The Jacques Cousteau NERR Teacher Modules.

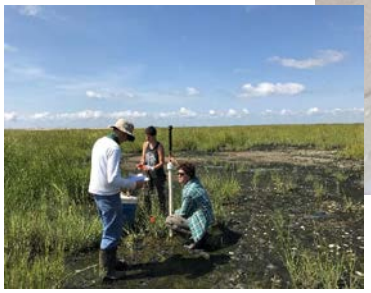
Inspired by the NERRS Science Collaborative project: "*Investigating the Interconnectedness of Climate Change, Nuisance Mosquito Populations, and Long-Term Resilience of Coastal Salt Marsh Systems*".

Overview of this NERRS Science Collaborative project

Understanding and monitoring changes to habitats within the estuary is one of the goals of the Jacques Cousteau National Estuarine Research Reserve (JC NERR). Salt marshes provide humans with many resources and services such as food, water filtration, and storm buffering. Therefore, monitoring marshes for natural and man-made change is a priority for the JC NERR research team. Of particular concern is whether there is a connection between rising sea levels and changes in mosquito populations. For this reason, the JC NERR, Rutgers University, and coastal county mosquito control agencies teamed up to examine the impact of rising sea levels on salt marsh mosquito populations, especially the possibility of highly productive sites (aka mosquito "hotspots") shifting upland closer to residential areas. Salt marsh mosquitoes can be a significant nuisance and have been implicated in the transmission of diseases to humans, livestock, and pets. To address this question Dr. Richard (Rick) Lathrop Jr. and Dr. Dina Fonseca co-lead this study that began in 2016 with a grant from the NERRS Science Collaborative. Dr. Lathrop is a Professor with the Department of Ecology, Evolution, and Natural Resources and Dr. Fonseca is the Director of the Center for Vector Biology at Rutgers University. Dr. Lathrop and Dr. Fonseca wanted to better understand the possible connections between rising sea levels and mosquito populations. This is particularly important to many coastal communities that face a future with more frequent flooding. The project team conducted mosquito surveys within various marsh habitats using methods such as soil coring (for presence of eggs), eDNA analysis, and drone technology. This project is being supported through the National Estuarine Research Reserve System (NERRS) Science Collaborative Program. More about this project can be found here http://www.nerrssciencecollaborative.org/project/Lathrop16

Project Partners

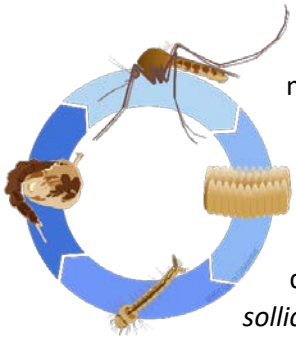
- Rutgers University
- The Jacques Cousteau National Estuarine Research Reserve
- Atlantic County Office of Mosquito Control
- Burlington County Office of Mosquito Control
- Monmouth County Mosquito Control Division
- Hudson Regional Mosquito Control
- Bergen County Mosquito Control Division
- Ocean County Mosquito Extermination Commission
- The Barnegat Bay Partnership
- New Jersey Division of Fish and Wildlife



Photos courtesy of Fonseca Labs

Background for Educators:
Mosquito biology, management and treatment

I think we can all agree that some mosquitoes can be a serious nuisance, but what role to they play in the ecosystem? Are they important in the food chain? Why do they bite us? Below is a brief introduction into the biology, ecology, and life cycle on one of the four mosquito species found in salt marshes, the eastern salt marsh mosquito, (*Aedes sollicitans).* Other salt marsh species include the brown saltmarsh mosquito (*Aedes cantator*), the unbanded salt marsh mosquito (*Culex salinarius*) and the black salt marsh mosquito (*Aedes taeniorhynchus*).



In mosquito species that require a blood meal (not all do), only the female mosquitoes bite. A blood meal provides protein and fat that are essential for egg production. But both male and female mosquitoes actually rely on sugar, such as the nectar of flowers, as a source of nutrition and can be important pollinators (eg. *Aedes vexans* is the primary pollinator of the orchid, *Platanthera obtusata*) (Source: Lahondère et all, 2019). Depending on the species of mosquito, eggs are deposited on standing water or damp soil. Eggs of the salt marsh mosquito, (*Aedes sollicitans),* are laid in the high marsh mud, away from predatory fish, and hatch only after being flooded by high tides, which happen during new or full moons (Source: "What is a mosquito?" 2020).

After hatching, mosquito larvae must be in water, although they cannot breath underwater and must come to the surface for oxygen. They feed on algae, fungi, bacteria and other microorganisms. While the exact length of the larval stage depends on temperature, adults of salt marsh mosquitoes will emerge in less than a week after eggs were flooded (Source: O'Meara, 1992). The larvae, as well as adult mosquitoes, are food sources for many species of animals that live in the salt marsh, especially other insects, spiders, fish, birds and bats (Source: Pers. comm. Fonseca, 2020).

