Cloacal vs. Jaw Swabs: A Novel Technique to Genetically Determine Diet of Sharks

https://en.wikipedia.org/wiki/Great_white_shark

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Abstract

Great white sharks in the New York Area have recently come back and have set a nursery in Montauk, NY. there have been theories concerning why they have returned, and one of the more common is that there are several invasive and native fish that have also returned, making New York a popular place to breed and raise young white sharks. However, common non-lethal methods to find diet can be stressful, so more uncommon, newer methods were tested to see if they can be as successful as previous techniques, such as cloacal and jaw swabs. Research conducted shows that they can obtain data from the samples, but because of several factors that changed the situation over the procedure, the hypothesis was not supported.

Introduction

While sharks are very well known in the popular media, these creatures are still quite a mystery to those who have tried to research them in the north eastern region. A factor of many sharks that is unknown, is the diet that sharks have during their adolescent years, especially off the coasts of New England and the Long Island Sound. During the earlier months of summer, sharks migrate north for breeding and delivery of pups. Through the months of April and through the beginning of October, the number of pups increase due to the moderate safety of bays and estuaries before migrating south as winter approaches. This is extremely important to coastal water ecosystems that contain sharks as predators. If there was a database with a list of species that young sharks in the New York Harbor consume, there could be new regulations concerning the safety of these species and the knowledge contributing animal safety and the ecosystem that these species live in. We know the diet of most adult sharks by using an extremely common and well known non-lethal method known as the gastric lavage, where the stomach would be flushed

out with all of the contents in it. After all content is expelled, identification of the creatures in the stomach would be through identifying the major body parts inside of the stomach, then sifting through the rest, using several procedures such as DNA barcoding to find out what was inside. However, while exceedingly successful, this procedure can cause a lot of stress inside of the sharks and can hurt many of the animals during this process. Therefore, other procedures that have been created to substitute this stress-inducing methodology are being suggested, much like mouth swabs (or Jaw) and cloacal swabs. Mouth swabs collect saliva, or oral fluids, while cloacal swabs collect feces from the anal cavity. However, they are not as popular, nor as widely used, as gastric lavage. The gastric lavage technique has proven reliable and may have more results to begin identification of stomach content, but, it is possible that these other non-lethal methods can be just as successful as the gastric lavage, and can dispense the same results, or specific information about the samples that the lavage can give concerning the adolescent sharks in the tri-state area because of the stress that gastric lavage creates. Mouth and cloacal swabs are used as less invasive methods towards the specimen to see if they are viable against the more common gastric lavage.

Hypothesis

It is hypothesized that mouth and cloacal swabs will prove to be competent enough to extract DNA. Researchers will be able to analyze results without having to use lethal, or stress inducing methods to extract gut content from marine animals. Mouth and cloacal samples would inflict less stress and bodily harm to the sharks that will be tested. Once bioinformatic procedures have been conducted, researchers should be able to trace back to what the captive sharks were fed before both procedures. Scientists will be able to determine what wild sharks have eaten, several days before they are captured momentarily for samples.

It is also believed that the information received from the methods will be as successful as gastric lavage for measuring gut contents for the estimate of 2 weeks, because of the variety of times and moments the shark creates excrement, and because of the ability that sharks have to store and not eat for up to several months at a time.

It is hypothesized that mouth swabs have a form of preservative because of research found that sharks have no enzyme inside of their saliva.

Background

Shark populations vary, depending on seasonal temperatures and migrations during the year**.** However, throughout the 1970's and 1980's, data indicated that sharks were declining dramatically due to extensive commercial, and recreational shark fisheries. throughout the 1990's and onward, the population of these carnivorous species has increased extensively (Dawicki, 2014). All depending on the species of shark, these animals have been known to eat various creatures. However, even though they are famous, basic information concerning average adolescent shark diet's in the northeast, is needed and mostly unknown. The first method that many scientists commonly resort to has been gastric lavage. Based on past experiments, gastric lavage has proven 50% effective for grass pickerel and 60% effective for largemouth bass (Andreasson,1971). So, with bigger fish with a body less tolerant to change in water chemistry and environment, lavage can yield better, more pure results. Gastric lavage, however, has had effects such as cardiac arrest and aspiration of fluid (Eddleston, 2007) something that could be detrimental to further analysis on the same specimen. Mouth Swabs, on the contrary, are different in the sense that they may not be as invasive or complicated. Research has been found that there is no digestive enzyme found in the saliva of sharks unlike humans, so it is possible

that there can be a natural preservative that can keep samples of their diet in previous days, and there would not be any need to analyze gut contents directly.

