

## Mystery Menace of the Sea: Fibropapillomatosis in Green Sea Turtles

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**Abstract:** Marine Science students will explore the concepts of emerging pathogens, biotechnology, scientific research, and the future of our oceans as they delve into the mystery surrounding the occurrence and aetiology of Fibropapillomatosis (FP), a deadly disease infecting Green Sea Turtles (*Chelonia mydas*). They will learn through direct instruction, readings and research, laboratory activities, and panel discussions. The unit will include a presentation by a research scientist from the Caribbean Conservation Council, student research projects on the natural history of sea turtles, the reading of Fire in the Turtle House by Osha Gray Davidson, and the modeling of on-going research of the Chelonid Fibropapillomatosis-Associated Herpesvirus. Laboratory experiences will include Bioinformatics exercises, an ELISA simulation, gel electrophoresis, and Polymerase Chain Reaction (PCR). Based on the analyses of the unit's cumulative results, the students will develop an activist's plan for public awareness of Fibropapillomatosis and making a positive impact on the ocean environment.

**Mission Statement:** Scientist may be on the cusp of discovering the culprit for Fibropapillomatosis, a devastating disease effecting Green Sea Turtle populations and the only known global disease of an animal. Marine Science students will gain and understanding of the impact of the disease and its possible link to environmental degradation. Through the utilization of the scientific process and biotechnology simulations students will model on-going research efforts to definitively isolate and identify the aetiological agent.

### Description of Teaching Unit:

#### Background

There are seven existing marine turtle species, all of which are currently either threatened or endangered. Severe declines in sea turtle populations have been attributed to overharvesting, entrapment by fishing lines and nets, boat collisions, and the destruction of nesting beaches and foraging habitat. (Herbst & Klein, 1995). In addition, a growing threat to the survival of sea turtle populations worldwide is Fibropapillomatosis (FP), an alarming neoplastic disease associated with infection by a novel alphaherpesvirus, fibropapilloma-associated turtle herpesvirus (FPTHV) (Greenblatt, *et al.* 2005).

This debilitating disease occurs primarily in green turtles (*Chelonia mydas*), but has also been reported in loggerhead (*Caretta caretta*), olive ridley (*Lepidochelys olivacea*), hawksbill (*Eretmochelys imbricata*), leatherback (*Dermochelys coriacea*), and flatback (*Natotor depressus*) turtles (Herbst, 1994; Huerta, *et al.*, 2002). FP is characterized by single to multiple epithelial tumors ranging from 0.1 cm to greater than 30 cm in diameter (Herbst, 1994). Externally, these tumors occur most commonly on the soft tissue areas of the sea turtle, but can also grow through the carapace and plastron (Jacobson, *et al.*, 1989). In some cases the tumors can grow internally on the lungs, kidneys, liver, and intestines and can disrupt normal organ function (Herbst, 1994).

In on-going research, a serodiagnostic assay has been developed for monitoring sea turtle populations for FPTHV exposure. FPTHV glycoprotein H (gH) expressed in recombinant baculovirus was used in an enzyme-linked immunosorbent assay (ELISA) to detect virus-specific 7S turtle antibodies. (Herbst, *et al.*, 2008). The etiological agent responsible for FP is unknown; however, recent research using viral metagenomics, consisting of viral particle purification and shotgun sequencing were used to examine viruses associated with sea turtle

FP resulting in the discovery of a novel single-stranded DNA virus, sea turtle tornovirus (STTV1) (Fei Fan Ng, *et al.*, 2009). A high incidence of FP also corresponds to coastal waters characterized by habitat degradation and pollution, a large extent of shallow-water area, and low wave energy, supporting speculation that one or more of these factors could serve as an environmental cofactor in the expression of FP (Folley *et al.*, 2005).

Teaching Unit

This unit will be presented in an integrated Marine Science course. Students will be required to draw upon previous knowledge of DNA, protein synthesis, genetics, evolution, and population and community ecology. The theme of emerging pathogens will be utilized to introduce students to biotechnology and role it plays in the study of emerging pathogens and species conservation.