Mosquitoes cause more death and disease than any other animal on the planet (Source: "Fighting the World's Deadliest Animal" 2019). Currently, in the eastern US, the most important mosquito-borne diseases are West Nile encephalitis and Eastern equine encephalitis (EEE) to humans and horses, as well as dog heartworm to pets. While the eastern salt marsh mosquito, *Aedes sollicitans,* has been associated with EEE transmission in epidemic years (Source: Crans 1977), salt marsh mosquitoes are primarily a significant nuisance, preventing enjoyment of coastal areas. This is why most coastal mosquito control in the past (and current day) treat marshes to reduce nuisance.

Organized mosquito control originated in NJ, driven by the detailed ecological research of John B. Smith. Dr. Smith was hired by the Rutgers Department of Entomology as the New Jersey State Entomologist based at the Rutgers New Jersey Agricultural Experiment Station (NJAES). Dr. Smith wrote a seminal book on mosquitoes and demonstrated that shallow grid ditching sped up the retreat of water that remained after high tides, preventing mosquito larvae from developing into adults. In 1912, Dr. Smith succeeded in convincing the NJ legislature to pass the County Mosquito Commission law mandating the creation of mosquito commissions in each of NJ's 21 counties under the guidance of the NJAES (Source:

"History of Mosquito Control at Rutgers: The John B. Smith Legacy", 2020). Grid ditching replaced harsher mosquito control practices that used oil and arsenic, however, it can be also be used to dry up marshes for real estate development (Source: Patterson, 2011). In the 1930's, as part of President Roosevelt's economic-rebuilding "New Deal" initiatives, the Civilian Conservation Corps (CCC) hand-dug thousands of miles of grid ditches in US coastal marshes (Source: Lesser, 2007). Many of these ditches can still be seen today (see Google Earth image of gride ditches near Mystic Island, NJ). With the development of DDT (dichloro-diphenyl-trichloroethane), synthetic insecticides replaced grid ditching for mosquito control. While Rachel Carson's publication "Silent Spring" brought awareness to the dangers and toxicity of DDT and this insecticide was banned in the US in 1972 (Source: "DDT- A Brief History and Status", 2017) other insecticides have been used to control salt marsh mosquitoes as well.

Image from Google Earth. Notice the faint grid-like pattern in the marshes

Nowhere is the "push-pull" of safeguarding the environment while being able to experience the environment as evident as on a coastal marsh.

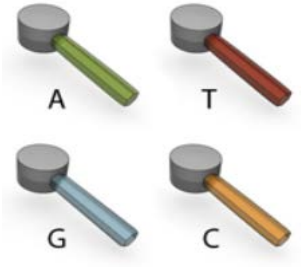
In an effort to avoid insecticides, other marsh modifications such as open marsh water management (OMWM) have been attempted to control mosquitoes. OMWM is designed to provide fish an aquatic habitat refuge during low tide and access to the flooded marsh at high tide so that they can more effectively disperse and feed on immature mosquitoes (Source: Pers. comm. Lathrop, 2020). However, like grid-ditching, OMWM also requires cutting into the salt marsh creating interconnected ponds that often are tied into existing grid-ditching. There is concern that accumulation of pools due to OMWM may limit the ability of marshes to respond to sea-level rise, but this has yet to be determined (Source: Elsey-Quirk, T., Adamowicz, S.C. 2016).

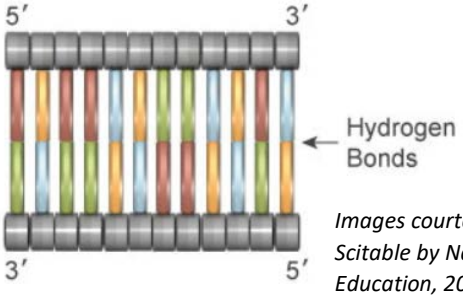
In NJ, current day salt-marsh mosquito management uses *Bacillus thuringiensis israelensis*, or BTI. BTI is a naturally occurring bacteria in soil which contains spores that produce toxins that specifically target detritivorous insects, such as mosquito larvae. This larvicide is not toxic to people or other vertebrates and is used in residential, commercial and agricultural settings, it is even approved in organic farming operations (Source: "BTI for Mosquito Control", 2020). While BTI is an effective larvicide, sea level rise creates challenges to salt marsh mosquito management by shifting mosquito breeding "hotspots". The objective of the Science Collaborative research project was to use methods like soil coring, environmental DNA (eDNA), remote sensing and drone technology, to pinpoint new locations were salt marsh mosquito females are choosing to deposit eggs. eDNA tools can also help recover historical patterns of mosquito distribution in salt marsh soil samples. This, along with marsh elevation maps, may allow us to provide detailed information about future salt marsh mosquito hotspots to help county mosquito control agencies develop more effective management approaches. To get an idea on how sea level rise will impact flood zones and where areas of new marsh may develop, explore the NJ Floodmapper website. https://www.njfloodmapper.org/

