

TITLE: Iridoviral Diseases in Tropical Fish

PRINCIPLE INVESTGATOR:

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ABSTRACT: The tropical fish industry is a lucrative business in Hillsborough County but is fraught with diseases that infect the fish to the point that they cannot be sold. Iridoviral diseases can and do infect the fish adding morbidity and mortality in many species. The students will inspect and perform DNA testing on several varieties of tropical fish to determine whether they carry the iridovirus. They will work with the University of Florida Tropical Aquaculture Laboratory, located in Ruskin, Florida, and professors located at the main campus of the University of Florida to help in developing a test that could be used to make a rapid diagnosis of this devastating virus. This set of lesson plans and experiments is meant to be utilized for both the upper level student and the student just beginning their science studies in high school.

RATIONALE: With the importance of the tropical fish industry in our backyard, and the presence of a fish farm on campus, one cannot exclude this as an important resource for Hillsborough County. The fact that this industry has many different diseases that can and do devastate the tropical fish is important for maintenance of this resource. Parasitic infections, fungal infections, true worm parasites (nematodes), bacterial infections, external arthropod parasites, miscellaneous benign tumors, and various viruses can devastate the “crop.” While not all of the students are interested in the tropical fish industry, several are interested in veterinary medicine, and quite a few are interested in pursuing science careers in college. By using experimental methods to attempt to diagnose Iridoviral diseases in the fish, the student will become familiar with techniques that are being used in the field of viral identification.

By exposing both the neophyte to science by using simulations and the advanced student by using actual techniques, including PCR and GenBank, this exposure will hopefully encourage the student to pursue scientific careers in the future.

DESCRIPTION OF TEACHING UNIT OR MODULE, INCLUDING EXPECTED OUTCOMES: It is anticipated that this teaching unit will take 2-3 weeks. All students will have been introduced to the scientific method and will follow the outline of that in formulating their data and conclusions. This unit will take place in the mid fall time period, when all of the students have been exposed to DNA, RNA, and PCR.

Initially, all students will be exposed to micropipetting techniques, utilizing the equipment on loan from the University of Florida. The advanced students (Advanced Placement Biology) will then work with tropical fish specimens provided by the University of Florida to extract DNA and amplify it by PCR. The specimens, after amplification by PCR, will be sent to the University of Florida for testing to determine the DNA make-up of the specimen, and attempt to link it to the iridovirus.

MODULE 1: Micropipetting techniques for both Honors Biology and AP Biology students utilizing the micropipette lab from the University of Florida

MODULE 2: Examination of tropical fish to determine if by observation viruses can be determined. This will be done using various specimens provided by the University of Florida Aquaculture Laboratory. The students will have previously been familiarized with the antigen/antibody concept and will understand that viral proteins are antigens and the response of the host is to produce antibodies to that antigen. If so, then an ELISA test should be positive and indicate that that fish has been exposed to the iridovirus.

Prior to performing the ELISA test, using equipment from the University of Florida, the students will view the Bio-Rad video on the ELISA Antibody Test to better understand this principle and testing process. This video will be shown to both the Honors Biology (neophytes) and AP Biology (experienced) students.

MODULE 3: Following the Elisa testing and identification of fish that are positive for a virus, the student will then submit fish positive for the virus for PCR amplification so that the DNA can be submitted for identification to determine if they are positive for iridoviruses.

MODULE 4: At the same time as the AP students are performing their advanced testing, the Honors Biology students will be using simulations (obtained from the University of Florida) for ELISA testing, DNA (and PCR) testing to determine whether their sample contains DNA positive for the iridovirus.

MODULE 4: Students can utilize Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>) to compare DNA specimens from fish positive for iridovirus and those not positive by comparing the known sequencing found in GenBank.

At the present time, there is no test that can easily determine whether a fish is positive for iridovirus. The advanced students will be working with researchers, both at the University of Florida Tropical Aquaculture Laboratory in Ruskin and Gainesville to attempt to develop such a test. Even if this is not accomplished, the students will learn and know the techniques for making a determination of positivity for iridoviruses in tropical fish.

