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You are what you eat!

A sandbar shark shows off all of her teeth, which she relies on to consume her favorite meal! Photo by David Hay Jones.

Why study diet?
When scientists use the word “diet” they aren’t referring to Atkins or Paleo. Instead, scientists are describing the items an animal eats, and even the items

it *prefers* to eat (yes, animals can be picky too!). As researchers, knowing what an animal eats is essential to our understanding of its biology, behavior, and role(s) in the food web. Prey availability is often responsible for large-scale animal migrations — like the southward migration of birds prior to food-scarce winter conditions. Understanding an animal's diet can also have implications for human health, particularly when it comes to seafood.

Ok that sounds pretty important…but how do we study diet?
Typically, the best way to understand what an animal eats is simply to open its stomach and examine the contents. However, this method is not without caveats. For example, consider the following scenario: you examine a blacktip shark's stomach and find parts of a crab, a heavily digested fish, and other various bones and hard parts. What does this tell you? Did that shark eat one or multiple crabs? What species of fish did the shark eat? What are the various bones and hard parts from? As you can imagine, examining stomach contents sometimes generates more questions than answers! That's why scientists have developed newer techniques to help answer some of these questions. One of these techniques is a process called DNA barcoding, which allows us to extract DNA from partially digested tissue to determine which species of prey the tissue is from.

Do you always have to euthanize an animal to examine diet?
No! In fact, we strive to use as many non-lethal and minimally invasive techniques as possible. One of these methods is Stable Isotope Analysis, or SIA. Briefly, we can examine the natural chemical tracers in an animal's tissue (typically muscle), which we collect in a swift and relatively painless manner, to obtain information about the diet of that animal. Unlike stomach content analyses, SIA doesn't provide us with species-level dietary information. However, we can get a general idea of the animal's food source location (e.g. far offshore in the open ocean or close to shore in a seagrass bed), and the animal's "trophic level" in the food web (e.g. top predator). Another non-lethal technique we can use is known as gastric lavage – read on for more information about this method!

Which technique is best?
Well, that depends on your study animal and your question. Say you're investigating a rare and potentially endangered species. In this case, you would likely choose a non-lethal technique like SIA or gastric lavage. Either of these techniques would allow for quick and minimally invasive sampling, without having to sacrifice the animal. Conversely, if you're examining the diet of a

species that has relatively healthy population levels, you may choose to investigate the stomach contents through DNA barcoding to obtain a more thorough interpretation of the species' diet. The best approach to answering questions about diet is to use a combination of techniques, wherever possible.

Studying diet is a huge part of understanding the biology of an animal and is a major aspect of our research at the Mississippi State University Coastal Research and Extension Center. Read on to learn about several studies we've conducted to investigate the diets of various fish and shark species off the coasts of Mississippi and Alabama!

Stomach contents and DNA barcoding

This sand seatrout was consumed by a smooth butterfly ray. It was so large that it did not completely fit into the ray's stomach, which left the tail undigested.

How do scientists investigate stomach contents?
Stomach contents have been analyzed by scientists for centuries as a means of quantifying the prey items consumed by particular organisms. Scientists generally obtain stomach contents via lethal means, through the surgical removal of the stomach. Once scientists obtain stomach contents from the species of interest, they sort the prey into categories (i.e. fish vs other animals like crustaceans, bivalves, and marine worms) and then count and weigh each prey type. Once enough stomachs have been

analyzed, the scientists can piece together the data to draw conclusions not only about the types of prey consumed, but potential changes in diet over time and across different regions or environments. However, conclusions can be deceptive if stomach contents are highly digested, because in this case prey identification by the scientist's trained eye alone is next to impossible.

How does DNA barcoding help scientists with diet analysis?
DNA barcoding is described as sequencing an unknown species' unique genetic marker, or barcode, and then matching that barcode with an already identified species using a barcoding database. With advances in technology, DNA barcoding has become a cost-effective technique to identify unknown prey to species (the most specific level possible). The increase in prey identification afforded by this technique provides additional information about the diet of the species of interest and can indicate whether commercially or recreationally important species are being predated upon. For instance, when an invasive predator is introduced into a system, it might choose to prey exclusively upon one native species, resulting in drastic declines of that native species. In addition, knowing whether a threatened or endangered species is being readily or even disproportionately consumed by other species is vital for creating and correctly implementing a rebuilding plan for the species in decline.