The Basics Behind DNA

The activities in this module look closer at DNA extraction and eDNA. But before we discuss the details, we need to start with the basics behind DNA. Deoxyribonucleic acid, or DNA, is one of two nucleic acids, which are information-containing molecules inside a cell or an organism (ribonucleic acid, or RNA, is the other nucleic acid). All living things have DNA within every cell. In fact, each cell in an organism's body has a complete set of DNA required for that living thing. DNA also is important in heredity, as when organisms reproduce, a portion of DNA from each parent is transferred to their offspring (Source: "Introduction: What Is DNA?", 2014).

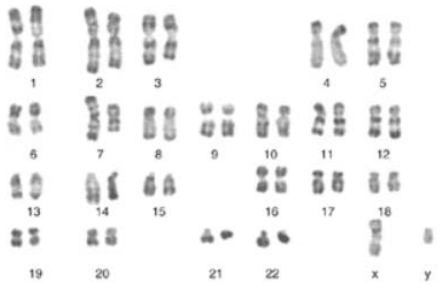
DNA is made up of a pattern of four different molecules called nucleotides. A nucleotide consists of a sugar base (deoxyribose) and a nitrogenous base, which contains the element nitrogen, together they create a nucleotide. There are four different kinds of nucleotides:
adenine (A) - a purine
cytosine(C) - a pyrimidine
guanine (G) - a purine
thymine (T) - a pyrimidine



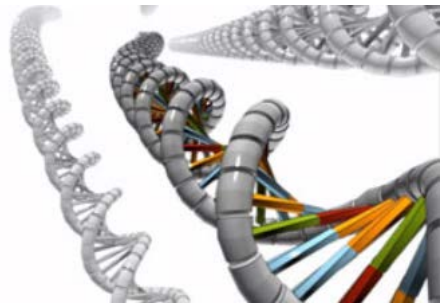


Images courtesy of Scitable by Nature Education, 2014

Imagine DNA like a code. The bonded pairs link together by hydrogen bonds then twisting forming a double helix which we commonly associate with when discussing DNA. DNA strands are long and complex. Think of your personal traits, the color hair or eyes you have, there's a DNA code for that. For that reason, DNA can be very long. If straightened out, one DNA strand within a single cell can be 2 meters long! So DNA is packaged tightly into a coiled form called chromatin**.** Highly compacted chromatin are known as chromosomes (Source: "DNA Is a Structure That Encodes Biological Information", 2014).

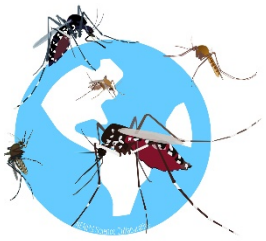


23 pairs of chromosomes from a human cell. Image courtesy of Scitable by Nature Education, 2014

