Rumen Protozoa

By Don Peterson

Introduction:

A bit of background information may help set the tone. My first encounter with rumen ciliates was during a MICROCOSMOS workshop in Fairbanks. The instructor, Dr. Elena Sparrow, had gotten a sample of rumen material for us to look at in context of different kinds of protozoa. My first response was just like the kids' responses. What an incredible amount of activity! The little buggers zoom all over the place and the sheer number of them in a drop of fluid is astounding.

As a teacher, I realized that this was pretty much a sure-fire way to get kids interested. As with pond water, it's virtually impossible not to find things. In the beginning, the objective was simply for kids to have a chance to look at the incredible numbers of organisms in the rumen chamber and gain an appreciation of the complexity of ruminant digestion. I also realized that Fairbanks kids have a unique setting; UAF's Large Animal Research Station (LARS) is home to four cannulated reindeer and the only cannulated muskoxen (2) in the world.

The Biotechnology Program uses CCD cameras mounted on microscopes to either digitize images, take digital video or take analog video. The first sample images/video were taken to Bill Hauer, LARS Manager. His initial response was also just like the kids' responses. Amazement! I must admit I was a bit amazed, too, as nobody from IAB had studied the protozoa. In fact, nobody I've talked with since had ever taken the time or had the opportunity to view the protozoa even though dozens and dozens of physiology/ metabolism studies have been done at LARS over the years.

In conversations with Bill Hauer and Bob White (then Director of the Institute of Arctic Biology), we realized this would be an excellent way to involve kids in genuine research. Biotech students worked on developing good fixatives /stains, culturing techniques, perfecting photography techniques, cataloging the protozoa, etc. These were not "formalized" in the beginning.

As I got more involved with the Learning Cycle Model, I realized this would be an excellent format to formalize students' efforts. By doing preliminary studies on anaerobic respiration, population estimation, wandering around the pens with the animals (NOT the bull muskox pen, however,) visiting with Bill Hauer and viewing "raw" rumen liquor, kids get a pretty good overview of arctic ruminants. Ahah! What a great way to get kids involved in *genuine* inquiry and process/ project-based science.

Blending these ideas resulted in the Rumen Ciliates LCM; this was the first year of the use of the formalized unit. Based on student interest and comments and the enthusiasm of the university folks, I believe more than ever that the LCM and inquiry/ process/ project-based science is the way to go.

A number of student projects and videos are on display at LARS and will be shown to elementary through university students. Several researchers have expressed interest in involving the high school students at some level. Student projects and videos will also be on display during the tourist season beginning next summer. Not bad for a bunch of high school kids!

Alaska Education Standards:

Science

- ✓ A14 & A14b) A student who meets the content standard should understand that the living environment consists of individuals, populations and communities.

- ✓ B1) A student who meets the content standard should use the process of science; these processes include observing, classifying, measuring, interpreting data, inferring, communicating, controlling variables, develop models and theories, hypothesizing, predicting and experimenting.

- ✓ B2) A student who meets the content standard should design and conduct scientific investigations using appropriate instruments.

Mathematics

- ✓ A4) A student who meets the content standard should represent, analyze, and use mathematical patterns, relations, and functions using methods such as tables, equations and graphs.

Technology

- ✓ C1) A student who meets the content standard should use technology to observe, analyze, interpret and draw conclusions.

- ✓ D1) A student who meets the content standard should convey ideas to a variety of audiences using publishing, multimedia and communications tools.

For a description of Alaska Education Standards visit the State of Alaska Department of Education & Early Development Website: www.eed.state.ak.us

Materials:

- ✓ Insulated containers (vacuum bottles)
- ✓ Source of CO_2 gas
- ✓ 125/250 Erlenmeyer flasks w/1-hole stoppers
- ✓ J-stem glass tubes
- ✓ Funnel

Materials continued**:**

- ✓ Cheese cloth/muslin
- ✓ Incubator
- ✓ Microscopes
- ✓ Preservative/Stain
- ✓ Small vials for students' samples

Teacher Preparation:
Rumen ciliates are extremely sensitive to thermal shock. Samples of rumen should be collected and transported in insulated bottles, which have been warmed with hot water. If possible, the bottles should be transported inside another insulated container (e.g. a cooler,) which has been warmed. The bottle cap should be left loose as the rumen is actively producing gases, which could explode the container.

