INTRODUCTION TO ORGANISMAL BIOLOGY

LAB MANUAL 2019

Fall 2019 - Biology Department - University of Ottawa
BIO1130 – Introduction to Organismal Biology
Lab manual - fall 2019 – www.biolab1.uottawa.ca

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My Info:
Name: ____________________________ Section: __________

Demonstrators Info:
TA 1: ____________________________ Office hours: __________
E-mail: __________________________

TA 2: ____________________________ Office hours: __________
E-mail: __________________________

Questions about absences, rescheduling a lab or schedule in general, please contact the BioLab team at: biolab1@uottawa.ca

Questions about the lab content, your marks, reports or any other concerns, please contact the lab coordinator: Dr. Fabien Avaron
Email: fabien.avoron@uottawa.ca
Biosciences Complex (BSC) Room 106
Office Hours: Friday 10:30-12:00 (open door the rest of the time).

www.biolab1.uottawa.ca
BIO1130 – Introduction to Organismal Biology

Lab Schedule – Fall 2019

Labs take place on the third floor of the BioSciences Complex (BSC), **except for lab 1, which is a field trip.** The room assignment is on the next page.

*Introductory session (attendance is mandatory):* The semester will start with an *information session* to the labs. The duration is **about 60 minutes.** Depending on your lab section, your intro session will start at 2:30 or 4PM:

<table>
<thead>
<tr>
<th>Date</th>
<th>Sections</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 09 Sep</td>
<td>A1, A6, C1</td>
<td>2:30-3:30</td>
</tr>
<tr>
<td>Tue 10 Sep</td>
<td>A2, A7, C2</td>
<td></td>
</tr>
<tr>
<td>Wed 11 Sep</td>
<td>A3, A8, C3, A11</td>
<td></td>
</tr>
<tr>
<td>Thu 12 Sep</td>
<td>A4, A9, C4</td>
<td></td>
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<tr>
<td>Fri 13 Sep</td>
<td>A5, A10, C5</td>
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<table>
<thead>
<tr>
<th>Section</th>
<th>Time</th>
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<tbody>
<tr>
<td>B1, B6, C6</td>
<td>4:00-5:00PM</td>
</tr>
<tr>
<td>B2, B7, C7</td>
<td></td>
</tr>
<tr>
<td>B3, B8, C8, B11</td>
<td></td>
</tr>
<tr>
<td>B4, B9, C9</td>
<td></td>
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<tr>
<td>B5, B10</td>
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*Regular labs (2:30-5:20 Monday to Friday for all sections)*

<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 16 Sep</td>
<td>A1, A6, C1 Mer Bleue Field trip (2:30PM)</td>
</tr>
<tr>
<td>Tue 17 Sep</td>
<td>A2, A7, C2 Field trip</td>
</tr>
<tr>
<td>Wed 18 Sep</td>
<td>A3, A8, C3, A11 Field trip</td>
</tr>
<tr>
<td>Thu 19 Sep</td>
<td>A4, A9, C4 Mer Bleue Field trip (2:30PM)</td>
</tr>
<tr>
<td>Fri 20 Sep</td>
<td>A5, A10, C5 Mer Bleue Field trip (2:30PM)</td>
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</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 23 Sep</td>
<td>B1, B6, C6 Mer Bleue (2:30PM)</td>
</tr>
<tr>
<td>Tue 24 Sep</td>
<td>B2, B7, C7 Mer Bleue (2:30PM)</td>
</tr>
<tr>
<td>Wed 25 Sep</td>
<td>B3, B8, C8, B11 Mer Bleue (2:30PM)</td>
</tr>
<tr>
<td>Thu 26 Sep</td>
<td>B4, B9, C9 Mer Bleue (2:30PM)</td>
</tr>
<tr>
<td>Fri 27 Sep</td>
<td>B5, B10 Mer Bleue (2:30PM)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 07 Oct</td>
<td>A1, A6, C1 Scientific Literature Week 1</td>
</tr>
<tr>
<td>Tue 08 Oct</td>
<td>A2, A7, C2 Scientific Literature Week 1</td>
</tr>
<tr>
<td>Wed 09 Oct</td>
<td>A3, A8, C3, A11 Scientific Literature Week 1</td>
</tr>
<tr>
<td>Thu 10 Oct</td>
<td>A4, A9, C4 Scientific Literature Week 1</td>
</tr>
<tr>
<td>Fri 11 Oct</td>
<td>A5, A10, C5 Scientific Literature Week 1</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 21 Oct</td>
<td>B1, B6, C6 Scientific Literature Week 2</td>
</tr>
<tr>
<td>Tue 22 Oct</td>
<td>B2, B7, C7 Scientific Literature Week 2</td>
</tr>
<tr>
<td>Wed 23 Oct</td>
<td>B3, B8, C8, B11 Scientific Literature Week 2</td>
</tr>
<tr>
<td>Thu 24 Oct</td>
<td>B4, B9, C9 Scientific Literature Week 2</td>
</tr>
<tr>
<td>Fri 25 Oct</td>
<td>B5, B10 Scientific Literature Week 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 28 Oct</td>
<td>A1, A6, C1 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Tue 29 Oct</td>
<td>A2, A7, C2 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Wed 30 Oct</td>
<td>A3, A8, C3, A11 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Thu 31 Oct</td>
<td>A4, A9, C4 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Fri 01 Nov</td>
<td>A5, A10, C5 Vertebrate Phylogeny Week 1</td>
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<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
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</thead>
<tbody>
<tr>
<td>Mon 04 Nov</td>
<td>B1, B6, C6 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Tue 05 Nov</td>
<td>B2, B7, C7 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Wed 06 Nov</td>
<td>B3, B8, C8, B11 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Thu 07 Nov</td>
<td>B4, B9, C9 Vertebrate Phylogeny Week 1</td>
</tr>
<tr>
<td>Fri 08 Nov</td>
<td>B5, B10 Vertebrate Phylogeny Week 1</td>
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<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 11 Nov</td>
<td>A1, A6, C1 Micro-evolution Week 1</td>
</tr>
<tr>
<td>Tue 12 Nov</td>
<td>A2, A7, C2 Micro-evolution Week 1</td>
</tr>
<tr>
<td>Wed 13 Nov</td>
<td>A3, A8, C3, A11 Micro-evolution Week 1</td>
</tr>
<tr>
<td>Thu 14 Nov</td>
<td>A4, A9, C4 Micro-evolution Week 1</td>
</tr>
<tr>
<td>Fri 15 Nov</td>
<td>A5, A10, C5 Micro-evolution Week 1</td>
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<table>
<thead>
<tr>
<th>Date</th>
<th>Section Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon 18 Nov</td>
<td>B1, B6, C6 Micro-evolution Week 2</td>
</tr>
<tr>
<td>Tue 19 Nov</td>
<td>B2, B7, C7 Micro-evolution Week 2</td>
</tr>
<tr>
<td>Wed 20 Nov</td>
<td>B3, B8, C8, B11 Micro-evolution Week 2</td>
</tr>
<tr>
<td>Thu 21 Nov</td>
<td>B4, B9, C9 Micro-evolution Week 2</td>
</tr>
<tr>
<td>Fri 22 Nov</td>
<td>B5, B10 Micro-evolution Week 2</td>
</tr>
</tbody>
</table>
Room assignment:

<table>
<thead>
<tr>
<th>Sections of week 1</th>
<th>Monday (Section &amp; room)</th>
<th>Tuesday (Section &amp; room)</th>
<th>Wednesday (Section &amp; room)</th>
<th>Thursday (Section &amp; room)</th>
<th>Friday (Section &amp; room)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC312</td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
<td>A4</td>
<td>A5</td>
</tr>
<tr>
<td>BSC330</td>
<td>A6</td>
<td>A7</td>
<td>A8</td>
<td>A9</td>
<td>A10</td>
</tr>
<tr>
<td>BSC335</td>
<td>C1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td>C5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sections of week 2</th>
<th>BSC312</th>
<th>BSC330</th>
<th>BSC335</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>B2</td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td>B6</td>
<td>B7</td>
<td>B8</td>
<td>B9</td>
</tr>
<tr>
<td>C6</td>
<td>C7</td>
<td>C8</td>
<td>C10</td>
</tr>
</tbody>
</table>

For more information, visit the lab website at www.biolab1.uottawa.ca

General Objectives of BIO1130 Laboratories:
The laboratory component of BIO1130 aims to:

- Familiarize students with the scientific method as used in biology,
- Develop a student's ability to effectively analyse and communicate scientific information and data obtained through observation and experimentation,
- Reinforce and complement the material presented in the lecture component of the course,
- Introduce students to the various ways of accessing scientific literature,
- Introduce students to the use of computer simulations in biology education.

Supplies:
You must bring to each lab session:
Your lab coat (except for lab 1: Field trip at Mer Bleue).
Your lab manual
Pen and a pencil / Eraser
Combination lock
Plastic ruler
Lined notebook paper (regular notebook)

The first lab is a field trip to Mer Bleue – Here is what you should bring:
Rain gear
Clipboard, plastic bag
Proper footwear
Lab manual
Paper to take notes

Online resources for BIO1130 labs:
Lab website: www.biolab1.uottawa.ca/BIO1130/
You will find many documents regarding the lab activities and assignments, as well as contact info of your TAs. Visit this site regularly, especially before having a new lab.

Guide Biolabo: http://salinella.bio.uottawa.ca/biolabo/
This web site was created by Dr Houseman and that contains interactive resources regarding techniques used in biology laboratories.

Introduction session:
The semester will start with an introductory session where you will meet your lab TAs. They will give you general information about the biology labs as well as instructions for the 1st lab, which is a field trip. This session will last about 90 minutes and attendance is mandatory. See the schedule on page 1 of the intro.
Laboratory Sessions:

Lab hours: 2:30 PM to 5:20PM
Be on time, preferably 10 minutes early (especially for the Mer Bleue lab: buses will leave at 2:30PM precise). Labs sessions commence with a presentation that introduces the day's work, therefore it is necessary to be there on time. You may not be allowed in the lab session if you arrive too late. No one can stay inside the labs after 5:30 PM. If you need to talk to your TA after the lab session, please go to the lobby located next to staircase B or during their office hours.
There are 4 labs in total for BIO1130. Refer to page 1 of this introduction to know when your labs will take place.

LAB1 is a field trip to Mer Bleue: Please arrive on Louis Pasteur Private between Learning Crossroads (CRX) and Brooks at 2:25 at the latest (earlier if you can) in order to embark the buses. Meet your TAs in front of your assigned bus.

Laboratory Rules:
Due to safety regulations and space limitations, no outerwear, briefcases, gym bags, etc. will be allowed in the labs. Cell phones and music players should not be used during the lab sessions nor kept on the benches. Lockers are available in the corridor outside the labs. Make sure you use a combination lock and never leave valuables in a locker if it is not locked. You cannot leave anything in the lockers overnight.

The 6 golden rules:
1. Lab coat is mandatory and must be worn at all times in the laboratory. You will not be allowed to enter the lab rooms without your lab coat.
2. No cell phone or MP3 player should be used in the labs.
3. You cannot bring food nor drinks into the labs.
4. No rowdiness. We work with expensive and sometimes dangerous equipment, as well as poisonous substances.
5. Discussion is encouraged, but general noise is disruptive.
6. Cleanliness. You must keep your own equipment and notes in order. Clean up your equipment and your bench at the end of each lab.

Safety Instructions:
What should you do if:
1. You break some glassware (beakers, pipettes, etc.). Notify your demonstrator, who will place the broken glassware in the broken glass container. If a mercury thermometer is broken, a technician (trained in hazardous waste cleanup) will come and deal with the situation.
2. You cut yourself or have any other medical problem. Notify your demonstrator, who will use the contents of a small First Aid Kit located in the designated drawer in each laboratory. A more complete First Aid Kit is available in BSC 331 and BSC 141. Emergency telephones at the front of each lab room are available for medical emergencies (dial 5411).
3. Your lab coat catches fire while in the lab. Let your demonstrator or lab partner bring you to the safety shower located in the hallway outside each room to extinguish the flames. Fire extinguishers located in each lab room beside the doors, may also be used for this purpose.
4. You hear the fire alarm.
Do not panic. You should leave your lab, in an orderly manner to the primary fire exit for your room. The primary and secondary fire exits for labs on the 3rd floor of the Biosciences centre are indicated on the evacuation plan posted on the corridor wall and are as follows:

<table>
<thead>
<tr>
<th>Lab</th>
<th>Primary Fire Exit</th>
<th>Secondary Fire Exit</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC 312 / 330</td>
<td>Stairwell A</td>
<td>Stairwell B</td>
</tr>
<tr>
<td>BSC 310/335/302</td>
<td>Stairwell B</td>
<td>Stairwell A</td>
</tr>
</tbody>
</table>

If the primary exit is not available, proceed to the secondary fire exit. Once you have reached the main floor, exit the building, move about 30 meters away from the door and await further instructions. Please make sure you know precisely where emergency exits are located.

5. You accidentally spray some toxic liquid into your eyes.
Notify your demonstrator, who will bring you to one of the two emergency eyewash stations located at the back of your lab room. Pull down on the water pipe and rinse your eyes in the two water fountains for at least fifteen minutes. An additional emergency eyewash station is found in the corridor. Thereafter, your demonstrator will take you to Health Services.

Field Trip Safety
The field trip to Mer will take place even if the weather is rainy, windy or cool. Be well prepared and bring appropriate clothing. The terrain may be uneven so make sure you wear appropriate shoes. Be aware that bugs and wildlife may be present at the Mer Bleue site. It is forbidden to disrupt and collect any sample during the field trip. Bring a waterproof plastic bag (e.g. “Ziplock”) to store your phone or wallet in case of rain. Let the coordinator (before the trip) or your TA (during the trip) know if you have any question or concern regarding your health or safety.

Evaluation
You will be evaluated based on: in-class performance and/or at home questionnaires and/or lab reports. Your TAs will give you instructions regarding the evaluation during the lab session. This information is also be available on the lab website (see the “Instruction file” on the webpage for each lab).

Each of the 4 labs is worth 25% of BIO1130 laboratories final mark. There is NO lab exam for BIO1130. The lab component of BIO1130 is worth 20% of the course and will be added to the 80% of the lecture component. There is no pass or fail grade for the labs. However, you must meet the attendance requirements to get a mark for the lab component.

Attendance to laboratories & Absence
With justification: Even with justification, you must be present to 3 out of 4 labs sessions AND hand in reports for all labs you attended. If not, the lab component will be worth 10% and the remaining 10% will be transferred to the lecture part.
In case of absence for medical reason, you must provide a medical note (give it to your TA or the lab coordinator).
All justification notes must be provided within 7 calendar days past your absence (except in the case of a long-lasting absence or medical condition). Absence justifications received after 7 days will not be considered and you will get a zero for the missed lab.
Without justification: You must attend at least 3 out of 4 labs and write 80% of assignments; otherwise you may get a zero for the lab component. If you missed a lab for a non-medical reason, please contact the lab coordinator ASAP in order to have your lab rescheduled. Any unjustified absence will result in getting a zero for the missed lab.

