OK**.



## Directions For Making Figures Using Minitab

A histogram will appear. Minitab automatically adds a title. If the figure is to be included in a paper, remove the title and use the text box tool to add a figure legend at the bottom of the histogram. In Minitab the text box button  is located on the tool bar. Also remove the background colors and lines by double clicking on the background of the figure and choosing white under the custom color options. Lines are removed in a similar fashion and bars may also be formatted by double clicking on them.



### Interval Plots and Bar Graphs

Interval plots and bar graphs are ways to present values for different categories you want the reader to compare. Your instructor may prefer one to the other. Some prefer interval plots; they use a symbol to convey the mean, while bar graphs depict the mean using a bar. Both kinds of figures should be presented with the variation around the mean conveyed by y-error bars representing one standard error (SE)

#### Presenting values of means

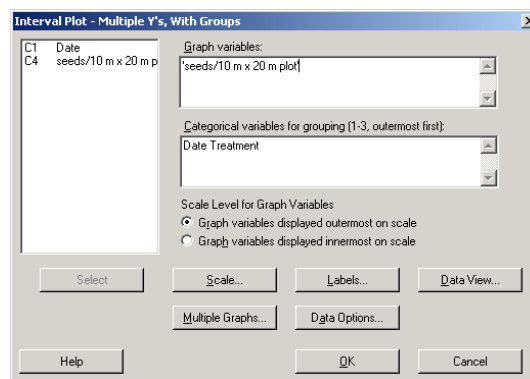
When values for different categories are means, you must ALWAYS indicate the precision of the estimate of the mean with an error bar. The convention in most disciplines is for error bars to represent +/- one standard error of the mean, or S.E. (see Statistics section for an explanation of what this is and how to calculate it). For clarity, the legend for such a figure should also state that values are means +/- S.E. Minitab's interval plot will add the y-error bars automatically as 95% confidence intervals so be sure to click on Options and change the y-error bars to Standard Error bars.

### Creating an Interval Plot in Minitab

Enter your data so that each variable is in a separate column. Label your columns appropriately.

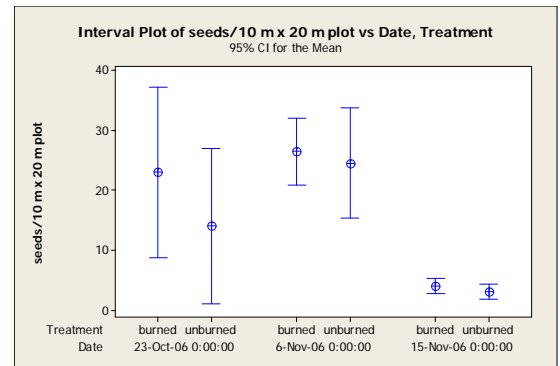
	C1-D	C2-T	C4
	Date	Treatment	seeds/10 m x 20 m plot
1	23-Oct-06 >>	burned	32
2	23-Oct-06 >>	burned	22
3	23-Oct-06 >>	burned	27
4	23-Oct-06 >>	burned	11
5	6-Nov-06 0>>	burned	29
6	6-Nov-06 0>>	burned	30
7	6-Nov-06 0>>	burned	24


Go to the menu **Graph >Interval Plot>Multiple Y's with groups**. A window will appear. In **graph variables** put the y-axis measurements (which are the dependent variables; what you measure). In **categorical variables** put the x-axis categories (the independent variables; what you manipulate).



## Directions For Making Figures Using Minitab

An interval plot will appear with a title and Y- error bars that indicate a 95% confidence interval. Change the 95% CI to SE bars. To do this, double click on the error bars and in the formatting window that appears go to **Options** and choose **Standard Error** rather than 95% CI. Your plot should look something like the one at right.



Remove the background colors and lines by double clicking on the background of the figure and choosing white under the custom color options. Finally, remove the title and subtitle by right clicking and choosing **delete**. Add a figure legend either by incorporating it into the text of the document, directly below the figure, or by dragging open a text box within the graph and typing in the appropriate text. In Minitab the text box button  is located on the tool bar.