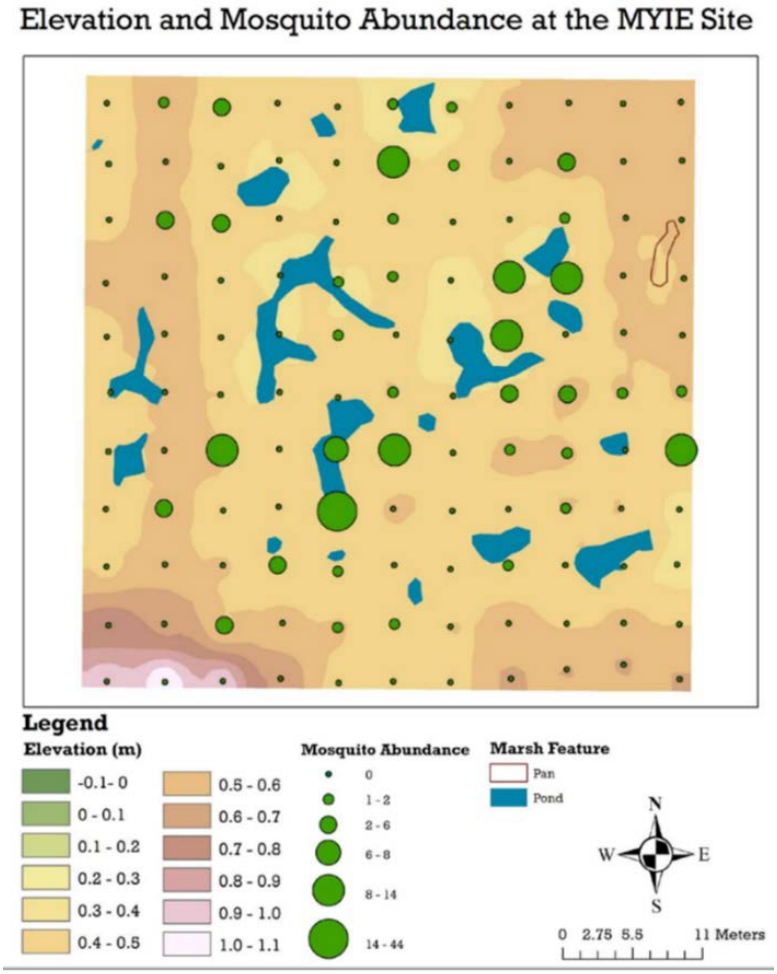


DNA double helix. Images courtesy of Scitable by Nature Education, 2014

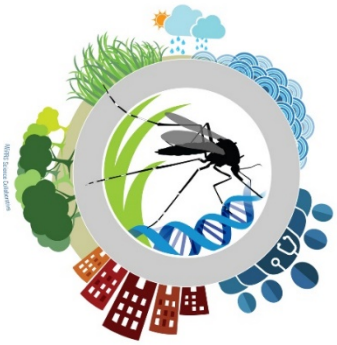
What is eDNA?



Environmental DNA (eDNA) is DNA from organisms that can be found in the environment. eDNA comes from cellular material shed by organisms (via skin, hair, excrement, etc.) into aquatic or terrestrial environments that can be sampled and monitored using highly sensitive molecular tools. This is important for the early detection of invasive species as well as the detection of presence or absence of other species, especially ones that may be rare or elusive (Source: Pilliod et all, 2013). eDNA can also give ecologists a complete species profile for that habitat, revealing which organisms are present. Scientists find eDNA by taking samples from the environment, like a water or soil sample, or from a surface swab. The sample is then taken to a lab where the DNA must be separated from any field contaminants (mud for example) and freed into a solution. The presence of specific sequences in the sample can then be assessed using a process called real-time Polymerase Chain Reaction or rtPCR in a dedicated machine that uses lasers and specific fluorescent probes. A probe is a sequence that has been shown to be completely unique for each species one is trying to detect. If the probe matches a stretch of DNA in the environmental samples, it will release a fluorescent signal detected by the laser, and the environmental sample is considered positive for the species of interest. Scientists must be very careful not to contaminate a sample with DNA from other sources, otherwise data will be skewed. Therefore, strict lab cleanliness practices are critical (Source: Pers. comm. Fonseca, 2020). As stated earlier, eDNA sampling and protocols were used in this project to detect presence or absence of the most important salt marsh mosquito species within marsh locations of interest.



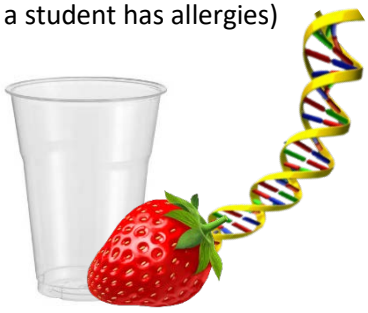
Activity 1: "What does DNA look like?"



Grade: 6th grade and up
Topic: DNA
Lesson Description: Students will perform a DNA extraction experiment.
NGSS Standards: MS-LS1-1, MS-LS3-1, HS-LS1-1, HS-LS3-1
Learning Objectives: All living things have DNA and all cells within the organism have the same amount of DNA. This isolation/extraction process mimics what scientists and other professional have to do to analyze DNA.

Materials. One single experiment will need the following materials:

- 1 re-sealable plastic bag
- Strawberries (use different fruit if a student has allergies)
- 2 tsp dish detergent
- ½ cup of water
- 2 plastic cups
- 1 piece of coffee filter paper
- ½ cup of COLD rubbing alcohol
- 1 tsp of salt
- 1 coffee stirrer or spoon



NERRD ALERT!! In a real-life scenario, scientists filter and isolate DNA before being analyzed. The process is similar to this strawberry DNA extraction activity!