LITERATURE CITED:

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7. Gibson-Kueh, S, Netto, P, Ngoh-Lim, GH, Chang, SF, Ho, LL, Qin, QW, Chua, FH, Ng, ML, and Ferguson, HW. *The pathology of systemic iridovirus disease in fish*. J Comparative Path 129:111-119 2003

BUDGET AND BUDGET JUSTIFICATION:

1. Purchase of book: Fish Diseases: Diagnosis and Treatment by EJ Noga. \$129.99 as reference for teacher and students.
2. 1 ELISA Immuno Explorer Kit BI-Rad 166-2400EDU \$155

SCIENCE LESSON PLAN

***TITLE:** Are Your Tropical Pet Fish Healthy? Iridoviral Diseases in Tropical Fish

KEY QUESTION (S): These lessons will explore the diagnosis of viral diseases in general, and in tropical fish specifically. What are viral diseases? How do they affect tropical fish? Can diagnoses of a viral disease be made by a specific test? Can Elisa testing diagnose a viral disease? Can PCR amplify DNA from a fish to identify a specific viral disease? Can using GenBank aid in this diagnosis? Can a lesson plan of this magnitude be modified for both Honors Biology and AP Biology classes?

SCIENCE SUBJECT: Biology

GRADE AND ABILITY LEVEL: Honors Biology: 9th grade; Advanced Placement Biology: 11th and 12th grade (prerequisite is Biology and Chemistry)

SCIENCE CONCEPTS: The students will understand the concepts of RNA, DNA, transcription, replication, protein synthesis, viruses, virus infections of normal tissue, polymerase chain reactions (PCR), ELISA testing and the antigen/antibody concept, scientific method testing in the lab, and computer research using GenBank.

OVERALL TIME ESTIMATE: This lesson should take between 7-10 days, depending on aid anticipated from the University of Florida and its extensions.

LEARNING STYLES: This lesson involves all three styles: visual, auditory, and kinesthetic.

VOCABULARY: DNA, RNA, replication, transcription, proteins, protein synthesis, viruses, virus infections, polymerase chain reaction, ELISA, antigens, antibodies, electrophoresis, scientific method, and GenBank.

LESSON SUMMARY: The lesson will cover methods relating to virus infestation of tropical fish and a means to diagnose this.

STUDENT LEARNING OBJECTIVES WITH STANDARDS:

SC.912.L.16.10

Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

SC.912.L.14.6

Explain the significance of genetic factors, and the pathogenic agents of health from the perspectives of both individual and public health.

SC.912.L.16.9

Explain how and why the genetic code is universal and is common to almost all organisms.

SC.912.L.16.3

Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.

SC.912.L.18.4

Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.

SC.912.L.16.7

Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.

SC.912.L.16.12

Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).

SC.912.L.17.15

Discuss the effects of technology on environmental quality.

SC.912.N.1.4

Identify sources of information and assess their reliability according to the strict standards of scientific investigation.

SC.912.N.1.6

Describe how scientific inferences are drawn from scientific observations.

MA.912.S.1.2

Determine appropriate and consistent standards of measurement for data to be collected in a survey or experiment.

MA.912.S.3.2

Collect, organize and analyze data sets. Determine the best format for the data and present visual summaries.

MATERIALS: There will be three honors biology classes with 25, 22, and 20 students respectively. There is one AP Biology class with 16 students. If there are enough materials, the students will work in groups of three. All students will first be exposed to the micropipetting lab provided by the University of Florida. Fish and fish specimens will be provided by the University of Florida Tropical Aquaculture Laboratory for use by the students. The ELISA antibody testing materials will be provided by the University of Florida and the students will use the Laboratory Quick Guide provided by CPET as used during the ICORE course. For the DNA extraction from the fish, the protocol used during the spring ICORE course will be used. The University of Florida again will provide materials. PCR will be utilized to increase the amount of DNA. Hopefully, a thermal cycler will be provided. If not, samples will be sent to the University of Florida for the thermal cycling step. DNA-Gel electrophoresis will be utilized to confirm the presence of DNA, which will then be compared to a control of known iridovirus DNA. If possible, and only if available, proteomics can be used to identify the polypeptide sequence of the control and unidentified sample to compare. The GenBank can also be used in this way, but only if time is available.

The Honors Biology students will perform the above (ELISA, DNA Extraction, PCR, and proteomics using the simulation labs provided by the University of Florida.

BACKGROUND INFORMATION: These labs are designed to expose both lower level and upper level students to DNA and its importance in identifying living organisms. Students should first become familiar with the properties of DNA, RNA, transcription, and replication. They should also understand the process of protein synthesis. Once the student understands these basic principles, they can advance to the laboratory where they will isolate DNA, increase its mass with PCR, and identify the DNA by electrophoresis. If time permits, the student will take the protein from the control and unknown fish specimens and utilize proteomics to identify the polypeptide chains.

