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Relationships Between Mercury and Trophic Level in Nine Coastal Pelagic Fishes off Southeastern Florida

Emily Akins
Nova Southeastern University

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Emily Akins

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Approved:
Thesis Committee

Committee Chair: David Kerstetter, Ph.D.

Committee Member: Amy Hirons, Ph.D.

Committee Member: Douglas Adams, M.S.

NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

RELATIONSHIPS BETWEEN MERCURY AND TROPHIC LEVEL IN NINE COASTAL PELAGIC FISHES OFF SOUTHEASTERN FLORIDA

By

Emily Akins

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Abstract

State and federal agencies have issued consumption advisories for various fish species for many years, including in Florida. Upper-level predatory fish, such as tunas and mackerels, are especially popular with anglers but are susceptible to high levels of mercury through bioaccumulation and biomagnification. This study used two data sets over two time periods, 2010-2012 and 2020-2021, to compare mercury and trophic level relationships in nine coastal pelagic fishes is Southeastern Florida. As these species are popular in recreational fisheries, charter and tournament catches formed the base of the samples that were analyzed for total mercury along with carbon and nitrogen stable isotope ratios. Total mercury values were not significantly different between the two data sets for species that were directly comparable. Fork length and mercury were positively correlated for Blackfin Tuna, Common Dolphinfish, King Mackerel, Little Tunny, Skipjack Tuna, and Wahoo, all of which had more than 5 samples per species. Fork length and trophic level were positively correlated in all the aforementioned species except Skipjack Tuna. Trophic level and mercury were positively correlated in all the aforementioned species except King Mackerel. The results of this study indicated mercury levels remained stable between the two time periods and further support that mercury and trophic level are positively correlated. This study not only provides valuable data on understudied small-bodied tunas, but it is also replicable to build a robust temporal analysis on bioaccumulation in these species.

Keywords: Mercury, Coastal Pelagic, Trophic Level, Bioaccumulation, Biomagnification

Introduction

One of the most notable impacts of mercury pollution is its presence in fish. As a naturally occurring element, mercury can be introduced to the environment through various routes. It is stored as a mineral in the Earth's crust, so events such as volcanic eruptions or erosion can release it into surrounding waters and the atmosphere (USGS, 1996).

According to the United Nations, 2.2 million kg of inorganic mercury were emitted globally by anthropogenic sources in 2018 alone (AMAP, 2019). The burning of fossil fuels and gold mining are the largest anthropogenic factors contributing to inorganic mercury emissions, with other sources such as battery waste and landfill leachate contributing less (EPA, 2020). The U.S. Environmental Protection Agency (USEPA) identifies coal burning factories, such as power plants, as the largest source of inorganic mercury emissions in the United States (EPA, 2020). As global energy demands increase and world climate continues to change, more mercury is introduced into the environment (Sunderland & Mason, 2007). Multiple studies indicate that warming temperatures associated with climate change are contributing to higher mercury levels in marine systems through excessive runoff and releases from reservoirs, in conjunction with already high levels due to anthropogenic industrial pollution (Booth & Zeller, 2005; Fahnestock et al., 2019). Regardless of original source, the majority of the inorganic mercury emissions will make its way into the world's oceans through atmospheric deposition (Sunderland & Mason, 2007).

Mercury

Inorganic mercury finds its way into the ocean through many avenues, but this study is particularly interested in mercury found in coastal pelagic fish. There are numerous identified routes by which mercury can enter organisms. In fish, inorganic mercury can enter the body through contaminated water passing through gills (Ribeiro et al., 2000). Inorganic mercury is less harmful than methylated mercury because inorganic mercury is less reactive due to its molecular structure. While inorganic mercury will accumulate in organs, its affects are not as harmful as methylmercury as it cannot pass the blood-brain lipid barrier (Dart & Sulliva, 2004; Park & Zheng, 2012). Methylated mercury can enter the body through ingestion or respiration and is lipid soluble. When methylmercury passes through the blood-brain barrier it acts as a neurotoxin

within the brain (Reardon & Bhat, 2007). Methylmercury has been linked to changes in behavior and neurological instability in fish (Wiener & Spry, 1996). The initial stage of toxicity is oxidative stress, where glutathione production is inhibited resulting in suppressed antioxidant protection of the gills (Cappello et al, 2016). Methylmercury achieves this by mimicking amino acids like L-methionine or enzyme substrates, reducing glutamate production (Unoki et al, 2016; Reardon & Bhat, 2007). Continued exposure to methylmercury also results in reproductive failure. As a neurotoxin, methylmercury is particularly active in the hypothalamus (Crump & Trudeau, 2009). It commonly alters the production of gonadotropins (Ram & Joy, 1988), resulting in gonadal disruption (Liao et al, 2006) and oogenesis repression (Ram & Sathyanesan, 1983). Some of methylmercury's disruptions lead to cell death due to ion transport increases and macromolecule damage (Reardon & Bhat, 2007).

Approximately 95% of total mercury in fish muscle tissue is in the monomethyl form (Adams & McMichael, 2007; Bloom, 1992). Human mercury intake primarily comes from the ingestion of fish and shellfish (Sunderland and Mason, 2007). Cases of mercury poisoning have become less common in recent decades, but when it occurs it is called 'Minamata Disease' after the town in Japan where severe mercury poisoning occurred (Hachiya, 2006). In humans, Minamata disease most heavily affects the senses, as it targets the nervous system, and can result in tremors, partial loss of feeling in limbs, gross and fine motor control loss, and other sensory issues (Harada et al., 2001; Yorifugi, 2008). Most notably vulnerable are pregnant women and children, as mercury not only targets the nervous system which interrupts early development, but it also builds up in the umbilical cord and transfers to fetuses more quickly than the mother's blood (Harada, 1978; Vahter et al., 2000). Given this information, it is important to explore the pathways mercury takes to enter not only the ecosystem, but organisms themselves.

Anthropogenic sources contribute much of the mercury found in the environment and most of that mercury will make its way to the ocean as inorganic mercury. Once in the ocean, inorganic mercury has the potential to turn into methylmercury, where it poses threats to organisms (Beckers & Rinklebe, 2017). Bacteria is the primary avenue in which mercury can be methylated and introduced into the food web. Sulfate- (SRB) and iron- (FeRB) reducing bacteria are the major producers of methylmercury (Gilmour et al., 2013), and these bacteria can live in sediments and potentially even within some marine species' intestines (copepods) (Gorokhova et al., 2020).

Coastal fishes can be exposed to methylmercury in areas of mixing fresh and saline waters. The discharge of terrestrial humic compounds (which are bound to clay to form colloids) and methylmercuric hydroxide from freshwater into seawater, in this case the Atlantic Ocean and Gulf of Mexico, increases methylmercury and methylmercuric chloride availability (Rolfhus et al, 2003). When freshwater mixes with seawater, the ionic composition of colloids are disrupted, releasing methylmercury (Guentzel et al., 1996; Stordal et al., 1996). Methylmercury binds to humic compounds in freshwater, but when introduced to cation abundant marine waters, the methylmercury unbinds from the organic materials and becomes available to organisms (Stordal et al., 1996; Rolfhus et al., 2003). Common to these intertidal mixing areas are mangroves, which harbor sediment trapped methylmercury in addition to humic compounds. The Florida Bay mangrove transition zone deposits more methyl and total mercury than canals redirecting water from the northern Everglades into Florida Bay (Rumbold et al., 2011). Mercury levels in the coastal waters of this study can also be affected by seasonality, the Loop Current, and the North Atlantic Current (Liu et al., 2008; Harris et al., 2012), giving numerous avenues of mercury distribution. Regardless of source, once in marine waters, there are various avenues in which mercury can enter organisms and build up over time.

Studies that include speciation of mercury stable isotope ratios have discovered that the age, and potentially the source, of mercury can be traced according to the degree of photo-degradation (Senn et al., 2010; Lepak et al., 2015). For example, while the original source cannot always be located, the amount of time the mercury has spent in open, well-lit waters can be determined by how degraded the mercury is, identifying if the fish acquired it in coastal or pelagic waters. After eating numerous methylmercury-contaminated prey – whether the original sources were from bacteria, periphyton, or phytoplankton – individual predator fish can accumulate dangerously high levels of methylmercury (Liao et al., 2006; Bank et al., 2007).

While bioaccumulation is the process by which mercury simply accumulates over time in individual organisms, the associated term of biomagnification refers to the process by which mercury is passed from one trophic level to the next (and thereby increase in concentration) within a food web (Sunderland, 2007; Dijkstra et al., 2013). Humans who consume predatory fishes are at risk of consuming methylmercury at concerning levels as higher trophic level fishes consume more lower level species and the methylmercury accumulates in the muscle tissue in increased levels. Globally, the World Health Organization (WHO) set the standard of provisional

tolerable weekly intake of mercury at 1.6 µg/kg body weight per week (WHO, 2016). As mercury toxicity is such a threat to human health, understanding how it moves through the ecosystem and into consumable fish is important.

Florida's multibillion-dollar saltwater fishing industry relies heavily upon upper-level predatory fish species, many of which have been shown previously to bioaccumulate high levels of mercury in their tissues (e.g., Adams et al., 2003; Adams et al., 2018). State and federal agencies, such as the Florida Department of Health, release annual fish consumption advisories that recommend limiting the intake of many fish due to high levels of mercury (DOH, 2022; EPA, 2022). As mercury's neurotoxic characteristics affect fish reproductive and cognitive functions (Panigrahi & Misra, 1978; Ribeiro et al., 2000; Liao et al., 2006) as well as humans (Reardon & Bhat, 2007; Unoki et al., 2016), the U.S. government has taken action to address mercury pollution levels.

The U.S. federal government has specifically regulated the release of mercury since 1980, but the most influential regulations began with the passage of the Clean Air Act of 1963 and the Clean Water Act of 1977. Mercury was added to the Clean Air Act as an air toxin in 1990 and the Clean Water Act listed waters impaired by atmospheric mercury as a subcategory in 2007, encouraging control measures from the EPA (EPA 2007; EPA 2022). In 2011, the EPA released new "Mercury and Air Toxics Standards" which led to an emissions reduction of 86% in seven years (EPA, 2018). The state of Florida adheres to current standards set and enforced by the EPA down to the state level (EPA, 2016). The state also has further regulations on mercury disposal from point source locations, such as industrial waste (FDEP, 2019). Multiple models have been created and used to understand trends in mercury over the decades and have successfully explained current declines in ocean surface water mercury levels based on reductions in mercury emissions (Sunderland & Mason, 2007; Soerensen et al., 2012). A decrease in anthropogenic emissions and mercury containing products since 1990 has already been linked to a decrease in atmospheric mercury levels (Zhang et al., 2016). It is plausible then, with enough reductions in anthropogenic mercury emissions and time, to see reductions of mercury concentration in organisms' tissues.

Study species

The targeted sportfish species in this study are not only critical to Florida's fishing industry but are also important predators in the coastal pelagic ecosystem. All the species from the original sample set (2010-2012) (Moore, 2014) were in the order Perciformes and from the families Carangidae, Coryphaenidae, and Scombridae. Seven species were in the family Scombridae. Many scombrids are known for their utilization of varying habitats, particularly coastal pelagic regions. These species include Blackfin Tuna (*Thunnus atlanticus*), Little Tunny (*Euthynnus alletteratus)*, Skipjack Tuna (*Katsuwonus pelamis*), Cero Mackerel (*Scombermorus regalis*), King Mackerel (*Scomberomorus cavalla*), Spanish Mackerel (*Scomberomorus maculatus*), and Wahoo (*Acanthocybium solandri*). The species belonging to the remaining families include Crevalle Jack (*Caranx hippos*) and Common Dolphinfish (*Coryohaena hipurus*) and are known to spend portions of time both inshore and offshore. Mean sizes, estimated life span, and general diet for each species can be found in Table 1.