Prep/Set up:
Before students begin, review the background information about DNA, eDNA, and this Science Collaborative Project, if not done so already. Each group of students should have a set of the materials above. For more assistance, watch this video: https://www.youtube.com/watch?v=ojGRBQ2FjP8

Instructions:

1. Wash and pull off any green leaves from strawberries. One or two pieces of fruit will work.
2. Place strawberries into the re-sealable plastic bag. Seal the bag, and gently crush strawberries in the bag for 2 minutes. *This part is fun, and it begins to break open the cells of the strawberries.*
3. In a plastic cup, begin to make your DNA extraction liquid: mix the detergent, salt, and water. *For inquiry-based learning, see if students can determine the correct combonation of ingredients for making the extraction liquid. If time allows, students can perform tests to determine this.*
4. After mixing, add the DNA extraction liquid to the plastic bag with the crushed strawberries.
5. Reseal the bag and crush the mixture for an additional minute.
6. Place the coffee filter paper inside the second cup. Take the strawberry/extraction mixture from the bag and begin to strain the mixture through the filter. Gently squeeze any remaining liquid.
7. Add the cold rubbing alcohol to the strawberry/extraction mixture in the cup. Do not stir! This helps isolate the DNA from the rest of the mixture. Wait a few seconds, you will begin to see a cloudy substance form at the top of the liquid.
8. Tilt the cup and use the stirrer to pick up the DNA that's now floating at the top. *THAT's DNA!*

Critical thinking questions:

- What did the DNA look like once the experiment was done? Can you make other observations?
- Who would need to extract DNA as part of their job/career? Why would they need to do that?

Teacher Copy for "What does DNA look like?"

Materials. One single experiment will need the following materials:

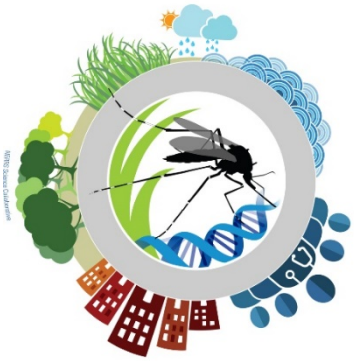
- 1 re-sealable plastic bag
- Strawberries
- 2 tsp dish detergent
- ½ cup of water
- 2 plastic cups
- 1 piece of coffee filter paper
- ½ cup of COLD rubbing alcohol
- 1 tsp of salt
- 1 coffee stirrer or spoon

Tip: Make sure to chill the rubbing alcohol in the fridge or freezer the day before or a few hours before the experiment.

Critical thinking questions:

- What did the DNA look like once the experiment was done? What other observations can you make? It was a stringy, milky-looking substance.
- Who would need to extract DNA as part of their job/career? Why would they need to do that? Forensic scientists in crime labs and ecologists/environmental scientists. Forensics for DNA matching in criminal cases and ecologists/environmental scientists to analyze eDNA.

Activity 2: "Muddy Genes"



Grade: 6th grade and up
Topic: Environmental DNA (eDNA) to study habitats and biodiversity
Lesson Description: Students will understand one way environmental DNA can be used to study an ecosystem.
NGSS Standards: MS-LS1-1, MS-LS2-1, MS-LS2-2, MS-LS2-4, HS-LS1-1, HS-LS2-6, HS-LS4-2, HS-LS4-5
Learning Objective: Students will be simulating how scientists obtain and analyze environmental DNA (eDNA) and how this information can be used to make informed decisions about species management.

Materials:

- 3 buckets (paper bags, or other deep containers)
- Multicolor beads
- 36 six-inch-long pieces of pipe cleaner in three different colors (12 in each color)
- eDNA probe worksheet

Set up/Prep:
Before students begin, review the background information about mosquito biology, management, DNA, eDNA and this 2018 Science Collaborative Project if you have not done so already. This is an important part when answering critical thinking questions at the end of the activity.

Students will have three buckets, each one represents a different marsh habitat zone (A, B, and C). The **Marsh A** bucket is an area of high marsh located near the upland forest edge. **Marsh B** bucket is an area of low marsh. **Marsh C** bucket is an area of marsh on the creek edge/mud flat. See the figure below for a visual on the different marsh habitat zones for reference. Each marsh zone (bucket) contains 12 eDNA sequences which are represented by 6 beads on a pipe cleaner (figure 1). The eDNA sequences each correlate to its respective marsh animal. Students will take "samples" and use the eDNA probe worksheet to determine what species eDNA is present in each marsh zone (bucket). If the class is being broken up into smaller groups, make sure to have multiple supplies of the materials above. *Use the teacher prep sheet to understand the details about this activity.