Expected Outcomes

The students will...

- § gain an understanding of the natural history of the Green Sea Turtle and the impact of Fibropapillomatosis on Florida and global populations.
- § define emergent pathogen.
- § be able to access GenBank and perform a basic local alignment search tool genesearch for the Chelonid Fibropapillomatosis-Associated Herpesvirus.
- § demonstrate an understanding of the most common methods for diagnosing viral infections: enzyme-linked immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR), and microarrays, and be able to describe their role in current research.
- § define retrovirus and explain how they are different from other virus types.

Activities

- 1Day Sea Turtle Presentation; Dan Evans, Research Scientist, Caribbean Conservation Council
- 3 Days Introduce Project Journal /WebQuest/Small Group Research Project; each group will be responsible for the research of a specific sea turtle species and creating an information poster depicting the turtle’s natural history, species status, and issues affecting their populations.
- 1Day Group presentations of Sea Turtle Informational Poster
- 5 Days Guided reading and discussions of Fire in the Turtle House: The Green Sea Turtle and the Fate of the Ocean by Osha Gray Davidson; reading unit will introduce the concept of emerging pathogens and the Fibropapillomatosis menace.
- 1Day Global Mapping Project: Students will track sea turtle migration and incidence of FP, project will continue through the school year; a report including data analyses and conclusions will be incorporated in the end of year assessment.
- 2 Days Lecture and discussion: Review of protein synthesis and concept of a genome; introduction to the concept of emerging pathogens, retroviruses and those belonging to the enveloped viruses which are sensitive to environmental factors, specifically Chelonid Herpesvirus.
- 1 Day WebQuest; a look at current GSTFP hypotheses and research
- 1 Day Bioinformatics Exercise: BLAST FPTHV genome  
Herpes simplex virus genes UL23 through UL36  
*Alphaherpesvirina*
- 1 Day Laboratory Exercise: ELISA Simulation

3 Days            Small Group Final Project: Students will create an information brochure of Sea Turtle Fibropapillomatosis and an activists' plan for improving the sea turtle's ocean environment.

Extension

Sea Turtle Watch in conjunction with a weekend field experience at the Barrier Island Institute / Archie Carr Wildlife Refuge

**Expertise of the PI:** Charlynn Watson Campbell is the principal investigator for this proposal. She is a current participant in the University of Florida's ICORE-HHMI 2009 Emerging Pathogens Institute. Ms. Campbell earned a BA in Biology and Chemistry from Milligan College, TN, and an MA with a concentration in Appalachian Flora and Fauna from East Tennessee State University. Throughout her 19 year teaching career she has taught AP Biology, Anatomy and Physiology Honors, PIB Biology, Marine Science Honors, and Environmental Science. Her laboratory experience in performing and teaching biotechnological procedures includes but is not limited to restriction enzyme digests, gel electrophoresis, PCR, and ELISA assays. As a resource teacher with the Lee County Department of Environmental Education, Ms. Campbell acted as an environmental liaison between the public and private sectors and taught the Environmental Science and Political Actions Research Seminar Class. Beyond the classroom Ms. Campbell's field research experience includes extensive work with the Six Mile Cypress Slough, the Bald Eagle, Burrowing Owl, and Manatee. She also served as a water quality agent for the South West Florida Water Management District (SWFWMD).

**Literature Cited:**

- Fei Fan Ng, T., C. Manire, K. Borrowman, T. Langer, L. Ehrhart, and M. Breitbart. 2009. Discovery of a novel single-stranded DNA virus from a sea turtle fibropapilloma by using viral metagenomics. *J. Virol.* 83(6): 2500-2509.
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National Research Council. 1990. Decline of the sea turtles: causes and prevention. Washington: National Academy Press.