What makes the species in this study considered 'coastal pelagics' is their tendency to spend large portions of their lifespans both in coastal and pelagic ecosystems. The Atlantic waters off southeastern Florida contain an ecotone connecting the shallow and deep regions of the continental slope, which starts with the shelf approximately only 3-4 km wide before the steep slope drop into the east Florida escarpment (Banks et al., 2008). The shallow region atop the shelf is the coastal zone which is enriched with terrestrial nutrients, such as organic material from mangrove forests, and have higher productivity (Castañeda-Moya, et al., 2013). The region of the slope that is off the shelf is characterized by deep water and lower productivity, enriched by introductions of nutrients such as rainfall, so is considered offshore and the pelagic zone (Paerl et al, 1999). A majority of the species in this study spend their adult lives in pelagic waters and spawn in coastal waters but variability among species is present. Some species spend time nearshore and offshore (i.e., King Mackerel, Skipjack Tuna) (Finucane et al., 1986; Andrade & Santos, 2004) and others may even live mostly nearshore as adults and spawn in pelagic waters (i.e., Crevalle Jack) (Berry, 1959). Highly migratory species, such as the Common Dolphinfish, are truly oceanic because they spend large amounts of time in the open ocean but still come nearshore as juveniles (Schwenke & Buckel, 2008). Coastal pelagic species share many common prey items, such as smaller fishes and crustaceans, most of which feed on phytoplankton or zooplankton, which in turn forms the base of the food web (Dittel et al., 2000; Rosa et al., 2008).

The following species are subject to state harvest regulations set by the Florida Fish and Wildlife Conservation Commission (FWC) Division of Marine Fisheries Management, which manages fishes in state waters: Blackfin Tuna, Common Dolphinfish, King Mackerel, Spanish Mackerel, and Wahoo. Crevalle Jack, Cero Mackerel, and Little Tunny are considered 'unregulated species' by FWC but have a limit of two fish or 100 pounds per person per day, whichever is more, for recreational fishing (FWC, 2022). Common Dolphinfish, King Mackerel, Spanish Mackerel, and Wahoo are additionally managed by the South Atlantic Fishery Management Council in federal waters (SAFMC, 2022). FWC also coordinates with NOAA and multi-state agencies as appropriate for shared stocks, such as King Mackerel.

Stable Isotope Ratios

Stable isotope ratios may be able to reveal habitat utilization and trophic level to give a better understanding of the mercury-trophic level relationship. As organisms consume prey, nutrients are ingested and retained within the organism, such as in organs or muscle tissue. Muscle tissue has a turnover rate of about 3-4 months in fish; therefore, stable isotopes give insight into the last 3-4 months of the organism's life (Fry, 2006; Buchheister & Latour, 2010; Richert et al., 2015). Most elements have multiple isotopic states that differ due to varying number of neutrons in the nucleus, affecting the atomic weight. Heavier isotopes are metabolically more costly so are retained in the organism while the lighter ones are preferentially used in biological processes as both are excreted or respired (Checkley & Entzeroth, 1985; Peterson & Fry, 1987). The ratio of heavier to lighter stable isotopes can be more positive or more negative. More positive ratios are more enriched in the heavy isotopes while more negative ratios are more depleted in the heavy isotopes (Fry, 2003).

Stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively) are particularly useful for trophic level and food web structure analyses. The carbon stable isotope ratio used in this study is $^{13}C/^{12}C$ and is denoted by $\delta^{13}C$. From the point of assimilation to the final predator, the ratio of carbon isotopes changes very little, making $\delta^{13}C$ useful in identifying the original carbon source. Since the enrichment of $\delta^{13}C$ is balanced by respiration and excretion, the composition of stable carbon isotopes in an organism reflects the composition of stable isotopes in its diet with a small enrichment of about 0.5 to 1 per mil (parts per thousand or ‰) per trophic

level increase (DeNiro & Epstein, 1978, Peterson & Fry, 1987). Inshore regions have higher nutrient levels due to upwelling, runoff, and erosion. Phytoplankton use CO_2 to convert sunlight into energy, making carbon available in the food web. More nutrients in inshore waters results in higher levels of photosynthesis, causing more productivity and availability of carbon. This process occurs in pelagic waters as well, but at a slower rate due to lower nutrient levels. Nearly all the available carbon in the ocean originates from marine plankton (Williams & Gordon, 1970). Therefore, in marine environments, inshore $\delta^{13}C$ levels tend to be enriched and have less negative ratios as compared to the more negative offshore ecosystem ratios (France, 1995). This more or less enriched $\delta^{13}C$ levels is reflected in the fish muscle tissue and allows for an understanding of what habitat has been primarily utilized in the past three to four months.

In contrast to carbon, stable nitrogen isotope ratios are typically enriched by 3-5‰ to an organism's dietary nitrogen per level, making them useful to track trophic level changes. The nitrogen stable isotope ratio used in this study is $^{15}N/^{14}N$ and is denoted by $\delta^{15}N$. The ^{15}N is more readily retained and accumulated in muscle tissues than lighter ^{14}N, both of which are assimilated by photosynthetic organisms and passed along the trophic level (DeNiro & Epstein, 1981). The higher the trophic level of an organism, the more positive the ratios tend to become to reflect higher enrichment of $\delta^{15}N$. In many ecosystems, there can be a 10 to 15‰ total increase from lower to higher levels (Minagawa & Wada, 1984). Previous work in southeast Florida revealed trophic groupings within coastal pelagic species (Moore, 2014). The lowest level, or those with the lowest ratios of $\delta^{15}N$, were young (small) Wahoo and Skipjack Tuna, reflecting species of limited diets that are preying on lower trophic level species (DeNiro & Epstein, 1981; Moore, 2014). Blackfin Tuna and Common Dolphinfish were considered mid-level as they consumed very diverse diets both inshore and offshore. Little Tunny and King Mackerel were highest in trophic level, selectively feeding on higher level prey in more pelagic waters, reflecting other species with these characteristics. In southeast Florida, because the pelagic and coastal ecosystems come together at the coastal pelagic ecotone due to the proximity of the edge of the continental shelf, $\delta^{15}N$ and $\delta^{13}C$ can be highly variable (Richert et al., 2015).

While the goal of this study is not to identify sources of mercury into the marine environment, it is important to understand how these species are exposed to mercury to fully comprehend the stable isotope-mercury relationship. All of these study fishes are higher level predators in the ecosystem and spend at least some time in inshore waters. As juveniles, many of

these coastal pelagic species will feed on zooplankton that consume periphyton or feed on invertebrates that consume zooplankton. Regardless of the prey, periphyton, bacteria, or phytoplankton have at some stage been consumed, passing on methylmercury. As coastal pelagic species mature, zooplankton will no longer be a dominant food source and will instead be replaced with small fish, cephalopods, and other invertebrates. However, small prey items still depend on zooplankton in the food web, meaning the predatory coastal pelagic species are now intaking an even more concentrated dose of methylmercury. The transference from coastal species is not the only way methylmercury enters pelagic waters, but multiple mercury 'hot spots' have been identified in the South Florida region, indicating large amounts of mercury are entering Florida marine waters via estuaries (Adams, 2018). Mercury concentrations increase in fish through bioaccumulation in tissues over time and through biomagnification with consumption of contaminated prey (Sunderland, 2007, Dijkstra et. al., 2013). Specifically, these species have been consuming methylmercury their entire lives and the levels with which it has accumulated in their systems poses a risk not only to themselves, but also to their predators, such as humans.

Despite the economic importance of these species, there is little research comparing mercury levels and stable isotopes over time in South Florida coastal pelagic species. Studies on Spanish Mackerel and Bottlenose Dolphin in the Atlantic have presented a decline in mercury levels in tissue and blood, respectively, over time, though neither study use stable isotope ratios (Adams & McMichael, 2007; Schaefer et al., 2015). As previously mentioned, mercury bioaccumulates through the ecosystem and can be positively correlated to $\delta^{15}N$, so establishing multiple datasets over time periods may allow these relationships to be tracked. Identifying and analyzing the relationship between $\delta^{15}N$, $\delta^{13}C$, and mercury between these two time periods may allow more insight into the movement of mercury in the environment, particularly in these fisheries. The data from this study will provide the basis for more temporal studies on these species and mercury in the future. Relationships may be revealed that can be applied in other similar ecotones across the world.

In this study, stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) are compared with methylmercury concentrations in nine coastal pelagic fish species that are commonly targeted by recreational and commercial anglers in Southeast Florida (defined as the geographic region encompassing Monroe, Miami-Dade, Broward, and Palm Beach Counties). These stable isotope

ratios are frequently assessed to understand food web interactions in fish species (e.g., de la Moriniere et al., 2003) because $\delta^{15}N$ is indicative of trophic level, and $\delta^{13}C$ can indicate original carbon sources (i.e., seagrass versus phytoplankton) within an ecosystem food web (Peterson & Fry, 1987; Post, 2002). Multiple studies have established a positive correlation between $\delta^{15}N$ and mercury levels in some species (Al-Reasi et al., 2007; Atwell et al., 1998; Jarman et al., 1996), indicating that further understanding of this relationship is needed.

Discrete datasets from two time periods – 2010-2012 and 2020-2021 – were used to compare and analyze stable isotope ratios and mercury in white dorsal muscle tissue of nine coastal pelagic species. The comparison of the two datasets allowed the analysis of temporal differences to better assess trends over time for mercury in the coastal pelagic ecosystem.

Table 1 . Families Scombridae, Carangidae, and Coryphaenidae species included in this study, along with their average size, general diet, and references used. Average sizes and lifespans presented are based on samples typically landed in recreational or commercial fisheries. Diet sources from FWC species profiles and studies referenced, when available (FWC, 2021).

Family	Name	Average Size	Lifespan	Diet	References
Scombridae	Blackfin Tuna (*Thunnus atlanticus*)	65-75 cm	5-7 yrs	Smaller fish, crustaceans, and squid	Ahbrabi-Nejad, 2014 Colette and Nauen, 1983 Adams and Kerstetter, 2014
Scombridae	Little Tunny (*Euthynnus alletteratus*)	90-120 cm	5-10 yrs	Smaller fish, crustaceans, and cephalopods	Manooch et al., 1985
Scombridae	Skipjack Tuna (*Katsuwonus pelamis*)	80-100 cm	6-7 yrs	Smaller fish, crustaceans, and cephalopods	Andrade & Santos, 2004 Ely et al., 2005 Barkley et al., 1978
Scombridae	Cero Mackerel (*Scomberomorus regalis*)	40-60 cm	Inconsistent Data	Smaller fish, crustaceans, and cephalopods	Finucane & Collins, 1984
Scombridae	King Mackerel (*Scomberomorus cavalla*)	70-100 cm	15-20 yrs	Smaller fish, crustaceans, and cephalopods	Finucane et al., 1986 Moore, 2014
Scombridae	Spanish Mackerel (*Scomberomorus maculatus*)	60-70 cm	15-25 yrs	Smaller fish, crustaceans, and cephalopods	Finucane & Collins, 1986 Powell, 1975 Nobrega & Lessa, 2009
Scombridae	Wahoo (*Acanthocybium solandri*)	70-170 cm	5-6 yrs	Fish, cephalopods	Oxenford et al., 2003
Carangidae	Crevalle Jack (*Caranx hippos*)	40-60 cm	17-20 yrs	Smaller fish, crustaceans, and squid	Caiafa-Hernández et al., 2018 Smith-Vaniz & Carpenter, 2007 Jefferson et al., 2022
Coryphaenidae	Common Dolphinfish (*Coryohaena hipurus*)	50-110 cm	5-7 yrs	Opportunistic feeders	Maggio et al., 2019 Oxenford, 1999 Shwenke & Buckel, 2008

Materials and Methods

The original specimens were collected from the waters off Brevard, Broward, and Miami-Dade counties from March 2010 through March 2012 (Tables 2-3). Palm Beach was not included in the original data set but fits into this project because of its adjacency to the rest of the counties and its similar habitat for coastal pelagic species. Skeletal muscle tissue samples and stomachs were taken when anglers provided their catches for measurement and tissue sampling at tournaments and opportunistic dockside sampling. Other samples were collected using NSU vessels with gillnets in addition to traditional rod-and-reel methods (Moore, 2014). Multiple analyses were run on these samples by the Fisheries Lab and FWC for stomach contents, stable isotope values $\delta^{15}N$ and $\delta^{13}C$, and mercury concentrations.