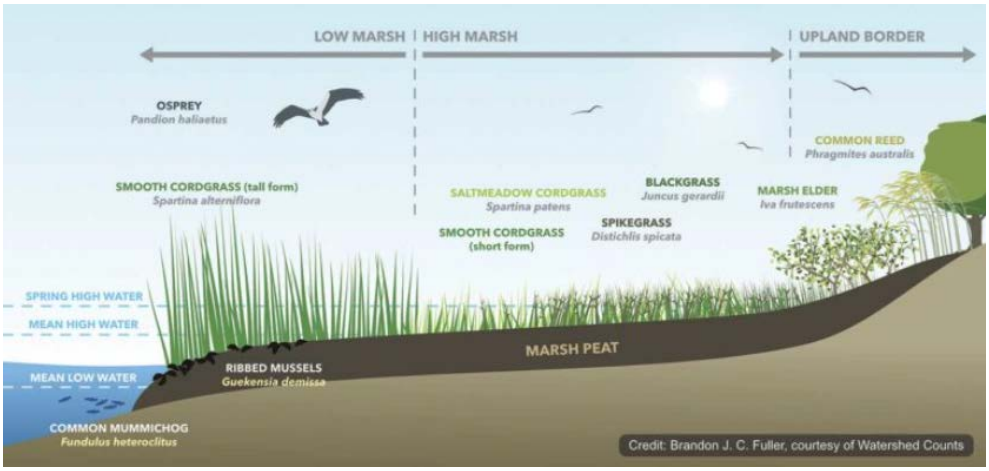
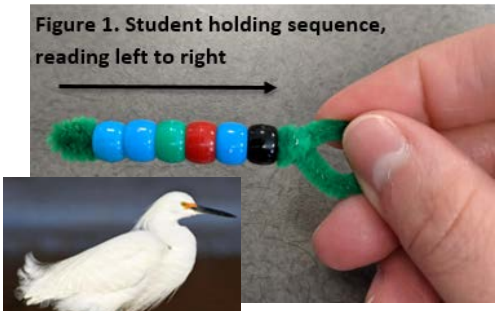


Figure above showing different marsh zones. Image from http://www.rimonitoring.org/saltmarshes/

Activity Instructions:
Students will be simulating how scientists obtain and analyze eDNA within marsh soil samples from three different salt marsh habitat zones.

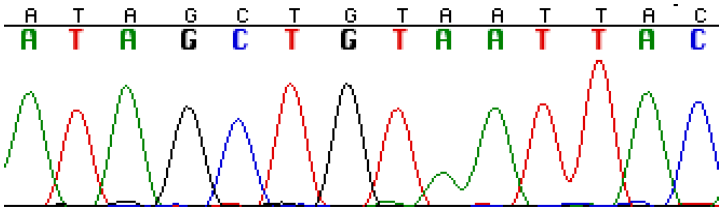
1. After reviewing the background information, have students hypothesize which marsh zone (bucket) they'd expect to see the most mosquito eDNA and why?
2. Students begin with one of the three buckets. Preferably beginning with the high marsh (marsh A) and working their way down to the creek edge (marsh C).
3. Without looking, have a student reach into the bucket and pick 3 random eDNA sequences. Students can take turns doing this.
4. Use the eDNA probe matching worksheet. Match and record what animal each eDNA sequence correlates to. See figure 1 on how to read the DNA correctly.
5. Repeat this three more times until the bucket is empty.
6. When the bucket is empty, total up the number of eDNA present from each animal. Which had the most? Which had the least?
7. Repeat this process (steps 3-6) for the other two marsh zones (buckets).
8. Answer critical thinking questions below.



This matches the probe for snowy egret.

NERRD ALERT!!
The bead colors in this activity were picked for a reason! In real eDNA analysis, scientists amplify a region of DNA in the presence of specific probes labeled with different fluorescence. If there is a match, the florescence is released and as more copies are made, that color signal accumulates. Guanine shows as black, adenine is green, thymine is red, and cystosine is blue.

Screen shot of a chromatogram



Critical Thinking Questions

1. What observations can you make right away when comparing eDNA found in Marsh A, B, and C?
2. Do you think more eDNA sequences found for a particular species means more of that species is present in the area? 2a. Do you think it's possible to find DNA from other animals not common in these areas? Why or why not?
3. Which area(s) of marsh had mosquito DNA? Which zone had the most and was it what you expected?
4. How can the information above be useful to county mosquito control professionals?
5. Analyzing DNA is like cracking a code. Give an example of something else that's a code (or has coding) we use every day.

Teacher Copy for "Muddy Genes"

Prepping the activity

Materials:

- 3 buckets (paper bags, or other deep containers)
- Multicolor beads
- 36 six-inch-long pieces of pipe cleaner in three different colors (12 in each color)
- eDNA probe worksheet

*If the class is working on this in smaller break out groups, make multiple sets of the materials above.

The buckets each represent a different salt marsh zone, high marsh near the forest edge, low marsh, and muddy creek edge. Different species are more common in some zones than others. For example, it is unlikely you will find killifish DNA in marsh A, near the forest edge (unless caught and brought over by a predator). Inside the buckets are 12 individual strands of DNA, these are represented by beads on a pipe cleaner, each one correlates to a different organism. Students will be provided with a probe matching sheet to show the sequence that corresponds to which particular organism.