Cloacal swabs are another great method as well. This involves using a swab to collect leftover feces from a specimen's anal cavity. Since excrement samples have long been used to analyze the diet of several species, this method was highly supported and well suggested. The process, while being the method that can be most effective, may still be hard to analyze because of the chemicals and bacteria in the digestive system that degrade the animals eaten and the process that would be used to purify the samples. This method is less stressful compared to gastric lavage. With this information, we can analyze a span of foods and receive the similar results we would from gastric lavage.

Project Design

The process of collecting samples would occur in two places: The South Hampton School Laboratory (SHSL) along the south shore of Long Island between the New York harbor and Montauk point, where several sharks have been caught in previous years. This project aims to identify samples by a technique called DNA barcoding. The SHSL already has several sharks and collecting and acquiring sharks in the area would be through throwing fish parts into the waters, then, once one specimen had been obtained, the animal would be placed into tonic immobility, where the shark can be sampled without any movement or extra danger to those sampling. After the sharks are caught, several samples would be taken so that there can be various ways to determine what these creatures consume.

Work would begin with 2 White spotted Bamboo sharks (*Chiloscyllium plagiosum*) in SHSL (South Hampton School Laboratory). The two would be constants for both mouth swabbing and cloacal swabbing techniques, since both are in a contained and regulated area.

They would be swabbed to identify food that they have eaten and would be compared to the samples taken from fish and animals used for their normal diet. Targeting diagnostic genes for vertebrates and invertebrates, this would be able to identify what the captive sharks have eaten.

Mouth Swab technique:

The first method tested is mouth swabs. This method of sampling analysis is an uncommon method of taking dietary samples, due to its difficulty identifying foods with the chemicals that all saliva has. Yet, since there is no secretion of digestive enzymes (Weebly) it is very possible their saliva is made for preservation and may have some storing agent. It is thought that mouth swabs could have some residue of past meals because of its lack of digestive enzymes.

Cloacal Swab Technique:

The Cloacal swab would take place in the anal cavity near the cloacal fins. We would use the swab to collect samples of feces to see if it can retain some sample of the food it has eaten This experiment also tests this methods effectiveness. Cloacal swabs are risky. After digestion there are traces of the diet, but it is mixed with high levels of ammonia and other chemicals and bacteria that are in the digestive tract that deteriorate foods so that it can be dispelled as excrement. If purification and amplification of diagnostic genes are successful, it is possible to have an array of results from the diet that these creatures have had.

DNA Barcoding Method

DNA barcoding would take place in the New York Harbor School Laboratory, using the mouth and cloacal swabs collected in both SHSL and the field. To complete the process, the Urban Barcode Project procedure for DNA Barcoding would be used for all samples, were the samples would be purified by reagents like Lysis Solution (GU-CHl), Wash Buffer, and Distilled

water. The samples would then be placed in a thermocycler to apply the polymerase chain reaction using vertebrate and invertebrate primers. After the amplification and purifying processes in the New York Harbor Laboratory would be completed, the samples would be sent for sequencing using a commercial sequencing facility and analyzed through DNA subway.

Field Work

The field work would be the same as the procedures carried out with the proxies of Great white sharks *(C. carcharias)*, the white spotted bamboo sharks *(C. plagiosum)*. Samples would be collected with wild sharks and captured sharks would be tested using mouth and cloacal cells.The expectancy of results between captured and wild will be much different due to the several species that could be caught in the waters between the Upper New York Bay and Montauk point. There are differences between adult spotted bamboo sharks, in the controlled lab area and the Atlantic, adolescent Great white sharks that are being monitored. It is expected to catch a large array of young-of-the-year great white sharks because of the nursery found in Montauk, NY and because they are migratory animals *(A.B. Block, I.D. Jonsen, 2011)*

Materials

For Each Procedure there will be a need for different materials in order to collect, purify and amplify the DNA. Each of the charts are organized for the procedure with the Material Name, the amount needed, and the purpose of the material.