If you know in advance you will not be able to attend a lab, please contact biolab1@uottawa.ca in order to have your lab rescheduled (do NOT contact your TAs, they cannot do that). You will need a justification for any rescheduling of your lab.

Lab reports format
Format: Text must be printed on 8.5 x 11 inch white paper (1.5 space, 12 pt), begins with a TITLE PAGE (see page 7), be stapled in the top left hand corner. DO NOT place your report in a binder or other plastic cover.
Refer to Appendix 1 for instructions regarding how to make a graph.
Lab reports must meet certain quality standards. For instance, reports written on (or containing) pages torn from a notebook or with large stains will receive a zero. Ask your TA or the lab coordinator if you have questions.

When is my report due?
Your TAs will tell you when to hand in your report. The due date will also be indicated on the instruction file posted on the web site. Reports must be handed in before 5:00PM on the due date. The BSC building is closed after 6:00PM as well as the whole weekend. Weekend counts as 1 day = if your assignment is due on Friday, hand it in before 5:00 pm on Monday or you will receive a zero.

Can I hand in my report after the due date?
Yes, but no more than ONE day past the due date. A 10% penalty (out of the maximum mark) will be applied. After that, you will receive a zero for your assignment. Please contact your TAs if you need to make arrangements regarding the submission of your lab report (justification required before the due date).

Where do I hand in my report?
In the drop off boxes located in the lobby of BioScience (see map on website): once in the hall, walk all the way toward the Husky courtyard and you’ll find the drop boxes on your right, behind the elevator shaft.
Hand in your report in the box that corresponds to your section. If you put your report in the wrong drop off box, a late penalty will be applied. For instance, if your report is due on Monday and you put your report in the box of a Wednesday section, you will get a 20% deduction (10% per day, up to 100%). If you hand in your report in a wrong box one the same day as your section, a 10% “wrong” box penalty will apply.
So many potential penalties…..Therefore, hand in your report at the right date in the right box!
If you attended your lab with another section (with permission only, see absence), drop off your work in the box that belongs to the section you had your lab with. It is your responsibility to place your report in the right drop-off box.

Plagiarism: Read this before handing in your first report.
Your report must be your own and not a copy of someone else’s. Even though you may collaborate with other students and share common results, your lab report must be written individually.
Plagiarism will be determined if two reports (or part of two reports) are identical, or correspond to the
description of “what is unacceptable” in the uOttawa guide to plagiarism (see below). By report, we
mean any text, graph, drawing, table, caption or anything else included in the assignment (but the title
page).
In case of plagiarism, all students involved will receive a ZERO for their assignment and a non-formal
warning will be issued by the lab coordinator. If plagiarism happens again, a formal report will be sent
to the Faculty of Science.
**Plagiarism from books, friends' work, etc., is considered academic fraud and will be dealt with
severely.**
Visit the uOttawa website page about academic integrity (https://www.uottawa.ca/vice-president-
academic/academic-integrity/resources-students/frequently-asked-questions-faq) and contact your
TA or the lab coordinator if you have questions about plagiarism.

**How to get additional help:**
Read all documents available on the lab section of the lab web site: The answers to 90% of all
questions asked are printed in the instruction documents provided for each lab, and/or the
corresponding pre-lab presentation
Contact your TA: Make sure you have all relevant information about your TA: Office hours time and
location, email... If you can't make it for your TA's office hours, you can talk to any other TA teaching
BIO1130 labs. In case of problems that cannot be solved by your TA, contact the lab coordinator (me)
by email at fabien.avaron@uottawa.ca or drop by my office (BSC106). Regular office hours will be
posted soon.

**Email etiquette:**
Always include the course code in the subject field. In the message itself, write down your name and
your student number as a signature. Messages lacking a signature as well as rude messages will be
ignored. Use your uOttawa account if possible to avoid technical difficulties. Teachers and TAs will use
this email to contact you if necessary.

**Special needs / concerns about the labs:**
Feel free to contact the lab coordinator if you experience difficulties regarding the laboratories. If you
need specific accommodation due to a medical condition or else, please visit the Student Academic
Success Service website (access service) at www.sass.uottawa.ca to get additional information, and/or
contact the lab coordinator. We will do our best to provide you the best learning conditions. Please
note that accommodations will not grant any extension for handing in your lab report. This is a matter
of time management.

I hope you will enjoy this term,

Dr. Fabien Avaron
First Year Biology Laboratories Coordinator
An example of a typical lab report title page

The rate of transpiration in tomato plants – effects of temperature and air movement

By John P. Smith
1234567

BIO1130 Section A1

Demonstrators:
Anthony Henry and
Cynthia Wyandot

September 24, 2019

Department of Biology

University of Ottawa
Distribution of plants at Mer Bleue

Objectives
After completing this laboratory, you should be able to:

- Describe the special features of the vegetation and habitats at Mer Bleue
- Recognize the dominant plant species at Mer Bleue
- Draw by hand a graph that accurately and efficiently represents quantitative data
- Understand how sampling error affects the ability to state and test scientific hypotheses
- Formulate a hypothesis with respect to a factor that limits the distribution of a plant species and
- Make a prediction regarding modifications to the distribution of this species following a change in water level at Mer Bleue.

Procedure
Before the field trip

1. Familiarize yourself with the sampling sites and the plant species** that you will see at Mer Bleue by reading carefully the lab manual.
2. Visit the lab website at biolab1.uottawa.ca and: 1) read the lab1 Powerpoint presentation and instruction file, 2) review colour pictures of Mer Bleue dominant plant species, and 3) find out what plant will be “Your plant” for the assignment*.
3. Plan to bring clothing appropriate for fall weather. The field trip will go ahead rain or shine. Read the introduction chapter about the field Trip.
4. Please visit the washroom, if necessary, before boarding the bus.
5. Arrive no later than 2:25pm on the sidewalk between CRX and Brooks on Louis Pasteur Private to embark your bus. Don't be late or you'll miss the bus. Go directly to your assigned bus. Give your name to your TA before getting on the bus. No other method of transportation will be allowed. You may not find your own way to Mer Bleue. All students must take the bus. There will be no exceptions.

* During the field trip, you will be asked to identify all plants listed in the lab manual, and not only your assigned species.
Lab 1 - Distribution of plants at Mer Bleue

**During the field trip**

6. Follow the instructions given by your demonstrators. It is important that you remain in your group. Each group will complete the series of 5 stations before returning to the bus (approximately 90 min).

7. Board the same bus on your way back and give your name to the demonstrator.

**Following your return from the field trip**

8. Within 18 hours after the field trip (i.e. before the next day at noon), **record your data on the lab website** (Lab1 page \(\rightarrow\) Mer Bleue data entry).

9. If you haven’t yet, visit the lab website to obtain the name of the plant species you have been assigned for the lab report. This can be done before the trip. Also, download the **group data pertaining to your species** (data will be posted about 24h after the field trip).

10. One week after the field trip, hand in your lab report (see Instruction file on the lab website) in the document drop off boxes, **before 5 PM**.

11. You will receive your corrected report (version 1) during your lab2.

12. One week after lab2, you may submit a second version of your graph **together with the corrected first version** (version 2 is optional).

13. For more details about the Mer Bleue reports, due dates and corrections, please visit the **lab website**.

**Site description**

The Mer Bleue conservation area is a protected wetland of a little more than 30 km\(^2\) and is located about 10 km southwest of Ottawa (see map on website). This area is particularly important for the conservation of its biodiversity, as it represents one of the last relatively intact bogs in southern Canada. It is a refuge plant and animal species, some of which are threatened by extinction (for instance, the spotted turtle, *Clemmys guttata*).

The vegetation found there is similar to what is found hundreds of kilometres to the north, in the boreal forest. About half of the conservation area's surface is a raised or domed bog. The sphagnum moss found therein releases acids that significantly reduce microbial activity. Since decomposition is slowed or almost stopped, dead moss accumulates slowly (over hundreds, even thousands of years) and eventually fills the ponds or lakes until it forms a dome, as in Mer Bleue. The semi-aquatic acid habitat that results is colonized by specific types of plants that you will see, such as: blueberries, black spruce, leatherleaf, larches, etc. The raised bog at Mer Bleue is adjacent to a marsh dominated by cattails and home to beavers and muskrats. At the edge of the wetland you will find sandy dunes or ridges.
that covered with vegetation normally found on drier soils (basswood, sumac, etc.) or by conifer plantations.

**Your task**

You will visually sample the vegetation at 5 stations in Mer Bleue by **recording which of the plants listed in the lab manual are present in your sampling area**. Back from the field trip, you will enter your observation in the BIO1130 lab website. This way, the observations of all students will be combined in one big file. One week after your lab, hand in your assignment using the combined data corresponding to the **species that has been assigned to you**. Your lab report will include:

1- A **Graphical analysis** of the data, by plotting a graph showing the **incidence of your plant throughout the 5 observation stations** (ordered in a dry to wet gradient).

2- Answers to the questions listed in the instruction file located on the lab website at: biolab1.uottawa.ca/BIO1130/

   Read the **instruction file** on the lab website for additional information about the Lab 1 assignment.

Adapted from a laboratory created by Antoine Morin
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*Flowers = you may see flowers on these species in September*
Dominant plant species at Mer Bleue

Plant descriptions were adapted from: Forest plants of Ontario - Chamber and Bentley, 1996.

Category: Tall trees

**Acer rubrum**

Red maple - Érable rouge

- Medium-sized tree, reaching 25 m in height.
- Young bark is smooth and grey. With age it becomes grey-brown, divides into crests that are sometimes free at the edges.
- Leaves with 3-5 lobes, having a distinct V-shaped notch between them (sugar maples have a U-shaped notch). Upper surface is light green. The petiole is often red.

**Acer saccharum**

Sugar maple - Érable à sucre

- Large tree, attaining 35 m in height.
- Young bark is smooth and grey. Mature bark has vertical ridges curled outward along one side.
- Leaves with 5 lobes with long blunt-pointed tips, a few irregular teeth, having a distinct U-shaped notch between them (red maples have a V-shaped notch).

Watch colour pictures of the Mer Bleue plants on the lab website
**Betula alleghaniensis**  
**Yellow birch - Merisier ou Bouleau jaune**

- Medium-sized tree, may reach 25 m in height.
- Leaves alternate, simple, 8-11 cm long, oval-shaped gradually tapering to a sharp pointed tip.
- Upper surface is yellowish-green, underside is paler, 9 or more veins per side.
- Bark is also characteristic; thin, smooth and shiny red-brown on young trees. On older trees, bark is yellowish-grey or bronze and peels off in curly strips.


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**Betula papyrifera**  
**Paper or White birch - Bouleau à papier**

- Tree may reach 30 m in height.
- Leaves alternate, simple, 6-8 cm long, heart-shaped, double-toothed. Upper surface is flat green, underside paler.
- Characteristic bark is thin, smooth and red-brown on young trees, white, papery and peeling on older trees.
**Fraxinus americana**  
**White ash - Frêne blanc**

- Tree may reach 30 m.  
- Compound leaves made up of 5-9 (usually 7) leaflets.  
- Leaflets 6-15 cm long, oval to lanceolate, underside of leaflets is hairless except along veins.  
- Twigs and petioles hairless.  
- Mature bark has ridges that form a regular diamond pattern.

**Larix laricina**  
**Tamarack or larch - Mélèze**

- Mid-sized tree (10-15 m).  
- Needles very soft, in clusters along the twigs, about 2-3 cm long, deciduous.
Figure 7.

**Picea mariana**  
Black spruce - Épinette noire

- Mid-sized tree, 7 – 10 m tall.
- Needles are short (1 cm), stiff, with rounded ends, arranged **all the way around the twigs**.
- Cones small, 2 – 3 cm long, often persisting on the trees.

Figure 8.

**Pinus resinosa**  
Red pine - Pin rouge

- Tall tree (25-35 m) with a very straight trunk
- Bark is furrowed and reddish.
- Needles **in groups of two**, stiff, quite long (10-15 cm).
- Cones are oval, 3-4 cm long
**Pinus strobus**  
White pine - Pin blanc

- Very large tree that can attain 50 m. Growth form irregular in older trees.
- Needles are soft, normally 6-10 cm long and in groups of five.
- Bark is grey-green in young trees becoming darker with deep crevasses in mature trees.

**Populus tremuloides**  
Trembling aspen - Tremble ou Peuplier faux tremble

- Tree 10 – 15 m tall
- Bark has a smooth, waxy appearance, pale green to almost white. Older bark becomes grey and forms long crests.
- Leaves are nearly round, with a sharp, pointed tip. The petiole is thin and flattened, which causes the leaves to tremble in the slightest breeze.
**Prunus serotina**  
**Black cherry - Cerisier tardif**

- Mid-sized tree (up to 22 m tall).
- Bark is smooth, dark reddish-brown with greyish horizontal lenticels. Older bark becomes scaly with lenticels still visible.
- Leaves lanceolate, with a sharp, pointed tip, upper surface is dark green, teeth sharp and incurved, underside of leaf is hairy (sometimes orange) on both sides of the mid-vein.

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**Tilia americana**  
**Basswood - Tilleul ou Bois blanc**

- Large tree reaching 20 – 25 m in height.
- Leaves deciduous, alternate and heart-shaped (10 cm long and 10 cm wide), ending in a sharp point, finely toothed. The base of the leaf is strongly asymmetric.
- Small (0.5-1.0 cm in diameter) fruits in clusters on a leaf-like bract.
- Bark is dark and shallowly grooved on mature trees.
**Ulmus americana**  
American elm - Orme d'Amérique

- Large tree reaching 35 m in height.
- Leaves alternate, about 10-15 cm long, ending in a sharp point. The base of the leaf is **strongly asymmetrical**. Upper surface is dark green and **rough**, while underside is paler and slightly hairy.
- Bark is dark greyish-brown, deeply furrowed, becoming spotted with ash-grey and scaly with age.

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**Alnus incana**  
Speckled alder - Aulne rugueux

- Shrub or small tree, 2-4 m tall.
- Leaves alternate, simple, oval, often thick, coarsely double-toothed.
- Smooth dark bark speckled with lenticels.
- Small woody cones.
**Cornus alternifolia**
Alternate-leaved dogwood - Cornouiller à feuilles alternes

- Shrub or small tree 1-10 m tall.
- Alternate leaves, but sometimes crowded toward end of branch where they may appear opposite or in whorls.
- Terminal flowers forming a flattened cyme. Dark-blue berry like fruit.

**Cornus stolonifera / Cornus sericea**
Red-osier dogwood - Cornouiller stolonifère

- Shrub 1-3 m tall.
- leaves opposite.
- Terminal flowers forming a flattened cyme. White or bluish fruit.
- Young branches red to purplish.
**Frangula alnus**  
*Glossy buckthorn – Neprun bourdaine*

- Small tree or shrub up to 6 m tall.
- Short trunk with pale elongated pores (lenticels).
- Leaves 5-8 cm long 3-5 cm wide. Mostly alternate, simple, smooth-edges.
- Leaves medium to dark green - glossy
- 5-10 prominent veins per side.
- Flowers: Greenish to yellow, less than 6 mm wide.
- Fruits green to red to purplish black, berry-like drupe. About 7 mm across. Fruits hang in clusters. Mature August-September.