**Budget and Budget Justification:**

Funding in the amount of \$200 is requested and will be used during the 2009-2010 school year toward the purchase of the following items:

- World Satellite Laminated Wall Map by National Geographic, 34"X85", \$85
- Fire in the Turtle House: The Green Sea Turtle and the Fate of the Ocean by Osha Gray Davidson, set of 15 (price varies with availability, to be determined at time of purchase)

**Theme: Emerging Pathogens**

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<b>Lesson Title</b>	Mystery Menace of the Sea: Fibropapillomatosis in Green Sea Turtles
<b>Grade Span</b>	11-12
<b>Content Emphasis</b>	Science
<b>Targeted Benchmarks</b>	
<b>Author</b>	Charlynn W. Campbell
<b>School</b>	Clay High School

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**Lesson Preparation**

**Learning goals:**

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- § define retrovirus and explain how they are different from other virus types.

**Estimated time:**

The study unit consists of a series of related lessons that are intended to span over the course of four weeks.

**Materials/Resources:**

- § Student project journals
- § Project journal student guidelines and rubric
- § Access to computer lab/library
- § Sea Turtle WebQuest activity sheet ([www.ccc.org](http://www.ccc.org))
- § Posterboard and art supplies
- § Class set of Fire in the Turtle House: the Green Sea Turtle and the Fate of the Ocean by Osha Gray Davidson
- § Guided reading study packet for Fire in the Turtle House
- § Global Satellite Wall Map with color coded markers and/or pins
- § Global Tracking and Mapping Project student guidelines and corresponding rubric
- § PowerPoint presentations:
  - DNA/protein synthesis/genome
  - Emerging pathogens/retroviruses
  - BLAST
  - ELISA
- § WebQuest activity sheet for current GSTFP research
- § Digital projector or overhead project and screen
- § BLAST student packet
- § ELISA lab student packet
- § Lab assessment instrument

- § Student unit evaluation and feedback instrument

**Teacher Preparation:**

- § Order map, markers, and class set of book
- § Coordinate Caribbean Conservation Council presentation
- § Prepare WebQuest student activity sheets
- § Create guided reading study packet and accompanying quizzes for Fire in the Turtle House
- § Create PowerPoint presentations
- § Create BLAST and ELISA student lab packets
- § Develop lab assessment
- § Develop rubrics for:
  - Project journal
  - Informational poster
  - Mapping project
  - FB brochure
- § Develop student unit evaluation and feedback instrument

**Introduction:**

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- 1 Day WebQuest; a look at current GSTFP hypotheses and research

- 1 Day            Bioinformatics Exercise: BLAST FPTHV genome  
Herpes simplex virus genes UL23 through UL36  
*Alphaherpesvirina*  
(Including pre and post-lab discussions)
- 1 Day            Laboratory Exercise: ELISA Simulation  
(Including pre and post-lab discussions)
- 3 Day            Small Group Final Project: Students will create an information brochure of Sea  
Turtle Fibropapillomatosis and an activists' plan for improving the sea turtle's  
ocean environment.

**Extensions:**

- § Fieldtrip to the University of Florida for Gel Electrophoresis and PCR lab experience
- § Sea Turtle Watch in conjunction with a weekend field experience at the Barrier Island  
Institute / Archie Carr Wildlife Refuge
- § Read: Wayward Wind by Archie Carr

**Applications:**

Through the course of the unit, the students will recognize that the concept of emerging pathogens is not limited to marine turtles but relevant to all plants and animals, potentially influencing the evolution of the affected organisms as well as having a social, economic, and/or political impact. Participation in lectures, discussions, and group research will provide numerous opportunities for considering and comparing other emerging pathogens (i.e. H1N1).

**Assessment:**

Assessment of the targeted benchmarks will be on-going. The students will demonstrate their understanding of key concepts and mastery of essential skills throughout the course of the study unit. Student products (project journals, information posters, brochures, and presentations) will be assessed by pre-determined rubrics. The students will have access to the rubrics at the introduction to the activity. Lab skills, data collection, and analyses will be assessed through teacher observation and evaluation of written laboratory reports. The reading unit and WebQuests will be assessed by the level of student participation and intermittent quizzes.

**Teacher Self-Reflection:**

-to be completed upon completion of study unit-