Mouth and Cloacal Swabs

Swab	100-200 swabs	To collect saliva and cloacal swabs
COPAN eswab Vial	To keep samples	Easier to order and preserve samples
Freezer	1 large	To contain and preserve samples
Latex Gloves	2 boxes (XL)	For protection of hands against skin of animal and unknown factors in environment

DNA Purifying(Primers and Reagents)

Guanidine Hydrochloride	300 ul	Sample purification
Silica Resin	3 ul	Sample purification
Wash buffer	1000 ul	Sample purification
Sterile Distilled Water	100 ul	Sample purification
NEB Quick-load Taq 2x	12.5 ul	Sample reactions
Barcoding Primer mix	472.5 ul	For PCR reactions
Positive DNA controls	90 ul	To test PCR machine
Practice loading dye	300 ul	Practice
pBR/BstNI marker or 1000bp ladder	75 ul	Molecular weight standards for Gel Electrophoresis
DNA Stain (SYBR Green)	30ul	To mark Gel during Gel Electrophoresis

DNA Purifying Materials (Rest)

Water Bath	1	Incubating DNA
Styrofoam Rack	2 (30 slots each)	Holding DNA in Water Bath and other like situations
Thermometer	4	Measure accuracy of water bath

Blue gel UV Box	1	Pictures of Gel electrophoresis
Blue gel Camera Hood	1	Pictures of Gel electrophoresis
Camera	1	Pictures of Gel electrophoresis
Microcentrifuge	1	Purification
Thermocycler	1	Purification
Micropipette(10 ul)	2	Purification
Micropipette (100 ul)	2	Purification
Micropipette (1000 ul)	2	Purification
10 ul tips	90 (Roughly)	Purification
100 ul tips	60 (Roughly)	Purification
1000ul tips	90 (Roughly)	Purification
Beakers	2	Containers for biohazardous materials
White Racks	1	Vial organization
Sharpies	3	Marking vials
1.5 ml microfuge tubes (4 per rxn)	150	Sample purification
1.5 ml microfuge tubes	150	Aliquots
0.2 PCR RTG bead tubes	7	PCR
0.2 strip tubes	3	For PCR samples going to be sequenced.
Styrofoam box and cold pack	1	Freezing of primers, reagents, and samples

Gel Electrophoresis

Chambers	1	Where gel is held

Trays	1 (For each chambers)	Gel pouring system
Combs	1 (for each tray)	Slots for the samples
Power Supply	1	Turn on chamber
2-1 Agarose gel	100ml(Each Tray)	Where DNA is placed for electrophoresis
Electrophoresis Running Buffer	100-150ml	Material to push or transport DNA
Petri Dish	2	Test strip for 2 ml tubes (Sequencing)
Envelope	1	Sending sequence

Procedures

Non-lethal methods will be used in order to discover least invasive methods and successful diet analysis for the wild, and spotted bamboo sharks. Procedures would be based off the most efficient and least stressful possibilities. The procedures listed are the two mentioned before, and the methods used after concern DNA Barcoding, which include purifying, amplifying, and sequencing DNA samples to have data concerning the DNA found. Gastric lavage will not be tested on wild or captured sharks and expected data will be based on the results we receive from DNA barcoding. Gel electrophoresis is included in the process for DNA specifically to test the accuracy behind each of the samples and to assure that which has to be sampled and sequenced can be expected and further analysis can be done from. Below is the list of procedures and steps taken for each method used in the experiments done. They include both Mouth and Cloacal Swabs, and The DNA Barcoding procedure from the procedure found in their protocol *(Cold Spring Harbor Laboratory, 2014).*

Cloacal Swab

1. Place shark into tonic immobility
2. Separate cloaca opening
3. Enter cloacal swab
4. Swab up excrement for 5 seconds
5. Place sample inside of preservative Vials
6. Mark Vials with type of sample, species, and date
7. Samples are preserved in ice