*Adapted from Zelimir Borzan, University of Zagreb. Bugwood.org 2001. Creative Common Attributions.*

**Photinia melanocarpa** *(Aronia melanocara)*  
*Black Chokeberry – Aronia noir*

- Perennial shrub
- Low or mid-sized - up to 2.5 m tall
- Leaves alternate, oval to elliptic, sharp pointed at tip
- Leaves 1-4 cm wide, 2-8 cm long, with fine teeth on margin
- Petiole 2-10 mm
- Leaf-like stipule at the base of petioles
- White flowers with white petals, 4-6 mm long in stalked clusters (5-15)
- Round, purple to black fruits, 6-10 mm in diameter, in small clusters

**Rhus typhina L.**

**Staghorn sumac - Vinaigrier sumac**

- Shrub or small tree - up to 6 m tall
- Leaves: alternate, compound divided into 11-31 leaflets.
- Central stalk reddish and hairy, 30-50 cm long.
- Leaflets **7-13 cm long**, slender-pointed, sharp toothed and stalkless.
- Fruits: Reddish fuzzy drupes 3-5 mm long in dense erect cone-shaped clusters located at branch tips.

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**Salix spp.*

**Willow - Saule**

- Shrub with multiple stems (1.5 - 2.5 m). Often forms large monospecific stands.
- Narrow leaves (7-15 cm long, 2 cm wide), ending in a sharp point. Finely toothed, alternate, and whitened beneath.

*spp means “species”. You will observe several species of the *Salix* genus in Mer Bleue.
Category: smaller shrubs and bushes

**Rubus idaeus**  
Raspberry - Framboisier

- Erect stems (100-150 cm) with fine spines.
- Alternate compound leaves with 3-5 leaflets.

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**Vaccinium myrtilloides**  
Blueberry - Bleuet

- Shrub (20-60 cm tall)
- Leaves alternate and often leathery.
- Twigs and leaves densely hairy.
- Toothless leaves
- Fruit is blue or red.

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Adapted from: Chambers B. Legasy, K and Bentley, C. - Forest plants of central Ontario. ©1996 Loner pine publishing, ON.
**Category: various small plants**

**Aralia nudicaulis**

*Wild sarsaparilla - Aralie à tige nue ou salsepareille*

- Herbaceous plant **20-40 cm tall**.
- Short single stem divided into **3 sections each carrying one compound leaf**.
- Compound leaf composed of 5 leaflets on a simple petiole.
- Leaflets oval, 5-12 cm long and finely toothed.
- Purplish-black fruit.


**Asclepias syriaca**

*Common milkweed - Asclépiade commune*

- Herbaceous hairy thick-stemmed plant, 100-150 cm tall.
- Very fragrant pink or purple flowers forming an umbel.
- Long opposite leaves (10-20 cm long) covered with fine white hair.
- Large ovoid fruit (5-10 cm), light green and covered by sharp outgrowth. Seeds are attached to long and silky hairs.

**Aster cordifolius**  
Heart-leaved aster – Aster à feuilles cordées  
- Perennial herb, 20-120 cm tall  
- Nearly hairless, flowering stems  
- Basal leaves heart-shaped, toothed, 3.5-12 cm long and 2.5-7 cm wide. Smaller stem leaves are lance-shaped and stalkless.  
- Flower heads organized in panicle. 10-16 outer rays florets (blue or purple), and 14-20 inner tubular florets 3-5mm long (yellow to purple). Yellow button-like central disk.

**Chamaedaphne calyculata**  
Leatherleaf - Cassandre caliculé ou faux bleuets  
- Small, upright shrub (about 60 cm high), irregular, asymmetric growth form.  
- Leaves alternate and upright, thick, leathery, oblong or oval, 1-5 cm long, very slightly toothed.  
- Terminal bell shaped white flowers present end of spring - early summer
**Cornus canadensis**
**Bunchberry - Quatre-temps**
- Perennial herb
- Flowering stems 10-20 cm high
- 4-6 opposite leaves
- Egg-shaped to oblong leaves, 2-7 cm long with 7-9 prominent veins
- Inflorescence composed of small greenish or purplish flowers surrounded by 4 white 1-2 cm long petal-like bracts (June-July)
- Fruits: bright red round berries (July-August)

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**Drosera rotundifolia**
**Round-leaved sundew - Rossolis à feuilles rondes**
- Small carnivorous plant, 2 cm to 15 cm (with flower) tall.
- Oval leaves covered with glandular dark red hairs secreting sticky liquid and digestive enzymes.

---


**Equisetum sylvaticum**  
*Woodland Horsetail – Prêle des bois*

- Perennial horsetail up to 50 cm tall.
- Green sterile stems in whorls of feathery-branched branches.
- Terminal spore-bearing cones (1.5-3 cm) on 2 to 6.5 cm long stalks.

---

**Eriophorum angustifolium**  
*Cottongrass - Linaigrette à feuilles étroites*

- Perennial with grass-like leaves (20-60 cm tall).
- Fruit with many white hairs (cotton-like).
**Kalmia angustifolia**

Sheep laurel - Kalmia à feuilles étroites

- Shrub (15-100 cm tall).
- Opposite leaves or in whorls of 3. Margins toothless.
- Underside of leaves is pale green, upper surface is darker and leathery.
- Pink or purple flowers in June.


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**Lycopodium obscurum**

Ground pine - Lycopode foncé

- Small herbaceous plant typically about 10 cm tall.
- Spreads by rhizomes (an underground stem).

**Lythrum salicaria**  
Purple loosestrife - Salicaire

- Herbaceous perennial from 60-150 cm tall.
- Base of leaves widely attached to stem.
- Purple flowers occur in terminal spikes.

**Matteuccia struthiopteris**  
Ostrich fern - Matteuccie fougère-à-l'autruche

- Very large fern (60-230 cm tall).
- Fertile fronds distinct from green fronds.
**Onoclea sensibilis**  
Sensitive fern - Onoclée sensible

- Fern with triangular fronds (45-60 cm tall). Unlike most ferns, the pinnae (leaflets) are only slightly wavy, rather than being well divided.

**Pteridium aquilinum**  
Bracken fern- Ptéridium des aigles

- Triangular fern (30-100 cm tall) with a stiff stipe (stalk). Frond **divided into 3 parts**.
**Rhododendron groenlandicum**  
Labrador tea - Thé du Labrador

- Small shrub, 30 – 120 cm tall.
- Leaves alternate, thick, waxy on the upper surface, the lower surface is densely covered with pale brown to orange cottony hairs.
- Leaf edges strongly curled under.

**Rudbeckia hirta**  
Black-eyed Susan - Rudbeckie hérissée

- All parts of this herbaceous plant are hairy (30-100 cm tall).
- Flower has a blackish central disk surrounded by yellowish petals.
**Silene vulgaris**  
*Maiden's tears* - *Silène ou Pétard*

- Perennial herbaceous plant 15-45 cm tall; branched from its base.
- White globular bell-shaped flowers.

**Solidago spp.**  
*Golden Rod (several species)* - *Verge d'or (plusieurs espèces)*

- Perennial herb 30-150cm tall
- Leaves alternate, stackless, lance-shaped, 6-13cm long and 0.5-1.8cm wide
- Leaves numerous and crowded on stem, margin sharply toothed to toothless
- Inflorescences: yellow florets (2-3mm high).
- Numerous florets (up to 30) on the upper side of flowering branches that often are curved, in triangular cluster
Sphagnum spp.
Sphagnum moss (several species) – Sphaigne (plusieurs espèces)

- Small nonvascular (1-10 cm tall) green plant composed of a head (capitulum, 1.5 cm wide), "stem" and "leaves".
- Color varies depending on sun exposition (from red-purple to green).

Spiraea alba/ latifolia
White meadowsweet - Spirée blanche

- Shrub 1-2 m tall.
- Small white flowers in narrow panicles (10-15 cm long)
- Leaves alternate, crowded, sometimes overlapping
- Long lanced-shaped leaves (3-6 cm long 1-2 cm wide): *Spiraea alba* (left)
- Wide lanced-shaped leaves (3-8 cm long 1-3 cm wide): *Spiraea latifolia* (right)
Typha latifolia
Cattail or Bulrush - Quenouille

- Herbaceous plant, often growing in dense stands.
- Leaves are grass-like (1 - 2.5 m long, 0.6 - 2.5 cm wide).
- In autumn, a characteristic cigar-shaped, dark-brown ear (clusters of flowers) is produced, which eventually liberate fluffy seeds.

Vicia cracca
Cow vetch - Vesce jargeau ou jargeau

- Perennial plant with climbing stems.
- Blue or violet flowers.
- Alternate leaves, almost stalkless, about 2.5 cm long
Lab 1 - Distribution of plants at Mer Bleue

Sampling sites

Figure 45. Aerial photograph the Mer Bleue trail.

1. **Station 1 - The field**
   Located on a sand dune, this abandoned field is probably the driest habitat. Plants here are exposed to both the sun, receiving lots of light, and to the drying winds. You will find many grasses, numerous perennials and some shrubs.

2. **Station 2 - The marsh**
   Home of the cattails, the marsh is a pseudo-aquatic habitat where water is always available. The inlet stream provides it with nutrients and oxygenates the water. This in turn allows the organic matter to decompose and prevents the accumulation of peat moss.

3. **Station 3 - The bog**
   At this location, water is covered by a carpet of sphagnum mosses, shrubs and roots between 1 to 7 metres thick. This water, which is most often found within the sphagnum, is very acidic and poor in oxygen. As a result, decomposition is inhibited. At the bottom of the bog, peat moss accumulates and will eventually fill in the lake.

4. **Station 4 - The ecotone**
   On the edge of the bog, close to the marsh, this station represents a transition zone. Drier than the bog and the marsh, the soil allows a better growth of trees, while remaining acidic.

5. **Station 5 - The forest**
   Located on a sandy dune, this station bordering the marsh has well-drained soil and many trees that form a closed canopy.

Scientific Literature

Objectives

After completing this module, you should be able to:

• Assess whether a printed or electronic publication can be considered an original scientific paper
• Search for references using the online resources of the University of Ottawa, including Web of Science
• Find an electronic copy of a scientific paper held by the library network of the University of Ottawa
• Correctly quote scientific publications in your own work or lab reports
• Detect plagiarism and learn about University of Ottawa policy regarding academic fraud.
• Build a bibliographic database, insert citations in a text and generate a reference list using a Reference management tool (Mendeley)

Introduction

Scientific knowledge, in order to be useful, must be transmitted in a credible way. Written communication, either on paper or in electronic form, facilitates the diffusion of scientific and other knowledge. However, the quality of the texts varies. Word processing and the Internet now allow nearly everybody to publish virtually anything they like. It may therefore seem difficult to evaluate the truthfulness of what is portrayed as a fact, and a healthy scepticism is always a worthy attitude in the scientific world.

A sound judgment is necessary, although not sufficient, to gauge the credibility or the truthfulness of any information. Errors, fraud, plagiarism and hoaxes exist in science as much as anywhere else. By using our judgment and carefully picking our sources of information, we can minimize the risk of error and improve the credibility of our writing. In the scientific world, the privileged source of information is the refereed scientific publication, which contains original scientific articles.

This module is aimed at showing you, if you are not already familiar with them, certain facets of the scientific endeavour. The first part covers original scientific publications, their characteristics and the usual mechanism leading to their publication in a refereed scientific journal. The second part, more practical, covers the online tools at your disposal at the University of Ottawa. These tools will allow you to search the available literature and to access original scientific publications. The third part describes when and how to quote references in order to avoid being accused of plagiarism and risk academic fraud sanctions.
Original scientific publications and refereed journals

1- Definition

An original scientific publication is a report or paper describing and interpreting the results of observations, experiments or statistical analyses. The publication must include a description of the scientific methodology used to obtain the results, putting the reader in a position to judge their validity. The term original not only refers to the contents of the publication but implies that the author(s) of the publication have themselves done the study, as opposed to simply reviewed the available literature, popular science books or journals that only report (and sometimes not quite accurately) the results of studies done by others. Furthermore, to be considered credible, a publication has to be evaluated by experts in the field before being published. This revision process is the distinguishing feature of refereed journals.

Daily newspapers (such as the Ottawa Citizen or Le Droit) and journals (e.g. National Geographic or Québec Science) do not publish any original scientific papers. Their articles are often written by journalists who are not experts in the discipline, typically do not include a detailed description of the experimental methodology, and their scientific contents are rarely evaluated by experts on the subject (even though the criticisms of the editorial process can be very harsh). Consequently, these sources, as stimulating as they may be, cannot be considered credible sources of scientific information.

The vast majority of websites are not considered credible sources of scientific information for several reasons: the author is not identified, no experts have reviewed the information offered on the site or the experimental methodology used to obtain the information is not described. There are however credible databases and electronic publications available on the Internet, revised as they are by experts using the same process, and as thoroughly as any article in a refereed publication.

Handbooks and general or specialized encyclopaedias are generally considered sources of reliable information, but not to the same degree as original scientific publications. These books offer a synthesis but rarely give any details on the methodology used to gather the information. They give a superficial coverage of several subjects and generally do not report on any new developments (printing an encyclopaedia is a major undertaking to say the least!).

In short, if the author is not given, or if the author was not directly involved in the reported work, if the detailed methodology (or raw data) are not available, or if the text was not refereed by specialists in the field, there is some risk in having absolute faith in the contents of a scientific publication.
2- Refereed scientific publications

There are several thousand scientific journals and most of them are specialized in a sub-discipline. Notable exceptions are Nature and Science, both are among the most prestigious scientific publications, which publish original articles in all scientific disciplines. The quality of journals varies a lot; some have more stringent requirements than others and have a better reputation.

Therefore, several alternatives are available to the author of an article who wants to publish in a refereed scientific journal. Since an article can only be published in one journal and as ethical considerations prevent submitting an article to several journals at the same time, the author will find it advantageous to pick the right journal.

How can one identify the best journals and weed out the second rate ones? The best journals have generally earned their reputation: over a rather long period, they have published and widely distributed excellent articles in a given discipline. These articles have not only been read but they have also been frequently cited in other articles published later in the same journal and elsewhere. Experienced research scientists have a good idea of the quality of scientific journals, which publish, in their own specialized field. For the others, the Institute of Scientific Information publishes statistics such as the Impact Factor based on the frequency of references to articles in a given journal.

The Impact Factor of a journal is a measure of the average number of citations that each article from a given journal has received in a given period of time. This is an objective measure but it should be used with caution as the results vary considerably from one type of article to another (review articles are more frequently cited) and the discipline concerned. Furthermore, as the number of citations can vary widely from one article to another in the same journal, the average number of citations does not take into account this variability. For these reasons, the Impact Factor is often criticized, but failing any other alternative, it remains a very useful tool.