DNA, RNA, replication, transcription, proteins, protein synthesis, viruses, virus infections, polymerase chain reaction, ELISA, antigens, antibodies, electrophoresis, scientific method, and GenBank have previously been identified as new vocabulary words associated with these lessons.

1. DNA (deoxyribonucleic acid)-Nucleic acid polymer produced from covalent bonding of nucleotide monomers that contain the sugar deoxyribose; the genetic material of nearly all organisms.

2. RNA (ribonucleic acid) –Nucleic acid produced from covalent bonding of nucleotide monomers that contain the sugar ribose; occurs in three forms: messenger RNA, ribosomal RNA, and transfer RNA.
3. Replication-the process where the DNA molecule copies itself.
4. Transcription- the process by which messenger RNA is synthesized from DNA.
5. Proteins-organic compound made of amino acids joined by peptide bonds; primary building block of organisms.
6. Protein synthesis-the process by which RNA is utilized to synthesize polypeptide chains that will become proteins.
7. Viruses-nonliving strand of genetic material that cannot replicate on its own, has a nucleic acid core, a protein coat, and can invade cells and alter cellular functions.
8. Polymerase chain reaction (PCR)-genetic engineering technique that can make copies of specific regions of a DNA fragment.
9. ELISA- Enzyme-linked immunosorbent assay, also known as an enzyme immunoassay (EIA), is biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.
10. Antigens-foreign molecules that provoke an immune response.
11. Antibodies-proteins in blood plasma that attach to a particular antigen.
12. Electrophoresis-also called cataphoresis, is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field.
13. Scientific method-the method by which all scientists propose and conduct research.
14. GenBank- GenBank® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences.

ADVANCE PREPARATION: The teacher has to have taught the principles of DNA to the students before beginning this experiment. Materials, as they are provided by the University of Florida, will contain specific instructions as to the preparations of any solution required for conducting the experiments. All material should be in the possession of the teacher at least one week prior to commencing the experiments.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

I will write two lesson plans, the honors biology will be brief while the AP biology will be more detailed.

AP Biology

1. The students will first be exposed to DNA through their textbook. This should take about one week because of all the enzyme reactions they are required to know.
2. Another week will be devoted to RNA and protein synthesis.
3. Following the classroom exercises, we will spend about two days reviewing PCR, ELISA, and GenBank.
4. I want to spend one or two periods with the micropipetting lab, with the students becoming familiar with this technique.
5. I plan to show the Bio-Rad film on PCR and ELISA to the students.
6. The students will then be exposed to the fish, hopefully both well and sick ones. They will note that there is very little difference seen by observation alone.
7. They will then use the scientific method to formulate a hypothesis, prediction, run the experiment, collect data, and form a conclusion.
8. The ELISA test will be performed first to see if there are any antibodies to supposed antigen introduced by the virus.
9. DNA will be extracted from the fish and then amplified by PCR.
10. Comparisons of these fish will be made with known Iridoviral fish to determine whether there are sick fish or not.

11. If time permits, determining the protein make-up of the fish and comparing it to both Iridoviral and normal fish will be done.

Including classroom time and utilizing Saturdays for some of the labs, I anticipate about three weeks to complete this lesson plan.

Honors Biology

1. Exposure in the classroom to DNA, RNA, protein synthesis ELISA, and PCR should take about 8 days.
2. These students will also have the micropipetting lab, and view the films (at least for now because I have yet to determine their capability of comprehending these films.
3. They will observe the fish and make observations just as the AP students did.
4. They will not be utilizing any of the advance equipment but will utilize the simulations for ELISA (I believe there was one) and PCR.
5. From these simulations, they will make a determination about their hypothesis.
6. I might expose them to GenBank, but again this will be determined by their progress up to that point.

These lesson plans should take about two weeks, with no Saturday labs.

ASSESSMENT SUGGESTIONS:

Since there are actually several labs, the students will have to write lab reports following their lab. Obviously, the AP students' lab report will be more detailed and will follow the protocol I have set forth for the other labs done. Honors bio students will also have to file lab reports which they will have done for all the labs that have been performed.

I usually have quizzes every Friday and a test every third week. This will not change and the quizzes and tests will include work from the text on the subjects covered, as well as the lab exercises.

Hopefully, at the conclusion of the lessons, with the assistance of the Tropical Fish professors, we might be further along in developing a simple test that can determine whether a fish has iridovirus.

EXTENSIONS: None

RESOURCES/REFERENCES: See protocol for references. Videos provided by Bio-Rad will be utilized for ELISA and PCR information.