Rumen ciliates are also sensitive to exposure to oxygen. Exposure to the atmosphere should be kept as minimal as possible.

It is possible to collect the rumen directly in warmed preservative/stain. However, the fluid contains a wide variety of vegetation and other foodstuffs, which make it difficult to isolate the ciliates.

When filtering the rumen to obtain rumen liquor, the flasks should be filled with carbon dioxide. Since CO_2 is denser than air, it will settle in the flask and displace the air.

Live samples must be incubated at 103-105˚C. With proper care, rumen liquor can be kept viable for up to five days.

Rumen liquor can be preserved in gluteraldehye (3%,) formalin (35%) or MFS. MFS has been found to be a superior preservative/stain because of its nuclear staining capability (nucleus shape and location is one of the factors used in classifying rumen ciliates). To minimize deformation of the protozoa, the preservative/stain should be warmed to 103-105˚F.

Have students prepare MFS. Observe strict safety precautions as formaldehyde is toxic. *See attachments for formulation of MFS.*

Gear-Up:
Students attend a field trip to UAF/Institute of Arctic Biology's Large Animal Research Station on Yankovich Road in Fairbanks. Under close supervision, students are allowed to walk in the pens and touch/pet the reindeer/caribou and musk oxen. Allow adequate time for students to interact with the animals. Draw attention to the fact that LARS is the home to the only cannulated musk oxen in the world.

Reindeer/caribou and musk oxen are unique to the arctic and touching/petting the animals allows a "bond" with the animals. LARS personnel draw rumen samples from cannulated animals (reindeer and/or musk oxen,) and allow students to assist. In some cases, mass peristalsis occurs during this process, resulting in a stream of rumen "shooting" out of the cannula -- an attention-getter for sure!

This is also a good time to pre-assess students' understanding of anaerobic respiration, fermentation, ruminant anatomy/physiology and protozoology.

Explore:
Have students filter the rumen fluid into warmed culture flasks, which have been filled with CO_2. Filtering should be done through several layers of cheesecloth or a layer of coarse muslin. This removes most of the debris and allows easier isolation of ciliates. Filtering must be completed quickly to minimize exposure to the atmosphere. The fluid will contain enough food to maintain viable cultures for up to five days.

Alternatively, foodstuffs such as ground, dried leaves, grass and twigs can be added. It is inadvisable to do this before students pose questions. Their experiments may include the addition of food to maintain viable cultures, the effects of adding different types of foodstuffs, etc.

The flasks should be moved to the incubator as quickly as possible.

Have students view the viable liquor under 400x magnification. There are a tremendous variety of ciliate forms and locomotion patterns. The variety and activity is astounding.

Note: 1000x oil immersion causes pressure on the cover slip, which inhibits locomotion of the ciliates.

Generalize:
After viewing live rumen ciliates, have students develop an appropriate question that viewing the sample has generated. Equipment availability will be a factor in the development of each student's investigation; a proposed question needs to be answerable.

Possible questions include:

- ✓ What is the taxonomy of the various isolated ciliates?
- ✓ What is the ratio of holotrichs to entodiniomorphs?
- ✓ Is the ratio of holotrichs to entodiniomorphs different in reindeer and musk oxen?
- ✓ Are the same ciliates found in reindeer and musk oxen?
- ✓ How many ciliates are in a cm^3 of filtered rumen liquor?
- ✓ Do the ciliate populations differ in summer and winter?

Possible questions continued:

- ✓ Does the addition of foodstuffs (ground, dried vegetation the animal would be feeding on) affect the viability of the cultures?
- ✓ How does the digestibility of various foodstuffs compare?

Apply and Assess:
Have students design and complete an experiment to answer the question developed.
Have students complete a report/presentation, which demonstrates their understanding of the question's answer.

Components may include:

- ✓ Cover sheet/Title Slide
- ✓ Abstract
- ✓ Clearly stated question that is answerable
- ✓ Background information needed to investigate the question
- ✓ Anaerobic respiration
- ✓ Ruminant anatomy/physiology
    a) Cannulation
    b) UAF/IAB Large Animal Research Station
- ✓ Replicable method used to collect appropriate data
- ✓ Results that are appropriate to developing a conclusion and labeled ciliate pictures
- ✓ Conclusion which clearly answers the question asked
- ✓ Discussion of problems encountered, factors affecting the outcome, possible extensions
- ✓ References

The student should develop the actual report/presentation format. Possibilities include a technical report, an electronic presentation, a poster (similar to poster sessions at scientific conferences.) a Web site, a video, etc. Encourage students to utilize a method that is meaningful to them.