Mouth Swab

1. Tonic Immobility
2. Open mouth or keep mouth open
3. Swab inside of the cheek for 5 seconds
4. Place swab inside of vial
5. Mark Vials with type of sample, species, date…
6. Samples are preserved in ice

Purification of samples

1. Add specimen to microcentrifuge tube
2. Add 300 ul of lysis solution (GU-HCL)
3. Incubate at 65oC for 10 minutes
4. Centrifuge for 1 minute
5. Transfer 150 ul supernatant into new microcentrifuge tube
6. Name new tube and store previous tube in -20^{o} C
7. Add 3 ul of Silica Resin to new tube
8. Vortex
9. Incubate at 57^{o} C for 10 min
10. Centrifuge for 30 seconds
11. Remove supernatant
12. Add 500 ul of wash buffer
13. Vortex
14. Centrifuge for 30 seconds
15. Remove 500 ul of supernatant
16. Add another 500 ul of wash buffer
17. Vortex
18. Centrifuge for 30 seconds
19. Remove 500 ul of remaining supernatant
20. Centrifuge for 8 seconds
21. Remove remaining supernatant

22. Add 100 ul of distilled water
23. Mix by pipetting
24. Incubate at 57^{o} C for 5 min
25. Centrifuge for 30 seconds
26. Transfer 50 ul of supernatant to new microcentrifuge tube
27. Name new tube and preserve previous tube in -20^{o} C

Amplification of DNA

1. Add 23 ul Primer mix to microcentrifuge tube
2. Add 2ul of DNA into microcentrifuge tube
3. Transfer mix into new PCR RTG bead tube
4. Name new PCR RTG bead tube
5. Enter sample into the Thermal Cycler for amplification of a specific gene

Gel Electrophoresis

1. Microwave Agarose gel in 30 second intervals until transparent
2. Cool for 5 minutes
3. Clean gel tray and place combs
4. Pour gel into tray
5. Set for 20 minutes
6. Take out combs from gel
7. Add 2 ul of SYBR Green to fresh microcentrifuge tube
8. Add 5 ul of DNA extract to new tube with SYBR Green
9. Load 5 ul of sample into slots in gel
10. Open Blue gel electrophoresis chamber
11. Place buffer in it, high enough that it can reach the cathode and anode
12. Place gel in buffer
13. Turn on, and electrophoresed at 130 volts for 30 minutes
14. Take pictures of end result
15. Send in samples for sequencing

Safety

Safety is important for both the specimen and students who will be involved in the experiment. Precautions must be taken by the students to ensure that the sharks are not harmed in any way according to the animal safety guidelines. Materials such as gloves for the sharks' rough skin and the possible microbes in the mouth that could be harmful for human beings, tail rope,

lab coats and safeguards for those handling the sharks should be provided. While placing the shark in tonic immobility, adult supervision and guidance would be required, and materials such as the mouth and cloacal swab would have to be prepared beforehand, to prevent any discomfort the shark may encounter when the sample is being collected. When in the lab it would be important to make sure that the students are using safety equipment as well. This would include; gloves, lab coats, closed toed shoes, goggles, and ice should be provided to keep samples like mouth and cloacal swabs at a constant, cool temperature. During the experiment, various reagents and primers will be handled for DNA Barcoding. All equipment reagents, primers, and unused samples must be frozen along with the samples at -4^{o}C for short periods of time, and -20^{o} C for longer periods of time.