Collection protocols for samples in this study reflect the original dataset techniques, but without the use of NSU vessels or gillnet gear. Samples were collected from recreational tournaments and ongoing collaborations with local charter vessels (e.g., *Lady Pamela II* in Dania Beach, FL). As whole fish became available, fish morphometric data such sex and fork length were recorded. Fish were returned to anglers once measurements were complete, sex was determined, and muscle tissue samples were taken. Alternatively, filleted carcasses ("racks") were provided by vessels and brought back to the Fisheries lab for processing. Once in the lab, a scalpel was used to remove a 15 g sample of white muscle tissue from the dorsal muscle, and viscera was removed to be sexed and stomach content inspected if possible (Adams, 2009). Scalpels, forceps, and any other instrument or surface area utilized were sanitized between each fish with 70% laboratory-grade ethanol. Muscle samples were stored in plastic cryogenic vials and frozen at -20ºC until processed.

Sampling was done to reflect the sizes of the specimens collected in the 2010-12 sample set and species were broken into size classes when sample numbers allowed. The first size class included all of the juveniles of the species set, as established by size at maturity (see Table 6 for sizes and references). Size at maturity was established by the literature for each species at 50% of the population reproductively mature with developed or ripe gonads, when available. In cases of multiple studies or there was a significant difference between male and female age at maturity, as cited within the reference, an average of the sizes available was taken. Species that had enough samples to be broken up into thirds included Blackfin Tuna and Little Tunny, and after

the juvenile grouping, were grouped into increments of 200 mm. Common Dolphinfish did not have a juvenile size class and were grouped with 100 mm increments because there were many samples on both the small and large ends of the size range, but few in the middle. This grouping division allowed for three size classes in the Common Dolphinfish samples. Size classes were implemented to account for potential changes in feeding ecology, for as predator gape increases, prey size tends to increase (Rudershausen, 2010).

Stable Isotope Analyses

Approximately 5 g of white muscle tissue from each fish was sampled to be analyzed for $\delta^{15}N$ and $\delta^{13}C$. Muscle tissue samples were dried at 60°C for a minimum of 72 hours, ground, and homogenized using a dental amalgamator (Wig-L-Bug, Crescent Dental Manufacturing Company). Muscle subsamples were weighed to 0.6-0.8 milligrams (mg) and pelletized in tin capsules for stable carbon and nitrogen isotope analysis. Stable isotope analyses for $\delta^{13}C$ and $\delta^{15}N$ was conducted at the Smithsonian Institution's Museum Conservation Institute in Suitland, MD (USA) using a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to a Costech 4010 Elemental Analyzer (EA) via a Thermo Conflo IV (CF-IRMS). A set of standards was run every 10-12 samples and included USGS40 and USGS41 (L-glutamic acid) as well as Costech acetanilide. All samples and standards were run with the same parameters; this includes an expected reproducibility of the standards < 0.2‰ (1σ) for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope values are expressed in terms of δ and reported in comparison to the respective standard reference materials: Pee Dee Belemnite (PDB) for carbon and atmospheric air (N_2) for nitrogen. The isotopic values are reported with the standard parts per thousand notation ("per mil" or ‰):

$$\delta X = [(R_{sample}/R_{standard})-1] * 1000$$

where X is the isotope being analyzed and R is the ratio of the heavy to light isotope. $\delta^{13}C$ was lipid corrected due to lipids in white muscle previously significantly decreasing $\delta^{13}C$ in white muscle results (Logan et al., 2008). Lipids have more negative $\delta^{13}C$ values than muscle tissue and can falsely indicate dietary or habitat shifts (DeNiro & Epstein, 1977; Logan et al., 2008). The $\delta'^{13}C$ equation is:

$$\delta'^{13}C = \delta^{13}C + D\,[\theta+3.90/(1+(287/L))]$$

where D is the isotopic difference between protein and lipid (6‰ assumed for fish tissues), θ is the standard for fish muscle tissue (-0.207), and L is the lipid content of the sample (McConnaughey & McRoy, 1979; Sweeting et al., 2006; Logan et al., 2008). L is calculated:

$$L = 93/[1 + (0.246\ C/N - 0.775)^{-1}]$$

where C/N is the percent weight Carbon to Nitrogen ratio (McConnaughey & McRoy, 1979; Sweeting et al., 2006; Logan et al., 2008).

Stable isotope ratio of nitrogen can be used to estimate trophic level. Trophic position was established by the formula:

$$TP = (\delta^{15}N_{consumer} - \delta^{15}N_{base})/(\Delta n) + 1$$

where $\delta^{15}N_{consumer}$ is the measurement of $\delta^{15}N$ for the target species, $\delta^{15}N_{base}$ is the $\delta^{15}N$ measurement of the chosen organism to represent the $\delta^{15}N$ base in the food web, and Δn is the enrichment in ^{15}N per trophic level (Richards et al, 2020). The primary consumer, *Pyrosoma atlanticum*, has been used as the base primary consumer in many studies and has a $\delta^{15}N$ measurement of 3.15‰ (Richards et al., 2020). The ^{15}N enrichment per trophic level (Δn) is 3.4 (Post, 2002). The $\delta^{15}N_{consumer}$ was calculated by averaging each entire species' $\delta^{15}N$ results and also averaging the aforementioned species size classes.

Table 2. The location, years sampled, species, and number of coastal pelagic fish species sampled in the 2010-2012 dataset.

Location	Years Sampled	Species	Number of Samples
Cape Canaveral	2011, 2012	Cero Mackerel	1
		Common Dolphinfish	6
		King Mackerel	23
		Spanish Mackerel	37
Yamaha Contender Miami Billfish Tournament	2010, 2011, 2012	Crevalle Jack	2
		Common Dolphinfish	45
		King Mackerel	43
		Little Tunny	32
		Skipjack Tuna	26
		Wahoo	19

Table 3. The location, years sampled, species, and number of coastal pelagic fish species sampled in the 2020-2021 dataset.

Location	Years sampled	Species	Number of Samples
Biscayne Bay REEF Tournament	2021	Common Dolphinfish	13
		Skipjack Tuna	2
Broward County Shoreline	2021	Blackfin Tuna	1
		Cero Mackerel	1
		Little Tunny	3
Fishing Headquarters Ft. Lauderdale Charter	2021	Little Tunny	3
		Spanish Mackerel	1
Key West Meat Mayhem Tournament	2021	King Mackerel	19
Key West PBA Tournament	2021	Common Dolphinfish	9
		Skipjack Tuna	1
Lady Pamela Dania Beach Charter	2020, 2021	Blackfin Tuna	36
		Cero Mackerel	3
		Common Dolphinfish	36
		King Mackerel	21
		Little Tunny	48
		Skipjack Tuna	3
		Spanish Mackerel	2
		Wahoo	5
Pompano Beach Ladies Fish Off Tournament	2021	Blackfin Tuna	2
		King Mackerel	7
		Little Tunny	8
Port Everglades NSU Boat Basin	2020, 2021	Crevalle Jack	8
West Palm Beach Meat Mayhem Tournament	2021	Blackfin Tuna	3
		King Mackerel	26
		Wahoo	1

Mercury Analysis

Total mercury was analyzed with a DMA-80 direct mercury analyzer by FWC-FWRI scientists (Adams et al., 2018). Approximately 10 grams of white dorsal muscle tissue was sampled with a scalpel (cleaned with 70% lab grade ethanol) and care was taken to keep the sample tissue from coming in contact with fish scales or other contaminated areas. Samples were immediately placed into cryogenic vials and frozen after sampling. All tools and surfaces were cleaned and rinsed with 70% lab grade ethanol between fish. Wet-muscle subsamples of 0.01-0.1 g were cut from the roughly 10 grams of white dorsal muscle tissue samples and analyzed directly against aqueous mercury standards prepared in 2% HCl. Quality control was conducted by running method blanks, duplicate samples, and matrix spikes. Method blanks are performed every 10 samples and between species to purge the analyzer and minimize residual mercury. Duplicate samples were also run every 10 samples and the results of duplicate or triplicate runs were averaged and used as one result for the corresponding sample. Certified reference materials (CRM) ERM 464, DORM-4, and TORT-3 were used to confirm the calibration curve of the DMA-80 every 10 samples. After running the CRM, a tissue sample was run, followed by a run of the previous CRM and sample combined. This matrix spike was analyzed with the following formula to ensure the nanograms of mercury were consistent and run every 40 samples (EPA, 1998; Adams et al., 2003; Adams, 2018):

Matrix spike recovery = ((matrix spike Hg ng – tissue sample Hg ng)/(CRM Hg ng))*100. Total mercury levels are reported as milligram per kilogram wet weight.

Statistical Analyses

Both datasets of mercury and stable isotope values of coastal pelagic fish species in southeast Florida were statistically analyzed to evaluate relationships among the data. Visual assessments of normality were done using boxplots, histograms, and the residual plots of some sets, but a Shapiro-Wilks test was run on every set to establish normality and parametric or non-parametric test selection. Statistical comparisons were made both for mercury and stable isotope values with the goal to identify any relationship between the values and over time. R, in

conjunction with Rstudio, were used to run basic analyses of the data with descriptive statistics (i.e., regressions, correlation tests) (R Core Team, 2020).

All the data, which included mercury, $\delta^{15}N$, $\delta^{13}C$, and fork length as individual sets, species sets, and species size classes subsets were evaluated for normality using a Shapiro-Wilk normality test in addition to histograms, boxplots, and residual plots. When these tests indicate the assumptions of normality were not met, attempts were made to transform the data with base-10 logarithms or the square root, when appropriate. For normally distributed data, Pearson's correlation was run to analyze the relationship between mercury, fork length, and $\delta^{15}N$. For non-normally distributed, or non-parametric even after transformations, data with more than 30 samples, Kendall's tau was run. For non-parametric sets with less than 30 samples, Spearman's rho was used. Comparisons between the 2010 and 2020 data sets for stable isotope ratios, mercury, and fork length were conducted with unpaired t tests for normally distributed, or parametric, data and Mann-Whitney (Wilcoxon) Rank Sum tests for non-parametric data set with over three samples.