3- Normal procedures for publication in refereed journals

The interval from submission to publication of an article in a refereed scientific journal may range from several weeks to even years. The delay differs between journals, the extent of requested modifications for the article to be accepted in the chosen journal, and the speed at which editors, printers and referees work. The normal steps are as follows:

• After multiple drafts and a revision by the co-authors, a manuscript is sent to the editor of a scientific journal.
• The editor or one of the assistant editors who specializes in the subject of the manuscript asks experts in the field (2 to 5) to act as referees and sends each of them a copy of the manuscript. (1 week to 1 month)
• The referees anonymously evaluate and criticize the manuscript (some journals, rather rare, use a double blind approach and arrange for the referees not to know the name of the author to prevent any bias), and return their evaluation to the editor (or assistant editor). (1 week to 3 months)
• The editor or assistant editor evaluates the relevance of the criticisms made by the referees and sends the author the comments received and the decision (accepted, accepted with some revisions or rejected). In practice, almost all manuscripts have to be revised: there are always some mistakes! (1 week to 1 month)
• The author revises the manuscript in light of the changes requested by the editor or withdraws his submission (in some cases, to send it to another journal). The revised version is sent back to the editor with a letter describing the revisions or justifying the status quo. (1 week to 1 year)
• The editor evaluates whether the necessary modifications have been made. In some cases he may ask the original referees to re-evaluate the revised article. Generally, a final decision is given at this stage. If the article is accepted, the editor typically asks for some minor corrections (presentation or style). (2 weeks to 2 months)
• The author submits a final version to the editor who sends the article to the printer. (1 week to 6 months)
• The printer sends the galley proofs of the article to the author to check the correctness of his transcription and to correct any remaining errors. The author returns the proofs to the editor who introduces the latest modifications and sends all the articles to the printer. (1 week to 2 months)
• The printer prints the issue of the journal which is mailed to subscribers. (2 weeks to 1 year).

Bibliographic research tools

This section describes some of the bibliographic research tools you can find on the library website of the University of Ottawa (http://biblio.uottawa.ca/en). A brief description of the various tools, of the bibliographic databases most commonly used in biology and a short user guide are presented to help you to complete the exercise associated with this module.

You don't need to be on site to start a bibliographic research: a good deal of your work can be done from home or on the internet using the search tools available at the library network web page.

The online library catalogue allows you to find out whether a book or a particular scientific journal is available through the library network. However, this catalogue is not often used to start a search since the contents of scientific articles are not indexed. A search using key words will only yield a list of books.
To search for original articles, you must connect to one of the systems that index the contents of refereed periodicals. The library network will give you access to many bibliographic databases you can access from the homepage by clicking on the link Databases A-Z. Many databases are used for searches on biology topics, among them Web of Science, Scopus and Medline.

**Web of Science** indexes the table of contents of more than 12,000 scientific publications. It is a multidisciplinary database that also contains a citation index, allowing the user to find articles that cite another article. **Scopus** is another major search tool that contains more references than Web of Science, but doesn't offer a citation index. It is however a very powerful search tool. **Medline** is a bibliographic database covering biomedical sciences (about 3,900 scientific journals). This database is accessible through the [PubMed website](https://www.pubmed.org) and is a service of the U.S national library of medicine.

We will not go over the use of PubMed or Scopus in this lab. However once you are familiar with the use of **Web of Science** tools, you will be able to use any bibliographic database.

**Connecting to the library network**

You can freely access the library network content from any computer on campus, or at home. For access from outside the campus, you must log in using your library credentials. For more info, visit the [off-campus help page on the library website](https://example.com).
Connecting to Web of Science

From the library homepage click on databases A-Z in the Research section, then select Web of Science from the list (there’s also a direct link in the frequently used section, on the right).

Example of keyword search in Web of Science

1. Selecting your keywords

When starting a study on a specific topic, for instance the effects of climate change on bogs in Ontario, you must proceed step by step. The first step is to perform a very general search in order to identify a large number of references of potential interest. Then you will have to narrow down our searches until the number of references becomes reasonable. This is an iterative process and you may have to follow several leads. A good approach consists of first defining the main topics or keywords within our subject area. In our example, we picked three possible keywords: 1: Climate, 2: Bog, 3: Ontario

Then we must think of possible synonyms or more specific keywords that might help to narrow our search:
1. Climate: climate change, temperature increase, warming
2. Bog: Moss, Sphagnum moss
3. Ontario: North America, Canada, Ontario

After completing this preliminary analysis, we selected our first sets of keywords:
1. bog or vegetation
2. “climate change”
3. Canada or Ontario

The OR statement in sets 1 and 3, called an operator, means that the search will retrieve articles containing either of the two terms. In the second set, the quotation marks mean that the search will look for the two words in that particular sequence.
2. Starting the search

In Web of Science, enter the first set of keywords in the top search field and make sure the pull-down menu at the right indicates **Topic**.

**Figure 2.** Web of Science General Search screen. Enter the two keywords in the search field and select Topic at the right to indicate what type of keywords you typed in.

Now click on the **Search** button. Results are shown in figure 3.

**Figure 3** Results of a keyword search in Web of Science.

This search yielded over 208,000 references on bog or vegetation. This is clearly too many references for us to go through.

Now click on the orange ‘Search’ link to go back to the main search page and use the two other sets of keywords: (“**climate change**”), then (**Canada or Ontario**).

You will obtain >292,000 and >252,000 references, respectively. This is still way too many references for us.

To increase the specificity of our search and reduce the number of results, we will now combine our sets of keywords: Click on the **Search History** tab. This will show you the previous searches you performed during this session and the results of each of them. To combine the searches, select all three searches by clicking the boxes under “Combine Sets”, choose **AND** as an operator and click **COMBINE**. The result (1583) is much more manageable (Fig. 4).
Figure 4. Search history screen after combination of searches 1, 2 and 3.

To access the results, click on the number shown in the Results column (1581, in this case).

One of the articles retrieved by the search is Boulanger et al. 2017 (note: you may choose any article appearing in your results if you don’t find this particular one). Click on the title to access the Full record of this article.

Figure 5. Full Record page of an article

This page contains key information about the article: the complete list of authors, the abstract, how many times this article has been cited etc... This will help you to assess whether the article is relevant or not for your study. If it is, you can click on Add to Marked List to retrieve it more easily in the future. You can also export this paper to an online Reference Manager System, such as Mendeley (we will see how later in this chapter). Click on Return to Search Results at the top of the page to select more articles of interest if needed.

Tips for selecting keywords

Selecting the correct keywords is a determining factor in bibliographic searching. In some cases you get lucky. Often, you don’t and you have to start your search all over again using a different subset of keywords. Here are a few tips:
• Examine the reference list of the best references obtained during your preliminary search.
• If you do not get enough references, try and widen your scope! For example, use climatology instead of climate change or North America or nearctic instead of Canada or Ontario.
• Conversely, if you get too many references, combine searches, add more specific keywords or refine your results (see paragraph below).

Notes: An efficient search takes time and sometimes a touch of cleverness. Keep in mind the following few remarks:
• A good strategy for an exhaustive search consists of doing several searches based on different keywords, or covering different databases (e.g. Web of Science and Scopus or Medline).
• To make life easier, consult the online help for the proper syntax. In particular, do not hesitate to use “wildcards”. These are codes used to replace prefixes and suffixes.

Refining Results
You can reduce the number of references by adding more keywords to your search, and you also can use the Refine Results panel, located on the left of the Result page. You can select references within your results using different criteria, such as the title of the journal they were published in, the year of publication etc... To do so, click on a criteria, select one or several possible choices (click on more options/values to display additional choices) and click on Refine.

Accessing the Full text online
The electronic version of most articles is available online as a PDF file and can be downloaded. You have several options to access it:
1- By browsing the library collection of electronic journal (E-Journals). For a complete list of available journals at the University of Ottawa library, click the “E-Journals A-Z” link located on the library homepage, below the database list link. This option lets you browse the current and past issues of many journals.
2- On the Full Record page of any article in Web of Science (see fig. 5). The Full Record page has a button labelled “Full Text Options”, located above the title of the article. Clicking this button will give you several options to get the article. Choose “Full Text From Publisher”, if present. You may also see a button labelled “Afficher /Get it” on some papers, and may use it as well to get the full text.

Finding articles which cite another article
Web of Science allows you to find references that cite previously published articles. This can be useful when you find a good reference, such as an article that gives a good review on a topic. You may be interested to know what papers have cited this article after its publication.
On the Full Record page of any article, you will notice a panel entitled “Citation Network” located at the right of the page. This panel contains information about who cited the article.

For example, the keyword search we previously conducted in Web of Science yielded several review articles, including:

Bunbury, Joan. Holocene hydro-climatic change and effects on carbon accumulation inferred from a peat bog in the Attawapiskat River watershed, Hudson Bay Lowlands, Canada. Quaternary Research. Volume: 78 Issue: 2 Pages: 275-284 Published: SEP 2012

It is possible to find articles published later and that cited this article. To do so, go to the Web of Science Core Collection search page and enter Bunbury J* in the first search field. Then, change the “Topic” item in the box at the right to “Author”.

Figure 6. Cited Reference Search in Web of Science

To make the search easier, click on “+Add Another Field” under the text box, enter 2012 and change the search field type from “Topic” to “Year Published” (fig. 6). Click Search to get the results.

The article “Holocene hydro-climatic change and effects (…)” appears at the top of the result page. Click on the title to enter the Full Record page (figure 7).

Figure 7. Full Record for the Article of Joan Burbury

On this page, you can read at the right end of the title that this article has been cited 27 times. Click on the link that says “27 Times Cited” to get the list of articles.
Finding articles citing the same references

The Web of Science also allows one to find articles citing similar sources that you might have missed using a keyword search. From the Full Record page, click on “View related records” on the right panel. This will display articles sharing the highest number of references with the article of interest.

Library catalogue

In addition to scientific articles, the library possesses a large collection of reference books. Books can be borrowed or read at the Morisset library. Moreover, many titles are available online and can be read on your home computer.

Searching titles using the library catalogue.

To do so, use the Search+ field located on the library homepage. Click on the Advanced search button located below the search field in order to display more search options. Then enter the keywords relevant to our new topic, vertebrate evolution.

Type “vertebrate evolution” in the keyword field (Fig. 8) and select “Book” as format (we are not interested in scientific articles this time).

The results appear on the next window: 612 books relevant to our search. You can add more specific keywords if you wish to narrow down your search, but the second result (as of June 2016) seems interesting.

Figure 8. Library catalogue Search+ with Advanced Search options

After clicking on the title you will find more information about the book, as well as its location and availability. Some book are available online. In this case you will see an “Online Access” button directly in the search result page.
Citing sources: when, which ones and how?

When to cite

In your reports and assignments, you should cite your sources when you are presenting precise facts, important points or interpretations that could be contested.

It is not necessary to have references for all the statements found in your text. Well-established facts and generally accepted premises require no reference. For example, in a report on the status of cod stocks in the Northern Atlantic, it is not necessary to cite a reference to support a statement such as "cod is a fish", nor for a declaration as vague as "cod is probably one of the overfished species in Canadian waters." However, a more precise statement, like "cod is the most endangered species of fish in Canada", would obviously warrant the support of a reference.

If you support your most important statements with good references, your text will be more convincing and better demonstrate your knowledge of the subject.

What to cite

You should cite the original source of the facts and opinions you are reporting. You should only cite references or sources you have personally read.

Most often, in your assignments and scientific reports, you should cite the original references that were published in refereed journals. Review articles from refereed periodicals are also excellent sources. Government reports can also be good references when referring to policies or statistics. However, these reports rarely give details of methodology, and hence are far less authoritative references in support of your assertions, as are original publications.
Handbooks, encyclopaedias, and daily newspaper articles are generally frowned upon. The web, unless the source is well identified and credible, and experts have revised the information, carries very little credibility.

**How to cite**

When citing your sources, you must respect two principles:

1. The source of the facts or opinions must be clearly identifiable.
2. The reference given must be sufficiently detailed to allow anybody to find the source without ambiguity.

In practice, details of the format vary. Each scientific journal has its own standards and professors may also have diverse requirements. However, the most common presentation in science is to include in the text the name of the first author and the year of the publication (e.g. Mercier *et al.* 1999). You also can use the name of the author(s) directly in a sentence, such as: Mercier and colleagues (1999) studied the effect of......

Finally you must include a complete list of references located at the end of the text, and formatted similarly to this:


**References exist in two forms in a research article:**

1. **In-text citations**, which are brief references to the source. The style may vary, but typically they show the first author's name as well as the publication year (e.g. Smith 2012). When two authors contributed to an article, both names should be written (Smith and Roberts 2012). If more than 2 authors contributed, the name of the first author followed by *et al.* ("and others") should be written (e.g. Smith *et al.* 2012).

2. The **reference list (or “bibliography”)** located at the end of the text. It contains a more complete record of the articles cited: names of all authors, journal issue and pages, sometimes the title (never the abstract). The information in the reference list let the reader to quickly find cited articles using a bibliographic search tool. There are almost as many reference styles as there are journals and most of them are available in reference management tools.

**Reference Management Tools**

You can use a Reference management tool, such as Mendeley or End Note, in order to organize your collection of articles. These programs allows you to store all your references in one convenient location (online). It also lets you access your references from anywhere, insert citations in a text, generate a reference list, and format all citations in various pre-defined journal styles.
Using a reference management software

You must cite your source each time you are presenting someone else's ideas or results (otherwise this would be plagiarism, or idea theft). In consequence, your collection of reference articles will increase rapidly, and managing a high number of references can become very complicated. You can overcome this problem by using a reference management program. Various commercial programs are available, and one of them is **Mendeley, a free tool supported by the University of Ottawa Library**. With this program you can keep all your references in one place, organize them as you wish, insert in-text citations and finally generate a formatted reference list. Although Mendeley contains a basic search tool, it is not meant to carry out a bibliographic research *per se*, but rather to retrieve articles you previously identified.

In this paragraph we will cover how to import references from a database to **Mendeley**, how to insert citations into a text document, and then how to format the in-text citations and generate the reference list. Please refer to the Mendeley homepage (**www.mendeley.com**) to learn more about its features.

1- Installing Mendeley

Mendeley consist of 3 elements:

- The standalone program to store and manage your bibliographic references
- The MS Word plugin to insert citations in your text documents and generate the reference list
- The internet browser bookmark to import reference from a search database (e.g. Web of Science) into your Mendeley online library.

Here’s the procedure to follow to install those tools:

1. The Mendeley program can be downloaded from the following URL (all links are in the Lab2 page of the lab website):
   ```
   https://www.mendeley.com/download-mendeley-desktop/
   ```
   Select the version corresponding to your computer then install the program.
   Run the Mendeley program. You will be asked to create an account the first time you run the program. Make sure you write down this information, you will need it later.

2. Microsoft Word plugin: In Mendeley, go to the **Tools** menu, click on “Install the MS Word Plugin” and follow the instructions. This will add a new tab in Word called **REFERENCES**.

3. Install the import bookmark: visit the following URL:
   ```
   https://www.mendeley.com/import/
   ```
   Then, follow the instruction corresponding to your browser. This will add a Bookmark to your Internet Browser.

You can now start using Mendeley.
2- Adding references to Mendeley

You can easily export references found on the web into your Mendeley library. In **Web of Science**, click on the title of an article of interest. This will bring you to the **Full Record** page, as described in Fig. 5. Then click on the **Import Bookmark** you installed in the previous step. If you're not logged in Mendeley, you will have to do so, and then the reference will be saved in your Mendeley online library (Fig. 10).