How have we (MSU Marine Fisheries Ecology) used stomach contents and DNA barcoding?
The smooth butterfly ray is a common coastal ray found in the northern Gulf of Mexico. We investigated the diet of this species through traditional stomach content analysis and found it to be a fish-specialized predator. Interestingly, the consumed fish were often quite large relative to the size of the ray and were generally consumed whole, headfirst! (see photo above). In most cases, the fish prey was heavily digested, making identification to species impossible. So, we sampled tissues from the unidentified prey and used DNA barcoding to identify most of the prey items to species. Therefore, DNA barcoding provided us with information about the diet of this species that we would not have attained through traditional stomach content analysis alone. For example, we discovered that smooth butterfly rays change their diet seasonally based on prey availability. We also found that larger female butterfly rays consume larger fish species than the smaller males. The results of this study indicate that the smooth butterfly ray likely plays an important role in the structure and maintenance of coastal food webs like those in Mobile Bay.

Stable Isotope Analysis (SIA)

(Top): An incision is made prior to using a biopsy punch (held by researcher) to collect muscle tissue from a great hammerhead. (Bottom) Blood is extracted

from the caudal vein of an Atlantic sharpnose shark for stable isotope analysis. Photos by David Hay Jones.

What is a stable isotope?

Stable isotopes have been used to examine the diet (or "trophic ecology") of numerous organisms, including birds, plants, mammals, insects, and even fungi. Stable isotopes are the naturally-occurring, slightly heavier, non-radioactive forms of atoms. These slightly heavier atoms react at different rates than their lighter counterparts, but both are incorporated into an animal's body from its diet. We can measure the ratio of heavy to light isotopes in an animal's tissue to calculate an isotopic signature. This signature varies depending on which isotope we are examining, as well as by region and by animal.

How can this help us understand diet?

The two most common isotopes used for understanding diet are the stable isotopes of the elements carbon and nitrogen. The stable isotope signatures of carbon remain relatively constant as they move throughout the food web, meaning they don't change much from prey to predator. Therefore, they are good indicators of the primary source of carbon into the food web (i.e. offshore-based or inshore-based). For example, animals living in the open ocean have a very different carbon isotopic signature from animals living in nearshore seagrass beds. Nitrogen is more useful in estimating what we call "trophic position," which is the animal's specific location in the food web (e.g. a top predator, or somewhere in the middle). Nitrogen isotopes increase as they move through each step of the food web, meaning that the higher the isotopic signature of nitrogen in an animal's tissue, the higher the predatory level. The carbon and nitrogen values can be used together in statistical calculations that allow us to estimate an animal's niche, or role in the food web.

What kinds of samples do you need to collect from animals?

Any tissue in an animal contains the stable isotope signatures from its diet. Studies have used everything from feathers of birds, to whiskers of sea lions, to livers of fish. Most commonly, researchers use muscle tissue because it can be easily and non-lethally obtained using a biopsy punch (see top photo). Other studies have shown that tissues like blood (see bottom photo) can be useful when looking at seasonal shifts in diet because they are more metabolically active tissues, meaning they are more representative of the recent diet of that animal.

How else can stable isotopes be applied?

Other stable isotopes, like those of oxygen and hydrogen, can provide information on the migratory behavior of animals because the specific signatures of oxygen and hydrogen vary at different latitudes. Click here to read one study that mapped the migration pattern of California gray whales by examining the oxygen isotope in the waters trapped within the barnacles that were attached to the whales! These invisible chemical tracers can provide a lot of valuable information to researchers at little cost to the animals that we study.

Gastric lavage

(a technique for the strong-stomached!)