Table 4. A summary of the eight coastal pelagic species and the sample size (N), fork length (FL), δ15N mean ± standard deviation, δ13C mean ± standard deviation, δ'13C mean ± standard deviation, and total mercury (THg) mean ± standard deviation from the 2010-12 dataset (Moore, 2014).

Species	N	FL Mean(mm) (±SD)	δ15N(±SD)	δ13C(±SD)	δ'13C(±SD)	T Hg(mg/kg) (±SD)
Cero Mackerel	1	326	13.46	-18.38	-18.37	0.16
Crevalle Jack	2	401.5(±23.2)	12.9(±0.01)	-16.8(±0.89)	-16.8(±0.76)	0.54(±0.19)
Common Dolphinfish	51	758.7(±159.1)	9.3(±1.12)	-16.8(±1.23)	-16.7(±0.93)	0.10(±0.09)
King Mackerel	66	1009(±146.0)	13.5(±0.99)	-18.5(±0.91)	-17.06(±0.46)	1.14(±0.65)
Little Tunny	32	620.9(±150.6)	12.3(±1.21)	-17.5(±0.74)	-17.4(±0.47)	0.93(±0.76)
Skipjack Tuna	26	572.5(±104.2)	8.70(±1.5)	-16.9(±0.41)	-16.8(±0.38)	0.42(±0.38)
Spanish Mackerel	37	389.3(±58.1)	13.4(±0.69)	-19.7(±1.13)	-17.80(±0.93)	0.19(±0.07)
Wahoo	19	911.5(±269.1)	8.2(±1.15)	-17.0(±1.04)	-16.8(±0.70)	0.15(±0.18)

Table 5. A summary of the nine coastal pelagic species and the sample size (N), fork length (FL), δ15N mean ± standard deviation, δ13C mean ± standard deviation, δ'13C mean ± standard deviation, and total mercury (THg) mean ± standard deviation from the 2020-21 dataset.

Species	N	FL Mean(mm) (±SD)	δ15N(±SD)	δ13C(±SD)	δ'13C(±SD)	T Hg(mg/kg) (±SD)
Blackfin Tuna	40	468.5 (±151.9)	8.9(±1.51)	-17.4(±0.63)	-17.5(±0.39)	0.25(±0.33)
Cero Mackerel	5	454.2(±58.4)	10.6(±0.50)	-16.6(±1.17)	-16.51(±0.96)	0.22(±0.11)
Crevalle Jack	5	328.4(±76.5)	12.4(±0.47)	-16.5(±1.09)	-16.57(±1.1)	0.56(±0.24)
Common Dolphinfish	38	678.5(±129.6)	9.1(±0.85)	-16.3(±0.36)	-16.45(±0.38)	0.08(±0.07)
King Mackerel	62	1006(±195.4)	13.1(±1.43)	-18.2(±1.10)	-17.47(±0.61)	1.31(±0.89)
Little Tunny	40	509.8(±136.9)	11.6(±1.43)	-18.1(±0.80)	-17.90(±0.36)	0.52(±0.58)
Skipjack Tuna	6	547.8(±63.5)	8.5(±0.76)	-17.5(±0.35)	-17.74(±0.40)	0.24(±0.07)
Spanish Mackerel	4	441.3(±61.0)	12.2(±0.41)	-17.9(±0.45)	-17.71(±0.22)	0.35(±0.06)
Wahoo	6	882.3(±289.7)	8.3(±1.16)	-16.8(±0.21)	-16.98(±0.23)	0.09(±0.06)

Results

Specimen Collection

A total of 264 coastal pelagic skeletal white muscle tissue samples were collected between August 2020 through October 2021 and comprised of nine species (Table 3). Species were segregated into size classes based on length at maturity and the previous dataset composition. Maturation, based on length at maturity, varied among the species (Table 6). For example, in the 2010-12 dataset, there were 23 Common Dolphinfish in the 500-700 mm size range, therefore an attempt was made to obtain 23 Common Dolphinfish samples in the 500-700 mm size range for this set. In some cases, too many or too few samples were collected per size range, and some of the samples were not sent off for analyses to preserve resources. A total of 206 samples, out of the 264 collected, were analyzed for both mercruy and stable isotope ratios in the 2020-21 dataset.

Fork length analysis (Table 9, Figure 5) were used to compare the fork lengths between 2010 and 2020 in Common Dolphinfish, King Mackerel, Little Tunny, Skipjack Tuna, Spanish Mackerel, and Wahoo. Common Dolphinfish showed a significant difference in fork length between 2010 and 2020, with Common Dolphinfish sampled collected in 2020 being shorter on average than those collected in 2010. Little Tunny also had a significantly different fork length between 2010 and 2020. Only two Little Tunny samples below 400 mm, or juveniles, were collected in the 2010 data set, leaving two Little Tunny size class sets of 340-600 mm and 600 mm or above. When the juveniles are removed, there was no significant difference between 2010 and 2020 fork lengths of Little Tunny in the two adult size classes. The significant difference between all Little Tunny fork lengths in 2010 and 2020 is due to the 15 Little Tunny below 340 mm that were sampled in 2020, skewing the 2020 dataset toward a shorter average fork length. King Mackerel, Skipjack Tuna, Spanish Mackerel, and Wahoo showed no significant difference in fork lengths between 2010 and 2020.

Stable Isotope Analysis

A total of 206 samples were analyzed for all $\delta^{15}N$, $\delta^{13}C$, and mercury (Table 5) from the 2020-21 set. Some samples were not analyzed for all three $\delta^{15}N$, $\delta^{13}C$, and mercury to preserve resources, which reduced the final number of samples analyzed from 264 down to 206. King Mackerel had the highest $\delta^{15}N$ mean at 13.1 ‰ and Wahoo had the lowest at 8.3 ‰. The range for $\delta^{13}C$ was -16.3 ‰ for Common Dolphinfish and -18.2 for King Mackerel. $\delta^{13}C$ was lipid corrected due to lipids affecting $\delta^{13}C$ of white muscle, however, 88% of the samples collected had a C:N ratio less than four, which has been shown to not need lipid correction (Logan et al., 2008). Nevertheless, the remaining 12% of the samples had a C:N ratio above four and needed correction, therefore the entire set was corrected by converting into $\delta'^{13}C$ for consistency between analyses. The $\delta'^{13}C$ range was -16.45 for Common Dolphinfish to -17.90 for Little Tunny.

A Kruskal-Wallace and multiple comparison test identified a significant difference between $\delta'^{13}C$ in all species with five or more samples, and then placed them into two groups (KW chi-squared =108.56, $p < 0.001$). The first group was most $\delta'^{13}C$ depleted, with a range of -17.47‰ to -17.90‰, and consisted of Blackfin Tuna, King Mackerel, Little Tunny, and Skipjack Tuna. The second group was less $\delta'^{13}C$ depleted, with a range of -16.45‰ to -16.98‰, and consisted of Cero Mackerel, Crevalle Jack, Common Dolphinfish, and Wahoo.

Comparisons for $\delta'^{13}C$ and $\delta^{15}N$ were run on species with more than three samples in both the 2010 and 2020 data sets which included Common Dolphinfish, King Mackerel, Little Tunny, Skipjack Tuna, Spanish Mackerel, and Wahoo (Tables 7 and 8). King Mackerel, Little Tunny, and Spanish Mackerel showed a significant difference in $\delta^{15}N$ between the 2010 and 2020 datasets. There were far less Spanish Mackerel samples taken in 2020 than in 2010, which may not adequately represent this species in this comparison. All of the Little Tunny samples combined displayed a lower average $\delta^{15}N$ for the 2020 data set than the 2010 data set (Figure 1), but when broken down into size classes there were 15 more juveniles (according to fork length) in the 2020 set than in 2010 and with those removed, there was no significant differences in the $\delta^{15}N$ between the two time frames. King Mackerel results (Figure 2) were similar to the Little Tunny in that all of the samples combined have a lower average $\delta^{15}N$ and when broken into size classes, there was smaller fork length samples taken in 2020 below 900 mm than taken in 2010.

Samples above 900 mm were more similar in fork length between the years and this is reflected in the $\delta^{15}N$ having no significant difference in samples above 900 mm between the 2010 and 2020 data sets.

Results for the comparison tests were similar for $\delta'^{13}C$ in that species that showed a significant difference in $\delta'^{13}C$ between the two time frames included King Mackerel, Little Tunny, and Skipjack Tuna. All of these species that had a significant difference between the 2010 data set and the 2020 data set were more depleted in $\delta'^{13}C$ in 2020 than in 2010. King Mackerel was an average 0.41‰ more depleted, Little Tunny was an average 0.50‰ more depleted, and Skipjack Tuna (Figure 3) was an average 0.90‰ more depleted.

Table 6. A summary of the nine coastal pelagic species' length at maturity, according to the sources sited at 50% of the population reproductively mature with developed or ripe gonads, along with the number of juveniles and adults sampled according to length at maturity in the 2020 data set.

Species	Length at Maturity	Juveniles	Adults	Citation
Blackfin Tuna	450 mm	22	19	Ahrabi-Nejad, 2014
Cero Mackerel	350 mm	0	5	Finucane & Collins, 1984
Crevalle Jack	650 mm	5	0	Snelson, 1992
Common Dolphinfish	400 mm	0	38	Schwenke & Buckle, 2008
King Mackerel	700 mm	3	59	Finucane et al., 1986
Little Tunny	400 mm	15	25	Mohamed et al., 2014
Skipjack Tuna	500 mm	2	4	Andrade & Santos, 2004
Spanish Mackerel	350 mm	0	4	Finucane & Collins, 1986
Wahoo	900 mm	2	4	Jenkins & McBride, 2009

Table 7. Results of comparison tests for $\delta^{15}N$ between the 2010 and 2020 data sets where W is the test statistic for the Mann-Whitney rank sum test, t is the test statistic for the T test, n10 represents the number of samples from the 2010 data set, and n20 represents the number of samples from the 2020 data set.

Species	Test and Significance	n10	n20
All Species	Mann-Whitney, significant difference, W = 28156, p = 0.002	234	206
Common Dolphinfish	Mann-Whitney, no significant difference, W = 1029, p = 0.6622	51	38
Common Dolphinfish <600 mm	T test, significant difference, t = 2.238, p = 0.040	8	14
Common Dolphinfish 600-700 mm	Mann-Whitney, no significant difference, W = 68, p = 0.640	15	8
Common Dolphinfish >700 mm	Mann-Whitney, no significant difference, W = 151, p = 0.077	28	16
King Mackerel	T test, significant difference, t = 2.048 p = 0.043	66	62
King Mackerel <900 mm	T test, significant difference, t = 3.156, p = 0.003	21	21
King Mackerel >900 mm	T Test, no significant difference, t = 0.765, p = 0.447	45	41
Little Tunny	T test, significant difference, t = 2.468, p = 0.016	32	40
Little Tunny 340-600 mm	T test, no significant difference, t = -0.512, p = 0.613	15	30
Little Tunny >600 mm	T Test, no significant difference, t = 0.001 p = 0.999	17	10
Skipjack Tuna	Mann-Whitney, no significant difference, W = 77, p = 0.981	26	6
Spanish Mackerel	Mann-Whitney, significant difference, W = 143, p = 0.003	37	4
Wahoo	T Test, no significant difference, t = -0.090 p = 0.930	19	6

Table 8. Results of comparison tests for $\delta'^{13}C$ between the 2010 and 2020 data sets where W is the test statistic for the Mann-Whitney rank sum test, t is the test statistic for the T test, n10 represents the number of samples from the 2010 data set, and n20 represents the number of samples from the 2020 data set.