Open the Mendeley program to manage your library. Click on the “Recently Added” section in “My Library” and you will see the article you just imported (if it doesn't appear, click the **Sync** button just below the menu bar in Mendeley).

Figure 10. Save a reference to Mendeley from Web of Science.

Another way of adding references to your Mendeley online collection is to use the “Literature Search” function within the Mendeley program (fig.11). This function allows you to quickly retrieve articles that you have previously identified as interesting. Type the name of the author, keywords and/or year published in the search bar and Mendeley will show you the corresponding results. Select the article of interest by clicking on it, then in the detail panel (located at the right of the results), click on “Save Reference”. This will add the reference to your Mendeley collection.

Figure 11. Literature Search bar in Mendeley program.
3- Inserting References from your Mendeley Library in a text document

Installing the Mendeley plugin for Word added a “REFERENCES” tab in the MS Word Ribbon (Fig. 12). It will allow you to insert in-text citations and generate a reference list to your Word documents.

3-1-Choosing your format style

The way references are formatted is called “Style” in Mendeley. This is how your citations and the reference list will look in the final formatted document. Every journal has its specific style and the most common journal styles have been pre-set in Mendeley and can be selected from the Style menu of the REFERENCES tab.

Figure 12. References tab in MS Word.

Although the format of citations can be changed at any time, it is better to set it before starting to insert citations*. Choose your Style from the menu.

If it doesn’t appear, click on “More Styles...”. This will open the Mendeley program where you can install new styles. Click on the Get More styles tab, type the name of the style you want to add, then Install. Then go to the Install tab and click on Use this style, then Done. The style will be used and it now will appear in the Style menu of the REFERENCES tab, so that you don’t have to reinstall it again.

3-2- Inserting in-text citations

In your Word document, place the cursor where you want the citation to be added. Then click the Insert Citation button in the REFERENCES tab. This will open the Mendeley search bar. Type any keyword, author name, publication year, that correspond to the reference you wish to add. The corresponding articles in your Mendeley Library will appear in the search bar (fig. 13). Select the article of interest and press the OK button.

*Note: You actually need to insert at least 1 citation in order to select a Style
If you wish to add several references at once, proceed as before, however select a second reference BEFORE you click the OK button. The citations will be inserted and automatically formatted in the style you selected.

3-3- Generating a Reference List

In Word, click on Insert Bibliography, located in the REFERENCES tab. The reference list will be added where your cursor is located. The style will match the in-text citations one, and can be changed at any time (both in-text citations and the reference list will be updated). You may add new in-text citations after generating the reference list, they will be automatically added to it.

Plagiarism

The academic integrity site of the University of Ottawa offers various documents explaining what plagiarism is and what its consequences are. It also gives concrete examples of what is acceptable and what is not acceptable. A copy of the “Integrity in Writing” pamphlet is printed next page. Carefully read this document, as well as the paragraph in the introduction of the lab manual dedicated to plagiarism. Helpful links have been posted in the Lab2 page of the lab website regarding this matter. Ask your TA or the lab coordinator if you have any questions regarding plagiarism and academic integrity.

Lab assignment

Visit the lab2 page of the lab website to learn about the assignment for this lab.
Says who?

Integrity in Writing: Avoiding Plagiarism

Sanctions for committing plagiarism include failing grade for the work concerned; a loss of credit; additional requirement or expulsion; suspension or expulsion; revocation of your degree.

As a student, it is your responsibility to apply the University’s policies and procedures.
Academic integrity and intellectual property

Integrity in Writing: Avoiding Plagiarism

Academic integrity is respect for the intellectual community in which you are participating as a student and the standards governing it. This means that you are accountable for the honesty and the quality of the work that you submit.

The rights of intellectual property must be respected by properly acknowledging the original author's ownership of any words, phrases and ideas that are used in academic writing.

ACADEMIC INTEGRITY AND INTELLECTUAL PROPERTY

REFERENCING

SOURCE MATERIAL

Always introduce the material with a leading phrase (e.g. In a recent article, Smith says…) and provide the reference information in the text and in the Bibliography, Works Cited or References list.

The style of presentation of the reference information often varies by faculty, program and professor. Ask your professor which style you should be using, then consult the Academic Writing Help Centre's documents on referencing (APA, MLA and CM style) or their resources on other referencing styles.

N.B. All images, statistics, charts and tables, including their format, cannot be used without proper reference. The same is frequently true for software and specific computer codes.

PLAGIARISM

Plagiarism in writing is the incorrect use of source material. Whether it is intentional or not, failing to give credit for words, ideas or concepts that you get from any source, including your own previously submitted work, is plagiarism.

Avoiding plagiarism requires learning two skills:

a) using source material correctly and
b) referencing that material.

Any information that you take from another source must be properly referenced, whether it is from a book, a journal, a movie, a friend, or from class notes or lectures.

Writing a paper is not only a matter of gathering and presenting information, it is an exercise in comprehension and critical analysis.

Sanctions for committing plagiarism include a failing grade for the work concerned or in the class concerned; a loss of credits for the year or an additional requirement of 3 to 30 credits; suspension or expulsion from your faculty; or revocation of your degree.

As a student, it is your responsibility to know and understand the University's policies on academic fraud. The rules apply whether the offence is intentional or not.

"I didn't know" will not be accepted as an excuse.

There are three different methods of using source material:

1. Quoting

2. Summarizing

3. Paraphrasing

The correct uses of source material:

- quotation
- summarization
- paraphrasing

There are three different methods of using source material:

- quoting
- summarizing
- paraphrasing

Quoting: the purpose of quoting is to support your own argument. Take the exact words of an author and place them between quotation marks. Quoting is different from paraphrasing or summarizing because it uses only the words of the original author.

Important:
- Be accurate in transcribing the original; there is no reason for mistakes appearing in quotes.
- Use quotes sparingly.

Summarizing: the purpose is simply to give a brief account of what an author says, without going into the specific details or examples. Condense the meaning of a larger text into a more concise format, using your own words. When summarizing, follow the same order of ideas as the original.

Important:
- Remain true to the original author's intent.
- Use only what is most important or relevant from the text.
- Put any of the author's key terms in "quotation marks" or italics to show that they are not your own.

Paraphrasing: the purpose is to reword what an author says in order to support your own argument. Condense material into a concise format, using your own words. Use your own style without changing the meaning of the original text.

Important:
- Be selective. Use only what you need for your own purposes.
- Put any of the author's key terms in "quotation marks" or italics to show that they are not your own.

There are many advantages to learning how to avoid plagiarism:

- Acquiring the ability to construct a paper and integrate sources properly
- Developing critical thinking skills
- Taking ownership of your own ideas by making a clear distinction between others' ideas and your own analysis
- Creating a good impression of yourself as a writer
- Preventing unintentional academic fraud
**Sources that DO NOT HAVE TO BE REFERENCED**

Your own ideas do not have to be referenced. Anything that you conclude from your research or that you think up on your own counts as your own idea.

The exception to this is work that you have previously submitted. This must be referenced like any other source.

If your idea is similar to another author’s, make it clear in your writing that you thought of this idea on your own, but you later discovered it in another source (e.g. Similar conclusions are found in…).

Common knowledge does not have to be referenced. If the information meets the following criteria, it can usually be considered common knowledge:

- It appears in several sources without reference.
- It is not controversial. This means that the information is generally considered as fact.
- It takes up only a minor part of your paper. If it is part of your thesis or main arguments, or it is the basis of your research, it must be referenced.

If you have any doubts as to whether the information constitutes common knowledge, cite the source or consult your professor.

**COLLABORATIVE WORK**

There are two types of collaborative work, and each has its own difficulties regarding plagiarism.

1) **Authorized Group Work:** in which the professor assigns more than one person to complete a project as a team. Each member of the team is equally accountable for the final product. If one of your partners commits plagiarism, you are responsible and you may be sanctioned equally.

2) **Unauthorized Group Work:** in which students work together on a project without the permission of the professor. Sharing data and copying another’s work, even a small section, are considered plagiarism. Each student involved may be sanctioned.

**IMPORTANT CONSIDERATIONS FOR GRADUATE STUDENTS**

As your level of study increases, the importance of being responsible for your work and the information you use in it, including the sources of that information, also increases. This issue becomes especially crucial in two situations:

1) **Having your thesis edited** can lead to complications. There is a fine line between what is acceptable and what is not. Ask your professor or supervisor what level of editing is allowed.

2) **Working collaboratively with a professor** has its own unique challenges. An agreement should be reached before the work begins about who will take the credit for the work accomplished. Your ideas are entitled to the same protection as any other author’s.
Vertebrate Phylogeny

Objectives of this lab

- Perform careful and detailed observations of morphological characters in several species of euchordates
- Determine polarization of each character with respect to the outgroup
- Understand the terminology associated with cladistic analysis
- Express phylogenetic information regarding the evolution of Vertebrates in a cladogram
- Identify homoplasies and determine whether they are the result of convergence or reversals
- Using your cladogram, postulate a hypothesis regarding the evolution of endothermy in vertebrates.

Introduction

In 1835, a ship was anchored off the Galápagos Islands. Aboard that ship, the HMS Beagle, was Charles Darwin, a man with a passion for natural history. He was about to make an astonishing discovery while observing the island's wildlife, particularly finches. These observations eventually led him to postulate that species evolve, that is, they are derived from earlier species through transformation. It was in 1859 that he presented in his book, “On the origin of species”, his theory of natural selection. Darwin used the word ‘Phylogeny’ (created by Ernst Haeckel in 1866) for the first time in the last edition (1872) of ‘On the origin of species’, and defined it as being the "genealogical lineage of all organized' beings". Phylogenetic analysis is aimed at determining the sequence and form of branching that occurred within the tree of life. There has been tremendous progress in this area of research in the last thirty years, due to the refinement of analytical methods and the appearance of new databases (at the molecular level).

At present, phylogenetic research occupies a prominent position within the general scope of evolutionary biology. This research is aimed at establishing the historic framework governing the way living beings have descended from one another. Such a historic framework is significant, because it promotes a better understanding of the evolutionary processes responsible for today’s biodiversity.

Your goal today will be to define what types of phylogenetic relationship link certain species of vertebrates. These species are suitable representatives chosen from the main groups of today's vertebrates. You will use the cladistic methodology to carry out analysis. The purpose of this methodology is to define monophyletic groupings. In order to establish monophyletism, the most convincing approach consists of demonstrating that the members of a group share one or more characters that are exclusive to them.

You must read and understand the appendices in this chapter before attending this laboratory session. This exercise has been modified from a laboratory approach to cladistic analysis by Brooks et al. (1994).
Methods
This lab is divided into three phases:
1- You will make a series of morphological observations (12 characters) on nine species.
2- You will code your observations and use the lamprey as outgroup to determine the polarization of characters (see Appendix 1 of this chapter for explanations).
3- You will proceed with the phylogenetic analysis of your data. That is, based on the information included in the coded matrix, you will construct a branching diagram (cladogram).

1. Data Collection
One of the thrills of systematics is the discovery of characters that allow one to explain parts of the tree of life. However, since your observations are limited to 90 minutes, you will be assigned 12 characters that you will use in your phylogenetic analysis. Your first task consists of finding the state of characters in each of your nine assigned species. You will determine this information from examining the specimens on display in the lab, reading the provided documents and search the internet.

Description of characters
The next paragraphs present the list of all characters included in today's study. Each character is briefly described, and the possible states for each character are indicated (e.g. ‘Present’ and ‘Absent’).

Adult nephridial system (kidney type). In fish and amphibians, the functional kidney (mesonephros or opistonephros) is found more posterior, draining via tubules to a common mesonephric duct. In reptiles, birds and mammals, the adult kidney (metanephros) is more posterior and empties via tubules into a common ureter. Character states for this trait are: mesonephros (Ms) or metanephros (Mt). Diagrams showing the different kidney types will be available in the laboratory.

Amnion. The amnion is an extra-embryonic membrane that forms the amniotic cavity. Cells of the amnion secrete the amniotic fluid that provides the aquatic environment necessary for the development of the embryo. Possible states for this character are present (P) or absent (A).

Elimination of nitrogenous wastes. Vertebrates eliminate nitrogen mainly as 3 different forms: ammonia and ammonia salts, urea, or uric acid.
Ammonia excretion is common in aquatic vertebrates. Ammonia is soluble in water, and a large quantity of water is required to eliminate it. On land, water conservation is crucial, and nitrogenous waste is eliminated in a less toxic form requiring less water: urea, or uric acid. Urea is the main product of nitrogen excretion in amphibians and mammals, but plays a role in osmoregulation of various other species, such as sharks. In terrestrial turtles, lizards, snakes, crocodiles, and birds,
uric acid is the main nitrogenous excretion product. Compared to urea, uric acid can be transformed into a salt that can be eliminated with very little water.

The states for this character are the following: **ammonia** (lamprey and bony fish), **urea** (Chondrichthyes, amphibians, mammals) and **uric acid** (turtles, lizards, snakes, crocodiles, birds).

**Forelimbs modified for flight.** The light bones of the anterior limbs are elongated, reduced in number, resulting from fusion and always containing numerous air cavities (pneumatized). Character states are modification present (P) or absent (A).

**Gizzard.** The stomach of some vertebrates has an area where food is broken down by a thick muscular wall and by stones that it sometimes contains. Possible states for this character are present (P) or absent (A).

**Hair (or Fur).** Possible states are: Present (P) or Absent (A).

**Jaws.** Jaws are used for biting, cutting and/or orienting a prey before it enters the digestive tract. Vertebrates with jaws are gnathostomes; vertebrates without jaws are agnathan. They may be either present (P) or absent (A).

**Lungs and derived structures (swim bladder).** In many vertebrates, the respiratory exchanges occur mainly in the lungs, but some aquatic animals use structures derived from the lungs (swim bladder) for flotation purposes. Character states for lungs and derived structures are present (P) or absent (A).

**Notochord (persistent or present at certain stage of development).** An elongated cellular and cartilaginous rod-like structure found dorsally in chordate embryos. It sometimes persists into adulthood and provides axial skeletal support. States are notochord present (P) or absent (A).

**Number of digits on hind limb.** Collect your information from diagrams and specimens in the lab, as well as outside reference sources. If no hind limbs are present, the number of digits is zero. Possible character states are 0, 4 or 5 digits.

**Number of sacral vertebrae.** The sacral vertebrae (the hip) act as an attachment point for the pelvic girdle (posterior limbs). Possible states correspond to the number of vertebrae: 0, 1, 2+. 

**Occipital condyle.** A post-cranial protrusion (or bulge) that acts as the articulation point for the spinal column. Animals that do not have a neck (no head movement in relation to the thorax) do not have occipital condyles. Animals that do move or bend their heads have one or two occipital condyles. Possible states for this character are: 0, 1 or 2 occipital condyles.

**Paired appendages (fins or limbs).** Paired organs projecting from the axial skeleton, usually involved in locomotion. These may or may not contain skeletal elements. The states for this character are: present (P) or absent (A).

**Paired limbs with tarsi and carpi.** The tarsi and carpi are the bones that form the ankle and the wrist, respectively. Please note the following states: present (P) or absent (A).