Notes:
Generally, the students will need to preserve a sample of rumen liquor. This can be accomplished by adding a known volume of rumen liquor to a known volume of warmed preservative/stain. MFS is recommended. Five to ten parts of preservative/stain to 1 part of filtered rumen liquor provides good preservation and staining of the nucleus without over staining protoplasm and external debris. If the number of cultures is limited, students needing small samples should obtain them before experiments involving the entire culture are begun. See attachments for MFS formulation.

See attachments for calculating ciliates/cm^3.

Use 1000x oil immersion magnification for questions dealing with taxonomy, ratios, etc. See attachments for a rumen ciliate key. Identification to genus is usually adequate as speciation is determined by rather minute differences (e.g., location of *micro*nuclei), which can be very frustrating for students.

These possible assessments can be utilized in any combination suitable for your groups. If prior examples exist, it may be advantageous to show examples of "the good, the bad and the ugly" as a gauge for students.

Students can be involved in assigned values to the various components of presentations.

Extensions:
Possible open-ended questions:

- ✓ What economic impacts might develop from these kinds of studies?
- ✓ How does a ruminant's digestive system differ from the gastric system in humans?
- ✓ How might ruminants be contributing to global warming?
- ✓ What competitive advantage(s) might ruminants have?
- ✓ Filtering produced an artificially high concentration of ciliates. What might the concentration have been before filtering?
- ✓ What other organisms can be found in the rumen chamber?

Students can assist LARS personnel or graduate students in research projects.
Students can assist LARS personnel in general animal husbandry.
Students can assist technicians in research projects involving nutrition, metabolism, etc.
Pictures of ciliated protozoa can be taken with 35mm micrography to increase resolution. (Pictures can then be scanned to digitize them.)

Collaborators:
Bill Hauer, Manager
UAF/IAB Large Animal Research Station

Suzie Pence, Technician
UAF/IAB Large Animal Research Station

Rich Kadrowski, Technician
UAF/Institute of Arctic Biology

Morgan Robertson, Graduate Student
UAF/Institute of Arctic Biology

Partners in Science/National Science Foundation

References:
Obimoto/Imai; *Atlas of Rumen Microbiology*; Japan Scientific Societies Press.

"Rumen Ciliates, An Introduction to Ciliated Protozoa in Arctic Ruminants" CD-ROM; Skog Biological.

The Ultimate Ungulate Homepage
http://www.pathcom.com/~dhuffman/homepage.html

General Anatomy of the Ruminant Digestive System
http://www.gnv.ifas.ufl.edu/~fairsweb/text/ds/2842.html

Bar Diamond
http://www.bardiamond.com/caninfo.html

Brock, Thomas; Madigan, Michael; Martinko, John; Parker, Jack; *Biology of Microorganisms*; Prentice Hall, Englewood Cliffs, New Jersey 07632

Respiration (Undergraduate course)
http://www.bae.ncsu.edu/bae/courses/bae235/1998/slides/en

Respiration (Undergraduate course)
http://bio2000.ucdavis/edu/bis10/respiration/respiration.htm

Rumen Investigation

After the field trip to LARS, and after observing an untreated sample of rumen fluid, determine a question that you would like to answer regarding rumen ciliates. There are literally an infinite number of questions that could be investigated. Keep in mind the limitations of time, equipment and expertise. Remember, the question needs to be "answerable."

Here are a few sample questions:

- ✓ What is the number of ciliates per cm3 in musk ox and/or reindeer rumen liquor?
- ✓ What is the ratio of holotrichs to entodiniomorphs in musk ox and/or reindeer rumen?
- ✓ How does the variety and/or number of ciliates compare in musk ox and reindeer rumen liquor?
- ✓ What is the taxonomy of ciliated protozoa found in musk ox and/or reindeer rumen liquor?
- ✓ Does adding food to the cultures keep them viable for a longer period of time?

Once you have decided on a question you'd like to investigate, decide which, if any, of the preservatives/stains you wish to use. Three types are available:

1) 3% Glutaraldehyde (preserves well but does not stain);
2) 10% Formalin (preserves well but does not stain); or
3) MFS (Methyl green-formalin-sodium chloride)-- preserves well & stains nuclear material.