Species	**Test and Significance**	**n10**	**n20**
All Species	Mann-Whitney, significant difference, W = 29702, p = 0.0002	234	206
Common Dolphinfish	Mann-Whitney, no significant difference, W = 807, p = 0.180	51	38
Common Dolphinfish <600 mm	Mann-Whitney, significant difference, W = 23, p = 0.024	8	14
Common Dolphinfish 600-700 mm	Mann-Whitney, no significant difference, W = 35, p =0.115	15	8
Common Dolphinfish >700 mm	Mann-Whitney, no significant difference, W = 239, p = 0.726	28	16
King Mackerel	T Test, significant difference, t = 4.2674, p < 0.005	66	62
King Mackerel <900 mm	T test, significant difference, t = 2.9855, p = 0.005	21	21
King Mackerel >900 mm	T test, significant difference, t = 3.1046, p = 0.003	45	41
Little Tunny	T Test, significant difference, t = 5.2667, p < 0.005	32	40
Little Tunny 340-600 mm	T test, significant difference, t = 3.4227, p = 0.002	15	30
Little Tunny >600 mm	Mann-Whitney, significant difference, W = 141, p = 0.004	17	10
Skipjack Tuna	Mann-Whitney, significant difference, W = 148, p < 0.005	26	6
Spanish Mackerel	Mann-Whitney, no significant difference, W = 73, p = 0.9831	37	4
Wahoo	T test, no significant difference, t = 1.1403, p = 0.267	19	6

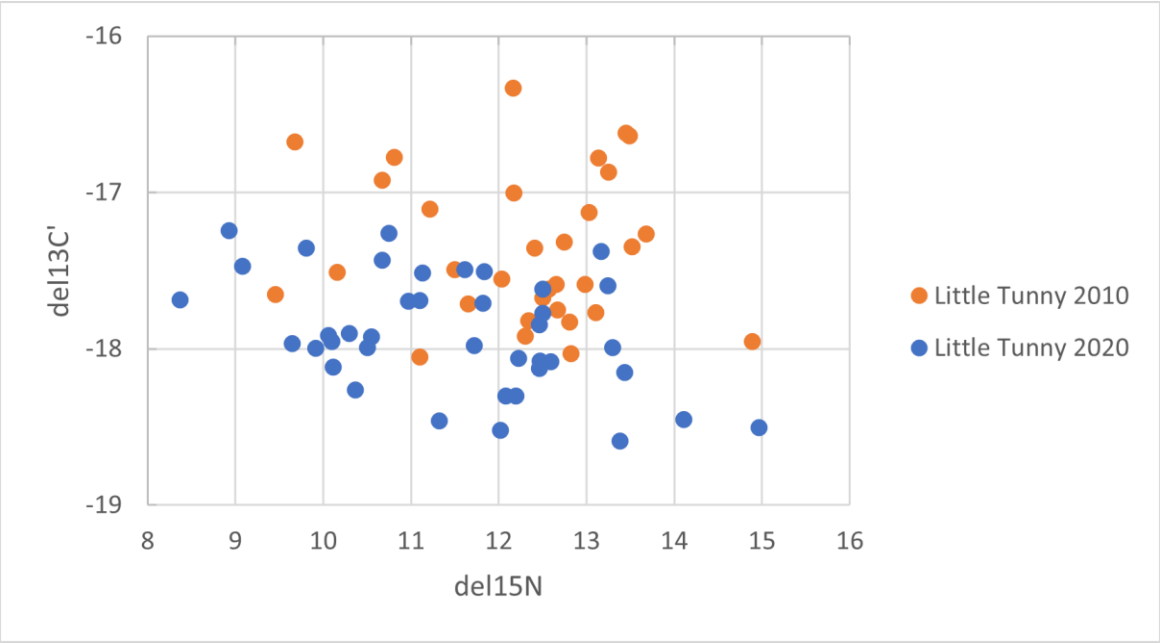

Figure 1. Scatter plot comparing the Little Tunny δ'^{13}C and δ^{15}N from the 2010 data set in orange to the Little Tunny δ'^{13}C and δ^{15}N from the 2020 data set in blue.

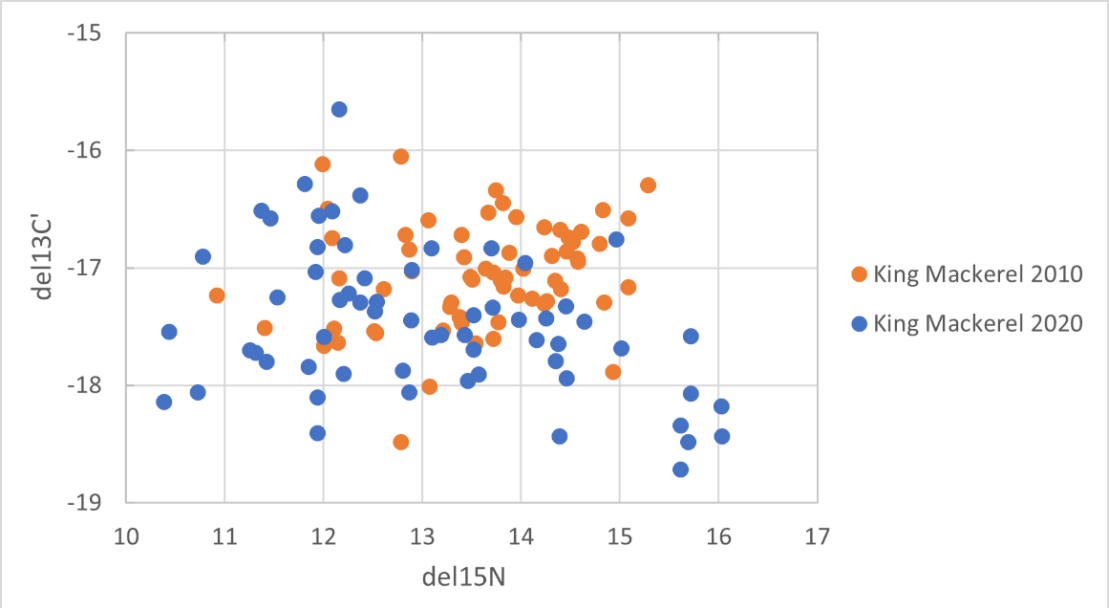

Figure 2. Scatter plot comparing the King Mackerel δ'^{13}C and δ^{15}N from the 2010 data set in orange to the King Mackerel δ'^{13}C and δ^{15}N from the 2020 data set in blue.

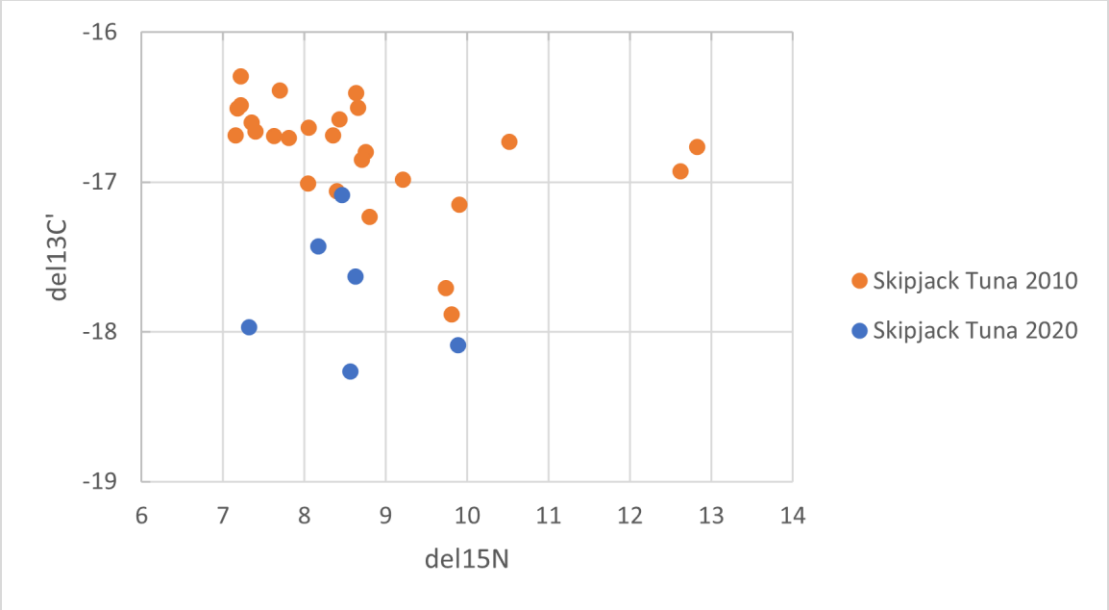

Figure 3. Scatter plot comparing the Skipjack Tuna δ'^{13}C and δ^{15}N from the 2010 data set in orange to the Skipjack Tuna δ'^{13}C and δ^{15}N from the 2020 data set in blue.

Trophic level

Using a hierarchical cluster analysis and confirming with Kruskal Wallace and multiple comparisons test (KW chi-squared = 140.19, $p < 0.001$), there were two groups based on $\delta^{15}N$. The groups are Blackfin Tuna, Common Dolphinfish, Skipjack, and Wahoo in the first and Crevalle Jack, King Mackerel, and Little Tunny in the second. Spanish Mackerel was grouped with the second group, but not enough samples were collected to confidently place with a Kruskal Wallace test. Cero Mackerel were in their own group between the two larger groups.

This preliminary Kruskal Wallace supports the results of the assigned trophic levels revealed by the Richards et al. (2020) trophic level formula. The first grouping had a trophic level range of 2.50-2.75 and included Blackfin Tuna (2.68, n = 40), Common Dolphinfish (2.75, n = 40), Skipjack Tuna (2.57, n = 6), and Wahoo (2.50, n = 6). The second grouping had a trophic level range of 3.45-3.92 and included Crevalle Jack (3.72, n = 5), King Mackerel (3.92, n = 62), Little Tunny (3.45, n = 40), and Spanish Mackerel (3.66, n = 4). Cero Mackerel was in the middle of these two grouping ranges, with a trophic level of 3.18 (n = 5).

The 2020 groupings are comparable to the 2010 groupings. The first grouping for the 2010 samples had a trophic level range of 2.49-2.82 and included Common Dolphinfish (2.82, n = 51), Skipjack Tuna (2.63, n = 26), and Wahoo (2.49, n = 19). The second grouping for the 2010 samples had a trophic level range of 3.69-4.05 and included Cero Mackerel (4.03, n = 1), Crevalle Jack (3.87, n = 2), King Mackerel (4.05, n = 66), Little Tunny (3.69, n = 32), and Spanish Mackerel (4.01, n = 37).

Mercury Analysis

A total of 234 muscle tissue samples were analyzed for total mercury in the 2020 set. This included samples that were not sent in for stable isotope analysis in addition to those that were. The 2010 data set also had 234 muscle tissue sample mercury results. King Mackerel had the highest mercury levels both years, with an average of 1.14 mg/kg total mercury in 2010 and 1.31 mg/kg total mercury in 2020 (Tables 4-5; Figures 4). Common Dolphinfish had the lowest mercury levels in both years, with an average of 0.10 mg/kg total mercury in 2010 and 0.08 mg/kg total mercury in 2020.