Making the eDNA

Each pipe cleaner with beads represent a specific DNA sequence for a different species found in the salt marsh. Make sure to use a different color pipe cleaners so it's easier for you to know which DNA sequences go in each bucket when setting up this activity. I chose **brown** for eDNA in Marsh A (high marsh near upland forest edge), **green** for Marsh B (low marsh), and **blue** for Marsh C (muddy creek edge).

- Take a 6-inch piece of pipe cleaner and string the beads through.
- Then at the beginning, loop pipe cleaner back through the beads. See Figure 1.
- Once threaded back through the beads, pinch at one end. See Figure 2.
- Make a large loop on the other end. This big loop indicates the end of the sequence. This is so students read and match the eDNA to the probes correctly, left to right. Figures 3 – 5.

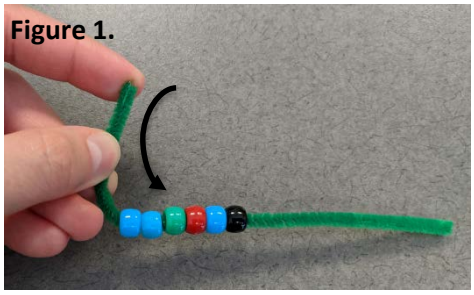


Figure 1.

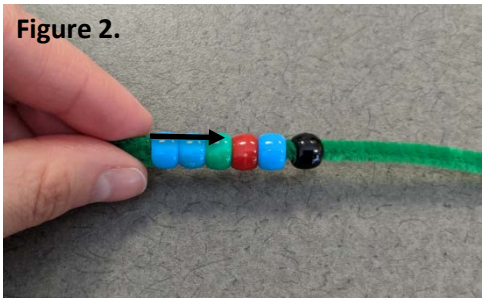


Figure 2.

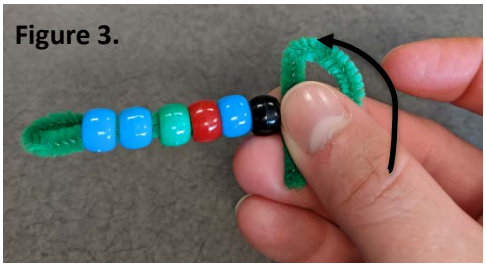


Figure 3.


Figure 4. Showing loop at end

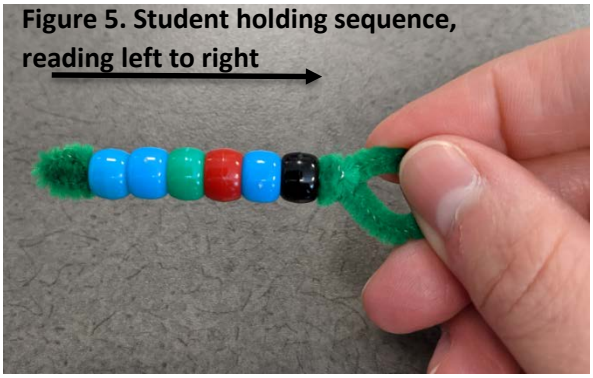


Figure 5. Student holding sequence, reading left to right

This matches the probe for snowy egret.

Use the eDNA probe worksheet to see which bead pattern represents which animal.
<u>In total</u>, make this amount:

- 4 DNA sequences for snowy egret (1 brown pipe cleaner, 3 green pipe cleaners)
- 4 DNA sequences for salt marsh mosquito (3 brown pipe cleaners, 1 green pipe cleaner)
- 2 DNA sequences for clapper rail (all brown pipe cleaners)
- 3 DNA sequences for white tailed deer (all brown pipe cleaners)
- 3 DNA sequences for marsh sparrow (2 brown pipe cleaners, 1 green pipe cleaner)
- 3 DNA sequences for diamondback terrapin (1 brown pipe cleaner, 2 blue pipe cleaners)
- 3 DNA sequences for laughing gull (all green pipe cleaners)
- 4 DNA sequences for fiddler crab (2 green pipe cleaners, 2 blue pipe cleaners)
- 6 DNA sequences for mud snail (2 green pipe cleaners, 4 blue pipe cleaners)
- 4 DNA sequences for striped killifish (all blue pipe cleaners)

When setting up this activity:
Marsh A bucket, (high marsh along the upland forest edge) will contain:

- 1 DNA sequence for snowy egret
- 2 DNA sequences for clapper rail
- 3 DNA sequences for salt marsh mosquito
- 3 DNA sequences for white tailed deer
- 2 DNA sequences for marsh sparrow
- 1 DNA sequence for diamondback terrapin

Marsh B bucket (area of low marsh) will contain:

- 3 DNA sequences for snowy egret
- 3 DNA sequences for laughing gull
- 1 DNA sequence for salt marsh mosquito
- 2 DNA sequences for fiddler crab
- 2 DNA sequences for mud snail
- 1 DNA sequence for marsh sparrow

Marsh C bucket (muddy/wet marsh area along the creek edge) will contain:

- 4 DNA sequences for mud snail
- 4 DNA sequences for striped killifish
- 2 DNA sequences for fiddler crab
- 2 DNA sequences for diamondback terrapin

European Green Crab
Image from http://eattheinvaders.org/green-crab/

***Extension idea:** Create one or two more DNA sequences that <u>are not</u> on the eDNA probe worksheet. Include them in one or more marsh zones (buckets). When doing the activity, students will realize it does not match any of the probes on their worksheet. These "mystery" DNA can represent invasive species in the marsh, like the European green crab. Discuss environmental issues that can occur with introduced invasive species.

Teacher Answer Key for "Muddy Genes" critical thinking questions

1. What observations can you make right away when comparing eDNA found in Marsh A, B, and C? Certain animal DNA is found within particular marsh zones. Some animals are more abundant in certain zones than others. This is probably preferred marsh habitat for these animals, though it looks like many cross between these zones at times.
   Specific observations:
   i. Terrapin DNA found near upland edge- this is possible during nesting season
   ii. White-tailed deer near upland forest edge only
   iii. Laughing gull DNA only found in low marsh. – common area for nesting within the marsh hay.
2. Do you think more eDNA sequences found for a particular species means more of that species is present in the area? 2a. Do you think it's possible to find DNA from other animals not common in these areas? Why or why not? It's likely that the more eDNA found, the more common this species resides in that area. However, many tests need to be done to determine that this is true and not a fluke. For example: It's possible to get DNA of a fish up in a tree- possibly an osprey just had its meal. When using eDNA to get full species profile of a habitat, animals and humans can "contaminate" sampling. This can happen through predator/prey interactions, fecal matter, humans not using proper sterilization methods, etc. That's why many samples need to be taken to make sure eDNA results are accurate.
3. 3. Which area(s) of marsh had mosquito DNA? Which zone had the most and was it what you expected? Marsh A and B but zone A, the high marsh area, had the most. Many students may not expect this since it's farther away from the water, and water is needed for larvae to hatch. However, killifish prey on mosquito eggs and larvae, so the female mosquitoes lay eggs in the higher areas of the saltmarsh to avoid predation. The very high tides during the new and full moons will come up high enough to allow for eggs to hatch.
4. How can the information above be useful to county mosquito control professionals? Knowing where mosquitoes are laying eggs is good information for current and future treatment for local mosquito control. This is especially of interest to county mosquito control professionals to predict where to treat now and into the future since many of our NJ salt marshes are changing due to climate change and sea level rise.
5. Analyzing DNA is like cracking a code. Give an example of something else that's a code (or has coding) we use every day. Computer programs, QR codes, bar codes on items for sale, apps, credit cards, the list goes on!

Student Activity Worksheet

eDNA Probes for Marsh Species. Match the eDNA found in the different salt marsh zones to the probes for each particular species

Striped Killifish (*Fundulus majalis*)

Image from Virtual Aquarium of Virginia Tech

eDNA probe

Clapper rail (*Rallus crepitans*)

Image from baynature.org

eDNA probe

Salt marsh mosquito (*Aedes sollicitans*)

eDNA probe

Snowy egret (*Egretta thula*)

Image from www.allaboutbirds.org

eDNA probe

Saltmarsh Sparrow (*Ammospiza caudacuta)*

Image from www.allaboutbirds.org

eDNA probe

Laughing gull (*Leucophaeus atricilla)*

Image from www.allaboutbirds.org

eDNA probe

Diamondback terrapin (*Malaclemys terrapin*)

Image from https://www.mdsg.umd.edu/topics/terrapins/diamondback-terrapins

eDNA probe

Fiddler crab (*Uca pugnax)*

Image from https://ocracokeobserver.com/2018/09/09/fiddlers-in-the-sand/

eDNA probe

Eastern mud snail (*Ilyanassa obsolete*)

Image from barnegatshellfish.org

eDNA probe

White-tailed deer (*Odocoileus virginianus*)

Image from https://www.nationalgeographic.com/animals/mammals/w/white-tailed-deer/

eDNA probe

Student Activity Worksheet. eDNA Log. Indicate which species are present in each marsh zone.

Species name	*Number of DNA probes matched*
Marsh A.	
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
Marsh B.	
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
Marsh C.	
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

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Sincerely,

Kaitlin Gannon
Education Coordinator
The Jacques Cousteau NERR

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