Discussion

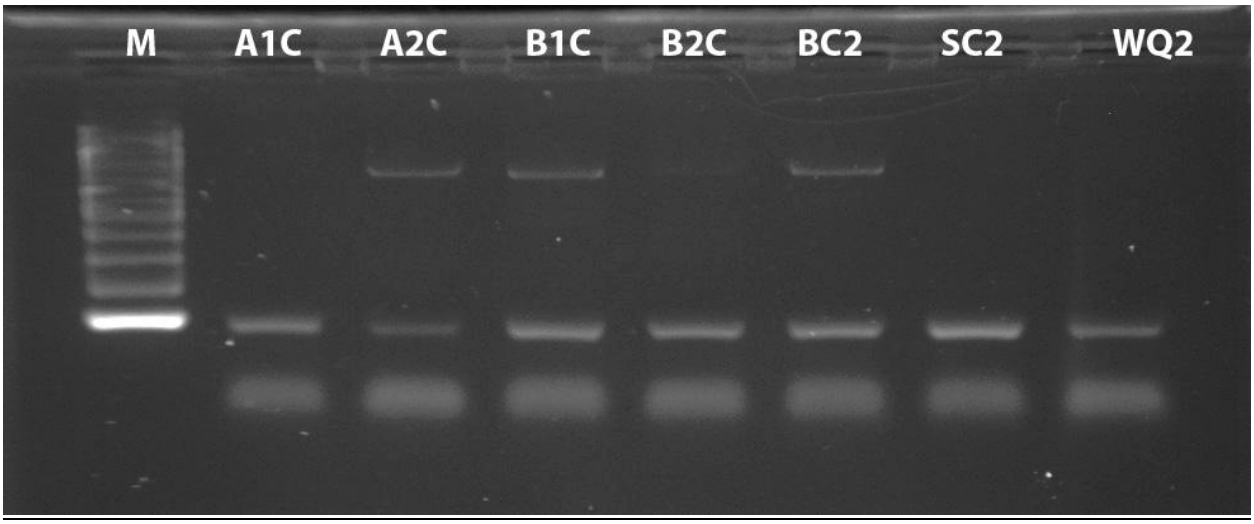

Figure .01: Each band represents the DNA captured through gel electrophoresis. M signifies the 10b ladder, a tool used to determine the success of DNA showing on a processed sample. A1C was the first round of mouth swabs. A2C represents the second round of mouth swabs. B1C represents the first round of cloacal swabs and B2C represents the second. BC2 was Bunker fish (control)and SC2 was shrimp(control). WQ2 represents the water quality that was taken the day that each shark was tested in the lab. The figure above shows that we have had success in all samples that were processed through DNA Barcoding. This shows that cloacal and mouth swabs do have the ability to contain samples of creatures.

7 samples were placed into DNA processing and it was found that all samples were purified and had some form of DNA on it through the bands that each of the samples created throughout the entire gel. However, 3 of these 7 had major bands above the secondary bands that lay at level with the reach of the 10pb Ladder. A fourth one produced a secondary band but it did not have the strength and was not as prominent as the other 3 samples that had secondary brands. These samples were A2C, B1C, B2C, & BC2. A2C showed a strong band, and originally was sampled from the first shark's cloaca, or anal cavity. The code to the right came from the second shark's mouth and cheek swab (B1C). the third band that came out, while faintly, showed signs of DNA from the cloaca, implying a pattern concerning effectiveness in the terms of the cloacal swab compared to the mouth swab. The last swab came from one of the controls, Bunker, which was one of the two foods that created his diet. This sign is significant but not expected because of the primer used with the process. There were three specific primers chosen for the experiment. The ITS invertebrate primer, the Vertebrate primer and the COI primer. The round done used the ITS Invertebrate primer, so it is unknown how bunker DNA was found considering it is not an invertebrate. Since no other primer was used and the invertebrates that the proxies ate (krill) was not prominent, they data seems to imply that the DNA found most commonly and that was shown in the samples was not a invertebrate but instead a fish, several implications were considered to why this result was found. For the samples that did not have any DNA within them, there is a plausible theory behind why they did not appear. The theory was that during the procedure, there may have been a 'goldilocks' effect with the samples, meaning DNA from the diet might be around for a certain time period before it was washed away during the barcoding procedure, which could imply more evidence to the hypothesis that DNA can be found with mouth and cloacal swabs. With this information, it can be said that the two sampling methods are

successful with DNA barcoding, though other DNA extraction procedures may be more accurate, and can lead to better results. For the other two hypothesis, one sample of saliva did receive a band during gel electrophoresis, showing that saliva is a valid method for extracting DNA contents, although more research has to be done to certify this, and, while it was not possible to measure the accuracy of these methods after a time span of two weeks because of the eating schedule and the short time frame used for this research, samples were taken days after these animals had eaten, so it is known that DNA from their diet is prominent within a few days, but it is not known if it is as strong within a longer span of time.

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