Initially, all of the mercury results were compared together with year the only factor of separation. Mercury between the 2010 dataset and 2020 dataset were not significantly different when using a Mann Whitney (MW) rank sum test (MW: W = 29489, $n_{2010} = n_{2020} = 237$, p = 0.268 two tailed). This set did not reflect that there was a significant difference in fork length between the 2010 data set and the 2020 data set, along with the unique characteristics of each species, therefore further analyses were run with species sets and species size class subsets. Mercury from both years from species with three or more samples were also compared using a T test for normally distributed data and a Mann Whitney (MW) rank sum test for not normally distributed data. Results were: Common Dolphinfish (MW: W = 1648, $n_{2010} = 51$, $n_{2020} = 54$, p = 0.08287), King Mackerel (T test with base-10 logarithm transformation, t=-0.661 $n_{2010} = 66$, n_{2020} = 67, p = 0.510), Little Tunny (MW: W = 932, $n_{2010} = 32$, $n_{2020} = 42$, p = 0.004195), Skipjack Tuna (T test with base-10 logarithm transformation, t=-8.276 = 92, $n_{2010} = 26$, $n_{2020} = 6$, p < 0.005), Spanish Mackerel (T test: t=-4.60, $n_{2010} = 37$, $n_{2020} = 4$, p = 0.010), and Wahoo (MW: W = 64, $n_{2010} = 19$, $n_{2020} = 6$, p = 0.687). Spanish Mackerel had significantly different mercury levels between the 2010 and 2020 data sets, with higher values in four samples from 2020. The large difference in sample amounts, with 37 in the 2010 data set and only four in the 2020 data set, may not be an adequate base for comparison between the two sets. A similar situation occurred with the Skipjack Tuna, in that there was 26 samples in the 2010 data set and only 6 in the 2020 data set, with a higher mercruy average in the 2020 data set. With the low, non-comparable sample number in the 2020 data set, there may not be an adequate base for comparison between the two sets. Little Tunny had a significantly different mercury level between the two time periods (MW: W = 875, $n_{2010} = 32$, $n_{2020} = 40$, p = 0.007 two tailed). However, the Mann Whitney test run on a comparison of the fork length of Little Tunny between the two data sets revealed there was a significant difference in fork length composition (MW: W =908, $n_1 = 32$, $n_2 = 40$, p = 0.002 two tailed) and the difference in mercury may be related to the difference in size composition. The 2010 data set contained two juvenile Little Tunny and the 2020 data set contained 15 juvenile Little Tunny. When the Mann Whitney rank sum test was used to compare only adult Little Tunny between the two data sets, there was no significant difference in mercury values (MW: W = 413, n_{2010}= 30, n_{2020}= 25, p = 0.5292).

Quality control of the DMA-80 and samples included the use of CRMs and method blanks. The mean mercury concentrations of the CRMs include: DORM-4 (n = 13) = 0.39

mg/kg, ERM-464 (n = 5) = 4.91 mg/kg, TORT-3 (n = 14) = 0.28 mg/kg. Matrix spike recoveries averaged 99.2% with a standard deviation of 0.53%. The method detection limit, as calculated by multiplying the standard deviation of the method blanks by three, was 0.1 ng. The average for the method blanks was 0.098 ng (n = 23) and only two samples were less than ten times the mean of the method blanks.

Bioaccumulation Analyses

Relationships between $\delta^{15}N$, fork length, and mercury were identified using correlation analyses (Table 10). There was a slightly positive significant relationship between $\delta^{15}N$ and mercury in all the species combined (Kendall's tau = 0.583, $p < 0.001$). There was also a slightly positive significant relationship between fork length and mercury in all the species combined (Kendall's tau = 0.487, $p < 0.001$). Nearly no significant relationship existed between $\delta^{15}N$ and fork length (Kendall's tau = 0.383, $p < 0.001$) in all the species combined. Species with more than 10 samples were divided into juvenile and adult groupings. Species with less than 10 samples that were left as one size class per species were Cero Mackerel, Crevalle Jack, Skipjack Tuna, Spanish Mackerel, and Wahoo. Some species had enough samples and variation in size so were grouped into three size classes and those species were Blackfin Tuna, Common Dolphinfish, and Little Tunny. King Mackerel was split into two size classes. Analyses were conducted on the species as a whole and the individual size classes. Blackfin Tuna, Little Tunny, and Wahoo all showed positive significant trends for bioaccumulation. Six samples may not be an adequate sample size to establish these trends, as Cero (n=5), Crevalle Jack (n=5), Skipjack Tuna (n=6), and Spanish Mackerel (n=4) all had no significant relationships in the bioaccumulation analyses.

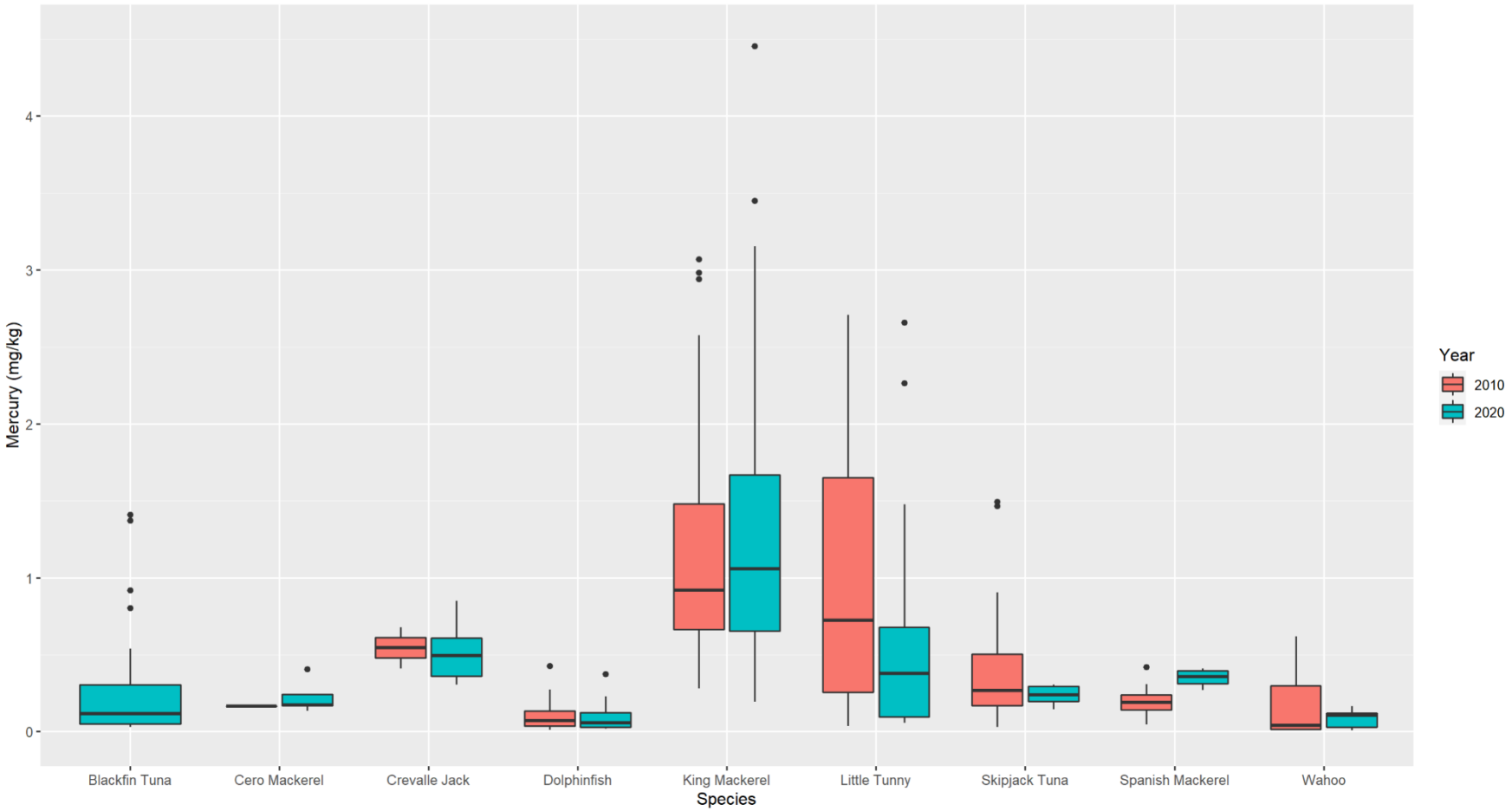

Figure 4. Box and whisker plot comparisons of the mercury between data sets, years 2010 and 2020, where the box indicates the range of the samples per species, the central line in the box is the average mercury value in mg/kg, the lines at the end of the box represent the samples that fall in the first and fourth quartiles, the dots before or after the lines represent outliers in the species samples, and the 2010 samples represented by the salmon color and 2020 samples represented by the blue color.

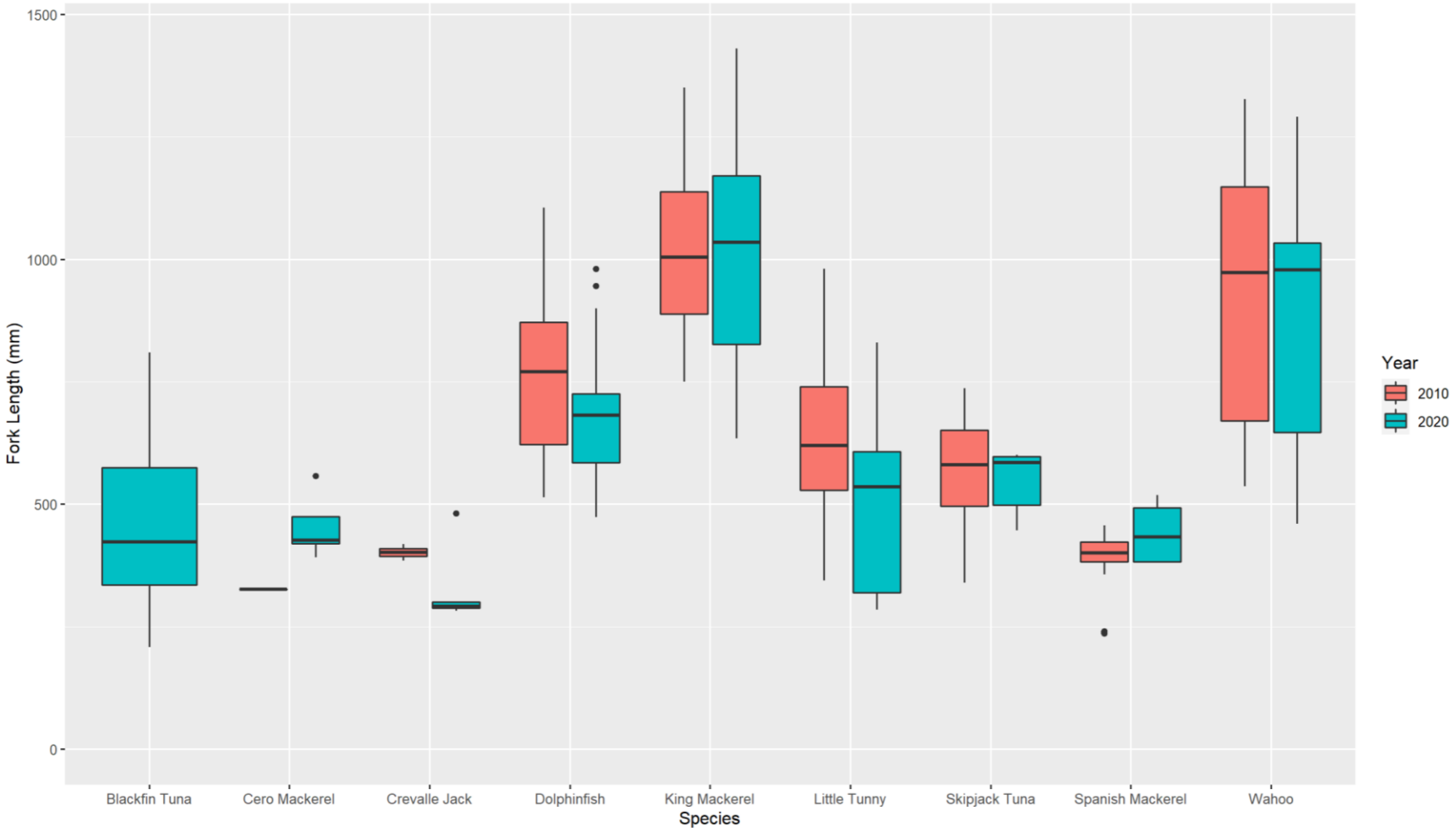

Figure 5. Box and whisker plot comparisons of the fork length between data sets, years 2010 and 2020, where the box indicates the range of the samples per species, the central line in the box is the average fork length in mm, the lines at the end of the box represent the samples that fall in the first and fourth quartiles, the dots before or after the lines represent outliers in the species samples, and the 2010 samples represented by the salmon color and 2020 samples represented by the blue color.