Figure 1 from Drymon et al. 2019. (A) Scientists obtain stomach contents from a baby tiger shark via gastric lavage. Avian remains recovered during this study included (B) thousands of individual feathers, along with (C, D) several whole, partially digested birds. (Tiger shark gastric lavage photo by David Hay Jones.)

What is “gastric lavage”?
Gastric lavage, also known as “stomach pumping” or “gastric irrigation,” is the process of flushing out the stomach using water or saline solution. Gastric lavage is commonly performed on humans, as well as household pets like cats and dogs, to remove ingested poisons from the stomach. However, this procedure is only used if it is unsafe or impossible to induce vomiting AND the toxic substance was consumed recently (within hours).

How does this relate to fish?
Glad you asked! Gastric lavage has been implemented globally in research studies as a nonlethal means of collecting stomach contents from various fish species, including bass, catfish, walleye, lingcod, and sturgeon. Gastric lavage has also been used to study the diets of elasmobranchs (sharks, skates, and rays) such as dogfish sharks, sandbar sharks, reef sharks, little skates, and yellow rays.

How have we (MSU Marine Fisheries Ecology) used gastric lavage?
In 2010, while conducting our shark monitoring survey off the Mississippi/Alabama coast, we caught a juvenile tiger shark that, surprisingly, coughed up some feathers! Through DNA barcoding, we determined that the ill-fated bird consumed by the shark was a brown thrasher (a land bird!). Intrigued, we began using gastric lavage to opportunistically and nonlethally acquire stomach contents from tiger sharks caught during our survey. To sample these tiger sharks, we would gently place a PVC pipe down a shark’s throat, fill it with saltwater, turn the shark upside down, and catch the flushed-out “shark barf” in a sieve. We would then safely release the somewhat confused, but otherwise unharmed, shark back into the Gulf to swim another day. We conducted this study for a total of 9 years (from 2010-2018).

What did we find?
Thirty-nine percent of the tiger sharks we examined (41 of 105) contained bird remains. Incredibly, all of the bird remains that could be identified to species using DNA barcoding belonged to land-based birds! No evidence of marine birds, such as pelicans or gulls, was found. This seemed counter-intuitive to us, so we decided to look at bird sightings data from *eBird*, a Cornell-run online database of bird observations logged by the birding community. We noticed that peaks in bird sightings along the Mississippi/Alabama coast for the species of birds consumed by the sharks aligned almost perfectly with the dates when the sharks consumed those same species of birds. It seemed like migration was a big piece of the puzzle… but we still weren’t sure how migratory, land-based birds were ending up in the water.

Interestingly, most of the shark/bird interactions occurred in the fall. The Mississippi/Alabama coast is the first stopover location for migratory birds flying north in the spring after crossing the Gulf of Mexico. It also serves as the final stopover location for migratory birds flying south in the fall. When preparing to leave the Mississippi/Alabama coast in the fall, migratory birds try to strategically time their departure to coincide with favorable winds. However, unforeseen inclement weather events, like big storms, can force the birds to stop their journey and land on, or even fall to, the water's surface. Once they land, they are unable to resume flight, making them easy meals for hungry baby tiger sharks.

Visit our Publications webpage, http://coastal.msstate.edu/drymon-publications, to access our *Ecology* publication entitled *Tiger sharks eat songbirds: scavenging a windfall of nutrients from the sky*.

Sea of Acronyms

Being an informed angler begins with understanding the terminology used in fisheries management. This series helps demystify the concepts hidden beneath a sea of acronyms.

IRI

Index of Relative Importance

In the context of a diet study, the IRI is a measure of how "important" a prey item is to the diet of a fish being studied. It takes into account how often the prey item occurs in the diet and how much of the prey item (by number and by weight) is consumed.

Marine Fisheries Ecology Lab

I'm Marcus Drymon, an Assistant Extension Professor at Mississippi State University and a Marine Fisheries Specialist at Mississippi-Alabama Sea Grant. Emily Seubert, Amanda Jefferson, Matthew Jargowsky, and I comprise the Marine Fisheries Ecology Lab. We'd love to hear from you! Please reach out to us at marinefisheriesecology@gmail.com, and check out the links below to access our website or follow us on Facebook.

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