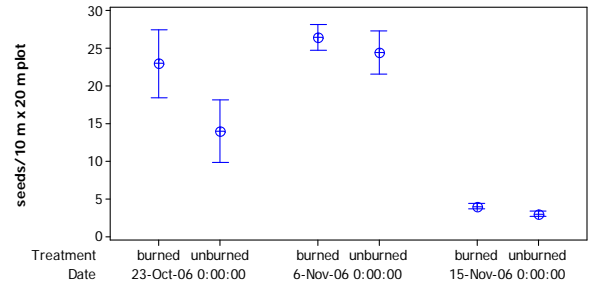


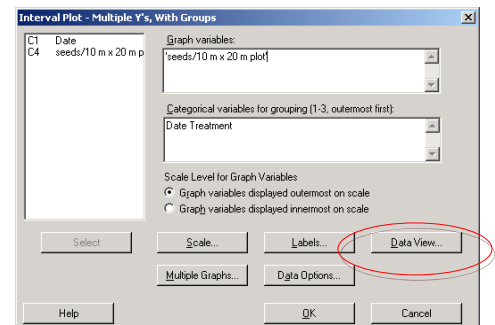
Figure 1. The effects of burning on mean number of seeds per 10m x 20m plot from three collection dates at Conard Environmental Research Area. Error Bars Represent 1 S.E. \* designates p 0.05.

### Creating a Bar Graph in Minitab

Enter your data so that each variable is in a separate column. Label your columns appropriately.

	C1-D	C2-T	C4
	Date	Treatment	seeds/10 m x 20 m plot
1	23-Oct-06 >>	burned	32
2	23-Oct-06 >>	burned	22
3	23-Oct-06 >>	burned	27
4	23-Oct-06 >>	burned	11
5	6-Nov-06 0>>	burned	29
6	6-Nov-06 0>>	burned	30
7	6-Nov-06 0>>	burned	24

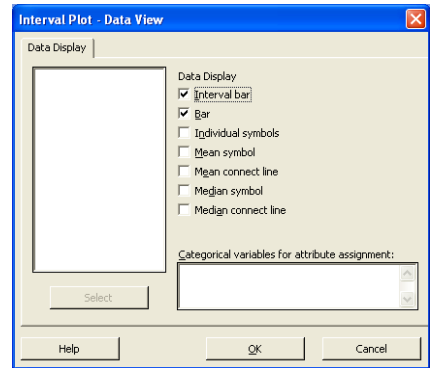
Go to the menu **Graph >Interval Plot>Multiple Y's with groups**. A window will appear. In **graph variables** put the y-axis measurements (which are the dependent variables; what you measure). In **categorical variables** put the x-axis categories (the independent variables; what you manipulate). *To create a bar style graph with bars for each category* click on the **Data View** button.



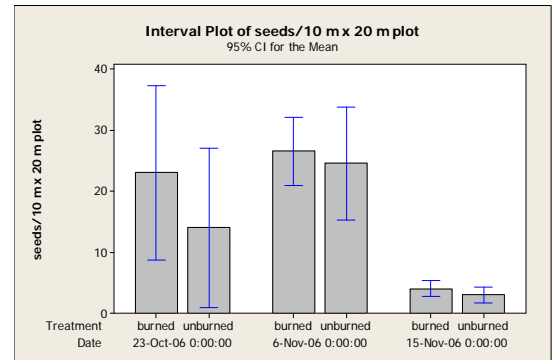


## Directions For Making Figures Using Minitab

When the Data View window appears, check **Bar and Interval Bar** as an option. Click **OK**.



A bar graph will appear with a title and Y- error bars that indicate a 95% confidence interval. Change the 95% CI to SE bars. To do this, double click on the error bars and in the formatting window that appears go to **Options** and choose **Standard Error** rather than 95% CI.



Remove the background colors and lines by double clicking on the background of the figure and choosing white under the custom color options. Bar colors may be changed by double clicking on the bars. Finally, remove the title and subtitle by right clicking and choosing **delete**. Add a figure legend either by incorporating it into the text of the document, directly below the figure, or by dragging open a text box within the graph and typing in the appropriate text. In Minitab the text box button is located on the tool bar.