Table 9. Difference in fork lengths between the data set from 2010 and the data set from 2020 in the six species that had more than three samples in each set along with the test used and the results of that test sets where W is the test statistic for the Mann-Whitney rank sum test, t is the test statistic for the T test.

Species	Significance
Common Dolphinfish	Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 1233, p = 0.029
Common Dolphinfish <600 mm	No Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 59.5, p = 0.838
Common Dolphinfish 600-700 mm	Significant Difference in Fork Length; T Test t = -2.64, p = 0.021
Common Dolphinfish >700 mm	Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 324.5, p = 0.015
King Mackerel	No Significant Difference in Fork Length; W = 2044.5, p = 0.996
Little Tunny	Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 908, p = 0.002
Little Tunny 340-600 mm	No Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 68.5, p = 0.071
Little Tunny >600 mm	No Significant Difference in Fork Length; T Test t = 1.337, p = 0.194
Skipjack Tuna	No Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 92.5, p = 0.499
Spanish Mackerel	No Significant Difference in Fork Length; Mann-Whitney Rank Sum W = 59, p = 0.524
Wahoo	No Significant Difference in Fork Length; T Test t = 0.203, p = 0.844

Table 10. Results of analyses comparing trophic level values (fork length and $\delta^{15}N$) and mercury, each species with the sample size (N), the statistical test used, and the results of that test.

Species	N	Relationship	Significance
Blackfin	41	Fork Length & Mercury	Highly Positive Significant; Kendall's tau = 0.802, p < 0.001
		Fork Length & $\delta^{15}N$	Positive Significant; Kendall's tau = 0.735, p < 0.001
		Mercury & $\delta^{15}N$	Positive Significant; Kendall's tau = 0.710, p < 0.001
Blackfin (<450 mm)	21	Fork Length & Mercury	Positive Significant; Pearson's cor = 0.629, p = 0.002
		Fork Length & $\delta^{15}N$	Slightly Positive Significant; Pearson's cor = 0.514, p = 0.018
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.432, p = 0.050
Blackfin (466-587 mm)	11	Fork Length & Mercury	Positive Significant; Pearson's cor = 0.645, p = 0.032
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = 0.581, p = 0.061
		Mercury & $\delta^{15}N$	Significant; Pearson's cor = 0.267, p = 0.428
Blackfin (>609 mm)	9	Fork Length & Mercury	Highly Positive Significant; Pearson's cor = 0.982, p < 0.001
		Fork Length & $\delta^{15}N$	Highly Positive Significant; Pearson's cor = 0.974, p < 0.001
		Mercury & $\delta^{15}N$	Highly Positive Significant; Pearson's cor = 0.944, p < 0.001
Cero Mackerel	5	Fork Length & Mercury	Not Significant; Pearson's cor = 0.840, p = 0.075
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = -0.546, p = 0.351
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = -0.670, p = 0.216
Crevalle Jack	5	Fork Length & Mercury	Not Significant; Spearman's rho = -0.2, p = 0.783
		Fork Length & $\delta^{15}N$	Not Significant; Spearman's rho = 0.1, p = 0.519
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = -0.545, p = 0.342
Common Dolphinfish	38	Fork Length & Mercury	Positive Significant; Pearson's cor = 0.781, p = 0.001
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = 0.278, p = 0.09
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.288, p = 0.80
	14	Fork Length & Mercury	Not Significant; Spearman's rho = 0.473, p = 0.09

Common Dolphinfish (<599 mm)		Fork Length & $\delta^{15}N$	Slightly Positive Significant; Spearman's rho = 0.6, p = 0.026
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.155, p = 0.60
Common Dolphinfish (600-700 mm)	8	Fork Length & Mercury	Not Significant; Pearson's cor = 0.241, p = 0.56
		Fork Length & $\delta^{15}N$	Not Significant; Spearman's rho = 0.595, p = 0.13
		Mercury & $\delta^{15}N$	Not Significant; Spearman's rho = 0.333, p = 0.428).
Common Dolphinfish (>700 mm)	16	Fork Length & Mercury	Not Significant; Spearman's rho = 0.418, p = 0.11
		Fork Length & $\delta^{15}N$	Not Significant; Spearman's rho = 0.59, p = 0.83
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.30, p = 0.26
King Mackerel	62	Fork Length & Mercury	Positive Significant; Pearson's cor = 0.790, p < 0.001
		Fork Length & N	Not Significant; Pearson's cor = 0.235, p = 0.07
		Mercury & N	Not Significant; Pearson's cor = -0.01, p = 0.94
King Mackerel (<900 mm)	21	Fork Length & Mercury	Slightly Positive Significant; Pearson's cor = 0.597, p = 0.004
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = -0.200, p = 0.39
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.113, p = 0.625
King Mackerel (>900 mm)	41	Fork Length & Mercury	Slightly Positive Significant; Pearson's cor = 0.573, p < 0.001
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = -0.07 p = 0.65
		Mercury & $\delta^{15}N$	Slightly Negative Significant; Pearson's cor = -0.40, p = 0.01
Little Tunny	40	Fork Length & Mercury	Positive Significant; Kendall's tau = 0.78, p < 0.001
		Fork Length & $\delta^{15}N$	Slightly Positive Significant; Kendall's tau = 0.50, p < 0.001
		Mercury & $\delta^{15}N$	Slightly Positive Significant; Kendall's tau = 0.59, p < 0.001
Little Tunny (<400 mm)	15	Fork Length & Mercury	Slightly Positive Significant; Pearson's cor = 0.56, p = 0.03
		Fork Length & $\delta^{15}N$	Slightly Negative Signficant; Pearson's cor = -0.58, p = 0.02
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = -0.36 p = 1.18
Little Tunny (400-600 mm)	15	Fork Length & Mercury	Positive Significant; Spearman's rho = 0.74, p < 0.001
		Fork Length & $\delta^{15}N$	Not Significant; Spearman's rho = 0.49, p = 0.07
		Mercury & $\delta^{15}N$	Positive Significant; Pearson's cor = 0.71, p < 0.001

Little Tunny (>630 mm)	10	Fork Length & Mercury	Positive Significant; Pearson's cor = 0.75, p = 0.01
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = 0.23, p = 0.52
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.26, p = 0.48
Skipjack Tuna	6	Fork Length & Mercury	Not Significant; Spearman's rho = 0.49, p = 0.36
		Fork Length & $\delta^{15}N$	Not Significant; Spearman's rho = 0.09, p = 0.92
		Mercury & $\delta^{15}N$	Not Significant; Spearman's rho = 0.83, p = 0.06
Spanish Mackerel	4	Fork Length & Mercury	Not Significant; Pearson's cor = -0.33, p = 0.67
		Fork Length & $\delta^{15}N$	Not Significant; Pearson's cor = -0.13, p = 0.87
		Mercury & $\delta^{15}N$	Not Significant; Pearson's cor = 0.39, p = 0.61
Wahoo	6	Fork Length & Mercury	Highly Positive Significant; Pearson's cor = 0.97, p < 0.001
		Fork Length & $\delta^{15}N$	Highly Positive Significant; Pearson's cor = 0.97, p < 0.001
		Mercury & $\delta^{15}N$	Highly Positive Significant; Pearson's cor = 0.94, p < 0.001

Discussion

Stable Isotopes and Trophic Level

When comparing the $\delta'^{13}C$ and $\delta^{15}N$ between the 2010 and 2020 data sets, King Mackerel were significantly depleted in both stable isotope ratios. The reduction in the smaller fork length set may explain the reduced $\delta^{15}N$ (Revill et al., 2019), but it would not explain the depletion shift seen in $\delta'^{13}C$. As depleted $\delta'^{13}C$ is more frequently seen in species feeding in more pelagic habitats (Miller et al., 2008), sampling location may explain the depleted $\delta'^{13}C$ values in the 2020 data set. Over half (54 of 73) of the King Mackerel sampled in the 2020 data set were collected between West Palm Beach and Fort Lauderdale, which is the smallest portion of the continental shelf and has the steepest slope opening to pelagic waters along the coast, roughly 5 kilometers or less. The samples collected in the 2010 data set were all from Cape Canaveral or Miami, where the continental shelf is roughly 20 kilometers from shore near Miami and over 50 kilometers from shore near Cape Canaveral. This same reasoning may be able to explain the significant depletion of $\delta'^{13}C$ in Little Tunny and Skipjack Tuna from the 2020 dataset, as these two species were sampled from the same region as King Mackerel.

All nine of these species are ranked in similar trophic levels. There is some variation between the ranked trophic levels of Blackfin Tuna, Skipjack Tuna, and King Mackerel, yet their $\delta'^{13}C$ ratios are similar, indicating similar habitats but different prey species. This is supported by previous work (Moore, 2014) indicating that while these three species may all rely more on offshore food sources, prey selectivity plays a role in trophic level (Miller et al., 2010). Blackfin and Skipjack Tuna school to target smaller prey items while this is less common in King Mackerel, who preferentially target larger ray finned prey items (Moore, 2014). King Mackerel's prey selection on larger teleost species is enabled by their adapted teeth and mandible, which allow this species to combine bite pressure with the speed at which they attack prey to target larger and faster prey items (Ferguson et al., 2015). Blackfin Tuna and Skipjack Tuna have also been reported to have higher feeding rates on crustaceans in pelagic waters along with high prey diversity, which are generally smaller and have lower trophic levels (Poland et al., 2019; Alatorre-Ramirez et al., 2017).

Common Dolphinfish are known as opportunistic feeders with great prey diversity and high growth rates, (Adams, 2009; Moore, 2014: Teffer et al., 2014) as supported by the lower

trophic level in this study. Common Dolphinfish typically feed on prey associated with floating structures (i.e. *Sargassum*) and their prey item size increases as the Common Dolphinfish fork length increases (Rudershausen et al., 2011). This relationship is important when considering the results of this study, because as Common Dolphinfish grow larger they rely less on floating structure prey and can move to target open water/pelagic prey items, which are larger and typically have higher trophic levels (Rudershausen et al., 2011). Species feeding in more pelagic waters have more depleted $\delta'^{13}C$, and $\delta'^{13}C$ in the 2020 dataset was less depleted for Common Dolphinfish than in the 2010 data set. This may be due to reduced size (on average 80 mm smaller) of the samples in 2020 or variation in sampling locations, but considering previous studies, this indicated smaller or younger Common Dolphinfish feeding in more nutrient enriched areas, such as closer to shore or in epipelagic regions, whereas larger Common Dolphinfish can expand to mesopelagic or more open water regions (Torres-Rojas et al., 2014). While there is no research yet, anglers at multiple tournaments and charter companies mentioned catching smaller Common Dolphinfish than in previous decades.