**Shell.** The body may be enclosed by either a dorsal carapace (composed of an outer keratinous layer and an inner layer of bone) and a ventral breastplate (plastron) or a
dorsal covering of keratinous and bony plates. The possible states for this character are shell present (P) or absent (A).

**Skeletal tissue.** The skeletal tissue may be mainly composed of cartilage or bony tissue. The states for this character are cartilaginous (C) or bony (B).

**Stomach.** The site where food is stored and digested, the stomach is characterized by its complex muscular wall that breaks down food, its acidic environment (HCl) and proteolytic enzymes. Character states are presence (P) or absence (A) of a stomach.

**Teeth on jaws.** They may be either present (P) or absent (A).

**Temporal fenestrae.** The temporal fenestrae are openings on the side of the skull. The evolution of these fenestrae is associated with the evolution of the jaw muscles in vertebrates. The holes (fenestrae) on the side of the skull provide more room for muscle action, increasing the power of the bite. Possible states for this character are: Absent (0), one pair (1) or two pairs (2) of temporal fenestrae.

### 2. Coding of Characters

You must transform the character states into numerical values. This will 1) make the matrix easier to read and 2) allow character polarization, that is determining which state represents the ancestral state and which one(s) represents the derived state. In order to do so, we will use the **lamprey** as the outgroup for all other. By definition, **the state observed in the outgroup is defined as plesiomorphic** and is coded as zero. Any different state observed for the same character is then apomorphic and coded by “1” (or “2” if there are more than 1 apomorphic state, see appendix 1 in this chapter).

### 3. Phylogenetic Tree Construction

You must successively add each character, one at a time, using the method explained in appendix 2 of this chapter. It requires defining a hierarchy of monophyletic groups based on shared derived characters (synapomorphies). The basic assumption is that the entire group studied (the ingroup) makes up a monophyletic group and that the lamprey is the sister group of all other vertebrates (=the closest relative to the group). The lamprey will then be used as the outgroup for the phylogenetic study and will help us to define the ancestral state of characters (see details in appendix 1).

**Steps to be completed**

**Step 1.** Conduct the phylogenetic analysis, graphically showing (by way of a cladogram) each step of your reasoning. (See Appendix 2).

**Step 2:** Once you have a cladogram that shows the phylogenetic relationships between the principal groups of vertebrates, you can use it to verify hypotheses concerning the evolution of other characters such as morphological and physiological characteristics. In order to avoid redundancy while interpreting the
evolution of these characters, they should not have been used for defining the cladogram.

For instance, in certain vertebrates, the heat produced by metabolism is too low to maintain a sufficient metabolic activity. These vertebrates are called **ectotherms**. Vertebrates with a sufficient metabolic heat production are called **endotherms**.

Conclusions to write: based on the distribution of endothermy on your cladogram, what can you conclude about the evolution of endothermy within vertebrates?

How to proceed: Consider endothermy as a character. Identify species that are endothermic and locate them on your cladogram. Then use your knowledge of the cladistic analysis to formulate a hypothesis regarding the evolution of endothermy within vertebrates: what type of character is it? Does it define a monophyletic group? What is the common ancestor of all endotherms?
Appendix 1
Phylogenetic research: the theory

Cladistics: a Simple Method

The main purpose of phylogenetic research is to reconstruct the evolutionary history of living beings based on similarities and differences that characterize them.

Over the last few decades, systematics has found a new purpose in science by adopting the cladistic methodology. This appendix explains how to express phylogenetic hypotheses by means of a branching diagram called a cladogram. A cladogram (Fig. 1) represents a natural hierarchy of monophyletic groups. These are defined based on consistency in the distribution of derived (or apomorphic) characters. The objective of this lab session is to acquaint you with the basic principles of phylogenetic research.

Characters: Terminology

The foundation for any phylogenetic hypothesis lies in the possibility that a researcher finds a sufficient number of characters to construct it. Such characters may be ontogenic, structural, behavioural, biochemical or hormonal elements that are consistent and that can be applied in comparative studies (Nelson & Platnick, 1981). This latter aspect is fundamental because the polarity of this character must be determined. Polarity implies a distinction, for a given character, between an ancestral state* (also identified as plesiomorphic) and a derived state* (also identified as apomorphic).

This step is crucial because only a group of organisms displaying an exclusive (apomorphic) state of a character can eventually be considered as monophyletic, based on this character.

Wiley (1981) stated a number of criteria that allow one to determine whether a character is homologous or not (in which case it is called homoplastic). For instance, a character observed in two or more taxa is deemed homologous for these taxa if it is also observed in one of their common ancestors. If the same character is present in different states in two taxa, these different states of the same character will nevertheless be considered homologous, if it can be shown that one is derived from the other. If this is the case, the original or ancestral state is considered as the plesiomorphic state whereas, the derived state is considered the apomorphic state of the character.

*by extension, we will call a character in its ancestral and derived states ‘ancestral character’ and ‘derived character’, respectively.
Once the apomorphic state of a character is shared by a group of species, this state is identified as a **synapomorphy** for these species. Hence, a group sharing a synapomorphy is **monophyletic**.

**Characters with more than one derived state:**

Sometimes characters possess several derived states: a character may have evolved several times during time (for instance, the number of digits on vertebrate hind limbs can be none, four or five). This may be problematic, since the sequence of transformation will have to be determined (which of the derived characters is the most recent?). We will see in appendix 2 how to solve practically this problem when building a cladogram.

**Monophyletic Groups**

A phylogenetic study only has scientific value if the group studied is recognized as being 'natural' (=monophyletic). According to Hennig (1950, 1966), the monophyletic status is attained only if the group contains **all the descendants, and only the descendants of a common immediate ancestor** (Fig. 2). In order to establish this monophyletism, the most convincing approach must demonstrate that the members of this group share one or many derived state(s) that are exclusive to them. Such features are called **synapomorphies**. Therefore, a hypothesis concerning the monophyletic status of a group of organisms is initially presented. This hypothesis will be refuted (rejected) or corroborated once new data has been obtained and analyzed. If the hypothesis is refuted, the main conclusion will be that the group studied is not monophyletic. This conclusion, valuable *per se*, may result in a reorientation of research for this group. If the hypothesis is corroborated, it will be concluded that the initial hypothesis is a reasonable approximation of historic reality.

On the other hand, a group of species including a common ancestor plus **only some** of his descendants (Fig. 3) is defined as **paraphyletic**. Such groups are not informative in term of cladistics and characters that lead to the formation of these groups are not homologous (see below, homoplasies).

Only one phylogeny can exist for a given group, but there is no way of knowing if the result of a phylogenetic study is a true reflection of historic reality. With this caveat in mind, cladists prefer a methodological approach that aims at invalidating a hypothesis (hypothetico-deduction), rather than confirming it (induction). This way, the phylogeny deemed to be closest to historical truth will be the one that remains intact after multiple attempts to refute it.
Graphic representation of phylogenetic hierarchy: the cladogram

Cladists incorporate information relative to the phylogeny of groups within a branched diagram: the cladogram. The cladogram is the simplest and most informative graphic expression of the distribution of derived characteristics (apomorphies) within a group of taxa.

How to read a cladogram?

A cladogram represents the distribution and the transmission of characters among species.

The vertical axis represents time, with the top of the cladogram being the present time, and the bottom being the most distant time.

The horizontal axis presents no information at all, merely a list of species. The order of species on the X axis is totally subjective (for instance, people like to place the outgroup on the left end, so that it is left out of the rest of the species).

Therefore, only the relation between groups on the Y axis is important, and no conclusion about the relationship between species can be made based on their proximity on the X axis.

Time is displayed on the Y axis in a non-quantitative way. You cannot measure time on the Y axis and you cannot determine the sequence of events that are located on different branches based on their position on the Y axis.

Character transformation events are symbolised by tick marks on branches and are labelled X(Y), where X is the character number and Y the derived state of this character (usually coded as “1” or “2”). The derived state is transmitted to all species located beyond (=above) the transformation point, until present day, or until a subsequent transformation occurs.

A cladogram represents groups that share a common derived character (a synapomorphy). Those groups are called monophyletic groups. On figure 4, groups [OG ABCD], [ABCD], [BCD] and [CD] are monophyletic groups.
A Method for Determining Polarity: the Outgroup Comparison

How do we establish if a character state is apomorphic or plesiomorphic? One way is to use a method called the **outgroup comparison**.

The outgroup comparison uses a comparison of the character state within the study group (or **ingroup**) with the state observed in a taxa outside of this group (**outgroup**).

Thus, when the state of a character is identical to the state observed in the outgroup, it is defined as ancestral (plesiomorphic), whereas any state different from that of the outgroup constitutes a derived (or apomorphic) state.

A good **outgroup** should be closely related to the ingroup otherwise it would be difficult to find homologous characters between taxa of these groups. Ideally, the outgroup should be a monophyletic group sharing a direct common ancestor with the ingroup (and therefore represents a sister group to the ingroup).

Conflicitive genealogy: The principle of parsimony

More often than not, the phylogeneticist will have to face conflicitive sets of character transformation (thus conflicitive phylogenies). This is when the principle of parsimony is invoked. This principle, also known as Occam’s razor, indicates that the solution that requires the minimum number of character states or transformations (evolutionary events) must be selected when different hypotheses are in conflict. In phylogenetic terms, this does not imply that evolution necessarily follows a parsimonious path but that the most acceptable cladogram, from a scientific standpoint, is the one that contains the lowest number of transformations.

One of the consequences of parsimony principle is that sometimes several cladograms that are just as parsimonious as others can be generated from the same character matrix. In such a case, the systematician must produce all the cladograms and discuss the differences.

Homoplasies: convergence and reversal

Causes of conflicts in a transformation series may be of two types. The first type is related to interpretation errors. Since there is no method in existence that can rectify human error, there is no point in wasting time on this issue. The second type, clearly more interesting from an evolutionary science standpoint, concerns nature's whims. Some characters are said to be **convergent**, which means that they are structurally similar even though they appeared independently in taxa that do not have an immediate common ancestor. The ability to fly in birds and bats is a character that is said to be convergent for both groups, since there is no direct common ancestor to both bats and bird that possessed the ability to fly.
Other characters show reversals. A reversal happens when a character that is unique to a taxon group reverts to more ancestral level in a taxon within this group. For example, the presence of wings is generally considered as a unique (apomorphic) character for insects. But many species of insects do not have wings (e.g. fleas, lice, etc.). These species have winged ancestors. Even though this character reverts to a state that looks more ancestral, winglessness is apomorphic for these species.

A convergence hypothesis cannot be established beforehand, but is rather the result of a phylogenetic analysis. For instance, in the case of bats and birds, many unique characters (synapomorphies) phylogenetically link each of these groups to different non-winged ancestors. Therefore, it is from this information that we can postulate that wings are the result of a convergence in both groups. The same reasoning is used in cases of reversal. Previous knowledge of phylogeny of the groups involved is therefore needed before a homoplastic hypothesis can be constructed.

As a result, based on the cladistic method, all characters are given equal value at the time of cladistic analysis. The quality of a phylogenetic hypothesis is rather derived from the addition of consistencies in the distribution of many apomorphic characters at each level of the phylogenetic hierarchy, than on the subjective interpretation of the adaptive or historical value of some characters.

Following this approach, it is therefore possible that similarities initially considered as apomorphic became non-homologous characters or homoplasies after all the characters were analysed.
Appendix 2

An Example in Phylogenetic Research

The following table contains observations (fictitious) that were carried out on 7 species of fish and one species that was chosen as the outgroup (OG) to the group that we want to study.

Table 1: Observation Matrix

<table>
<thead>
<tr>
<th>Species</th>
<th>Branchial chamber</th>
<th>Reproduction</th>
<th>Scales</th>
<th>Number of dorsal fins</th>
<th>Parental behaviour</th>
<th>Orientation of mouth</th>
<th>Position of eyes on the head</th>
<th>Teeth</th>
<th>Caudal fin</th>
</tr>
</thead>
<tbody>
<tr>
<td>OG*</td>
<td>Slits</td>
<td>Eggs</td>
<td>Absent</td>
<td>1</td>
<td>No</td>
<td>Dorsal</td>
<td>Dorsal</td>
<td>Present</td>
<td>Round</td>
</tr>
<tr>
<td>A</td>
<td>Operculum</td>
<td>Viviparous</td>
<td>Present</td>
<td>1</td>
<td>Yes</td>
<td>Terminal</td>
<td>Dorsal</td>
<td>Present</td>
<td>Pointed</td>
</tr>
<tr>
<td>B</td>
<td>Operculum</td>
<td>Viviparous</td>
<td>Present</td>
<td>1</td>
<td>Yes</td>
<td>Terminal</td>
<td>Dorsal</td>
<td>Present</td>
<td>Square</td>
</tr>
<tr>
<td>C</td>
<td>Operculum</td>
<td>Eggs</td>
<td>Present</td>
<td>2</td>
<td>No</td>
<td>Terminal</td>
<td>Lateral</td>
<td>Absent</td>
<td>Pointed</td>
</tr>
<tr>
<td>D</td>
<td>Operculum</td>
<td>Eggs</td>
<td>Present</td>
<td>1</td>
<td>No</td>
<td>Terminal</td>
<td>Lateral</td>
<td>Absent</td>
<td>Pointed</td>
</tr>
<tr>
<td>E</td>
<td>Operculum</td>
<td>Eggs</td>
<td>Absent</td>
<td>1</td>
<td>No</td>
<td>Ventral</td>
<td>Dorsal</td>
<td>Present</td>
<td>Round</td>
</tr>
<tr>
<td>F</td>
<td>Operculum</td>
<td>Eggs</td>
<td>Present</td>
<td>1</td>
<td>No</td>
<td>Ventral</td>
<td>Dorsal</td>
<td>Present</td>
<td>Pointed</td>
</tr>
<tr>
<td>G</td>
<td>Operculum</td>
<td>Viviparous</td>
<td>Present</td>
<td>2</td>
<td>Yes</td>
<td>Terminal</td>
<td>Dorsal</td>
<td>Present</td>
<td>Square</td>
</tr>
</tbody>
</table>

*Outgroup

I- Polarization Using the Outgroup Comparison

In this example, the species (OG) will be used for the outgroup comparison. This comparison will enable you to polarize the state of each of the characters that you have found. When a character state is present in the outgroup, it is coded "0". When this state is also present in a member of the ingroup (the group studied), it is also coded "0". If a state is different from that observed within the ingroup, it is apomorphic and must be coded "1" (when that character is binary: 0, 1) or "1" or "2" when that character displays three states (0, 1, 2). At the end of your analysis, your matrix should be the following.

Table 2: Polarized Matrix

<table>
<thead>
<tr>
<th>Characters Species</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
<th>(8)</th>
<th>(9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OG</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Each new character must be added up to the cladogram from the previous step. This way, the cladogram increases in complexity at each step as well as the resolution of your phylogenetic analysis.

**II- Construction of the Phylogenetic Tree**

General principle: you will progressively add each character on your cladogram while following three fundamental rules:

1- The cladogram must present the distribution of derived character states.
2- Species that share the same derived character states must be grouped together (=you create monophyletic groups).
3- When several solutions are possible, you must choose the one that implies the fewer number of steps or transformations (=use the principle of parsimony).