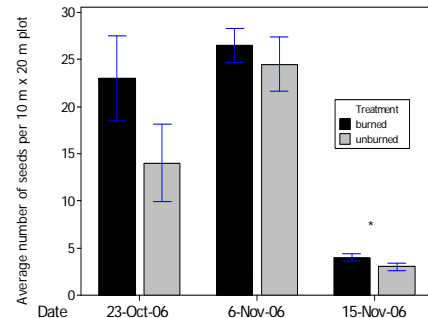


Figure 1. The effects of burning on mean number of seeds per 10m x 20m plot over three collection dates at Conard Environmental Research Area. Error bars represent 1 S.E. \* designates  $p < 0.05$ .

## Appendix C

### Scientific Paper Evaluation Forms

#### Biology Scientific Paper Evaluation Form (example 1)

Name \_\_\_\_\_

Your grade on this paper reflects my evaluation of how you've done in 5 major areas, as listed below. The "bottom line" is an integration of these factors expressed in a fashion similar to how journals evaluate manuscripts. I've also added a numerical grade.

#### Explanation of logic and design of study

1. Question or design not evident.
2. Both question and design stated, but not developed effectively.
3. Question clearly stated and design appropriately described.
4. Significance of question clearly stated and design elegantly described.

#### Data Analysis and Presentation

1. Inappropriate or inadequate.
2. Adequately analyzed and presented.
3. Efficiently and clearly analyzed and presented.
4. Elegantly analyzed and presented.

#### Data Interpretation

1. Conclusions inadequate and/or inappropriate.
2. Conclusions are explained and justified.
3. Conclusions are clearly related to the question.
4. Demonstrates intellectual creativity by putting conclusions in a larger context and/or proposing *interesting* directions for future research.

#### Use of the primary literature

1. No citations or inappropriate citations.
2. Citations used perfunctorily.
3. Citations used appropriately.
4. Significant synthesis and integration of citations.

#### Overall quality of writing

1. Unacceptable.
2. Acceptable.
3. Well-written.
4. Elegant.

ACCEPTED  
REVISION

NOT ACCEPTED

ACCEPTED WITH MINOR REVISION

ACCEPTED WITH MAJOR

## Appendix C

### Biology Scientific Paper Evaluation Form (example 2)

#### Title

\_\_\_ Does the title give an accurate preview of what the paper is about? (*i.e. Is it informative, specific and precise?*) 3 pts

#### Abstract

\_\_\_ Are the main points of the paper described clearly and succinctly? 3 pts

#### Introduction

\_\_\_ Does the Introduction have a logical organization? *Does it move from the general to the specific?* 5 pts

\_\_\_ Has sufficient background been provided to understand the paper? *How does this work relate to other work in the scientific literature*  
5 pts

\_\_\_ Has a reasonable explanation been given for why the research was done? *Why is the work important? What is its relevance*  
5 pts

\_\_\_ Is the final paragraph a brief description of the hypothesis/goals and findings of the paper? 5 pts

*Note: In cell and molecular papers, the final paragraph of the introduction is a brief summary of the findings of the paper. This format may be a different from that of other areas of biology or chemistry.*

#### Materials and Methods

\_\_\_ Could the study be repeated based on the information given here? 4 pts

\_\_\_ Is the material organized into logical categories? 4 pts

The materials and methods should be a source of detail about the experimental approaches of the authors. Procedures that have been repeated by the authors should only be listed once. Variations to the procedure should be briefly summarized. (*The M&M should not read like a recipe.*)

#### Results

\_\_\_ Is the content appropriate for a results section? 10 pts

- Simple introduction to the scientific question
- Brief description of the methods
- Clear description of the results for each experiment
- analysis of those results

\_\_\_ Are the results/data analyzed well? 5 pts

- Given the data in each figure, is the interpretation accurate and logical?
- Is the analysis of the data thorough or are some aspects of the data ignored?
- Does the author make connections between different sets of data within the text?
- Are the data interpreted in a larger context?

\_\_\_ Figures 5 pts

- Are the figures appropriate for the data being discussed?
- Are the figure legends and titles clear and concise?

*Note: The entire experimental findings of a paper should be apparent from reading the results section. It should be possible to understand the question the authors are asking, the experimental approach they use to answer the question, the results of those experiments, and basic analysis of the data. Larger issues of what the research means, how it relates to other work, etc should be included in the discussion.*

#### Discussion

\_\_\_ Does the author clearly state whether the results answer the question? (*i.e. support or disprove the hypothesis?*) 5 pts

\_\_\_ Were specific data cited from the results to support each interpretation? *Does the author clearly articulate the basis for supporting or rejecting the hypothesis* 5 pts?

\_\_\_ Does the author make connections between data sets within the paper? 5 pts

\_\_\_ Does the author adequately relate the results of the current work to previous research? 10 pts

## Appendix C

### References

- \_\_\_ Are the references appropriate and of an adequate quantity? *5 pts*  
\_\_\_ Are the references cited properly (both within the text and at the end of the paper)? *5 pts*

### Writing Quality

- \_\_\_ Is the paper well organized? (Paragraphs are organized in a logical manner) *7 pts*  
\_\_\_ Is each paragraph well written? (Clear topic sentence, single major point) *7 pts*  
\_\_\_ Is the paper generally well written? (Good use of language, sentence structure) *7 pts*

## Scientific Poster Evaluation Forms

Example 1, a tripartite scheme, has been used for many biology courses in the past. Parts one and two, which address the "science" of the experiment, carry more weight than part three, which addresses the aesthetics of the poster. Example 2 is used by Biology 150 students to review other student's posters during the Biology 150 Poster session.

### Biology Scientific Poster Evaluation Form (example 1)

1. Scientific merit:
  - Is the hypothesis stated clearly and is it discussed in light of what is already known about the question?
  - Is sufficient background material presented to adequately understand the rationale for the experiment?
  - Are the methods appropriate for answering the experimental question and carried out accurately?
  - Is the Materials and Methods section sufficiently clear so that someone else in the class could repeat the experiment?
  
2. Presentation and discussion of results:
  - Are the data clearly and accurately presented in the tables and/or figures?
  - Are the results discussed clearly in the context of the hypothesis? That is, do they indicate an answer to the experimental question?
  - Are the results discussed in relation to other published findings?
  - Are the statements and results of others referenced properly?
  
3. Overall quality of the poster presentation:
  - Is the visual quality of the poster adequate?
  - Are the sections of the poster organized in an appropriate and meaningful manner?

Bio 150 students may be asked to evaluate posters at the poster session using a form similar to the one shown below:

### Biology Scientific Poster Evaluation Form (example 2)

**Poster Authors:**

**Poster Title:**

**Course title (Check one):**

The Effects of Climate Change on Organisms

What Does it Mean to be a Plant?

The Language of Neurons

Prairie Restoration

1. Does the poster have adequate background information allowing you to understand why the investigation was done?  
strongly disagree / disagree / agree / strongly agree  
Explain:
  
2. Does the poster have a clearly stated hypothesis or question?  
strongly disagree / disagree / agree / strongly agree  
If so, what is it? If not, explain.
  
3. Does the methods section adequately detail how the investigation was carried out?  
strongly disagree / disagree / agree / strongly agree  
Explain:
  
4. Does the poster present the results in figures and tables that are easy to understand and appropriate for the data?  
strongly disagree / disagree / agree / strongly agree  
Explain:
  
5. Did the presenter do a good job explaining the investigation?  
strongly disagree / disagree / agree / strongly agree  
Explain:
  
6. Summarize the major conclusions and relate these conclusions back to the original hypothesis or question.



## Biology Oral Presentation Evaluation Form (example 2)

Presenter(s):

Reviewer:

Comments on scientific merit of the project

Comments on depth of understanding

Comments on team work

**Introduction. The study's rationale was**

comments

1. not clearly evident
2. evident, but not developed
3. clearly stated and appropriately developed
4. stated and developed elegantly

**Materials and Methods. The study's design was**

1. inappropriate and/or inadequate
2. evident, but not developed
3. stated clearly and efficiently
4. stated elegantly

**Results. Data analysis and presentation were**

1. inadequate and/or inappropriate
2. adequate
3. clear and efficient
4. elegant

**Discussion & Conclusions. Data interpretation was**

1. inadequate and/or inappropriate
2. explained and justified
3. clearly related to question
4. intellectually creative, in placing conclusions in larger context and/or proposing *interesting* directions for further research

**Visual aids were**

1. not clear and/or not helpful
2. adequate
3. clear and efficient
4. elegant

**Overall quality of speaking (eye-contact, volume, clarity, organization) was**

1. unacceptable
2. adequate
3. clear and articulate
4. elegant and entertaining

**Responses to questions were**

1. Unacceptable
2. adequate
3. clear, respectful, and informative
4. exceptionally insightful

Example of a good laboratory notebook

Your notebook may not be as tidy as this example but you must be sure it is legible and that it includes everything your professor requires. Notice how the calculations and diagrams are on the left side of the notebook pages. Tables and figures have been taped in on the left side also.

The best way to do a lab notebook is to prepare it ahead of time, make notes as you go, and then go back as soon as possible to add in details you didn't have time for during lab. Almost everything can be done ahead except for the data, discussion, and conclusions. You may even want to make data tables that can be filled in.

This example is written in past tense but usually for field work, and many times for lab work, you will prepare your notebook ahead of time, and in that case it should be in future tense.

(5)

BARLEY (PROTEIN SYNTHESIS) PROJECT

REFERENCE

S. Heimovaara-Dijkstra, J. C. Heistek, and Al. Wang. 1994. Counteractive Effects of ABA and GAs on Extracellular and Intracellular pH and Malate in Barley Aleurone. *American Society of Plant Physiologists, Plant Physiology* 106 (10): 359-363

S. J. Swanson and R. L. Jones. 1996. Gibberellic Acid Induces Vacuolar Acidification in Barley Aleurone. *American Society of Plant Physiologists, Plant Cell* 8 (12): 2211-2221

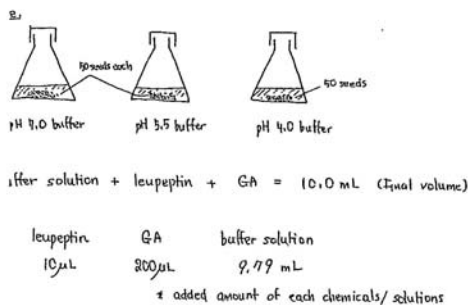
Y. M. Drozdowicz and R. L. Jones. 1995. Hormonal Regulation of Organic and Phosphoric Acid Release by Barley Aleurone Layers and Scutella. *American Society of Plant Physiologists, Plant Physiology* 108 (10): 769-776

INTRODUCTION

This experiment is to figure out the optimal pH for barley seeds exposed in 20 μM GA (20 GA), and the effect of pH on production of proteins.

\* Hypothesis \*

- the optimal pH for the barley seeds in 20 GA will be around 5.0 to 6.0.



(6)

- The high pH and the low pH will reduce the production of proteins, because of the pH sensitivity in proteins.

PROCEDURE (Week 1)

- We used razor blade to cut seeds (150) in half. Half that contained embryos were discarded.
- Seeds were sterilized by washing them in a solution of 1.5% hypochlorite for 10 minutes.
- Seeds were rinsed with dH<sub>2</sub>O for 5 min, and the water was decanted.
- Washing step was repeated for 6 more times.
- 10 mL of incubation buffer supplemented with the appropriate hormones. (Refer to Figure on the left page)
  - 3 different incubation buffers were prepared (different pH)
- Leupeptin (1 mg/1 mL stock) & a protease inhibitor were added to a final concentration of 1 μg/mL to the incubation buffer.
- Flask was placed in a reciprocating shaking water bath at 180 rpm, 35°C.



⑦

Na Succinate stock solution

$$C_1 V_1 = C_2 V_2$$

$$(1M)(x) = (0.05M)(100mL)$$

$$x = 5.0 mL$$

CaCl<sub>2</sub>

$$C_1 V_1 = C_2 V_2$$

$$(0.1M)(x) = (0.02M)(100mL)$$

$$x = 20 mL$$

## &lt; Preparation of Different incubation buffer (pH) &gt;

In order to have enough amount of buffer throughout the experiment, we set the final volume of <sup>10mL</sup> buffers, each, to be 100 mL.

1. 5.0 mL of Na Succinate buffer, 20 mL of CaCl<sub>2</sub> was added in the 150 mL-beaker.

2. We (approximately) added <sup>dH<sub>2</sub>O</sup> 65 mL into the beaker to leave some extra spaces available to add acids to make appropriate pH buffers. Repeat steps 1 & 2 to make some 3 beakers with solutions, <sup>10mL available for the acid addition</sup>

3. By using the digital pH meter, we added HCl or NaOH to bring to the appropriate pH: (4.0, 5.5, 7.0)

4. Add dH<sub>2</sub>O until the final volume reached 100 mL

## &lt; Collecting supernatants containing secreted proteins &gt;

Every 24 hours, the incubation buffer was replaced in the flask. The buffer was decanted and saved in a cold test tube. These tubes were kept in the refrigerator.

Once the buffer was decanted off the botley seeds, <sup>we</sup> added 100 mL of appropriate buffer solution to wash the seeds briefly. Flasks were swirled gently for about 10 seconds, and the wash buffer was discarded. Finally, 10 mL of fresh appropriate incubation buffer,

⑧

leupeptin, and 200  $\mu$ L of GA. The flasks were then returned to the shaking platform.

PROCEDURE (Week 2)

1. Each tubes that contained collected solutions were vortexed,

⑨

## &lt; Gel Loading and Staining &gt;

1. Set up the apparatus used to run gels,

2. 10  $\mu$ L of each sample was loaded into wells, using micropipettor and a long, narrow tips. One lane was loaded with a mixture of size standards of known molecular weight.

3. The gel was run by adjusting the power supply to 200 V.

4. The run was stopped when the blue tracking dye in the sample buffer reached the bottom of the gel.

5. After electrophoresis, the gel was placed in 100 mL of dH<sub>2</sub>O in a loosely covered container and it was microwaved on High for 1 min.

To save space, pages 8 and 9 of this example are only partially shown.

10

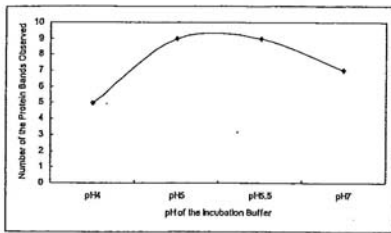


Figure 4: pH optimum curve for barley aleurone. The values were determined based on the pH at which the seeds were incubated and the number of bands present on the polyacrylamide gel. The number of bands was measured conservatively in that only bands that were fully visible by all members of the group were counted for the purposes of protein production. The number of bands was counted for the data collected after 72 hours.

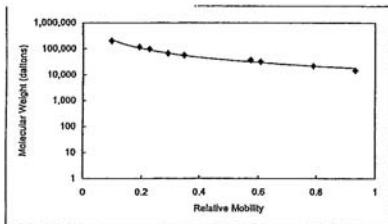


Figure 5: The relative mobility (R) and the known molecular weights of the proteins in the standard that were observed in the gel electrophoresis in Fig. 1 (7<sup>th</sup> lane). The following is the formula for the best fit line:  $y = 16584x^{-1.2393}$

8. The gel was shaken on an orbital shaker for 5 min.
9. The gel was then washed in 100 mL of dH<sub>2</sub>O for 10 min on a shaker.
10. 40-50 mL of 20% NaCl was added, and it was stored in the bag, sealed.

RESULT

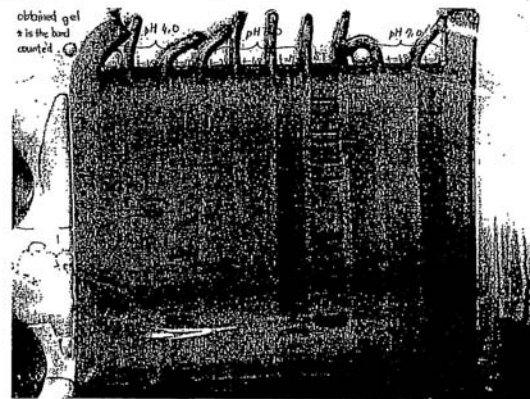


Table 1a: Relative mobility and the size of synthesized proteins in pH 4.0 incubation buffer.

Relative Mobility	Molecular Weight (daltons)
0.3365	56749
0.5769	30869
0.7019	24736
0.7740	22149
0.9519	17534

Table 1b: Relative mobility and the size of synthesized proteins in pH 5.0 incubation buffer.

Relative Mobility	Molecular Weight (daltons)
0.2692	79667
0.3558	56217
0.4952	37187
0.5288	34258
0.5673	31377
0.6202	28068
0.6875	24676
0.7500	22133
0.9327	16854

Table 1c: Relative mobility and the size of synthesized proteins in pH 5.5 incubation buffer.

Relative Mobility	Molecular Weight (daltons)
0.1442	147788
0.2115	95885
0.2596	76037
0.3365	56749
0.3750	50213
0.4183	44383
0.5625	31763
0.6154	28697
0.6875	25322

Table 1d: Relative mobility and the size of synthesized proteins in pH 7.0 incubation buffer

Relative Mobility	Molecular Weight (daltons)
0.1442	147788
0.2260	88965
0.3413	55849
0.4231	43815
0.5288	34059
0.6731	25934
0.7500	22951

11

Discussion

In comparison to the previous experiment done in the pH 5.0 buffer solution, pH 5.0 produced protein the most, then pH 5.5. This result supported our hypothesis, being within the range of our hypothesized optimal pH of around 5.0 to 5.5. The protein synthesis occurred the most in 6-9R lanes, regardless of the pH difference. This could be representing the time that will take for barley seeds to induce the enzyme synthesis after GA has been added. When we compare between the basic (pH 7.0) and the acidic (pH 4.0) condition, basic condition was able to produce more proteins overall, and also in different sizes. The barley seeds that were incubated in pH 4.0 were somehow not able to synthesize bigger proteins.

*Handwritten notes:*  
 $W_{pH 7.0} > W_{pH 5.0} > W_{pH 5.5} > W_{pH 4.0}$   
 $S_{pH 7.0} > S_{pH 5.0} > S_{pH 5.5} > S_{pH 4.0}$

## Tips and Information about Poster Making

When you are making your poster for Biology 150 you will be using Power Point templates that have been set up on the Projects Server at: <\\Storage\projects\Bio\Bio150>. Each Bio 150 class has a folder at this location and within the class folder will be a folder with the names of your group members on it. Open the template you wish to use and save it in your group's folder. Now you are ready to begin working on your poster in a place where everyone in your group can access it and make changes. When you finish your poster save it in the same group folder only with the word FINAL in the title of the Power Point document. Do not put the word FINAL in the document title until you are done, as this denotes posters that are ready for printing.

Instructions for getting to the Projects folder from a Macintosh Computer:

- Under the Go menu drag down to Connect to Server.
- Log in on the pop up window.
- In the next window scroll down to the server you want (**Projects**), then click and navigate to the folders you need. Within Projects go to Bio>Bio150>your class name>your groups names.

Instructions for getting to the Projects folder from a PC computer:

- Under the Start menu go to Run.
- In the box type: <\\Storage\PROJECTS\Bio\Bio 150>
- Once you are in the Bio 150 folder find your class folder and then your group folder.

### Tips for Making Posters using Microsoft PowerPoint with the Bio 150 Poster Templates

Do NOT alter the Page Setup... it is set up for the correct poster size.

We ask you to use a white background for your posters. It saves significant amounts of ink, print time, and wear and tear on the plotter printer we use to print posters. But your poster doesn't have to be drab! Color use is encouraged on all other parts of the posters to enhance its esthetics and content.

*To enable you to see what you are typing:*

Find the Zoom display on the toolbar (displayed as %). Use the pull-down menu from the small arrow just to the right of the Zoom display to enable viewing of smaller text as you are composing your poster.