Despite all samples being juvenile, Crevalle Jack were second from the highest ranked in trophic level and third most enriched in $\delta'^{13}C$. Crevalle Jack juveniles that do come inshore, as not all do, have been shown to heavily utilize estuarine habitats, which is reflected here in the $\delta'^{13}C$ results (Gonzalez et al., 2021; Jefferson et al, 2022). All Crevalle Jack samples were caught within Port Everglades, which may explain the enriched stable isotope ratios and mercury, as Crevalle Jack was also third highest for total mercury in the tissue samples. Port everglades is a coastal region above the shelf and it has been recorded to have high mercury levels (White, 2021).

Trophic Level and Mercury Bioaccumulation

Overall, species with higher trophic levels, as established by $\delta^{15}N$, also had higher mercury. No comparisons could be made with Blackfin Tuna, Crevalle Jack, Spanish Mackerel, and Cero Mackerel between the years due to low or nonexistent sample sizes, but they could be included in general comparisons. King Mackerel and Little Tunny had highest trophic levels and mercury averages, followed by Blackfin Tuna and Skipjack. Common Dolphinfish trophic level was between Little Tunny and Blackfin Tuna, yet mercury level average for Common

Dolphinfish was lower than the Blackfin and Skipjack Tunas. This may be due to the fast growth rate and high prey diversity (Adams, 2009; Teffer et al., 2014). Crevalle Jack was also second highest for both mercury and trophic level in the 2020 data set. Wahoo had the lowest trophic level and second lowest mercury average, which may be due to smaller Wahoo in this study as compared to in other studies (Cai et al., 2007; Adams, 2010).

As compared to other studies, the results from this study are, in general, typical. Blackfin Tuna in this study, with an average 0.25 mg/kg mercury value, were comparable to samples taken in Brazil between 2009 and 2010, but lower than samples taken in the Florida Atlantic between 1999-2002, in comparable size ranges (Adams, 2004; Moura Reis Manhaes et al., 2020). Although the sample size was small, Crevalle Jack in this study were very comparable to other Crevalle Jack sampled in Everglades estuaries between 2006 and 2008, with this study having an average fork length of 328 mm and average 0.56 mg/kg mercury, and Crevalle Jack sampled in the Everglades having an average fork length of 329 and average 0.60 mg/kg mercury (Adams et al., 2018). Common Dolphinfish in this study, with an average of 0.9 mg/kg mercury, were comparable to those samples from Atlantic waters between 1995 and 2005 in the same size range, but higher than those sampled in the northern Gulf of Mexico between 2002 and 2003, where samples were on average 100 mm larger than this study, but had an average of 0.7 mg/kg mercury (Cai et al., 2007; Adams, 2009). Alternatively, a study conducted in the Bahamas between 2015 and 2016 showed a higher mercury average of 0.2 mg/kg for Common Dolphinfish as compared to this study, although the $\delta^{15}N$ in this study was an average 9.4‰ and the Bahamas study 9.7‰ (Shipley et al., 2019). However, only six samples in all the Common Dolphinfish samples in this study exceed 0.2 mg/kg, suggesting higher mercruy levels in Bahamian Common Dolphinfish, though fork length information was not available for further comparisons (Shipley et al., 2019). The Common Dolphinfish from the Bahamas had a slightly more depleted $\delta^{13}C$, with an average of -17.1‰, compared to -16.3‰ in this study, indicating a difference in habitat utilization between the two locations, with the species in this study potentially being closer to shore (Shipley et al., 2019). King Mackerel in this study, with an average of 1.23 mg/kg, were slightly higher than those sampled off the Florida Atlantic and Gulf of Mexico coasts between 1990 and 2002, with an approximate average of 1 mg/kg (Adams & McMichael, 2007). However, King Mackerel samples collected in the northeastern Gulf of Mexico between 2007 and 2010 displayed a very similar $\delta^{15}N$ average of 13.43‰, as compared

to an average of 13.3‰ in this study, but showed over double the amount of total mercury, with an average of 2.8 mg/kg (Thera & Rumbold, 2014). Comparing the $\delta^{13}C$ averages, with -18.3‰ from this study and -17.3‰ from samples taken in the Gulf of Mexico, indicates a different carbon source, with species in this study potentially utilizing more pelagic environments (Thera, 2011). Little Tunny in this study were comparable to those sampled off the Atlantic Coast of Florida between 1999 to 2002 (Adams, 2004). Juvenile Little Tunny (below 400 mm) in this study, with an average of 0.1 mg/kg mercury from the 2020 data set, had less total mercury than those sampled in the Atlantic Ocean off the coast of Brazil between 2009 and 2010 in the same size range (Moura Reis Manhaes et al., 2020). Skipjack Tuna mercury values were comparable to, if not just slightly higher, than those sampled in the Atlantic coast of Brazil between 2009 and 2010, with values ranging 0.2-0.4 mg/kg in this study and values ranging 0.1-0.3 mg/kg in Brazil in comparable size ranges (Moura Reis Manhaes et al., 2020). Wahoo mercury values in this study were comparable to Wahoo sampled from the Atlantic waters of Florida to the Bahamas between 1997 and 2006 in the same size range (Adams, 2010).

King Mackerel and Little Tunny ranked highest in total mercury; however, the average for King Mackerel total mercury was over double Little Tunny average, 1.3 mg/kg versus 0.54 mg/kg, respectively. The significant positive correlation between fork length and total mercury for King Mackerel species, in addition to their selective feeding strategy, most likely contribute to this (Adams & McMichael, 2007). This species is also one of the longest lived in this study and a bias for large, mature adults may have presented itself by using angler preference. Interestingly, while grouped together for habitat utilization ($\delta'^{13}C$), Skipjack Tuna and Blackfin Tuna are not assigned as high of trophic levels as King Mackerel and Little Tunny. Similarly, Skipjack Tuna and Blackfin Tuna have roughly half as much total mercury as Little Tunny do on average, yet fork lengths for the species are similar. Previous studies have also shown that Little Tunny have higher mercury values than Blackfin Tuna and reports on Skipjack Tuna mercury levels are lower than both Blackfin Tuna and Little Tunny in both this study and other studies (Adams, 2004; Cai et al., 2007; Torres et al., 2016). While prey selectivity has already been mentioned as a contributor for the difference between the Tunas and the King Mackerels for trophic level, the Little Tunny samples still have higher averages of total mercury than the other two tuna species, even though diets are similar (Moore, 2014). Little Tunny and King Mackerel share the most common prey items of fish species in families Clupeidae and Carangidae while

Skipjack Tuna and Blackfin Tuna share the most common prey items of cephalopods and crustaceans (Manooch et al., 1984; Godcharles & Murphy, 1986; Garcia & Posada, 2013; Alatorre-Ramirez et al., 2017; Poland et al., 2019). This doubling of total mercury may be explained by the longer lifespan of Little Tunny and more adult Little Tunny being sampled in this study.

Using the diet data provided from the 2010 data set did reveal a potential trend in prey diversity and mercury levels. Species with higher prey diversity tended to have lower mercury levels, with Common Dolphinfish having the greatest prey diversity (17 different prey taxa) and lowest mercury level average. King Mackerel (7 prey taxa) and Little Tunny (6 prey taxa) had the least prey diversity and highest mercury level averages. Little Tunny and King Mackerel share common prey items of higher level teleost fish species, including *Decapterus* sp. (e.g., scad *Decapterus punctatus*), *Caranx* sp. (e.g., blue runner *Caranx crysos*), and *Eucinostomus* sp. (e.g., mojarra *Eucinostomus argenteus*), which have higher mercury values than lower level teleost fish species, such as *Anchoa* sp. (e.g., bay anchovy *Anchoa mitchilli*) (Manooch et al, 1985; Godcharles & Murphy, 1986; Simons et al., 2013; Senn et al., 2010). Prey items sampled in the Gulf of Mexico between 2005 and 2006 showed that prey species in the family Carangidae (*Caranx crysos*) have well over double the mercury values as prey species in the family Clupeidae (*Anchoa mitchilli*) (Senn et al., 2010). Little Tunny, Blackfin Tuna, and Skipjack Tuna have some overlap in consuming prey items from family Clupeidae, but Skipjack and Blackfin Tuna typically have higher consumption of crustaceans (Poland et al., 2019; Alatorre-Ramirez et al., 2017; Manooch et al., 1985). Food web analysis in the Gulf of Mexico sampled prey items between 2007 and 2010 and identified, in general, that crustaceans had lower mercury values than most mid-to-high-trophic level fish species (Thera & Rumbold, 2013). Skipjack Tuna (7 prey taxa) had similar mercury level average as Blackfin Tuna (12 prey taxa), reinforcing the similar utilization of habitats and shorter lifespans, though there was no mercury for Blackfin Tuna to compare in 2010. This trend reflects the well-established relationship between prey and predator bioaccumulation in that species with prey items with high mercury levels will in turn have higher mercury values as well (Cabana & Rasmussen, 1994; Teffer et al., 2014).

Wahoo consistently did not fall into this pattern, with only six prey taxa and the second to lowest mercury level averages. Wahoo had a statistically lower sample size and sample fork length in the 2020 data set than in the 2010 data set and also had the highest standard deviation

for average fork lengths of all the species in this study both years. Average fork lengths of both time frames are still below average for most Wahoo in other studies, but mercury values for the average fork length of Wahoo in this study are comparable to the mercury values in another study on Wahoo from Florida and the Bahamas in the same size range (Adams, 2010).

All species with more than six samples were found to have a significant positive correlation between mercury and trophic level ($\delta^{15}N$). The total mercury averages between the two time periods overall and in individual species did not significantly change except in Little Tunny, Skipjack Tuna, and Spanish Mackerel. The reduction in Little Tunny total mercury average was most likely due to the smaller average size of the Little Tunny sampled in the 2020 dataset versus the 2010 dataset. The size reduction between Little Tunny in the 2020 data set was due to 15 juveniles being sampled in 2020 versus only two being sampled in 2010. When the juveniles were removed from the data sets, there is no significant difference between the mercury values in Little Tunny between the two time frames.

While these results do not necessarily reveal any new relationships and the mercury levels did not change significantly over the years, there is no discounting the value in creating datasets like the ones established in years 2010 and 2020. These results did not show a decrease in mercury between the years, but it also did not show an increase. This may indicate that mercury levels have stabilized in this region and supports the information available on the relationship between mercury and trophic level in coastal pelagic species. Perhaps ten years is not an adequate amount of time for these changes to trickle down into marine ecosystems, and additionally some of these species can live longer than a decade. Other studies with similar time frames or largely increased time frames show changes of both decreased and increased mercruy values in a wide array of species, speaking to the complexity of mercury in fish and marine environments (Grieb et al., 2020; Bank et al., 2021). As mercury is eliminated from muscle tissue extremely slowly, these species will continue to increase their already high total mercury levels until they die (Amlund et al., 2007). These trends are particularly important to follow for fish that are consumed, like these nine species in Florida.

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