**Step 1.** The first hypothetical cladogram (with no resolution) is the following:

![Cladogram 1: No resolution.](attachment:image.png)

**Step 2.** Then, we start refining the cladogram by progressively adding more characters to it. The choice of the first character to start with is somehow subjective, but as a general rule you should always start with binary characters (those with only 0 or 1 as a value) and characters that appear derived in a large number of species (coded “1” in the matrix). Such characters define large monophyletic groups. In our example, character #1 indicates that all fish species of the ingroup have an operculum (bone that covers the branchial chamber) while the outgroup possesses slits on the sides of the branchial cavity. Therefore, the no resolution cladogram can be modified to reflect the distribution of character 1 states and well as the transformation point of character 1(0) to 1(1). To do so, a new branch is created between the OG that carries the state (0), and taxa [A-G] that possess the derived state (1). The transformation of character 1 is represented with a tick mark on the new branch, which is labelled with the character number followed by the new (derived) state of this character between brackets: 1(1). This creates a new monophyletic group: [A-G].
About parsimony: Creating a common ancestor to species A-G is the solution we chose, because it involved less evolutionary events than alternate solutions (such as 7 independent transformation events happening to A). This is the parsimony principle. It doesn’t tell you that your hypothesis is necessarily true, but that it is the most likely (in term of odds) to represent the correct sequence of events that actually took place.

Step 3. The next character to be added is character 3. It is found in its ancestral state in species OG and E, and is derived in the other taxa. Once again, we’ll try and regroup species possessing the derived state of this character (A, B, C, D, F and G) using the principle of parsimony. This will require the use a fundamental property of cladograms: the fact that the order of branches that are directly connected to a node (representing a common ancestor) can be rearranged:
We can see on cladogram 2b that the branch that carries species E has been moved to the left of the ingroup. Remember, the information presented on cladogram 2b is strictly identical to cladogram 2a. The $3(0) \rightarrow 3(1)$ transformation, can be added easily, thus creating the monophyletic group [ABCDFG] (cladogram 3).

**Step 4.** The same reasoning can be made for characters 2 (reproduction), 5 (parental behaviour), 7 (position of the eyes), and 8 (teeth)*. Adding the transformation points for these characters creates 2 additional monophyletic groups [CD] and [ABG], both defined by 2 synapomorphies. This concordance in the distribution of derived characters reinforces the hypothesis presented on your cladogram.

*Note: several characters have been added during step 4 to keep this chapter shorter (intermediate step are available on the lab website). However, when writing your report, only ONE character will be added at each step.

**Step 5.** Characters with several derived states.
How do we analyze a character that presents more than two states? First, we need to encode the states: the plesiomorphic state is coded 0 as usual and apomorphic
states are coded 1, 2, 3... The numbers used for apomorphic states do not reflect the sequence of character modifications. They only indicate that there are several apomorphic states for this character.

In other words, in a character that possesses 3 states (0, 1 and 2) the sequence of events can be $0 \rightarrow 1 \rightarrow 2$, $0 \rightarrow 2 \rightarrow 1$ or $2 \leftarrow 0 \rightarrow 1$.

To determine which sequence is correct, you need to have certain knowledge of the phylogeny of the ingroup. You can achieve that by analysing binary characters that show a clear polarization, like we did in this chapter. Once you have placed all binary characters you may continue with multistate characters.

To do so, you will follow the same method as for binary characters, except that each derived state has to be placed independently on the cladogram. Generally, one of the 2 derived states defines a monophyletic group and is the most recent state. The other derived state defines a paraphyletic group and is, by comparison, more ancestral. Sometimes, both derived states will define monophyletic groups and the exact sequence of events cannot be determined (this corresponds to the $2 \leftarrow 0 \rightarrow 1$ hypothesis).

Species A, B, C, D and G possess 6(1). We can see on cladogram 4 from previous step that these species can be grouped within one monophyletic group by creating a branch between [F] and [ABCDG]. Similarly, species E and F possess 6(2) and the transformation point can be placed on the existing branch between OG and E:

![Cladogram 5](image)

Once we have successfully placed both transformations on cladogram 5, we can see that the earliest derived state to appear is 6(2), followed by 6(1). Thus, the sequence of events is $6(0) \rightarrow 6(2) \rightarrow 6(1)$.

**Step 6.** Character 9 (type of caudal fin) poses another interpretational challenge. We can use the same reasoning as in step 5 and use the information already available on the cladogram. It is then clear that the transformation of $9(0) \rightarrow 9(1)$ that occurred after species E is earlier than character $9(2)$, which only exists in species B and G located higher in the cladogram. These two transformations can be placed easily on the cladogram, which becomes:
Step 7. We have not discussed character 4 until now. The apomorphic state of character 4 (presence of 2 dorsal fins) is found in species C and G. This represents another interpretation challenge since C and G do not share a common ancestor that is specific to them. Therefore, character 4 is a non-homologous character (=homoplasy). In order to determine whether it is a reversal or a convergence, we will test both scenarios and select the one that involves the fewer evolutionary events (principle of parsimony). Adding 4(1) at the level of the common ancestor of C and G creates a monophyletic group [CDAVG]. 4(1) would be transmitted to C and G and we would have to “remove” the derived state from D, A and B by adding 3 reversals [noted 4(0)*] at the level of these 3 species. The convergence scenario would add 4(1) twice, once at the level of C and once at the level of G.

The convergence scenario involves 2 events [2 x 4(1)*], whereas the reversal scenario involves 4 event [4(1) plus 3 x 4(0)*]. Therefore, we will select the convergence and add 4(1)* twice. The asterisk “*” added to the transformations indicates that character 4 is not a regular homologous character.
The final cladogram is the following:

Note that for your report you will need to include one additional cladogram with the actual species' names on the phylogenetic tree.

References
Microevolution

Objectives

- Understand the Hardy-Weinberg principle
- Calculate allele and genotype frequencies
- Determine if an evolutionary event is taking place within a population
- Understand how population size affects genetic drift
- Understand how an allele frequency affects its fixation rate
- Observe the cumulative effects of drift and selection

Part I: Live simulation of a fish population

I- Introduction

In this lab, you will be part of a population of fish that will live the time of the session. You will witness and measure changes that occur in this population when it is subjected to various conditions and events. This series of exercises will give you an opportunity to have a practical approach to a variety of concepts of microevolution and population genetics, such as: allele frequency, the Hardy-Weinberg principle, genetic drift, selection and evolution. To be well prepared, you should read your lecture notes and textbook chapters relevant to these topics.

First, you have to remember the basic laws of Mendelian inheritance of characters: We will consider today a species of fish that is diploid (i.e. possesses two copies of their chromosomes) and reproduces sexually.

Typical representation of a cross between two diploid individuals:
The Punnet square is the most common representation of the Mendelian transmission of characters and it is used to predict genotype frequencies resulting from a cross between two particular individuals. The example below shows the genotypes of the offspring resulting from the cross between two heterozygous individuals:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AA</td>
<td>Aa</td>
</tr>
<tr>
<td>a</td>
<td>Aa</td>
<td>aa</td>
</tr>
</tbody>
</table>

Figure 1: Punnet square showing a cross between two heterozygous individuals.

This simple table shows that 3 different genotypes will be produced during this cross. Their relative frequencies are 25%, 50% and 25% for the AA, Aa and aa genotypes, respectively. These are called genotype frequencies.

Now if we are not interested in just two individuals but rather a whole population, we enter the domain of population genetics. In this case we have to consider the alleles carried by all individuals, and calculate the allele frequency (= the relative abundance) of each of these alleles in the population.
Locus A, in our previous example, possesses two alleles (A and a) that are both present in the population in different proportions. The frequency of A is designated by p and the frequency of a is designated by q (with p+q=1).

Since we know the allele frequencies in the present population, we can calculate the **allele and genotype frequencies** in the next generation using a Punnet square (figure 2).

Given that the probability for an individual to possess allele A is p, then the probably of being homozygous for A is \( p \times p = p^2 \) (fig. 2). Similarly, the probability for an individual to be homozygous for the other allele is \( q^2 \), and the chance to be heterozygous is equal to \( 2pq \). The sum of the frequencies of all 3 genotypes is equal to 1: \( p^2+q^2+2pq=1 \).

![Figure 2](image)

**Figure 2.** Punnet square for a population. p and q represent the frequency of alleles A and a, respectively, in the population. Genotype frequencies can be predicted from allele frequencies: \([AA]=p^2\), \([aa]=q^2\) and \([Aa]=2pq\).

In the next generation, the A allele will be present in the offspring who have the AA and the Aa genotypes. Their frequency is \( p^2 \) and \( 2pq \), respectively. The frequency of A is equal to \( p^2+\frac{1}{2}(2pq) \) (since only half the alleles of genotype Aa contributes to the frequency of A). Thus, we can write that \( p' \), the frequency of A in the next generation will be: \( p' = p^2 + \frac{1}{2}(2pq) = p^2 + pq \).

Since \( p+q=1 \), then it is true that \( q=1-p \). If we substitute this value in the previous equation we will obtain: \( p' = p^2+p(1-p) = p^2+p-p^2 = p \) or \( p' = p \)

We can conclude that the frequency of A will not change in the next generation. The same is true for the frequency of a, which means that allele frequencies remain the same between generations.

**For example:** in a population of 100 individuals (representing 200 alleles since they are diploid), if there are 120 alleles of type (A) and 80 of type (a) then p=0.6 and q=0.4 (and p+q = 0.6+0.4 = 1). The genotype frequencies in the next generation will be: \([AA]=0.6 \times 0.6 = 0.36\), \([Aa]=2 \times 0.6 \times 0.4 = 0.48\) and \([aa]=0.4 \times 0.4 = 0.16\). Therefore, the frequency of A in the next generation \( (p') \) is equal to \( [AA] + 1/2[Aa] = 0.36 + 1/2 \times 0.48 = 0.6 \). The frequency of A didn't change.

This is the Hardy-Weinberg (HW) principle, from which two fundamental conclusions can be made:

1. Allele frequencies, as well as genotype frequencies, will not change between generations.
2. Genotypes frequencies in the next generation can be calculated from allele frequencies in the present generation.
However, this principle is only true under certain circumstances. A population in which the two conclusions of the HW principle hold is said to be in HW equilibrium. The equilibrium of a population relies on several fundamental assumptions:

a- There is no selection
b- There is no mutation
c- There is no migration inside or outside of the population
d- Chance does not cause some alleles to be transmitted more than others (no genetic drift)
e- Mating is random

If any of these assumptions is violated, the HW equilibrium is broken, the allele frequencies will change during time and genotype frequencies can't be predicted from the allele frequencies. Thus, the HW equilibrium represents a null hypothesis that can be used to test the presence of an evolutionary force within a population: if the actual (measured) genotype frequencies in a population are different than what the HW principle would predict, it is a sign that evolution may be acting within this population.

II-Methods

A- Introduction

The following exercises will illustrate different aspects of population genetics and microevolution. All steps have to be completed sequentially and simultaneously by the whole class. Keep a record of your own results as well as the combined results of the class. You will be asked to answer a series of question during the lab session and hand in the questionnaire at the end of the lab to your TA.

B- Diving into the lake...

In the lab today, each student will become a fish that lives in a lake represented by the lab. There should be around 30 to 40 fish in the lake. We will study the transmission of alleles located at 2 loci: the Blue locus and the Green locus. Each locus possesses two alleles (the dark one and the light), and they are located on distinct chromosomes, which means that alleles at the blue locus and the green locus are transmitted independently.

In addition to the blue and the green loci, each fish has a mating type (either XX or XY). For practical reasons, fish will keep the same mating type throughout the simulation. When you will start the lab today your fish will be assigned with a certain genotype. Then your fish will exchange alleles with another fish in the class and it will produce offspring. Then the offspring will produce their own offspring, twice. This way you will study the genetics of the fish population by observing and measuring genetic variations that may occur, and will lead to the evolution of the population.
1- Characterization of the population:

It's time to meet the fish you will be for the first exercise. As mentioned before, its genotype is characterized by three features:

- The blue locus (dark: B or light: b alleles) controlling the shape of the fin.
- The green locus (dark: G or light: g alleles) controlling the size of the mouth.
- The mating type (white cards): XX or XY. Individuals can only exchange genes with individuals of the other mating type.

Write your genotypic information at the top of the card on the “Initial Genotype” line.

Your TA will write on the black board the genotypic composition of the population. Use these numbers to calculate the genotype and allele frequencies.

Your task is to follow step-by-step instructions and answer questions on the provided questionnaire. You should exchange ideas and comments with the other students and your TA.

Answer Questions 1-5.

2- Hardy-Weinberg proportions:

Answer Question 6.

Since you know the frequencies of each allele present in the population, you can calculate the expected genotypes frequencies using the HW principle.

Answer Questions 7-10.

3- Genetic drift

Before you start to create new generations of fish, consider these questions:

- Do allele frequencies change from one generation to another?
- Do genotype frequencies change from generation to generation?

Allele exchange procedure:

Bring your fish to one of the spawning grounds located at the end of each bench (you will find supplementary allele cards there). You can go to any spawning ground in the lab. Then you must locate another fish with a different mating type than yours. If you can't find a fish with a compatible mating type, wait out this turn until the second round. You will exchange alleles at the Blue and the Green locus, however you will keep the same mating type for the whole exercise.

Then follow this procedure:

1. Take 2 alleles cards that match your genotype at the Blue locus (e.g. BB, pick 2 “B” allele cards) from the allele tray.
2. Put the two alleles from the blue locus in each of your fists. The person representing your mate will blindly pick one of your fists. The allele chosen will be the first allele of
the blue locus of the first offspring. Write this information on the genotypic card (Offspring 1, Allele 1, blue locus).

3. Repeat step 2, except this time YOU will randomly pick one allele from your fish's mate. This is the first offspring's allele 2 of the blue locus (Offspring 1, Blue locus, allele 2).

4. Now the 1st offspring possesses a full genotype at the blue locus.

5. Repeat steps 2-4 for the green locus.

6. The mating type of the offspring will be determined later.

7. Congratulations, your first offspring (#1) is developing. It won't take long before it is born.

8. Repeat the procedure above (steps 1-7) for the second offspring. Don't forget to use the parental genotype to carry on the allele exchange, not that of offspring #1.

9. When done, both offspring have a genotype at the blue and the green loci.

10. Allow your parental fish to die. Bring back parental alleles in one of the allele containers near the spawning grounds.

11. **Now allow the offspring to be born**: flip a coin to determine who between you and the other students (your fish's mate) will become offspring #1 and who will be offspring #2. Your new fish may have a different genotype. In order to keep the male/female ratio constant throughout the lab, **you will keep the same mating type** during the whole experiment. Write down the genotype of your new fish in the “Your Genotype” line in the next round table on the genotype card.


13. When the whole class has reached the next generation, return to one of the spawning ground and find a new mate. You cannot select a fish with which you have exchanged genes in the previous generation to prevent inbreeding.