*Recommended sizes for your text are:*

Title: 90-120 pt.

Author & advisor: 72-90 pt.

Headers: 60 pt.

Text: 18 pt, though 24 pt is recommended for your conclusions.

*To enter text:*

Move the pointer arrow over the text – the pointer changes to an I-beam to indicate that you can click and then type. The size of the textbox should automatically adjust as you type. Many of the icons on the toolbar for formatting text are the same as those in Word.

*To paste text from Word:*

Copy the selected text in Word.

Insert the cursor into a textbox in your PowerPoint poster. Paste. You may need to readjust the font size of the pasted text.

Alternatively, you can choose Paste Special from the Edit menu, then choose "unformatted text." The pasted text should take on the formatting already selected in your textbox.

*To resize a textbox (or image, or table):*

If you need to resize a textbox, click on the outline of it (the appearance of the outline will change from striped to shaded, with resizing handles on the corners), then click and drag a resizing handle to make the area larger or smaller. This resizing technique will work for pictures and tables as well.

*To insert an image:*

From the Insert menu, choose Picture>From File. You can browse and select the image to insert. Types of images you can insert: JPG, GIF, EPS, TIF

*To insert a graph from Excel or Minitab:*

Copy the selected graph. Preferably, choose Paste Special from the Edit menu, then choose “picture.” Alternately, you can Paste the graph in the normal way into your PowerPoint poster. A regular paste will allow you to edit the graph by double-clicking on it, but quite often the text, especially the vertical text on the y-axis, gets distorted and may be unreadable when you get your poster printed. By pasting your graph into PowerPoint as a picture you give up being able to edit the “picture” of your graph but you are more assured of it looking exactly the way you made it after the poster is printed.

*To create a table (other than Excel data):*

From the Insert menu, choose Table. You will be able to specify the numbers of rows and columns. You will have to resize the table, because PowerPoint makes it very large! If it's not resizing easily, you can decrease the font size while the table is selected, and try the resizing handles again. Alternately, you can Copy a Word table, and Paste it into PowerPoint.

*To align objects:*

Under the “Home” tab go to the Drawing menu and pull down the arrange button. Several options are available to help align selected objects. Also, under the “View” tab, choose to display the ruler and grids. These will not show on the poster, but will help you with alignment

*To save your poster:*

Go to Save as: and save your poster to [\\Storage\projects\Bio\Bio150\your class\your group](#). Save with the word FINAL in the name of the document.

*To print an 8-1/2 x 11” preview of your poster:*

Choose Print from the File menu (don't use the shortcut on the toolbar). In the print dialogue box, check the small box near the bottom for “Scale to fit paper,” then “OK.” Remember, you don't have to change the Page Setup.

## **Frequently asked questions about the Bio 150 Poster Session**

### ***How will I get my poster printed?***

Each group of students has a folder on the storage server in the following location [\\Storage\PROJECTS\Bio\Bio 150](#) (your class)\(your groups names)\. **By Monday December 10<sup>th</sup> at 5 pm you must have your completed poster saved to this folder with the word FINAL in the title of the Power Point document.** Once you have done this, the science secretaries print the poster before the poster session.

### ***How do I get my poster?***

After your poster has been printed it will be stored in room your lab room until you are ready to put it up for the poster session. Posters usually are ready by 4:00 p.m. Wednesday afternoon.

### ***What should I do with my poster once I get it?***

There will be a preliminary program posted in your lab room near the posters, so you can find out what easel number and time slot you are assigned to. On Thursday go to your assigned easel in the “glass elbow” and put up your poster any time BEFORE the poster session. At your easel you should find your nametag, four tacks for hanging your poster, and a program. Please wear your nametag during the poster session.

### ***What should I do when I am not at my poster?***

Visit other posters and fill out Poster Peer Reviews. There will be Poster Peer Review forms available from your professor. Unless otherwise instructed by your professor, all students are responsible for reviewing one poster from each of the other sections of Bio 150. The poster session is a social event as well as an opportunity to share scientific research so have a good time enjoying refreshments and visiting about Bio 150 research with your peers and visitors from around campus.