Remember: Use preservative that has been warmed to 38˚C!

Whatever the question, it is strongly recommended that you keep *very* careful records regarding dilutions, etc. Have a well-thought-out plan of attack before you begin. It may not be possible to redo this because the ciliates will most likely be dead and decomposition will have started before several class periods are over -- except, of course, for the preserved specimens.

The product will be a technical paper complete with background information, an abstract, methods, results, etc. (See the scoring guide for details.) Other options include electronic presentations, production of a video, construction of a poster or authoring a Web site. If you have other ideas, I would be *happy* to discuss them! Regardless of the method of presentation, the product must include labeled pictures of all genera of ciliated protozoa found in the sample(s.)

MFS

Application: Nuclear staining, preservative

"Ingredients:"

100 mL 35% Formaldehyde solution

900 mL Distilled water

0.6 g Methyl green

8.0 g Sodium chloride (Lab Grade)

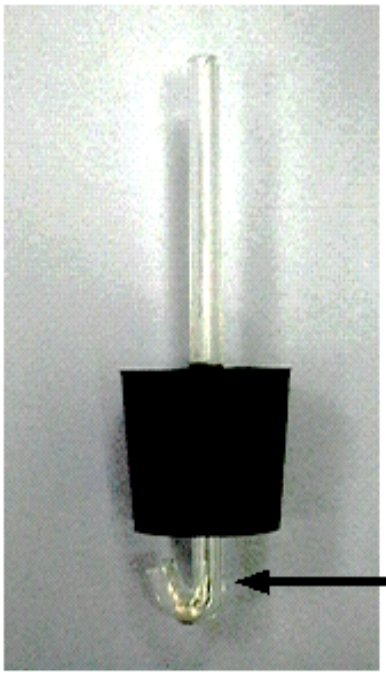
Preparation:

Mix ingredients well and store in the dark. Light exposure degrades methyl green into methyl violet resulting in poor staining.

When specimens are added to 5-10times the volume of MFS solution, only the nuclei of the ciliates are stained. One part rumen liquor and 9 parts MFS will provide a 10% dilution which is easy to work with when calculating ciliates per cm^3.

Specimens should be observed at least 30 minutes after the addition of MFS in order for staining to occur.

Specimens can be stored in the dark for at least 4 years.



J-Stem glass tube

When bending the glass tubing, observe safety precautions as the glass will be very hot.

The J-bend must be open enough to allow gases produced by the ciliates to escape. The bend helps prevent air from entering the culture.

Calculating Ciliates per CM^3

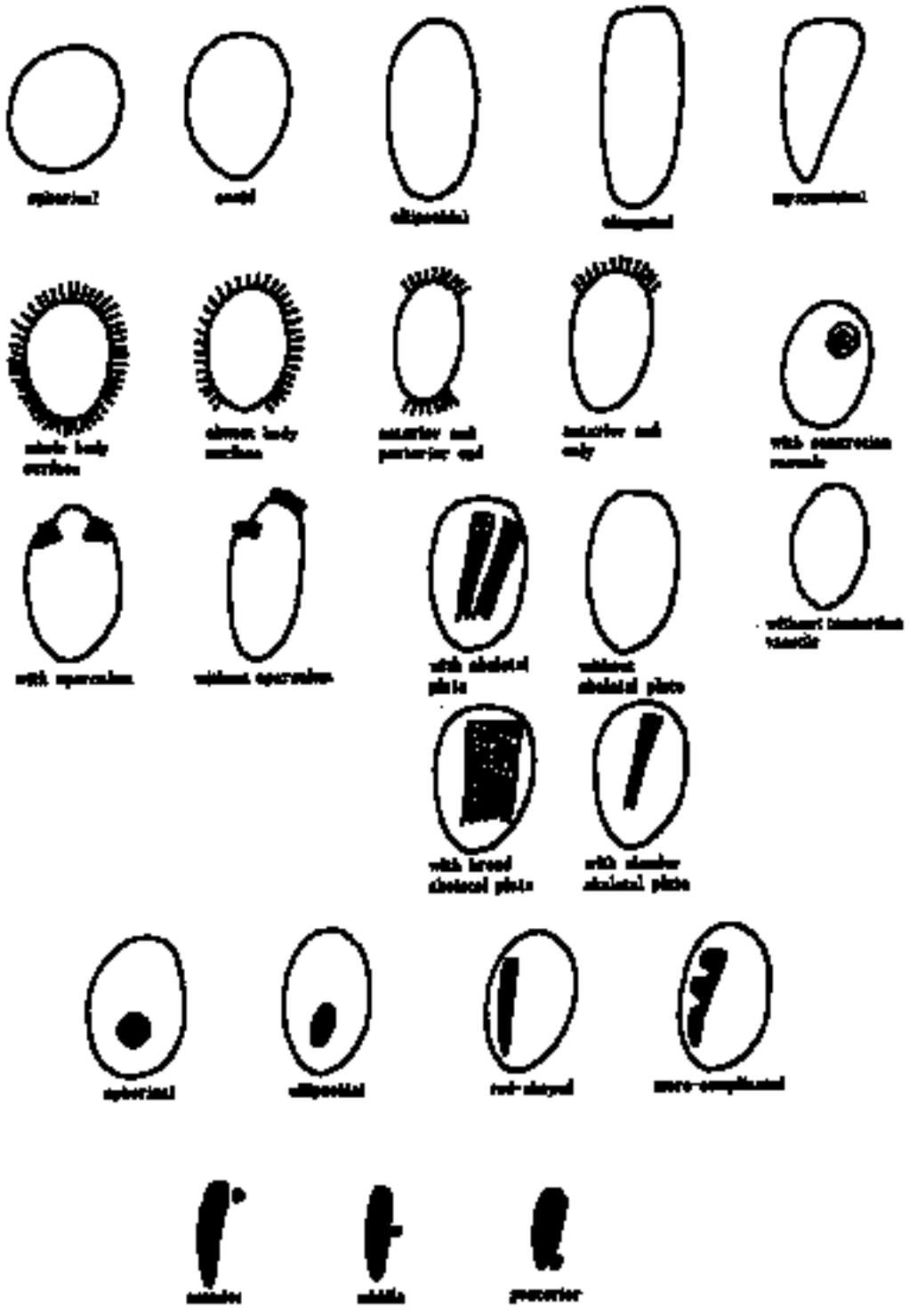
Example:

A drop of diluted rumen liquor with a diameter of .5 mm (.05 cm) was placed on a slide and a 22x22 mm (2.2x2.2 cm) coverslip was placed over it. An average of 6 ciliates were observed in each field of view using 400x magnification. The diameter of the field of view is 460 µm (0.046 cm)

The original rumen liquor was diluted using 1 part of liquor to 9 parts of MFS.

A Area of the coverslip $= s^2$	$= (2.2\ cm)^2 = 4.8\ cm^2$
B Area of the microscope field of view (FOV) $= \pi r^2$	$= \pi (0.046\ cm)^2 = 6.6 \times 10^{-3}\ cm^2$
C FOV/coverslip $= \frac{A}{B}$	$= \frac{A}{B} = \frac{4.8\ cm^2}{6.6 \times 10^{-3}\ cm^2} = 7.3 \times 10^2$
D Ciliates in the drop = (Ciliates in FOV)(C)	$= 6\ \frac{Ciliates}{FOV} \times (7.3 \times 102\ FOV) = 4.4 \times 10^3\ Ciliates$
E Volume of the drop $= \frac{4}{3}\pi r^3$	$= \frac{4}{3}\pi (0.025\ cm)^3 = 6.5 \times 10^{-5}\ cm3$
F $\frac{Ciliates}{Dilute\ cm^3} = \frac{D}{E}$	$= \frac{4.4 \times 10^3\ Ciliates}{6.5 \times 10^{-5}\ cm3} = 9.0 \times 10^8\ \frac{Ciliates}{Dilute\ cm^3}$
$\frac{Ciliates}{cm^3} = \frac{F}{Dilution\ Factor}$	$= \frac{6.7 \times 10^7}{1 \times 10^{-1}} = 6.7 \times 10^8\ \frac{Ciliates}{cm^3}$

Rumen Ciliates Morphology



(from Atlas of Rumen Microbiology; Oblmoto/Imai; Japan Scientific Societies Press

Score Cards: Rumen Ciliates Unit

Rumen Ciliates Researchers: Self Assessment	
Cover/Title	5
Abstract	10
Background	15
Question	10
Method	10
Results Pictures included!	25
Conclusion	10
Discussion	10
References	5
Mechanics	10
TOTAL	110

Comments:

Rumen Ciliates Researchers: Instructor Assessment	
Cover/Title	5
Abstract	10
Background	15
Question	10
Method	10
Results Pictures included!	25
Conclusion	10
Discussion	10
References	5
Mechanics	10
TOTAL	110

Comments:

Scoring Guide: Rumen Ciliates

	Outstanding	Successful	Completed	Attempted	Score
Cover/Title	-Title conveys content -Authors identified -Layout captures attention -Appropriate graphics to supplement text	-Title conveys content -Authors identified -Layout captures attention -Graphics missing or inappropriate	-Title conveys content -Authors identified -Layout does not capture attention -Graphics missing or inappropriate	-Title conveys content -Authors not identified -Layout does not capture attention -Graphics missing or inappropriate	(5)
Abstract	-Conclusion clear and concise -Results clear and concise -Method clear and concise -Background clear and concise	-Conclusion clear and concise -Results clear and concise -Method clear and concise -Background unclear or not concise	-Conclusion clear and concise -Results clear and concise -Method unclear or not concise -Background unclear or not concise	-Conclusion clear and concise -Results unclear or not concise -Method unclear or not concise -Background unclear or not concise	(10)
Background	-Anaerobic respiration explained clearly -Ruminant anatomy/physiology explained clearly -Cannulation explained clearly -LARS explained clearly	-Anaerobic respiration explained clearly -Ruminant anatomy/physiology explained clearly -Cannulation or LARS explanation unclear	-Anaerobic respiration explained clearly -Ruminant anatomy/physiology unclear -Cannulation and LARS explanation unclear	-Anaerobic respiration unclear -Ruminant anatomy/physiology unclear -Cannulation and LARS explanation unclear	(15)
Question	-Clearly stated with measurable outcome -Answerable based on student expertise -Answerable based on equipment/time	-Clearly stated with measurable outcome -Contains expertise or equipment/time constraints	-Clearly stated with measurable outcome -Contains expertise and equipment/time constraints	-Stated with unclear outcome -Contains expertise and equipment/time constraints	(10)

Scoring Guide: Rumen Ciliates *(continued)*

	Outstanding	Successful	Completed	Attempted	Score
Method	-Appropriate for obtaining desired results -Collection clearly described -Staining, etc. clearly described -Replicable as written	-Appropriate for obtaining desired results -Collection clearly described -Staining, etc. clearly described -Replication difficult	-Appropriate for obtaining desired results -Collection or staining, etc. not clearly described -Replication difficult	-Appropriate for obtaining desired results -Collection and staining, etc. not clearly described -Replication difficult *scoring guide continued*	(25)
Results	-Appropriate for developing conclusion -Quantified data -Labeled picts of all genera	-Appropriate for developing conclusion -Quantified data -Labeled picts of 4 genera	-Appropriate for developing conclusion -Data not quantified -Labeled picts of 2 genera	-Appropriate for developing conclusion -Data not quantified -Labeled pict of 1 genus	(10)
Conclusion	-Addresses question directly -Clearly written -Concise	-Addresses question directly -Not clearly written or "wordier" than necessary	-Addresses question directly -Not clearly written and "wordier" than necessary	-Indirectly addresses question -Not clearly written and "wordier" than necessary	(10)
Discussion	-Addresses problems encountered -Describes factors affecting outcome -Includes possible extensions	-Three references identified (May include books, articles, interviews and/or Web sites	-Addresses problems encountered -Affecting factors and possible extensions missing	-Problems encountered briefly described -Affecting factors and possible extensions missing	(10)
References	-Four or more references identified (May include books, articles, interviews, and/or Web sites)	-Three references identified (May include books, articles, interviews, and/or www sites)	-Two references identified (May include books, articles, interviews, and/or www sites	-One reference identified (May be a book, article, interview or www site	(5)
Mechanics	Bkgrounds/builds/ transitions consistent (PPT) or layout attractive -No errors in spelling, grammar	Bkgrounds/builds/transitions inconsistent (PPT) or layout not attractive -No errors in spelling, grammar	Bkgrounds/builds/transitions inconsistent (PPT) or layout not attractive -One spelling, grammar error	Bkgrounds/builds/ transitions inconsistent (PPT) or layout not attractive -Two or more spelling, grammar errors	(10)