14. Repeat the allele exchange procedure times.

15. When you're done, write down your final offspring genotype on the blackboard to perform class calculation.

**Answer Questions 11-16**

### 4- Mutation and Selection

In this exercise, we will study the transmission of new allele types in the population. These alleles appeared in the population by **mutation** of one of the previous alleles. In addition to increasing genetic diversity in the population, these new alleles will introduce a new factor: **selection**. So far, all alleles at the blue or green loci had the same **relative fitness**, which means that they did not influence the reproductive success of the fish that carry them. Now, the new allele of the blue locus (called B1) confers an advantage to the fish that carry it. We mentioned before that locus B controls the shape of fins, and mutant fish with the B1 allele (B1 mutants) have more efficient fins that allow them to escape from predators. Thus, these individuals have an increased chance of surviving until reproduction time compared to the non-mutants (wild type).

**Answer Question 17.**

A new allele also appeared at the green locus (which controls the mouth size). Fish that carry this allele (G1 mutants) possess a larger mouth that allows them to catch larger pieces of food. However, the lake is very rich in nutrients of various sizes so that
all the fish can feed themselves as needed. Thus, this mutation does not increase the fish fitness and is considered neutral.

Answer Question 18.

Methods:

Similarly to the previous exercise, the population will go through three rounds of gene exchange. Once again, if you cannot find a fish to mate with, wait until the next turn. During the first allele exchange turn, your TAs will introduce randomly a mutation in a certain number of arbitrarily selected fish. However this time the rules have changed somehow due to the introduction of the mutation. Follow the new procedure that follows:

1. Write down your initial genotype on the genotype card.

2. Selection of blue locus alleles:
   Randomly select the blue locus alleles of the offspring as described before. Then:
   - If the offspring has one of the mutant (B1) alleles it survives.
   - If the offspring lacks one of the mutant alleles (no B1), it may or may not survive.
     To determine the fate of the offspring, flip a coin (heads=lives, tail=dies).
   - If the offspring dies, go back to step 2 and re-select randomly a new set of alleles for the blue locus.
   - If the offspring lives or has the mutant allele, write the new offspring genotype your genotype card.

3. Selection of green locus alleles:
   Randomly select the green alleles as described before.
   Since the mutant does not have higher chances of survival, the offspring lives regardless whether it carries a mutant allele or not.
   Write down the offspring genotype for the green locus in table 4.

4. Select the genotype of the second offspring by repeating steps 2-3.

5. Allow your fish to die, and allow the offspring to hatch by taking the identity of one of the offspring as it has been determined in the previous steps (flip a coin to determine who will become offspring #1 and #2).

6. Once everybody in the lab is finished with the first round of allele exchange, find a new mate (different from the mate you have exchanged alleles with during the previous generation) and repeat steps 1-6 for two more generations. Note: no more mutant alleles will be introduced by your TAs in the subsequent rounds of gene exchange.

7. Your TA will count write the number of each genotype at the end of the 3 rounds of reproduction.

   Answer Questions 19-23.

This exercise was modified from Winterer (2001). Am. Biol. Teach. 63(9) pp678-687.
Part II: Introduction to computer simulation of Population genetics using Populus.

I- Populus basic instructions:

Populus is a simulation of population biology software developed by Don Alstad at the University of Minnesota (http://www.cbs.umn.edu/populus/). Follow the instructions given by your demonstrators to run the software.

The simulations we will use during this exercise are accessible from the “Model” menu. Each simulation model consists of two windows: the input windows in which you enter values for various parameters of the simulation, and the output window where the results are displayed. Both windows are resizable. You also can increase the number horizontal lines in the output window by clicking on options then finer grid. It is possible to zoom in any region of the display window by selecting the region of interest with the left mouse button (drag the selection rectangle around the region you want to zoom in). Right–clicking anywhere on the graph will reset the zoom to 100%.

Ask you TA for more information about the output window if necessary.

II- Genetic drift:

In the Model menu, select Mendelian Genetics then Genetic Drift. The input window will appear. Make sure the Monte Carlo tab is selected.

By default, you can enter values for four parameters: population size (N), initial frequency (p), number of loci and number of generations.

Simulation 1-1:
First, let’s simulate a population of 250 individuals and observe what will happen to an allele which frequency is initially 0.5.

In the input window enter these parameters: N=250, p=0.5 and number of loci=6. The number of loci set to 6 allows us to observe 6 simulated populations at a time, since all loci have the same parameters. Set the Runtime to 300 generations.

Click on “View” at the top of the input window to open the output window. A new window appears, showing a graphic representation of the variation in allele frequency over time (expressed in generation number). You will notice that a certain number of coloured broken lines, each representing a locus (i.e. a population in this case) may have reached the value 0 or 1. This phenomenon is called allele fixation. If an allele frequency reaches 0, the allele is lost in the population. On the other hand, if the frequency reaches 1 it is said to be fixed.

Answer Questions 1-2

Run the simulation again (in the same conditions) by pressing the “Iterate” button in the output window.
Lab4 - Microevolution

**Answer Question 3-6**

**Simulation 1-2**
Now let's go back to the input window and **reduce the population size to N=100** (don't change the other parameters). Run the simulation.

**Answer Questions 7-8**
To test your hypothesis, run the simulation 5 times with 6 loci each time and write down the number of alleles that have been either lost or fixed after **100 generations** with the following population size: **N=25, 75 and 150**.

You will use a quick statistical test to help you to compare your results for each population size: the 95% confidence interval. The 95% interval tells us that we can be 95% confident that the average is located between its lower and upper limits. Thus if the 95% intervals limits of two averages don't overlap, it is a good indication the averages are actually different. Use your calculator and/or Excel to calculate the 95% CI:

1- Calculate the average number of fixed allele \( \bar{m} \).
2- Calculate the standard deviation (SD) of the mean by choosing the **STDEV** function in excel (your TA will demonstrate how to at the beginning of the session).
3- Calculate the standard error (SE) using this formula:
   \[
   SE = \frac{SD}{\sqrt{n}}
   \]
   where SD represents the standard deviation and \( n \) the sample size (number of measurements).
4- The 95% confidence interval is centered on the mean value and has two limits:
   \[
   \text{Upper limit } L1 = \bar{m} + 2 \times SE \\
   \text{Lower limit } L2 = \bar{m} - 2 \times SE
   \]

Enter your results in table 5 of the questionnaire.

**Answer Question 9.**

**Simulation 1-3**
**Set the population size to N=10** and run the simulation for **50 generations**. Try different values of \( p \) (initial frequency) and run the simulation several times for each value tested. Count the number of fixed alleles, and the number of lost alleles.

**Answer Question 10.**

**III- Drift and selection**
In previous simulations, we observed how genetic drift can affect a population by changing allele frequencies. We will see now what is happening if we introduce a new parameter in our simulation: **selection**. Selection is controlled in the simulation by assigning fitness values to the different genotypes.

In the real world, both selection and genetic drift act simultaneously. The goal of this exercise is to observe the effect of selection on allele frequency and the combined effect of both genetic drift and selection.
Close the previous window(s) and now select Model>Mendelian Genetics>Drift and Selection.

**Simulation 2-1**
In the input window, enter the following parameters:
Set the fitness values to $w_{AA}=0.8$, $w_{Aa}=1$, $w_{aa}=0.8$, population size to $N=500$. Start with an initial frequency $p=0.5$ and run the simulation for 100 generations.
Answer Question 11

**Simulation 2-2**
Change the population size to $N=250$, then to $N=50$
Answer question 12.

**Simulation 2-3**
Now let's give a selective advantage to one of the genotypes and set fitness values to $w_{AA}=1$, $w_{Aa}=1$, $w_{aa}=0.9$ in a population of 200 individuals and an initial frequency $p=0.1$.
Answer Question 13 and 14

**Simulation 4**
Change the population size to $N=25$ and run the simulation several times (at least 20 times).
Answer Question 15 and 16

This exercise (part II) was modified from a laboratory proposed by Dr J. Brown at Grinnell College in 1998.

Don't forget to hand in your questionnaire to your TA before you leave the class
APPENDIX
Graphical representation of quantitative data

Use a graph when you wish to:

- see overall trends, patterns or relationships in the data
- compare two or more factors in a general or quantitative fashion,
- present large data sets in a comprehensible way and analyse data

General Principles of Graph Construction

A- Plot type

Here are some examples of different graph or plot types:

**Bar graphs (horizontal or vertical)**
These graphs consist of proportional bars of equal width and variable length. Quantitative variables are placed **along one axis only** (the x-axis for horizontal bar graphs and the y-axis for vertical bar graphs). Bars can represent discrete values (for example locations, categories, time periods…) and are separated by empty spaces. Bar graphs should not be confused with **histograms**, which have quantitative scale along both axes.

**Histograms**
Histograms are similar to bar graphs in that they consist of a number of proportional bars of equal width and variable length. They differ in that **histograms are used to analyse and study distributions**. When building a histogram, the data range must first be divided into a number of intervals and the number of observations falling into each interval recorded. The percent of observations in each interval can be then calculated and plotted on the y-axis.

**Two panel bar graph or histogram (vertical layout):**
Les règles générales des histogrammes ou graphiques à barres s'appliquent, plus les suivantes :
General rules regarding histograms or bar graphs apply, plus
- The Y axis spreads over the 2 panels.
- The scale of the Y axis on both panels is the same (even though the range of the data may be different).
- Label for the X panel is located below the lower panel
- Tick marks are placed on both X axes
- Only one symbol key is used for both panels.

**Pie charts**
A pie chart shows the relationship or proportion of parts to a whole. It is useful if one element makes up a significant portion of the whole. Since generally no scale is provided one must judge the sizes of the angles to infer percentages or proportions represented by a given slice. This type of chart is poor for determining exact values should be avoided.

**Straight-line graphs**
They are generally used when many data points (n>30) are available at constant intervals, in order to see trends or changes in a variable through time. Points are connected by straight lines to indicate
the fluctuation in values through time.

**Scatterplots**

Scatterplots are used to investigate the relationship between two different sets of data. In these graphs variable quantities are scaled along both axes. The independent is always presented on the x-axis, whereas the dependant variable (the one whose value depends on the independent variable) is presented on the y-axis. With these graphs, it is possible to evaluate quantitatively the relationship between two variables.

**B- Presentation**

**I - Page layout**

Layout should consider the size, placement and orientation of the graph. Typically the graph uses the top 2/3 of the page (in portrait orientation), the last 1/3 being used for the caption.

**II- Data to ink ratio**

The data to ink ratio tries to emphasise the importance of the data itself relative to the other elements of the graph. Maximise the data to ink ratio by reducing the amount of non-data ink (grids, borders and unnecessary text...).

**III- Data**

1. **Symbols**
   - Use visually prominent plotting symbols to show the data. The size and appearance of the symbols should be considered. Open or filled circles, squares and triangles are, for example, appropriate. Data symbols should be more prominent than any line linking them or drawn through them.
   - Lessen the visual impact of data labels so they do not interfere with the data or disorder the graph.
   - Axis labels must be concise and precise.
   - Symbols that overlap must be distinguishable. Use distinct symbols (e.g. different shapes or numbers in superscript) to indicate how many data points overlap.
   - If data sets superpose, they should be visually separable.
   - If more than one series of data is plotted and/or you use several symbols, a key for the symbols should be used. Place the symbol key within the plot area of the graph in a space devoid of data. If this is not possible, place it either immediately above or to the right of the x-y axis area. The use of colours should be avoided.

2. **Error Bars**
   - If means are plotted, error bars must be traced. Error bars may be either the sample standard deviation, the standard error of the mean or the 95% confidence interval (indicate what error bars represent in the caption).
   - The error bars should be slightly less prominent than the data points.
   - If a bar graph is used, only present the means + the error bars.

3. **Plotting area**
   - This is an *imaginary* rectangle which encompasses all the data points and error bars. A larger plotting area means a clearer and more easily readable graph (see also page layout).
IV- Axes

1. Axes scale
- Choose a scale for the axis so that the data uses most of the available space (see also page layout)
- Choose an interval that comprises your whole data set (including error bars, if present)
- Only when necessary use a scale break (a break in the x- or y-axis). Do not connect the data points on each side of the break.
- Add 3 to 5 tick marks on quantitative axis. Tick marks should be evenly spaced and all plotted data should be contained between the lower tick mark and the higher tick mark.
- Tick marks number is not limited on non-quantitative axes. Put one tick mark for each category.
- Each tick mark must be labelled with the value or the category it represents.

2. Axis identification (labels)
- Axes labels must be appropriate for the variables plotted. Units between brackets must be included after the text of the label.
- Appropriate SI abbreviations for units must be used (see appendix, next)
- The label text should be brief and precise.
- If you plot logarithms of a variable, the axis label should correspond to the tick mark labels.

V. Caption

A graph must have a caption, located right below the graph

1. What should be included in the caption?
- The caption starts with “Figure 1” or “Graph 1” (“Table 1” is fine for a table). “Figure 1” is accepted for all captions, regardless the type of figure that is presented (graph, table, drawing etc...).
- The first sentence in the caption should be a specific and informative title. For instance, indicate what data is presented on each axis, as well as the complete name of the studied organism (see binomial nomenclature below), if applies.
- The body of the caption should be enough to explain briefly the mechanism by which the data were collected and analysed.
- If only two symbols are used in the graph, they may be explained in the caption as an alternative to a symbol key. For more than two symbol types use a key (see Symbols).
- If several observations were combined or if a mean average was calculated, indicate the number of observations (= sample size). The abbreviation for sample size is ‘n’ (e.g. n=20).
- Do NOT describe trends in your data. The graph is right above the caption and speaks by itself.

2. Binomial nomenclature of Organisms
In addition to common name(s) given to organisms, each of them possesses a unique name based on phylogenetic relationships it shares with all other organisms. The most common way to name an organism is to use the binomial nomenclature initiated by the 18th century biologist Linnaeus. This nomenclature indicates the genus and species, the most specific characteristics in the hierarchy of designations. Specific typography rules apply: only the first letter of the genus is capitalized (not the species name) and both genus and species are printed in italics (or underlined when hand written). Ex: dog (common name) is Canis domesticus or Canis domesticus. Binomial nomenclature must be used in captions.
3. Description of the type of data presented on the graph

- If means are presented, this should be stated, as well as the sample size.
- If data were transformed (if you did the calculation), the type of transformation should be indicated.
- Briefly explain the error bars. For instance: mean average ± standard deviation, mean average ± standard error, mean average ± 95% confidence interval, etc...

C- Example of a graph:

Figure 1: Population growth of *Paramecium aurelia* at 20°C in a sterile solution of 1% (w/v) peptone versus time. Means ± standard error (n=20 counts) are presented.

D- Evaluation Criteria

Evaluation criteria for graphs (not inclusive):

**Presentation:** Graph type, data: ink ratio, layout, clarity and tidiness.

**Data:** Symbols, error bars, axes units and labels

**Caption:** Description, nomenclature, sample size...

We recommend you do your graph by hand. It must be drawn on millimetre paper.

You may chose to do your graph using a computer. It is fine but it may take more time that doing it by hand. In this case, print your graph on white plain paper. Same criteria will be used to mark graph done either by hand or with a computer.

References:

