FORAGING ECOLOGY OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN GALVESTON BAY, TEXAS

by

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Dedication

I would like to dedicate this research to my parents. They have supported my dream of researching dolphins ever since the first time I saw one as a child. They never discouraged my goals even though we lived in the panhandle of Texas and the nearest coastline was over 600 miles away.

Acknowledgements

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ABSTRACT

FORAGING ECOLOGY OF COMMON BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN GALVESTON BAY, TEXAS

Sherah Ann McDaniel
University of Houston-Clear Lake, 2022

Thesis Chair: George J. Guillen, Ph.D.

The overall goal of this research was to develop a better understanding of the trophic ecology of the Galveston Bay common bottlenose dolphin (*Tursiops truncatus*) stock that would provide critical data needed to manage this species. The specific objectives of this study were to: (1) estimate areas used by dolphins for foraging, (2) estimate factors contributing to foraging behaviors of dolphins, and (3) estimate proportions of different prey consumed by bottlenose dolphins in Galveston Bay. From 2015-2017, two survey methods (behavioral data from photo-identification surveys and stable isotope data from biopsy surveys) were used for objective one and two, while stable isotope ($\delta^{13}C$ and $\delta^{15}N$) mixing models were used for objective three. Dolphins were observed foraging for 62.3% of all photo-ID sightings. Dolphins were observed foraging most often in the channel (52.3%), followed by open bay (41.4%), and nearshore (6.3%) but there is no evidence that the odds of observing foraging behavior was different between the habitats.

For the sightings where foraging behavior was observed, 68.7% of the time a shrimp trawler was present. It is estimated that when approaching a trawler during a survey, the probability of observing dolphins patrolling is 60.8% of the time (95% CI: 55.6% to 100.0% , one-sided, one-sample porportion test, p-value <0.05). Foraging significantly decreased as time passed throughout the day in sightings from 2015-2017 (beta regression: pseudo $R^{2=}$ 0.8726, p-value <0.05). Potential prey of dolphins were collected in 2015 and 2016 for stable isotope analysis. Data from those sampling events and select nekton from Barcenas (2013) were used to model proportions of prey consumed by dolphins using a Bayesian isotope mixing model, Stable Isotope Analysis in R (MixSIAR version 3.1.10). Ward's hierarchical cluster analysis was used to group 19 nekton species into six groups based on their mean C and N isotopic values. Overall, group six which contained only one species, White Mullet (*Mugil curema*), was estimated to contribute to the highest proportion of nekton prey consumed by dolphins (median: 25.3%) based on MixSIAR analyses. The second highest proportion consumed by dolphins overall was group two (Atlantic Brief Squid [*Lolliguncula brevis*], Hardhead Catfish [*Ariopsis felis*], and Striped Mullet [*Mugil cephalus*]) at 21.0%. There was a significant difference between the δ^{15}N (‰) values in Upper Galveston Bay (UGB) and Lower Galveston Bay (LGB) (Wilcoxon Rank Sum Test, W=105.5, p-value≤0.05). This difference may suggest that dolphins in UGB and LGB are foraging on different prey or may support the notion that the upper portions of the bay are more heavily influenced of elevated anthropogenically produced δ^{15}N (‰). This research contributes to baseline data that can be used for further analysis in future studies. The results from the stable isotope analysis may be used in combination with mercury and organochlorine contaminant analysis to examine trophic level biomagnification in the Galveston Bay ecosystem.

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Introduction

Galveston Bay

The Galveston Bay (GB) ecosystem is the largest estuary in Texas and covers approximately 600 square miles (Lester & Gonzalez 2011). On average, Galveston Bay receives about 11 million acre-feet of freshwater from the Trinity River, San Jacinto River, and runoff from the rest of the watershed (Batchelor & Guthrie 2008). The Galveston Bay watershed covers approximately 24,000 square miles and extends up to the Dallas-Fort Worth metroplex (Lester & Gonzalez 2011). Runoff from the watershed is an important contributor of nutrients, organic matter, and contaminants into GB. It is estimated that freshwater inflow from the major drainages inputs more than 80% of carbon and nitrogen and 95% of the phosphorus to Galveston Bay (Lester & Gonzalez 2011).

Galveston Bay exchanges the majority of saltwater with the Gulf of Mexico at Bolivar Roads, San Luis Pass and prior to closing in 2020 and to a lesser extent, Rollover Pass (Lester & Gonzalez 2011, The Texas General Land Office 2021). The average depth in the Galveston Bay ecosystem is six to ten feet (1.8-3.0 m) excluding the dredged ship channels. The Houston Ship Channel (HSC) is dredged to approximately 45 ft (13.7 m) by the Army Corps of Engineers and extends 52 miles (83.6 km) from the juncture of the Texas City Channel to the turning basin in Houston. The HSC is 530 ft (161.5 m) wide with plans to widen it to 700 ft (213.4 m) by 2025 (Port Houston 2020). Bayport Ship Channel (BSC) and Barbour's Cut are 300-400 ft (91.4-121.9 m) wide with plans to widen them to 455 ft (138.8 m) (Port Houston 2020). Galveston Bay is commonly divided into four sub-bays: Galveston Bay, Trinity Bay, East Bay, and West Bay.

Galveston Bay can be further split into Upper Galveston Bay (UGB) and Lower Galveston Bay (LGB) with an artificial line drawn from Eagle Point from the west to Smith Point to the east (Lester & Gonzalez 2011).

The Galveston Bay ecosystem is comprised of typical estuarine habitats including wetlands, mud flats, submerged aquatic vegetation (SAV), oyster reefs, open bay bottom and open water. Historically, SAV was found in the nearshore habitats of UGB and in Trinity Bay but is now only present almost exclusively in West Bay and Christmas Bay (Pulich & White 1991, Lester & Gonzalez 2011). The rest of the open bay bottom is primarily bare mud apart from oyster reefs. Human alterations such as the dredging of channels, which increase the flow of saltwater into the bay, likely holds more influence on the circulation than natural processes (Wilber & Clarke 1998, Wilber & Clarke 2001, Steichen 2018).

Dolphin Research in Texas

Under the mandates of the Marine Mammal Protection Act of 1972 (MMPA), all marine mammal populations should not fall below their optimum sustainable levels (MMPA 16 USC §§ 1361-1423h). According to the MMPA an optimum sustainable population is defined as, "a population size which falls within a range from the population level of a given species or stock which is the largest supportable within the ecosystem to the population level that results in maximum net productivity" (MMPA 16 USC §§ 1361-1362). The National Marine Fisheries Service (NMFS) is tasked with providing an annual stock assessment report for all strategic stocks of marine mammals. To be considered a strategic stock, the level of direct human-caused mortality must exceed the potential biological removal (PBR) level. The PBR level defined by the MMPA is "the maximum number of animals, not including natural mortalities, that may be removed from a marine mammal stock while allowing that stock to reach or maintain

its optimum sustainable population" (MMPA 16 USC §§ 1361-1362). NMFS considers all stocks of common bottlenose dolphins (*Tursiops truncatus*) in the northern Gulf of Mexico's bays, sounds, and estuaries (BSE), except for the Sarasota Bay/Little Sarasota Bay Stock, to be strategic according to the 2018 assessment. The assessment determined a vast majority of stock sizes are unknown but are assumed to be small and therefore few mortalities would exceed PBR (NOAA 2019). The stock sizes in Galveston Bay were unknown or undetermined for management purposes due to outdated stock estimates that were several decades old at the inception of the current study. Abundance estimates based on data more than eight years old are not considered acceptable for management purposes under the MMPA and estimates more than five years old change the status of the stock assessment from adequate to inadequate under the NOAA Fisheries Stock Assessment Improvement Plan (Rosel et al. 2011). Based on these criteria, data available at the origination of this study for GB were extremely outdated and there was a need for further research to determine stock status (Hubard & Swartz 2002, Mullin et al. 2007, Balmer et al. 2013, Vollmer & Rosel 2013, Marine Mammal Commission 2015). Recently, Ronje et al. (2020) estimated the abundance of bottlenose dolphins in GB to be 842-1,132 based on surveys conducted in January and July of 2016.

Individual common bottlenose dolphins can be tracked long-term using markings that occur on their dorsal fins (Würsig & Würsig 1977). Markings can be natural in origin from conspecific interactions, such as biting, or caused by anthropogenic factors such as propeller strikes or fishing line entanglement. Data obtained from these markings, also termed nicks and notches, can be used to estimate population size using modified mark-recapture methods. However, fin markings may change, so other features like scarring and color patterns can play an important role in identifying individuals.

Common bottlenose dolphins (hereafter dolphins) are the only cetacean that enter Galveston Bay. A cetacean is from the order Cetacea and includes an entirely aquatic group of mammals commonly known as whales, dolphins, and porpoises (Mead 2020). Most dolphin research in Texas has been conducted in nearshore ocean coastline and in ocean to bay passes as opposed to the open bays. Boat-based and helicopter surveys were conducted in Galveston Bay and coastal waters of the Gulf of Mexico in 1990 (Henningsen & Würsig 1991). During their study a total of 1,002 different individuals were identified and 867 dolphins were only sighted once. During a different study in 1990 and 1991, boat-based, photo-identification (photo-ID) surveys were conducted in a northern section of coastal Texas and over 1,000 individuals were identified but because the majority of the dolphins were only sighted once, it was concluded that only about 200 use the area for extended periods of time (Bräger 1993). According to Fertl (1994), a seasonality peak in abundance occurs in spring and autumn based upon photo-ID surveys in the Galveston Ship Channel. From 1995 to 2001 West Bay was surveyed and it was determined a small community (28 to 34) of dolphins demonstrated evidence of site fidelity (Maze & Würsig 1999, Irwin & Würsig 2004, Irwin 2005).

Four unusual mortality events (UME) in Texas have been documented since 1992 (Fernandez & Hohn 1998, Colbert 1999, Fire et al. 2011, NOAA 2020). NMFS sponsored a live dolphin capture effort in response to a 1992 die-off from an undetermined cause in Matagorda and Espiritu bays and collected physiological information from 36 dolphins (Würsig & Lynn 1996, Lynn & Würsig 2002). UMEs that occurred from 1993 to 1994 were caused by an infectious disease. The cause of a UME during 2007 and 2008 remained undetermined. A UME which also occurred along the Texas coast in 2011 to 2012 was caused by biotoxins produced by harmful algal blooms. Foundational knowledge of the dolphins involved in these UMEs gathered from long

term tracking studies, such as where they spend most of their time, would have been useful to understand the cause of the UMEs.

Several natural and anthropogenic risks exist for dolphins in Galveston Bay. Hurricanes and heavy rainfall influence the salinity in the bay and prolonged low salinities have been documented to have negative effects on dolphins including skin lesions (Fazioli & Mintzer 2020), immune deficiencies, and mortality (Toms et al. 2021). Galveston Bay is a highly trafficked estuary. Port Houston ranks highest in the nation for total foreign and domestic waterborne tonnage, 70% of which is liquid bulk (Port Houston 2021). Several collisions have occurred between tankers or ships resulting in the release of chemicals on board. Dolphins do not avoid areas where chemical spills have occurred (Henningsen & Würsig 1992, Smultea & Würsig 1995). Dolphins in Barataria Bay, LA suffered from petroleum hydrocarbon exposure and toxicity following the *Deepwater Horizon* oil spill affecting dolphin's adrenal hormones, lungs, survivability, and fecundity (Schwacke et al. 2014, Schwacke et al. 2021).

The Environmental Institute of Houston (EIH) at the University of Houston-Clear Lake (UHCL) partnered with Galveston Bay Foundation (GBF) in 2014 to form the Galveston Bay Dolphin Research Program (GDRP) and started conducting regular photo-ID surveys in upper Galveston Bay to further extend the limited knowledge of the Galveston Bay dolphin population. Since then, over 950 individual dolphins have been identified and about 192 exhibit year-round or seasonal site fidelity to the upper-western portion of Galveston Bay (K. Fazioli, personal communication, April 24, 2022). Based on data collected to date, the program has found that dolphin encounter rates increase in the upper portions of the bay during warm months where water temperatures are typically >23°C (May-October) with a decline in cooler months

where water temperature is typically <23°C (November-April) (Fazioli et al. 2017, Mintzer & Fazioli 2021).

Foraging

One of the most important factors affecting bottlenose dolphin movement patterns is the spatial and temporal distribution of prey resources (Shane et al. 1986, Hanson & Defran 1993, Hart 1998, Browning et al. 2014b), therefore determining the foraging ecology of these animals is crucial to understanding their life history. Data regarding the feeding ecology of dolphins throughout Galveston Bay was deficient at the beginning of this study. Most of the previous research was conducted in the lower portions of Galveston Bay and the Galveston Ship Channel (GSC) (Henningsen & Würsig 1992, Fertl 1994, Moreno 2005, Piwetz 2019).

Various dolphin foraging behaviors exist around the world which can be population or site specific (Sargeant et al. 2005). Connor (2001) postulated that the variations in foraging behaviors are due to ecological variations. Since calves stay with their mother for years, it is likely that they are socially learning foraging behaviors between generations (vertical culture) (Whitehead et al. 2004). The behavior can also be ascertained by other conspecifics (horizontal culture). The definition of culture to which this paper refers is, “Information or behavior shared by a population or subpopulation-which is acquired from conspecifics through some form of social learning” (Rendell & Whitehead 2001). Variations in foraging specializations can be a product of different habitat types, different prey availability, and vertical and horizontal culture transmission (Berens McCabe et al. 2010).

In the genus *Tursiops* foraging specializations have been observed that can occur in association with bottom substrate, in the water column, on the beach, and in the air. In the northwestern Bahamas, Rossbach and Herzing (1997) observed common bottlenose

dolphins crater feeding where craters are left in the sand after the dolphin has dug into the sand (sometimes burying itself up to its pectoral fins) seeking buried prey. A specialized form of crater feeding using sponges has been documented in Shark Bay, Australia where dolphins carry sponges on their rostrums to likely prevent abrasions on their rostrums during bottom grubbing (Smolker et al. 1997). A behavior seen with cetaceans around the world is fluke-out dives. This behavior is exhibited when dolphins dive down and their flukes come out of the water, assumingly to feed on prey located in the water column at deeper depths (Acevedo-Gutiérrez & Parker 2000). Kerplunking is the act of the peduncle being lifted high out of the water before bringing the flukes down into the water creating a high vertical splash that is estimated to startle prey hiding in seagrass beds (Nowacek 1999, Connor 2001). Beaching behavior has been observed on a steep beach at the tip of the Peron Peninsula, Australia where dolphins surge partially or fully out of the water and onto the beach in order to catch fish (Berggren 1995, Sargeant et al. 2005). These animals are foraging individually and not as a social unit. In contrast, strand-feeding occurs in South Carolina and Georgia and differs from beaching in that a group (range 1-6 but most frequently 4 individuals) of dolphins cooperatively swim in unison and create a surge wave to strand the fish onto the mud banks before feeding upon them (Duffy-Echevarria et al. 2008). Dolphin mud plume feeding has been documented since 1999 in the lower Florida Keys (Lewis & Schroeder 2003). During this behavior mud plumes were created by a single animal and then that animal would lunge through the plume (Lewis & Schroeder 2003). Individuals created their own plumes but multiple groups (consisting of 1-10 individuals) were as close as 20 to 100 m of each other engaging in the same behavior (Lewis & Schroeder 2003). Begging is a behavior where dolphins elicit food from a human such as head out of the water and opening mouth at the surface and is commonly observed in Sarasota, Florida (McHugh 2015). Fish whacking

has been observed in Sarasota Bay, Florida and also in Galveston Bay. Fish whacking is when a dolphin is side-swimming and quickly and forcefully strikes a fish with a dorsal or ventral thrust with its flukes (Wells et al. 1987, Nowacek 1999). Pinwheeling is also a behavior seen in Sarasota, Florida (Nowacek 2002) and Galveston Bay and is performed by an individual tucking its head and spinning in side-swim orientation, rotating around the midpoint of the body to herd fish (Leatherwood 1975, Nowacek 2002). Another dolphin foraging behavior observed in Galveston Bay since the 1990s is following fishing trawlers (Henningsen & Würsig 1991, Bräger 1993, Fertl 1994), with reports of this association dating back to the 1930s in other areas of Texas (Gunter 1942). Trawler foraging (patrolling) is a foraging technique that includes following behind the deployed nets of a shrimp trawler presumably feeding on nekton stirred up from the nets (Leatherwood 1975), nekton escaping through the turtle exclusion device (TED), directly getting into the net by lifting the lead line and feeding on discarded bycatch thrown overboard by the shrimper. Furthermore, the trawlers can concentrate the prey in one area, likely making it easier to catch. This behavior has been observed in Galveston Bay since the 1990s (Henningsen & Würsig 1991, Bräger 1993, Fertl 1994). Trawler foraging has also been documented off of the Atlantic coast of Florida, Baja California, the Mississippi Sound (Leatherwood 1975), and Moreton Bay, Australia (Chilvers & Corkeron 2001). In Moreton Bay, both sexes and all age groups of dolphins (*Tursiops aduncus*) engage in trawler foraging (Chilvers & Corkeron 2001). Sympatric conspecifics are also present and do not associate with trawlers (Chilvers & Corkeron 2001). Chilvers and Corkeron (2001) identified 242 dolphins that had been seen eight or more times, of which 154 animals were trawler dolphins and 88 animals were non-trawler dolphins.

Previous studies have shown bottlenose dolphins around the world exhibit a generalist foraging strategy consuming a wide variety of prey, including demersal and

pelagic fish species, cephalopods, and crustaceans (Norris 1961, Gaskin 1982, Shane et al. 1986, Barros & Odell 1990, Barros & Wells 1998, Barros et al. 2000, Santos et al. 2001). Since bottlenose dolphins have been shown to consume a wide variety of prey, they have often been called opportunistic feeders (Leatherwood 1975, Gaskin 1982, Shane et al. 1986); however, some studies have shown that certain populations exhibit selective feeding (Corkeron et al. 1990, Santos et al. 2001, Berens McCabe et al. 2010). Dolphins that spend most of their time offshore or in open coastal habitats are exposed to different prey species, such as cephalopods, while BSE dolphins typically depend more heavily on fish as prey (Berens McCabe et al. 2010). Barros and Wells (1998) found that between 1984-1996, Pinfish *(Lagodon rhomboides)* accounted for nearly 70% of all prey items ingested in stranded dolphins near Sarasota, Florida. Berens McCabe et al. (2010) analyzed stomach contents from 15 dolphins near Sarasota, Florida from 1996-2006 and found that the most abundant species preyed upon was Gulf Toadfish (*Opsanus beta)* which represented 34.8% of the total prey ingested, followed by Pinfish, Ladyfish (*Elops saurus*), and Spotted Seatrout (*Cynoscion nebulosus)* at 9.4%, 7.9%, and 7.5%, respectfully. Barros (1993) suggested that passive listening for soniferous fishes may be an important strategy for prey detection. This was supported by the Berens McCabe et al. (2010) study which found soniferous fishes made up 6.3% of available prey, while they were 51.9% of total prey consumed.

The feeding rate of dolphins is difficult to estimate but data is available from captive dolphins, dead specimens from the wild, and live captured dolphins. Dolphins in captivity were fed 8-15 kg of fish per day throughout 3-5 feeding sessions (Wells et al. 2013). Gunter (1942) captured an adult dolphin with about 40 pounds of fish in its stomach. Female dolphins do not eat more while pregnant but double their consumption during lactation (Kastelein et al. 2002). Twenty-nine recently dead bottlenose dolphin

stomachs were analyzed from Aransas Bay and St. Charles Bay, Texas from 1939-1941 (Gunter 1942). Over 83% of the fishes were Striped Mullet (*Mugil cephalus*) (Gunter 1942). The next most abundant fish was the Gizzard Shad (*Dorosoma cepedianum*) which made up about 8% of the stomachs (Gunter 1942).

Dolphin and Prey Movements

Abiotic variables such as temperature, salinity, depth, and distance from shore may be correlated with dolphin distribution: however, it is likely due to prey distribution (Torres et al. 2008). Upper portions of estuaries are warmer than offshore waters in the summer and cooler in the winter (Cowan Jr. et al. 2012). In subtropical estuaries such as Galveston Bay, the rainy season may be critical for immigration of juvenile fishes, freshwater discharge, and pulses of primary productivity (Cowan Jr. et al. 2012).

Bräger (1993) observed a diurnal feeding pattern of dolphins during the summer in Galveston Bay and Gulf of Mexico, with most feeding occurring in the morning and another small peak in the afternoon. In the winter, more observations of foraging were recorded throughout the day with less traveling and socializing (Bräger 1993). This increase in feeding throughout the day was also observed in Florida when temperatures declined from 26.7-31.7ºC to 15.6-20.6ºC in the fall (Shane 1990, Bräger 1993). Dolphins may have higher energy requirements when the water temperature decreases (Bräger 1993). There is also likely less prey abundance in the cooler months based on many nekton life histories (Steichen 2018). Many species of nekton move about the estuary based on changes in salinity (Cowan Jr. et al. 2012). A myriad of estuarine species move offshore to spawn at various times of the year, presumably because the eggs are buoyant in saltwater and are intolerant to large changes in salinity (Cowan Jr. et al. 2012).

Multiple species of finfish previously documented as dolphin prey utilize Texas estuaries and nearshore coastal waters including Striped Mullet, White Mullet (*Mugil curema*), Sand Seatrout, and Southern Flounder (*Paralichthys lethostigma*). Striped Mullet will often leave the estuaries in autumn (Spiller 1982, TPWD 2015a), presumably to move to warmer waters where they can spawn and avoid cold winter temperatures (Wallace 1975, Whitfield et al. 2012). Some adults return to estuaries following spawning (Whitfield et al. 1978, Funicelli et al. 1989, Ditty & Shaw 1996) and others may remain within the marine environment (Whitfield et al. 2012). Striped Mullet spawn from late October to December along the Texas coast (Sheridan 1983, TPWD 2015a) and larvae become abundant in northern Gulf of Mexico between November and December (Ditty & Shaw 1996) in water temperatures between 23-25°C (Hill 2004). Chang et al. (2004) reported mullet in higher frequencies offshore as they matured. Adult Striped Mullet can survive in freshwater (0 ppt) to hypersaline conditions as high as 90 ppt (Lee & Menu 1981). They can tolerate temperatures from 6°C-33°C (Apekin & Vilenskaya 1978, Marais 1978, Whitfield et al. 2012). The average adult standard length of Striped Mullet is 50cm (Thomson & Luther 1990). White Mullet also migrate offshore beyond the outer continental shelf to spawn, primarily from April to September (Ditty & Shaw 1996).

Sand Seatrout (*Cynoscion arenarius*) spawning occurs March-September with peaks in March and April and August and September in the lower estuary and shallow coast (7-15m depth) (Ditty et al. 1991). Larvae drift into upper estuaries and prefer shallow marshes and channels during their early life stages moving to deeper areas as they mature (Conner & Truesdale 1973, Moffett et al. 1979, Benson 1982, Ditty et al. 1991). Early life stages are found over soft bottom (Conner & Truesdale 1973) while adults are found over most substrates in estuaries and offshore (Sutter & McIlwain 1987).

Trent et al. (1968) suggested that the distribution of Sand Seatrout is more dependent on temperature than salinity. Sand Seatrout tolerate a wide range of temperatures (6°C-37°C) but prefer temperatures of 20-24°C or greater (Simmons & Hoese 1959, Roessler & Zieman 1970, Copeland & Bechtel 1974, Gallaway 1978, Moffett et al. 1979). They are observed to tolerate a wide range of salinities (0-45 ppt), but larvae and juveniles are more tolerant in lower salinities (<15 ppt) (Gunter 1945, Benson 1982, Warren & Sutter 1982, Ditty et al. 1991).

Adult Southern Flounder concentrate at Bolivar Roads during the fall migration (November to January) from Galveston Bay as they move from the bay to spawn in the Gulf of Mexico (Spiller 1982). Dolphins have been seen fish tossing flounder in fall months as far back as the 1970s (Shane 1977, personal observation).

Both subadult and juvenile white shrimp (*Litopenaeus setiferus*) and brown shrimp (*Farfantepenaeus aztecus*) move from shallow seagrass beds, tidal creeks and wetlands as they grow into deeper open bay waters and then move offshore to spawn (TPWD 2002a). However, these two species emigrate to the Gulf at different times of the year (TPWD 2002a). Brown shrimp exit the estuary from May through August with peaks from May to July while white shrimp exit the estuary from September through December (TPWD 2002a). Decreasing water temperatures in the fall are the main driving force for white shrimp emigrating to the Gulf (TPWD 2002a).

Seasonal movements of dolphins in UGB coincide with Southern Flounder, Striped Mullet, and white shrimp emigration from the bay to spawn offshore (Spiller 1982, TPWD 2002b, TPWD 2015a, Mintzer & Fazioli 2021). Dolphins in Galveston Bay have been observed fish tossing American Gizzard Shad, Striped Mullet, Southern Flounder and anecdotal communications with fishermen on shrimp trawlers have reported Atlantic Cutlassfish to be a favorite prey from bycatch thrown back from the trawlers.

Shane (1977) reported observing dolphins tossing Atlantic Cutlassfish exclusively in summer months.

Objectives

The overall goal of this research was to develop a better understanding of the trophic ecology of the Galveston Bay common bottlenose dolphin stock that will provide critical data needed to manage this species. The specific objectives of this study were to: (1) estimate areas used by dolphins for foraging and (2) estimate factors contributing to foraging habits of dolphins in Galveston Bay. It is impossible to determine the effects of environmental disturbances such as chemical spills, UMEs, or major freshwater influxes without development of prior baseline data on these critical life history attributes.

Methods

The following methods involving dolphin research were performed under the University of Houston-Clear Lake Institutional Animal Care and Use (IACUC) Protocol #F14.005 SEFSC permit #14450, NMFS permit #18881 and (Appendix A and B).

Study Area

This research was part of the GDRP's long-term population study where the main study area in Upper Galveston Bay was attempted to be covered every month by photo-id surveys (Figure 1). Other areas of the Galveston Bay ecosystem were surveyed when time and weather conditions allowed.

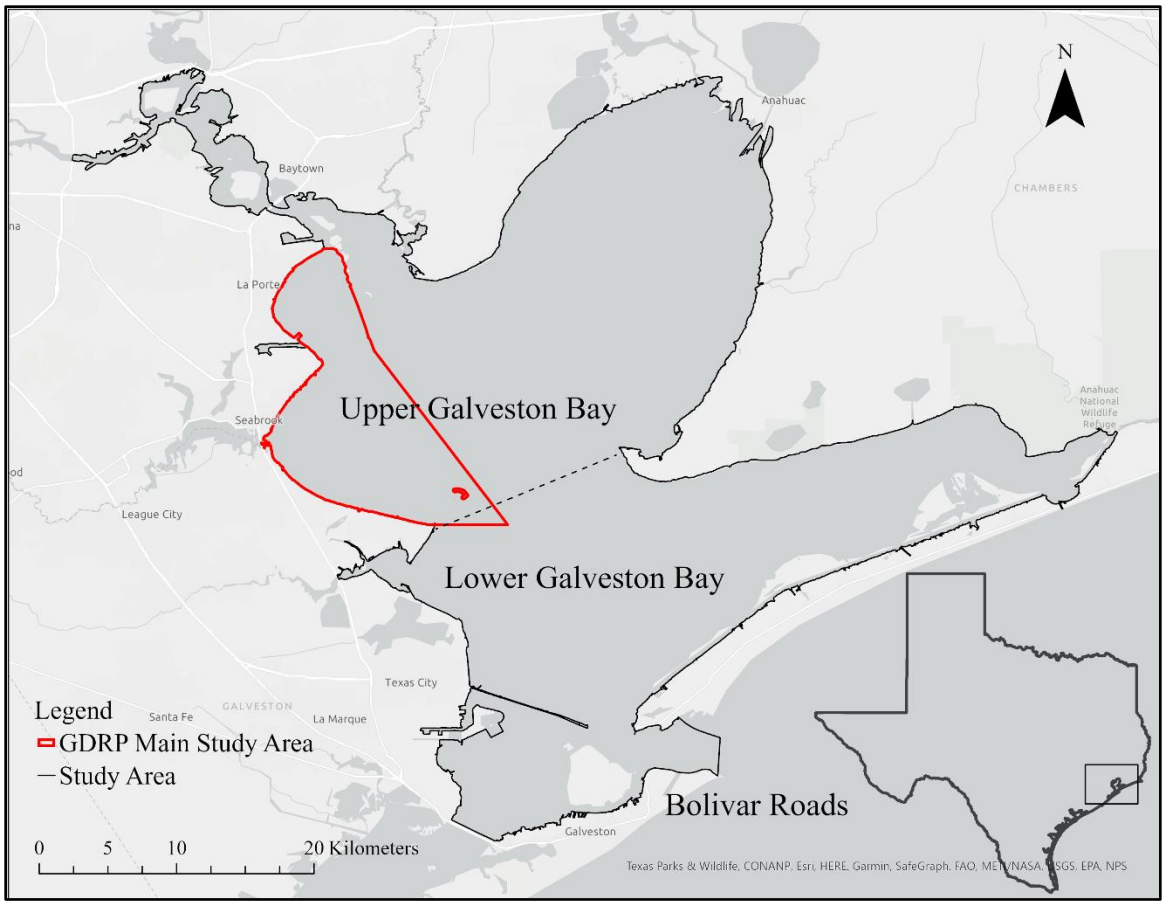

Figure 1:

Map of study area in Galveston Bay, TX. The red outline is the main study area for the Galveston Bay Dolphin Research Program (GDRP); however, surveys were conducted throughout the rest of the Galveston Bay, inshore of the Intracoastal Waterway.

Habitat Categories

Galveston Bay was split into three habitat categories: nearshore, open bay, and dredged channels based on general trends in bathymetry. Nearshore was considered the shoreline extending 500 m out where the depth generally slopes down to approximately 6 ft (1.8 m). A buffer of 300 m was applied to the edges of the Houston Ship Channel (HSC), and this was considered the channel habitat where the depth averaged about 13.7 m. A buffer of 200 m was applied on the edges of the Bayport Ship Channel (BSC) and a

buffer of 400 m was applied on the Texas City Channel. Open Bay was considered all areas between nearshore and channel where the average depth is 1.8 to 3.0 m excluding Red Fish Island which was defined as nearshore habitat where a 100 m buffer was applied based on bathymetry.

Sampling Design: Photo-ID Surveys

Boat-based, photo-ID population surveys were conducted following standardized procedures (Sarasota Dolphin Research Program 2006, Melancon et al. 2011, Rosel et al. 2011, Urian et al. 2015). Surveys were conducted on three vessels including a 22 ft (6.7 m) catamaran-style vessel (Twin Vee), a 25 ft (7.6 m) v-hull Boston Whaler, or on rare occasions, a 22 ft (6.7 m) v-hulled JH Performance. Boat crews were comprised of at least three people (a boat operator, a port observer, and a starboard observer), who were all actively searching for dolphins while on effort (Rosel et al. 2011). The vessel traveled at a speed between 11.5-20 mph (18.5-32 km/hr) along a transect with a randomized starting direction towards a randomized habitat type (nearshore, open bay, or channel) until dolphins were observed initiating the start of a sighting. A sighting is an observation of a single dolphin or group of dolphins interacting with one another, moving in the same general direction, or engaging in the same activity, usually within 100 m of each other. Once groups were encountered, the vessel was slowed to match the speed of the dolphins and the vessel moved parallel to the group within ~6-20 m to capture photographs using a digital camera with a zoom-telephoto lens (150-300 mm) of dorsal fins of individual dolphins for identification. While the photographer attempted to obtain photos of all the dorsal fins, the other observers helped keep track of dolphins and recorded data. The coordinates of the beginning and end of the sighting was recorded via GPS. Overall sighting conditions for detecting dolphins were determined using the combined attributes of lighting (cloud cover), sea state and glare. Cardinal directions were recorded for the

initial, general, and final heading of the dolphins. Dolphin activities were noted and ranked by prevalence during the sighting. Dolphin behavior indicative of probable feeding activity were swirling, fish tossing, following a shrimp trawler (patrolling), fluke out dives, or chasing fish, described as quick and variable direction of movement. Feeding activity was recorded when a fish was observed in the mouth of a dolphin. Herein, the term "foraging" will refer to the observation of either probable feeding or feeding activity. Human activity occurring within 100m of a dolphin group was recorded, along with any observed interactions. Human interactions of interest in this study were classified as patrolling, scavenging, and probable scavenging. Patrolling is defined as, "following, milling, or traveling within 20 m of boats, lines, or piers." The most common observation of patrolling was behind shrimp trawlers. Scavenging is, "observed feeding on bait or catch throwback, when the fisherman did not intend to feed the dolphin." Probable scavenging is when indications of scavenging behavior was observed without confirmation of feeding. The total number of dolphins and composition of the group (adults, calves and young of year) was estimated.

Surface (~0.3 m deep) water temperature (°C) and salinity (psu) were recorded for each sighting using a YSI model multiparameter probe (sonde). If sightings were in close proximity with little time passed between the end of a previous sighting and the start of the next sighting (typically less than an hour), then water quality from the previous sighting or environmental point would be used. Once all the data and photographs were obtained for a sighting, the vessel continued the survey until another group of dolphins were encountered and the process was repeated. Additional environmental points were sampled at predetermined locations. Water quality parameters taken at environmental survey points included Secchi depth (m), water temperature (°C), salinity (psu), pH, and dissolved oxygen (% and mg/L) at three profile depths (0.3 m

above the bottom, middle of water column, and 0.3 m from the surface). Tides were recorded using the nearest NOAA buoy for sightings and environmental points. If an active trawler was encountered along a survey route and dolphins were not observed, the location of the trawler was noted along with its coordinates. An active trawler was defined as a trawler that is preparing to put nets in the water, pulling nets in the water, pulling up nets, processing catch or throwing bycatch overboard.

Lab Methods

Survey data was entered into the FinBase Photo-Identification Database System (Adams et al. 2006). Photographs from the field were sorted and established photo-identification methods as described in Sarasota Dolphin Research Program (2006), Urian et al. (2015), and Rosel et al. (2011) were used to grade photos for quality and fins for distinctiveness. A catalog of all individuals were stored in FinBase, and fins were matched to previously identified dolphins. If a dolphin had not been previously observed or if it was not successfully matched, a new catalog number was created and assigned to that individual. All identifications were verified by a second qualified photo-identification researcher.

Data Analysis

A one-sided, one-sample, proportion test was conducted in R (version 3.6.3) to test the probability that when a shrimp trawler was approached, dolphins were observed following the trawler more than 50% of the time (R Core Team 2021). A significance level of $\alpha = 0.05$ was used for all statistical analyses.

An odds ratio test was used to determine if the proportion of dolphins foraging was different between each habitat (channel, open bay, and nearshore). The odds ratio defined for each group used the formula (Ramsey & Schafer 2013):

$$odds\ ratio = \omega_2/\omega_1 \quad (1)$$

where ω_2 was the odds of foraging for group 2 and ω_1 was the odds of foraging for group 1. For example, let ω_1 be the odds of foraging in channel habitat = 100 foraging / 59 not foraging or 1.69. For every dolphin observed not feeding an estimated 1.69 dolphins are feeding. Let ω_2 be the odds of foraging near shore = 12 foraging / 5 not foraging or 2.4. The odds ratio of ω_2/ω_1 = 1.42. The log of the odds ratio then can be used with the corresponding estimated standard error to calculate the z-statistic which can be compared against a standard normal distribution (Ramsey & Schafer 2013). The standard error is calculated:

$$SE[log(\omega_1/\omega_2) = \sqrt{(\frac{1}{n_1\pi_c(1-\pi_c)} + \frac{1}{n_2\pi_c(1-\pi_c)})} \quad (2)$$

The proportion of foraging behavior was calculated for each hour (0700-1500) of the survey day for each sighting and fitted with a beta regression model (betareg) in R (Ferrari & Cribari-Neto 2004, Cribari-Neto & Zeileis 2010). The beta regression analysis provides a robust alternative to simple linear regression when data are restricted between 0 and 1 (i.e., proportional data where the data cannot take the values of 0 or 1). The hour data were transformed to account for the inherent cyclical pattern of time with the equation:

$$sin\ (2\pi\left(\frac{time}{24}\right)) \quad (3)$$

Tide stages (low, rise, high, low) were compared to frequency (%) of foraging behavior observed for each sighting.

Results

Photo-ID Surveys: Habitat Analysis

From 2015 to 2017 a total of 464.9 hours were spent on the water during 75 photo-ID surveys covering 6,684.5 km (Table 1). During the 75 photo-ID surveys, 303 sightings were recorded, and 2,604 dolphins were observed. Dolphins were observed

probable feeding or feeding (foraging) for 62.3% of the sightings (Figure 2). Of the sightings of dolphins foraging, 52.3% were observed in the channel followed by 41.4% in open bay, and 6.3% nearshore. Although dolphins were seen most often foraging in the channel, we failed to detect a statistically significant difference of the odds of observing foraging behavior between habitats (Table 2). Each tide stage was evaluated for the proportion of foraging behavior observed for each sighting (Figure 3). Foraging occurred 58.8% of sightings during low tide, 61.0% during a rising tide, 64.6% during high tide, and 65.3% during a falling tide.

Table 1:

Photo-ID survey summary from 2015-2017.

Year	**# of Surveys**	**# of Sightings**	**Total Distance (km)**	**Total Hours**
2015	21	73	1623.5	118.0
2016	26	99	2542.4	164.3
2017	28	131	2518.6	182.7
Total	75	303	6684.5	464.9

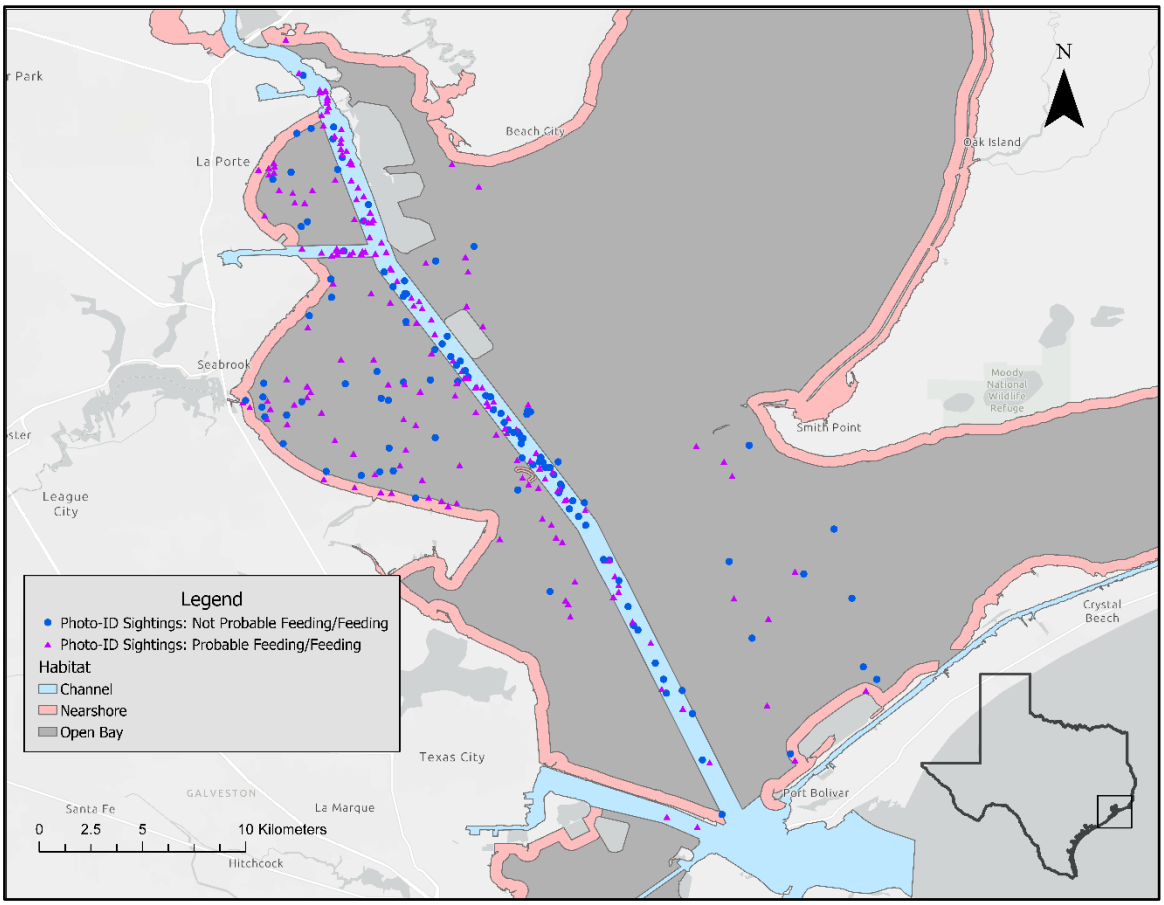

Figure 2:

Map of all dolphin photo-ID sightings from 2015-2017. Blue circles depict dolphins not observed probable feeding/not feeding and purple triangles depict dolphins observed probable feeding/feeding (62.3% of all sightings).

Table 2:

Odds ratio of observing dolphins foraging between each habitat.

Habitat Comparison	Odds Ratio	Lower 95% CI	Upper 95% CI	z-statistic	p-value
Channel to Open Bay	1.03	0.64	1.67	0.12	0.90
Nearshore to Channel	1.42	0.24	2.10	0.66	0.51
Nearshore to Open Bay	1.46	0.48	4.39	0.70	0.48

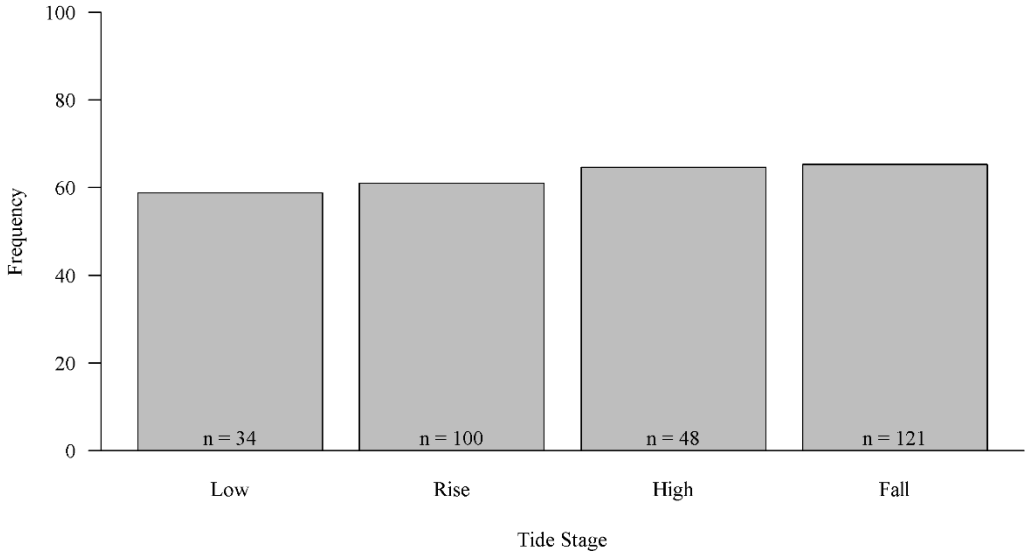

Figure 3:

Frequency (%) of foraging behavior observed during each tide stage for sightings 2015-2017.

Shrimp Trawler Association

For the sightings where foraging behavior was observed, 68.7% of the time a trawler was present. All dolphins that have been seen nine or more times (n=200) to date have been associated with a shrimp trawler at least one time. It is estimated that when approaching a shrimp trawler during a survey, the probability of observing dolphins patrolling is 60.8% (this includes biopsy surveys i.e., not randomized transects; see Chapter 2) of the time (95% CI: 55.6% to 100.0% , one-sided, one-sample, proportion test, p-value <0.05). There were only three sightings in which a trawler was present within 100 m of a dolphin group and no interaction was observed. However, it is unknown if the dolphins from those sightings were interacting with the trawler prior to the start of the sighting. Of all photo-ID sightings, a trawler was present 42.9% of the time. Of all sightings during biopsy surveys (Chapter 2), a trawler was present 55.4% of the time. Trawlers were present for 46.8% of all sightings including biopsy and photo-ID surveys.

Diel Foraging Behavior

Foraging significantly decreased as time passed throughout the day in sightings during 2015-2017 (beta regression: pseudo $R^{2=}$ 0.8726, p-value <0.05) (Figure 4). Foraging was observed in 91% of the sightings from 0700 to 0759 (n=11) and was observed in 69% of the sightings from 0800 to 0859 (n=42). From 0900 to 0959, foraging behavior increased to 81% (n=42). From 1000 on, foraging decreased to 30% by 1559. Three sightings occurred in the 1600 hour but foraging was not observed and was excluded from the beta regression analysis.

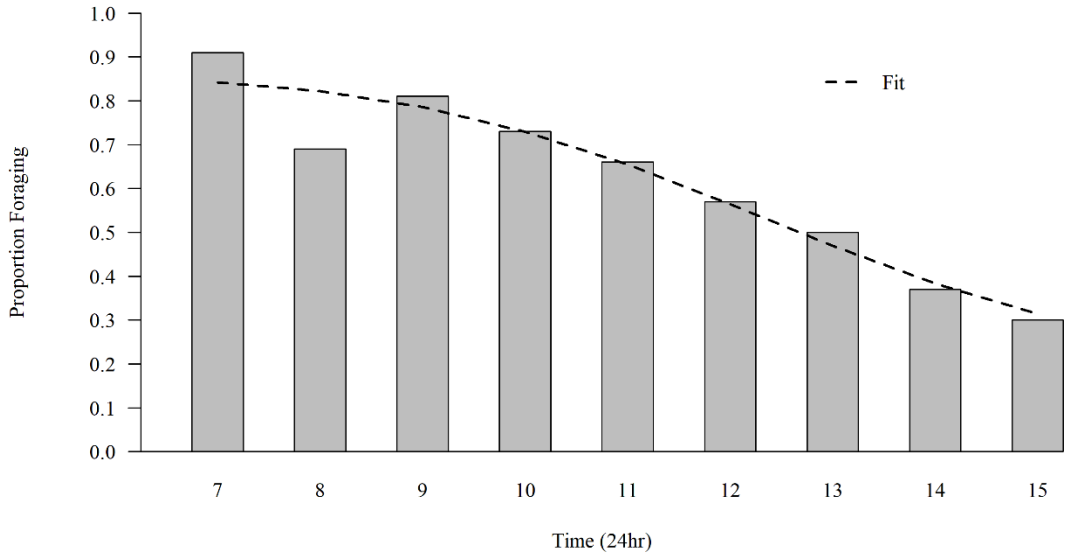

Figure 4:

Proportion of dolphins foraging from 0700 to 1500 for all photo-ID surveys from 2015-2017 with a fitted beta line. Pseudo $R^{2=}$ 0.8726, p-value <0.05

Discussion

Dolphins were observed foraging individually and in groups, and with and without shrimp trawlers. Dolphins were observed foraging during 62.3% of the sightings. This finding is similar to previous observations of overall dolphin foraging (57%) in Lower Galveston Bay (Moreno 2005, Piwetz 2019). During the current study for the sightings where foraging behavior was observed, 68.7% of the time a trawler was also present. Piwetz (2019) found foraging associated with trawlers 52% of the time in the GSC. Similarly, Fertl (1994) reported dolphins with trawlers 54% of all observations in the GSC from 1990-1992. Henningsen and Würsig (1991) observed 42% of all sighted dolphins behind trawlers during a seven-month study in Galveston Bay and nearshore Gulf of Mexico.

Chilvers and Corkeron (2001) were able to distinguish two distinct sympatric communities based on trawler associations as a proxy. The non-trawler community relied heavily on sea grass habitats for foraging. Chilvers and Corkeron (2001) suggest trawling activities are an important factor for their habitat requirements, but dolphins are capable of foraging in the absence of trawlers. The current study encountered 200 dolphins that were seen nine or more times and found that they all have been associated with a shrimp trawler at least once. Based on low numbers of sighting histories (i.e., less than nine sightings) there is not enough evidence to show that there are dolphins in Galveston Bay that do not associate with trawlers and more data is needed.

Piwetz (2019) found that dolphins were more likely to be foraging near trawlers in the morning and more likely to be foraging in the afternoon in areas without trawlers in the GSC. During the current study, the frequency of foraging during sightings declined throughout the day (0700-1600). The number of sightings also decreased as time passed. Trends in the number of individuals per sighting were not evaluated during this study. Although the number of sightings decreased, this does not imply that the number of dolphins observed decreased throughout the day. Dolphins were frequently observed in large groups primarily socializing in the afternoon throughout this study, which concurs with Piwetz (2019) finding of increased social behavior and decreased foraging behavior later in the day.

Shrimp trawler bag limits, seasons, and fishing durations are regulated by TPWD. Prior to September 1, 2015, commercial bait-shrimp boat bag limits were regulated using two seasons: August 15 to March 31 fishing was permitted 30 minutes before sunrise to 30 minutes after sunset with a 200-pound bag limit and April 1 to August 14 when fishing was permitted 30 minutes before sunrise to 2pm (TPWD 2014). As of September 1, 2015, trawling is allowed 30 minutes before sunrise to 30 minutes after sunset year-

round with a 200-pound limit for commercial bait-shrimp boats in major bays and bait-bays (TPWD 2015b, TPWD 2016). For the sightings where foraging behavior was observed, 68.7% of the time a trawler was also present. Since dolphin foraging behavior was highly prevalent when trawlers were present, perhaps, the number of trawlers fishing throughout the bay declined as they met their weight limit. In addition, during 2015, the time limit for trawling from April 1 to August 14 occurred earlier in the day at 1400. Dolphins in Sarasota Bay, Florida forage throughout the day and peaks are observed in the morning and late afternoon (Wells et al. 2013). Individuals from the Sarasota Bay dolphin population were often found interacting with recreational boats (begging, patrolling, provisioning, depredating lines, scavenging) (Powell & Wells 2011). These behaviors with recreational boats are not commonly reported in Galveston Bay and perhaps recreational boat interactions are higher in Sarasota Bay due to a lack of commercial trawlers within the bay system and the higher number of recreational boats in Sarasota Bay. More research would be necessary to test this hypothesis.

Dolphins were observed foraging most often in the channel (52.3%), followed by open bay (41.4%), and nearshore (6.3%). Moreno (2005) reported higher densities of dolphins in deeper waters in Lower Galveston Bay. Although dolphins were most observed foraging in the channel, a statistically significant difference was failed to be detected in the odds of observing foraging behavior between the habitats. The detection rates in different habitat types may vary for a variety of reasons such as the depth, the different areas of each habitat type, and dolphin behavior. This limitation should be explored further.

Past studies in Sarasota Bay, Florida have found that when sea grass beds are present as a habitat, it is preferentially used as a foraging habitat over other areas (Barros & Wells 1998, Wilson et al. 2017). With the lack of extensive sea grass beds in most of

the Galveston Bay ecosystem, dolphins may instead take advantage of easy prey sources such as high concentrations of nekton associated with shrimp trawlers.

CHAPTER II:

STABLE ISOTOPE ANALYSIS

Introduction

Stable Isotopes

Stable isotopes of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) are commonly used in estuarine ecology to aid in identifying the primary source of carbon incorporated into an organism and estimating trophic position (Fry 2006). Isotopes are the same element consisting of the same number of protons, yet a different number of neutrons. Isotopes change in a predictable manner as elements cycle through the biosphere (Peterson & Fry 1987). The ratio of the heavy isotope to the light isotope for a consumer is influenced by the organic matter that it consumes. Isotopic fractionation occurs because the lighter isotope usually proceeds slightly faster than the heavier isotope in reactions (Hagy III & Kemp 2012). Isotope ratios are calculated as a deviation from a standard reference material using a ratio of the heavy and light isotope and are expressed in delta (δ) notation and have units of per mil (‰).

Stable isotope analysis (SIA) is a commonly used method for understanding the trophic ecology of various species of animals including mammals (Fry 2006). Specifically, SIA has been used around the world to determine marine mammal community structure (Barros et al. 2010, Kiszka et al. 2011, Browning et al. 2014b), foraging habitat (Fernández et al. 2011, Rossman et al. 2013), and feeding ecology (Ramsay & Hobson 1991, Ames et al. 1996, Walker et al. 1999, Davenport & Bax 2002, Kurle & Worthy 2002, Yamamuro et al. 2004, Lusseau & Wing 2006, Reich & Worthy 2006, Knoff et al. 2008, Alves-Stanley & Worthy 2009, Kiszka et al. 2010a, Newsome et al. 2010, Worthy & Browning 2011, Wilson et al. 2013b).

Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ are the most used isotopes to study foraging ecology such as trophic status and habitat use. Isotopic signatures from the consumer and food source are needed to construct potential trophic links. The $\delta^{13}C$ value provides information on the type of ecosystem or trophic pathway the prey came from (e.g., terrestrial vs. aquatic). Whereas the $\delta^{15}N$ value provides information regarding what trophic level the predator and prey inhabit. Nearshore production from attached algae and detritus results in a higher (enriched) $\delta^{13}C$ value while pelagic production from phytoplankton results in a lower (depleted) $\delta^{13}C$ value (France 1995, Post 2002, Fry 2006). An increased (enriched) $\delta^{15}N$ value is indicative of higher trophic levels. Also, artificially elevated $\delta^{15}N$ values can occur in urban estuaries where nitrogen is discharged from anthropogenic sources such as wastewater effluent (Fry 2006, Barcenas 2013). Nitrogen pathways in upper Galveston Bay are dominated by anthropogenic sources from the San Jacinto and Trinity Rivers (Barcenas 2013). The next bay most affected by anthropogenic nitrogen loading was Trinity, followed by East, and West Bays (Barcenas 2013).

Terrestrial and freshwater organic material are more ^{13}C depleted ($\delta\,^{13}C$= -28‰) while marine phytoplankton is more ^{13}C enriched ($\delta\,^{13}C$= -21‰) (Hagy III & Kemp 2012). The most enriched ^{13}C come from seagrasses and marsh plants ($\delta\,^{13}C$= -13‰ to -10‰) (Hagy III & Kemp 2012). Estuarine plants such as *Spartina spp.* can have a mixture of carbon sources from the terrestrial derived organic matter and may have the same $\delta\,^{13}C$ as marine phytoplankton (Hagy III & Kemp 2012). Evaluating stable isotopes of sulfur ($\delta\,^{32}S$ and $\delta\,^{34}S$) concurrently with $\delta\,^{13}C$ has proven useful in further differentiating primary producer food webs supporting upper trophic level consumers (Hagy III & Kemp 2012).

Many studies have used stranded (deceased) marine mammals opportunistically to determine diets and possible trophic relationships (Davenport & Bax 2002, Borrell et al. 2006, Knoff et al. 2008, Barros et al. 2010, Fernández et al. 2011, Worthy & Worthy 2011, Rossman et al. 2013, Browning et al. 2014b). However, the use of stranded animals exclusively can be problematic for several reasons. Gut contents from stranded animals can provide biased and limited information since it is based on what diseased or injured individuals are capable of capturing and eating prior to death. Using tissue samples from stranded animals may also generate biased estimates of population levels of stable isotope composition since injured or sick dolphins may have ceased feeding or shifted their feeding behavior towards habitats containing easier to catch prey or towards easier to capture prey within a specific habitat. Also, stranded animals that are not part of a population that is being studied provide only limited information about their life history. However, the isotopic signatures do not change over time in deceased animals (Payo-Payo et al. 2013). Payo-Payo et al. (2013) did not find any statistical differences in stable isotope signatures from deceased striped dolphins (*Stenella coeruleoalba*) tissues sampled over various increments during a 62-day study. Although the isotopic signatures do not change over time, caution should be taken when conducting SIA on dead individuals because they may provide a biased estimate of the trophic relationships of healthy dolphins. However, the use of SIA on deceased animals can complement stomach content data and observational feeding in long-term studies.

Prey

In order to characterize the trophic ecology and diet of bottlenose dolphins, it is necessary to obtain data on potential prey items. Barcenas (2013) collected extensive data on the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of potential prey of dolphins within Galveston Bay. Barcenas (2013) collected $\delta^{13}C$ and $\delta^{15}N$ values for 49 different species from 32

families totaling 623 samples in 90 different sites in the Galveston Bay ecosystem. Recently published data indicated diet-tissue discrimination factors (differences in the prey and consumer) of 1.01±0.37‰ (mean±sd) for $\delta^{13}C$ and 1.57±0.52‰ (mean±sd) for $\delta^{15}N$ are appropriate to use for bottlenose dolphins (Giménez et al. 2016). Similarly, Browning et al. (2014a) suggests diet-tissue discrimination factors of 1‰ for $\delta^{13}C$ and 1.5‰ for $\delta^{15}N$ as opposed to 3‰ commonly used for $\delta^{15}N$ values. Isotopic turnover rates of 2-3 weeks for bottlenose dolphins suggest that there is potential for assessing recent feeding ecology and habitat usage in wild populations (Browning et al. 2014a, Browning et al. 2014b). Half-life turnover rates for captive bottlenose dolphins suggests 24.16±8.19 days for carbon and 47.63±19 days for nitrogen (Giménez et al. 2016).

Data collected by Texas Parks and Wildlife Department (TPWD) over a 23-year period (1992-2015) found 30 species of juvenile to subadult life stages accounted for 97% of total catch with a bag seine within 15.2 m of shore. The top ten species were Gulf Menhaden (*Brevoortia patronus*), white shrimp, brown shrimp, Florida grass shrimp (*Palaemonetes sp.*), Atlantic Croaker (*Micropogonias undulatus*), Spot (*Leiostomus xanthurus*), Bay Anchovy (*Anchoa mitchilli*), Pinfish, White Mullet, and Inland Silverside (*Menidia beryllina*). Gulf Menhaden made up 47% of the total catch from their study period (Steichen 2018). Seasonal cyclical trends were evident, with the highest number of individuals captured (catch per unit effort = CPUE) in the summer (June-August), decreasing as the year progressed, with the lowest CPUE in winter (December-February). The CPUE started to increase in the spring (March-May) (Steichen 2018). During winter and spring Gulf Menhaden and Atlantic Croaker exhibited the highest relative abundance. Gulf Menhaden were the most abundant species in the spring and white shrimp were the most abundant species in the fall throughout the Galveston Bay ecosystem. Based on the TPWD bag seine data, changes in the biotic composition were

driven by seasonal patterns and did not appear to respond to extreme events such as droughts or floods.

Some of the most diverse of the benthic nekton are the croakers, mullets, stingrays, shrimps, and crabs which eat benthic invertebrates (Cowan Jr. et al. 2012). Mobile predators such as the sea trouts (*Cynoscion* sp.) feed on more motile prey such as pelagic fishes and penaeid shrimps and are loosely associated with the bottom (Cowan Jr. et al. 2012). These type of fishes or secondary consumers are not limited to a certain habitat and are widely distributed throughout the estuary (Cowan Jr. et al. 2012). Marine catfishes (Ariidae) are omnivorous benthic foragers often feeding on many different types of prey (Yåñez-Arancibia et al. 1988). Anecdotal evidence of dolphins eating just the bodies of sea catfish and leaving the heads, dorsal and pectoral fins dates back to 1942 (Gunter 1942). Ronje et al. (2017) documented eight individual dolphins in the Mississippi Sound, Mississippi, Pensacola Bay, Florida, St. Joseph Bay, Florida and Sarasota, Florida associated with severed catfish heads. Hardhead Catfish (*Ariopsis felis*) were also observed in dolphin's mouths (Ronje et al. 2017). Records of trauma and death of dolphins have been attributed to fish spines in the Gulf of Mexico, including four instances in Texas (Ronje et al. 2017). Catfish otoliths occur in relatively low abundance in dolphin stomachs, and it may be that the decapitation of catfishes may be underestimating their consumption (Ronje et al. 2017).

In one study in Sarasota Bay, Florida, Striped Mullet was among the largest prey consumed, but overall contribution of biomass was second to Pinfish, (and similar to Pigfish, *Orthopristis chrysoptera*) due to their low numbers in stomachs of stranded dolphins (Barros & Wells 1998). On the East Coast of Florida in the Indian River Lagoon, Striped Mullet ranked third in prey species consumed in terms of importance by frequency of occurrence and total numbers taken (Barros 1993). Average standard

lengths for prey consumed by dolphins in Sarasota Bay ranged from 69 mm to 640 mm with most prey measuring generally within 124 mm to 168 mm (Barros & Wells 1998).

Dolphin Biopsy Methods

Over the past 25 years, scientists from the Northeast Fisheries Science Center have collected thousands of biopsy samples using a variety of methods from wild cetaceans (Wenzel et al. 2010). The processes that are used to collect skin tissue from live animals include biopsy poles (Bilgmann et al. 2007), modified 0.22 caliber rifles (Krützen et al. 2002, Kiszka et al. 2010a, Browning et al. 2014b), capture-release efforts (Rossman et al. 2013, Browning et al. 2014b), exfoliation (Lusseau & Wing 2006), ex-situ sampling (Browning et al. 2014a), and crossbows with modified darts (Weller et al. 1997, Gorgone et al. 2008, Kiszka et al. 2011).

The use of the biopsy pole is limited to bow-riding dolphins (Bilgmann et al. 2007). Bilgmann et al. (2007) found that the biopsy pole system resulted in mild behavioral responses when hit or missed by the biopsy pole. This mild behavioral response was described as the individual accelerating under water and leaving the bow (Bilgmann et al. 2007). Dolphins in Galveston Bay bow-ride on large ships and barges. At the beginning of this study, it was assumed dolphins would not bow-ride on a small vessel travelling at 2-6 knots and therefore this method was not utilized. Taking exfoliated skin samples also requires bow-riding dolphins and so this method was not an option.

Capture-release efforts cause stress to cetaceans because the handling process is prolonged and the risk for injury is higher. Capture-release methods also require many skilled professionals and was not a feasible option at the beginning of this study. Since this study focused on wild common bottlenose dolphins any type of ex-situ sampling was not a viable option.

The International Whaling Commission has found that remote biopsy darting is an acceptable method of obtaining tissue samples based on lack of evidence of chronic negative effects on sampled small and large cetaceans (International Whaling Commission 1991). The method that was used to obtain skin samples for this study was a remote biopsy darting technique using a crossbow and modified bolt that has been demonstrated to be safe technique with only limited short-term behavioral responses and minor transient injury to the animal (Weller et al. 1997, Krützen et al. 2002, Noren & Mocklin 2012, Tezanos-Pinto & Baker 2012). Gorgone et al. (2008) found that this type of sampling had minimal behavioral effects on the target and non-target animal(s) that were within close proximity to the sampled dolphin. Of the thousands of remote biopsy samples taken from cetaceans, only one case of mortality has been reported (Bearzi 2000). In this case, the dart hit in the desired location, but the wound did not appear to be the direct cause of death. The dolphin's blubber was thin and the dart penetrated the muscle layer. Bearzi (2000) postulated that death was induced by vagal shock with ceased breathing leading to heart failure. Since this is the only reported case of death related to remote biopsy darting, it is anomalous.

Objectives

The objectives of this study were to: (1) estimate areas used for foraging, (2) estimate factors contributing to foraging behaviors, and (3) estimate proportions of different prey consumed by common bottlenose dolphins in Galveston Bay.

Methods

Sampling Design: Dolphin Biopsy Surveys

Remote biopsy sampling was conducted following standardized protocols developed by the National Institute of Standards and Technology (NIST) and NOAA (Wenzel et al. 2010, NOAA 2014). A Barnett BCR crossbow, *CETA-DART*®

aluminum/carbon fiber bolts, and sterilized stainless steel tip cutter heads developed by Dr. Finn Larsen were used to obtain biopsy samples (Figure 5 and 6). The custom cutter heads are stainless steel measuring 10 mm wide x 25 mm deep and are built with three retention barbs affixed internally to obtain maximum sampling retention (Figure 6). The custom designed bolts are built with a high-pressure polyethylene flotation molded on the forward portion of the bolt. A GoPro camera was fixed to the forward shaft of the crossbow to record all shots and to attempt to record the behavior following the shot. Ready to use sample kits were provided by the NIST. Personnel sampling with the crossbow completed training with experienced samplers from the Chicago Zoological Society-Sarasota Dolphin Research Program (CZS-SDRP) in Sarasota, Florida and in Galveston Bay. Biopsy surveys were conducted similar to photo-id surveys, but routes were not randomized, rather directed with intent to find as many dolphin groups as possible within targeted regions of the bay. Dolphins were sought in areas with the highest encounter probability, such as following shrimp trawlers. Attempts were made to sample dolphins throughout various locations in Upper Galveston Bay (UGB), Trinity Bay, East Bay, and Lower Galveston Bay (LGB). Biopsy darting was also attempted in West Bay, but it was very difficult to get a sample due to the low number of dolphins encountered and high number of mom/calf pairs. Irwin and Würsig (2004) estimated dolphin abundance in West Bay to be 28 to 38, including nonresident individuals.

Figure 5:

Photo of crossbow and modified dart used to take remote biopsy samples of dolphins.

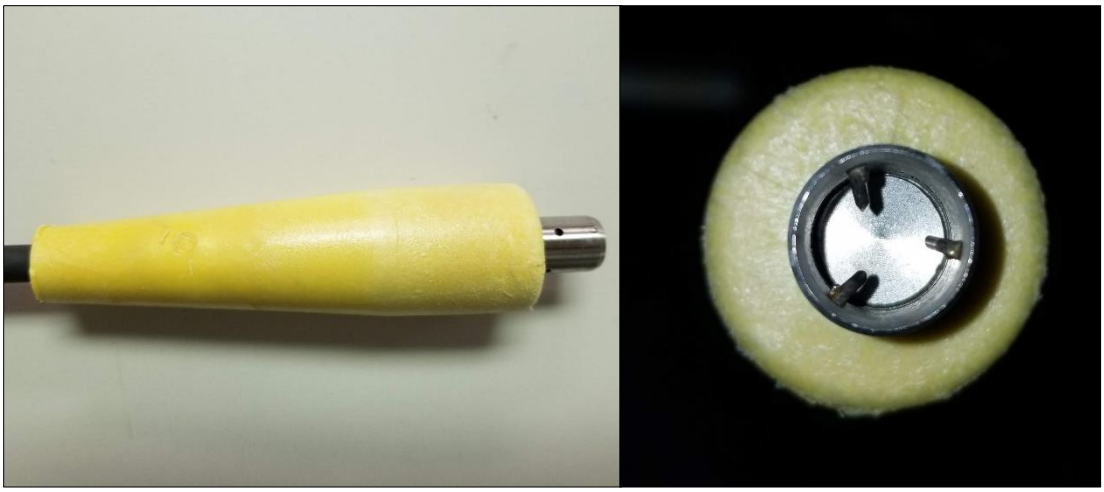

Figure 6:

Left: Close up of the flotation device and 10x25mm stainless steel sampling tip. Right: The three retention barbs inside of the sampling tip.

The biopsy crew consisted of at least a coxswain (boat operator), an arbalester (crossbow operator), a photographer, and when available, a data recorder. If a fourth person was unavailable, then the datasheet would be filled out by one of the other three crew members after the shot was taken. When a dolphin or group was sighted, the coxswain slowed the vessel down to match the speed of the dolphin. The photographer determined if a dolphin was suitable for sampling. If the animal had been previously biopsied it was not targeted to be biopsied again. Other circumstances that eliminated a dolphin from being sampled were animals that looked emaciated or ill and dolphins that displayed evasive behaviors. Evasive behaviors defined by NOAA are prolonged diving, underwater exhalation, underwater course changes, or rapid swimming at the surface. Only adults were targeted for sampling. A group that contained a calf that is known or estimated to be less than two years of age (<50-75% of the presumed mother's body length) (Urian & Wells 1996) or adults that had an animal surfacing in echelon (adjacent

to and slightly behind) position were also avoided. If the individual dolphin was deemed a good candidate, the arbalester would attempt to collect a biopsy sample from the region of the body immediately below the dorsal fin (Gorgone et al. 2008) and above the lateral mid-line (Figure 7). The sample attempt was only taken when the arbalester was perpendicular to the target animal. Biopsy darts were not reused in the field if the shot resulted in a sample or if the dart hit the water or hit the animal without collecting a sample. In most cases, the photographer took pictures of the dorsal fin of the target dolphin in addition to the dart impact. The bolt was immediately retrieved from the water. The data recorder documented the spatial location using a GPS and completed the biopsy datasheet. The group behavior (pre-biopsy and post-biopsy) was recorded in addition to the estimated distance from boat (Wenzel et al. 2010, NOAA 2014).

Figure 7:

Photo taken right after dart sampled dolphin tissue below the dorsal fin and above the lateral midline.

A full sample included skin and blubber and weighed approximately 0.8 g. Although this study focused on the stable isotope analysis, the sample was subsampled into pieces to be archived and used for genetics (gender determination and genomic DNA), toxicogenomics, histopathology and immunohistochemistry, persistent organic pollutants, and mercury analysis for other projects (Figure 8). The photographer took a photo of the sample with a scalebar to document sample length (mm). The sample was divided into the epidermis layer and blubber layer using sterile forceps. The epidermis layer was sectioned additionally, and a small piece of skin was placed in cryogenic vial (Cryovial®) for stable isotope analysis. The blubber was further divided for other analyses. The stable isotope sample was placed in a liquid nitrogen dry shipper while on

Figure 8:

Photo of sample split into sections for different analyses. The smallest piece of skin was used for stable isotope analysis.

the sampling vessel. Upon returning from the field, the samples were moved to a -80°C freezer prior to analysis.

During sample processing, the other crew members completed the biopsy datasheet and watched the target animal to record post-biopsy behaviors. After the sample was processed and all datasheets were completed, another biopsy attempt may have been attempted in the same group if the dolphins were still approachable. No more than three sampling attempts were made from each group and no more than two samples were collected from individuals of the same group.

In addition to the remote biopsy darting of live dolphins, four samples were obtained from stranded (deceased) dolphins under a NMFS parts authorization and in collaboration with the Texas Marine Mammal Stranding Network (TMMSN). Samples

were transferred to UHCL and stored in a -80°C freezer until all samples were ready to be analyzed.

Fish Sampling

All fish were collected according to IACUC (#11.001.R1) protocols (Appendix C). Fish sampling was conducted throughout Galveston Bay in June and August of 2015 and in August of 2016 for potential dolphin prey. In 2015, fish were opportunistically taken during sampling for the National Coastal Condition Assessment (NCCA), a project contracted to EIH by the Texas Commission on Environmental Quality and the United States Environmental Protection Agency. Sampling sites were chosen using a Generalized Random Tessellation Stratified survey design (USEPA Office of Water and Office of Research and Development 2015). Two additional sites were added to the August 2016 sampling to get a more representative sample throughout Galveston Bay.

Fish were collected from a 22 ft (6.7 m) catamaran-style vessel using an otter trawl with an opening of approximately 3.1 m by 0.3 m and a stretch mesh size of 38.2 mm. One to four tows were pulled for 5-15 minutes each at a speed of about 2-3 kts. Three specimens of Atlantic Croaker, Sand Seatrout, and Spot were targeted at each site. If those three species were not captured, then other species were kept. Fishes were put in a Ziploc bag and placed on ice and upon arrival to the lab transferred to a -80°C freezer. When the fish were processed, they were weighed (grams) and standard length (mm) was measured. Scales were removed (if applicable) with a scalpel and epaxial muscle was taken using a biopsy punch. The muscle was placed in a Cryovial® and placed back into the -80°C freezer.

Lab Methods

Genetic analysis of dolphin skin samples was conducted by NOAA Fisheries (Southeast Fisheries Science Center). Dolphin and fish samples were freeze-dried

(Labconco Inc. Model #7750020), sealed in Cryovials® and sent to the Stable Isotope Geosciences Facility at Texas A&M University (College Station, TX) to be ground, weighed, and analyzed by mass spectrometry. A Thermo Scientific Delta^{plus}XP isotope ratio mass spectrometer with Carlo Erba NA 1500 Elemental Analyzer was used for stable isotope analysis. Standard reference materials that were used included carbon from Vienna Pee Dee Belemnite (V-PDB) and atmospheric nitrogen gas. Data are expressed as per mil (‰) using delta (δ) notation based on the equation:

$$\delta X = (\left(\frac{R_{sample}}{R_{standard}}\right) - 1) * 1000 \qquad (4)$$

where "X" is ^{15}N or ^{13}C and "R" is ^{15}N/^{14}N or ^{13}C/^{12}C. A ±1σ instrumental uncertainty of ±0.2‰ for both isotopes was reported by the lab. Many studies use lipid extracted tissues because high lipid content decreases the signal for δ^{13}C. However, lipid extraction alters the apparent δ^{15}N in tissue. Therefore, Wilson et al. (2013a) recommended against lipid extraction if C:N ratios were <4.5 for dolphins. All dolphin sample C:N ratios were <4.5 and therefore lipid extraction was not conducted.

Data Analysis

Data were statistically analyzed using R (version 3.6.3). A significance level of α=0.05 was used for all statistical analyses. Prior to statistical analysis a Shapiro-Wilk test was used to check for normality for dolphin isotope samples. A Wilcoxon Rank Sum Test was used to determine if there were differences between δ^{13}C (‰) and δ^{15}N (‰) values of the stranded (deceased) dolphin samples and live dolphin samples. A Kruskal-Wallis Rank Sum Test was used to determine if there were differences between δ^{13}C (‰) and δ^{15}N (‰) values by year (2015, 2016, 2017) or by habitat (nearshore, open bay, channel). If differences were detected a Pairwise Wilcoxon Rank Sum Test was used to determine which variable differed. A Wilcoxon Rank Sum Test was also used to

determine if there were differences between $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values between sexes and by location (UGB and LGB).

MixSIAR

Mixing models use a formula that utilizes isotope values from the consumer and potential prey (Phillips 2012). A Bayesian isotope mixing model, Stable Isotope Analysis in R (MixSIAR version 3.1.10), was used to estimate proportions of dolphin prey consumption (Stock & Semmens 2016). MixSIAR is an open-source R package that users create and run Bayesian mixing models to analyze biological tracer data (e.g., stable isotopes). MixSIAR uses a model fitting algorithm called Markov Chain Monte Carlo (MCMC) that repeatedly estimates values of proportions of prey (Parnell et al. 2010, Phillips et al. 2014). The new estimates produced from the algorithm are compared to old estimates and values that are not similar are discarded (Parnell et al. 2010, Phillips et al. 2014). The similar values create a Markov chain and as the number and length of MCMC chains is increased, the chains will converge on the true posterior distribution for each variable (Stock & Semmens 2016). MCMC estimate a posterior distribution for each variable and the mean, median, standard deviation, and Bayesian credible intervals can be calculated (Stock & Semmens 2016). Two diagnostic tests are produced with MixSIAR results: the Gelman-Rubin and Geweke (Stock & Semmens 2016). The Gelman-Rubin convergence diagnostic should be <1.05 (Gelman & Rubin 1992, Brooks & Gelman 1998, Stock & Semmens 2016). The Geweke diagnostic test is a two-sided z-test comparing the mean of the first part of the chain to the mean of the second part of the chain (95% confidence) (Geweke 1991, Stock & Semmens 2016). At convergence of the chains, the means should be the same (Stock & Semmens 2016). MixSIAR models for this study were developed with three MCMC chains and on the “very long” (1,000,000 chain length and 500,000 burn in value) for optimal convergence of chains.

MixSIAR requires data from the consumer (dolphins), the source (nekton), and fractionation values (difference between the consumer and source) to estimate proportions of each source to the consumer. Fractionation values from past studies were used for analysis (1.01±0.85‰ [mean ± sd] for $\delta^{13}C$ and 1.57±0.52‰ [mean ± sd] for $\delta^{15}N$) and are assumed constant across all nekton prey sources (Giménez et al. 2016, Wilson et al. 2017). Since samples were not lipid extracted, an uncertainty of 1.7‰ was incorporated into the $\delta^{13}C$ value (Wilson et al. 2013b, Wilson et al. 2017). Isotopic data on potential prey organisms were obtained from a food web study in Galveston Bay (2008-2009) and were combined with fishes sampled in the current study (2015-2016) (Barcenas 2013). Phillips et al. (2014) recommends the number of sources used in the MixSIAR model should be limited without excluding any sources and that the discriminatory power of mixing models starts to decline above six or seven sources. In order to include a broad range of probable prey sources, Phillips et al. (2005) recommends grouping sources to reduce the total number. Therefore, Ward's hierarchical cluster analysis was used to group 19 nekton species into six groups based on their mean C and N isotopic values (Phillips et al. 2005, Giménez et al. 2017). A cluster dendrogram was generated using Ward's hierarchical cluster analysis and Euclidean distance in R (Murtagh & Legendre 2014). Ward's agglomerative clustering methods are unique in that it is based on a classical sum-of-squares criterion which produces groups that minimize within-group dispersion at each binary fusion (Murtagh & Legendre 2014).

Results

Isotopic Analysis of Dolphin Biopsy Samples

From 2015 to 2017 a total of 239.9 hours were spent on the water during 35 biopsy surveys covering 2,692.5 km (Table 3). A total of 36 live dolphin biopsy samples were taken and four samples were provided by the TMMSN from stranded dead dolphins.

Of the 40 samples, 13 were collected in 2015, 12 were collected in 2016, and 15 were collected in 2017 (Figure 9).

Table 3:

Biopsy survey summary table from 2015-2017.

Year	# of Surveys	# of Sightings	Total distance (km)	Total Hours	# of Shots Taken	# of Biopsy Samples	# of Stranded Samples
2015	11	55	754.6	84.2	22	13	0
2016	20	65	1648.5	130.0	16	10	2
2017	4	19	289.4	25.6	17	13	2
Total	35	139	2692.5	239.8	55	36	4

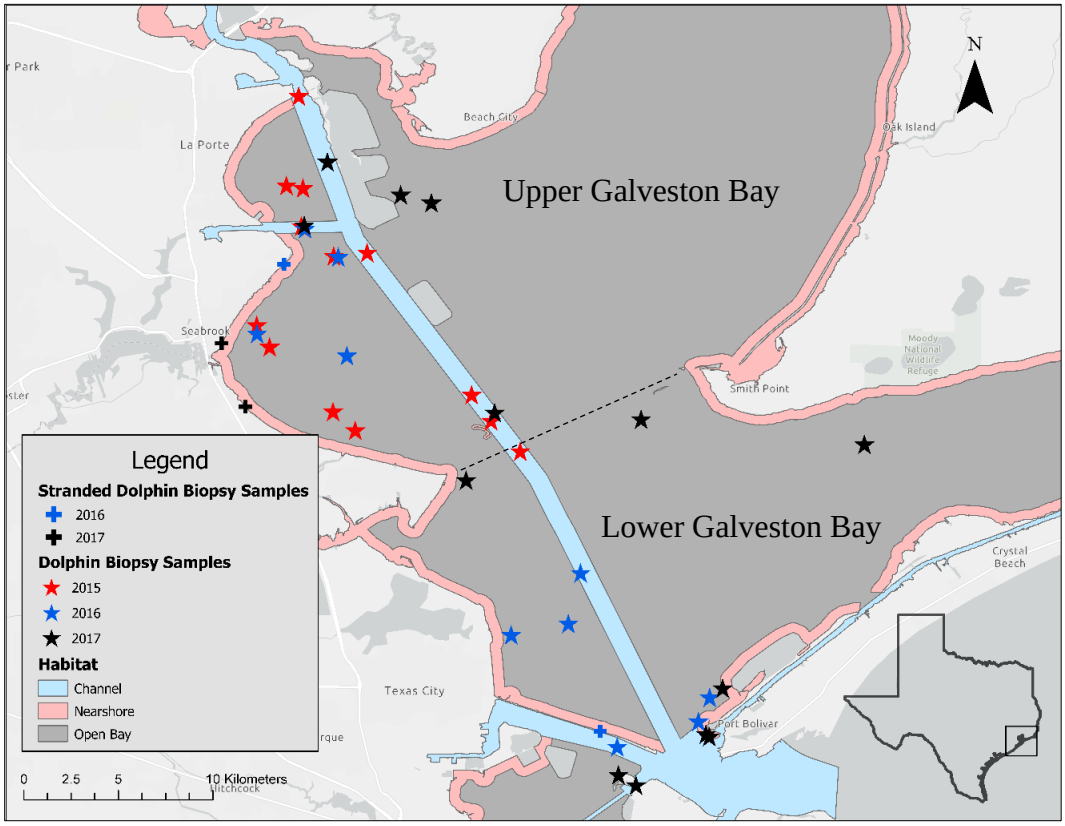

Figure 9:

Map of dolphin biopsy sample locations by year throughout the Galveston Bay ecosystem.

Twenty-four samples were taken in UGB, and 16 samples were taken in LGB (Table 4). Overall, the $\delta^{13}C$ values ranged from -20.59‰ to -15.95 ‰ and the $\delta^{15}N$ values ranged from 15.45‰ to 22.78‰ (Table 4). The median $\delta^{13}C$ value overall was -18.68‰ and the median value overall for $\delta^{15}N$ was 19.73‰. The mean $\delta^{13}C$ value overall was -18.67‰ and the median value overall for $\delta^{15}N$ was 19.43‰. Environmental variables measured during biopsy surveys are summarized by year in Appendix D.

Table 4:

Descriptive statistics of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) based on dolphin tissue samples collected by location from 2015-2017.

		$\delta^{13}C$ (‰)				**$\delta^{15}N$ (‰)**			
Location	**n**	**Min**	**Med**	**Mean**	**Max**	**Min**	**Med**	**Mean**	**Max**
Upper Galveston Bay	24	-20.59	-19.10	-18.80	-15.95	15.67	20.01	19.79	21.4
Lower Galveston Bay	16	-20.46	-18.45	-18.47	-16.76	15.45	18.86	18.88	22.78
Upper & Lower Combined	40	-20.59	-18.68	-18.67	-15.95	15.45	19.73	19.43	22.78

Genetic analysis of skin samples was conducted on all samples that were analyzed for stable isotopes except one (Catalog ID #69) where enough sample was not obtained to analyze. For analysis involving sex, #69 was presumed to be a male based on its coefficient of association (seen 24 times together out of 26 sightings=0.923) with another large animal (Catalog ID #79) who have been seen flanking a female (anecdotal field observation). Coefficient of association values range from 0.00 for two dolphins never

sighted with each other to 1.00 for dolphins always seen with one another. Lifelong male pair bonds are common in bottlenose dolphins around the world and presumed to be beneficial for both males when courting a female (Wells et al. 1987, Parsons et al. 2003, Diaz-Aguirre et al. 2018). Assuming #69 is a male, 31 samples were obtained from males and nine samples from females (Table 5).

Table 5:

Summary of individual dolphin $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values by ascending date, sex, and location. All sex data confirmed by genetics except for #69 who is assumed male based on coefficient of association with another large individual (denoted by "").*

Date	Catalog ID	Sex	Location	Latitude	Longitude	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N Ratio
24-Aug-2015	302	M	UGB	29.56738	-95.00000	-18.05	20.94	3.36
25-Aug-2015	282	M	UGB	29.51749	-94.95311	-20.20	19.77	4.28
25-Aug-2015	288	F	UGB	29.55714	-94.99387	-17.44	18.87	3.31
26-Aug-2015	11	M	UGB	29.61448	-94.97879	-19.91	20.47	3.86
26-Aug-2015	209	M	UGB	29.63261	-94.97800	-19.17	20.41	3.85
26-Aug-2015	234	M	UGB	29.67639	-94.98004	-16.42	19.45	3.17
26-Aug-2015	284	M	UGB	29.63387	-94.98589	-19.71	19.80	3.61
28-Aug-2015	393	M	UGB	29.52640	-94.96370	-18.27	20.37	3.58
20-Sep-2015	427	M	UGB	29.60192	-94.94746	-15.95	18.73	2.95
05-Oct-2015	211	M	UGB	29.60037	-94.96336	-19.55	20.46	3.64
07-Oct-2015	118	M	UGB	29.52204	-94.88850	-19.02	20.26	3.47
07-Oct-2015	428	M	LGB	29.50749	-94.87462	-19.93	18.73	3.75
13-Oct-2015	429	M	UGB	29.53440	-94.89774	-19.61	19.99	3.37
17-May-2016	284	M	UGB	29.59640	-94.98708	-20.42	19.97	3.58
28-Jun-2016	73	F	UGB	29.55303	-94.95710	-18.82	19.23	3.39
28-Jun-2016	146	M	UGB	29.56345	-94.99990	-17.84	20.24	3.20
01-Jul-2016	1	M	UGB	29.61334	-94.97702	-19.40	19.42	3.51
01-Jul-2016	570	M	UGB	29.59985	-94.96121	-18.53	15.67	3.63
25-Jul-2016	572	M	LGB	29.36703	-94.82846	-17.57	16.51	3.32
28-Jul-2016	296	M	LGB	29.37906	-94.78981	-16.76	15.45	3.53
19-Aug-2016	445	M	LGB	29.42565	-94.85171	-18.56	17.92	3.37
19-Aug-2016	542	M	LGB	29.42025	-94.87893	-18.05	20.46	3.30
19-Aug-2016	573	M	LGB	29.44969	-94.84591	-18.24	19.29	3.29
25-Aug-2016	124	M	LGB	29.39052	-94.78447	-19.37	18.88	3.76
05-Dec-2016	UNK1	M	LGB	29.37438	-94.83662	-20.46	17.41	4.27
21-Aug-2017	213	M	LGB	29.37190	-94.78466	-19.17	19.36	3.50
21-Aug-2017	689	F	LGB	29.39488	-94.77829	-19.01	18.06	4.23
21-Aug-2017	752	F	LGB	29.37310	-94.78624	-18.53	19.68	3.34
21-Aug-2017	753	F	LGB	29.34882	-94.81954	-18.79	18.81	3.22
21-Aug-2017	754	M	LGB	29.35369	-94.82789	-17.76	19.94	3.53
22-Aug-2017	69	M*	LGB	29.52274	-94.81709	-17.61	18.83	3.30
22-Aug-2017	160	F	LGB	29.51079	-94.71084	-18.36	20.03	3.42
22-Aug-2017	744	F	UGB	29.52586	-94.88677	-17.90	18.62	3.01
23-Aug-2017	323	M	UGB	29.62945	-94.93150	-19.56	20.32	3.57
23-Aug-2017	480	F	LGB	29.49377	-94.90038	-17.27	22.78	3.09

Date	Catalog ID	Sex	Location	Latitude	Longitude	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N Ratio
23-Aug-2017	700	F	UGB	29.62566	-94.91689	-19.68	20.03	3.74
24-Aug-2017	1	M	UGB	29.61490	-94.97737	-20.59	21.12	3.59
24-Aug-2017	27	M	UGB	29.64509	-94.96635	-18.34	18.22	3.52
21-Nov-2017	UNK2	M	UGB	29.55887	-95.01678	-19.30	21.40	3.46
02-Dec-2017	80	M	UGB	29.52870	-95.00550	-17.59	21.12	3.03

Isotopic Analysis of Nekton Samples

A total of 12 sites were sampled for fish from 2015 and 2016 (Figure 10). Nine sites were sampled in 2015 and eight sites were sampled in 2016. Six of the eight sampled in 2016, were revisits from 2015. A total of 128 fish representing nine species were sampled from 2015 and 2016 (Table 6). Of the 128 fish collected, 53 were sampled in 2015 and 75 were sampled in 2016. The smallest $\delta^{13}C$ recorded was -28.39‰ from an Atlantic Croaker collected in LGB in 2015. The highest $\delta^{13}C$ was -18.07‰ from a Spot sampled in LGB in 2015. The smallest $\delta^{15}N$ was 9.94‰ from an Atlantic Croaker sampled in UGB in 2015 (Appendix E). The highest $\delta^{15}N$ was 22.88‰ from a Spot sampled in UGB in 2015.

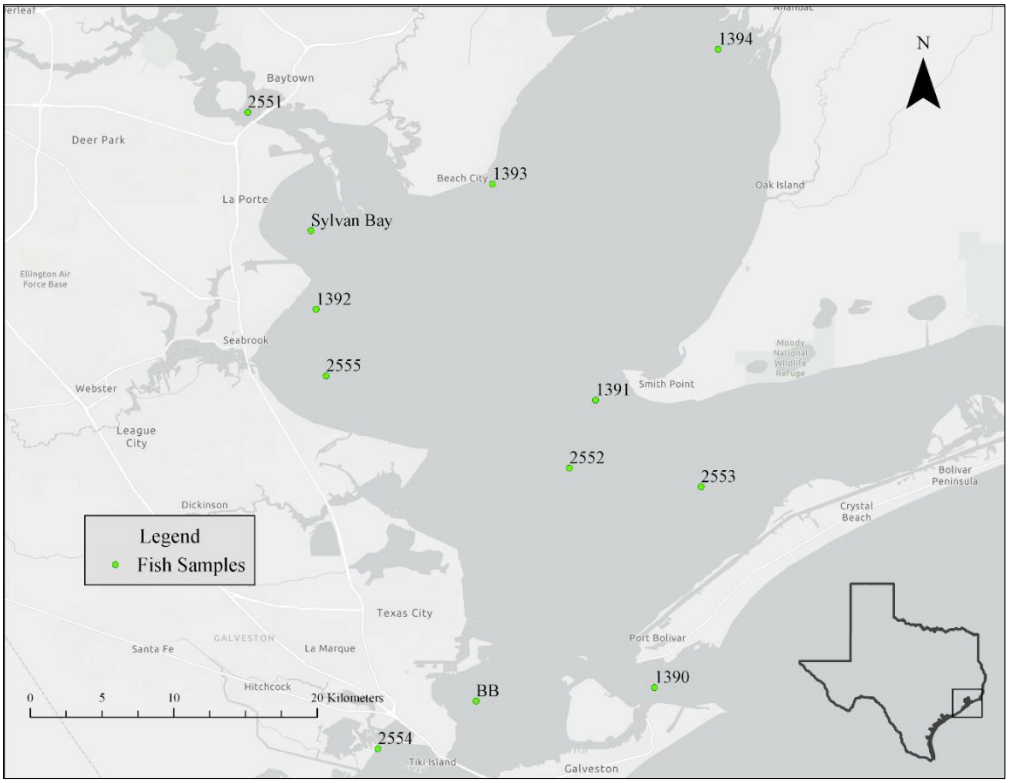

Figure 10:

Map of sites sampled in 2015 and/or 2016 for fish tissue.

Table 6:

Descriptive statistics of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values measured in fish collected during 2015-2016.

Fish			$\delta^{13}C$ (‰)						$\delta^{15}N$ (‰)					
Scientific name	**Common name**	**n**	**Min**	**Q1**	**Med**	**Mean**	**Q3**	**Max**	**Min**	**Q1**	**Med**	**Mean**	**Q3**	**Max**
Elops saurus	Ladyfish	1	-25.21	-25.21	-25.21	-25.21	-25.21	-25.21	17.82	17.82	17.82	17.82	17.82	17.82
Ariopsis felis	Hardhead Catfish	6	-21.02	-20.03	-19.43	-19.45	-18.56	-18.23	16.70	17.03	17.49	17.37	17.54	18.09
Bagre marinus	Gafftopsail Catfish	11	-26.79	-23.77	-22.84	-22.90	-21.91	-20.29	17.17	18.00	18.68	18.72	19.38	20.33
Cynoscion arenarius	Sand Seatrout	36	-26.46	-24.67	-23.17	-23.26	-22.74	-18.88	15.20	17.46	17.83	17.95	18.58	20.60
Leiostomus xanthurus	Spot	22	-26.00	-24.20	-21.39	-22.19	-20.70	-18.07	14.43	16.16	17.84	18.02	19.39	22.88
Micropogonias undulatus	Atlantic Croaker	45	-28.39	-24.37	-23.41	-23.36	-21.77	-18.89	9.94	17.53	18.54	18.24	19.28	22.03
Pogonias cromis	Black Drum	1	-22.38	-22.38	-22.38	-22.38	-22.38	-22.38	20.67	20.67	20.67	20.67	20.67	20.67
Trichiurus lepturus	Atlantic Cutlassfish	4	-22.07	-21.93	-21.29	-21.19	-20.55	-20.13	15.86	16.00	17.36	17.48	18.85	19.35
Chloroscombrus chrysurus	Atlantic Bumper	3	-23.57	-21.46	-19.34	-20.42	-18.85	-18.36	15.92	16.45	16.98	16.78	17.21	17.44

Spatial, Temporal, and Gender Stable Isotope Comparison

A significant difference was not detected between isotopic values of stranded dead and live dolphin samples (Wilcoxon Rank Sum Test, p-value >0.05). Therefore the stranded dolphin samples were used in all subsequent statistical analyses except between habitat comparisons. The location (UGB or LGB) of where the stranded dolphins were found was used with the assumption that is where they were prior to death. Two of the stranded dolphins were unable to be matched to the GDRP catalog. The other two stranded dolphins had multiple sightings in UGB, where they stranded.

There was a significant difference in $\delta^{15}N$ (‰) between UGB and LGB (Wilcoxon Rank Sum Test, W=105.5, p-value ≤0.05) (Figure 11). There were significant differences between $\delta^{15}N$ (‰) values for at least one of the years (2015, 2016, 2017) (Kruskal-Wallis Rank Sum Test, p-value ≤0.05). Although, the Pairwise Wilcoxon Rank Sum Test only suggested a difference between 2015 and 2016, it was not statistically significant at a p-value of 0.05 (Figure 12). There were no significant differences in $\delta^{15}N$ (‰) values between sexes. No significant differences were detected between $\delta^{13}C$ (‰) values between any of the variables.

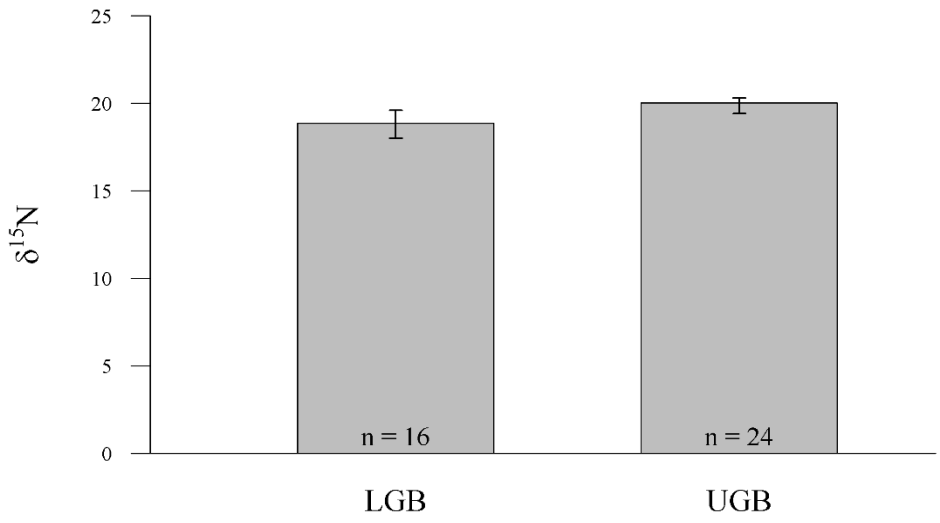

Figure 11:

Bar graph of dolphin $\delta^{15}N$ (‰) from Lower Galveston Bay (LGB) and Upper Galveston Bay (UGB). LGB: median: 18.9, 95% CI: 18.0-19.5. UGB: median: 20.0, 95% CI: 19.4-20.3.

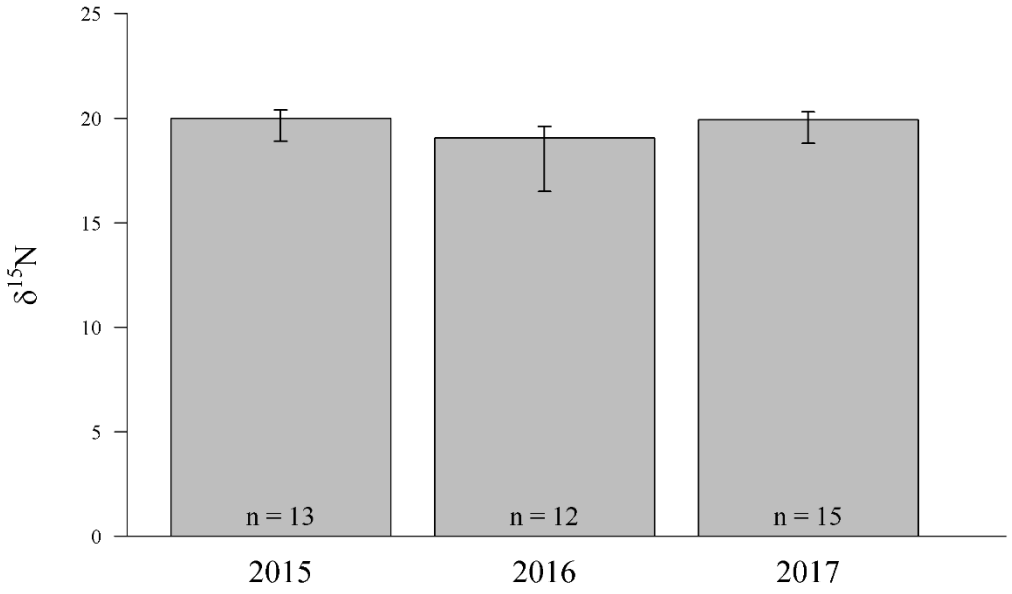

Figure 12:

Bar graph of dolphin $\delta^{15}N$ (‰) values from 2015-2017. 2015: median: 20.0, 95% CI: 18.9-20.4. 2016: median: 19.1, 95% CI: 16.5-19.6. 2017: median: 19.9, 95% CI: 18.8-20.3.

Stable Isotope Mixing Model

Ward's hierarchical cluster analysis was used to group 19 nekton species into six groups based on their mean isotopic values (Figure 13). Data collected from the current study during fish sampling in 2015 and 2016 and from nekton from 2008 to 2009 from Barcenas (2013) were used in the groupings (Table *7*). Dolphin isotopic values were generally within the constraints of the prey on the isoplot which is required to meet assumptions of the MixSIAR model (Phillips et al. 2014) (Figures 14 and 15). The Gelman diagnostic should be <1.05 and all variables were below 1.01. The Geweke diagnostic also indicated that chains were well converged (95% confidence).

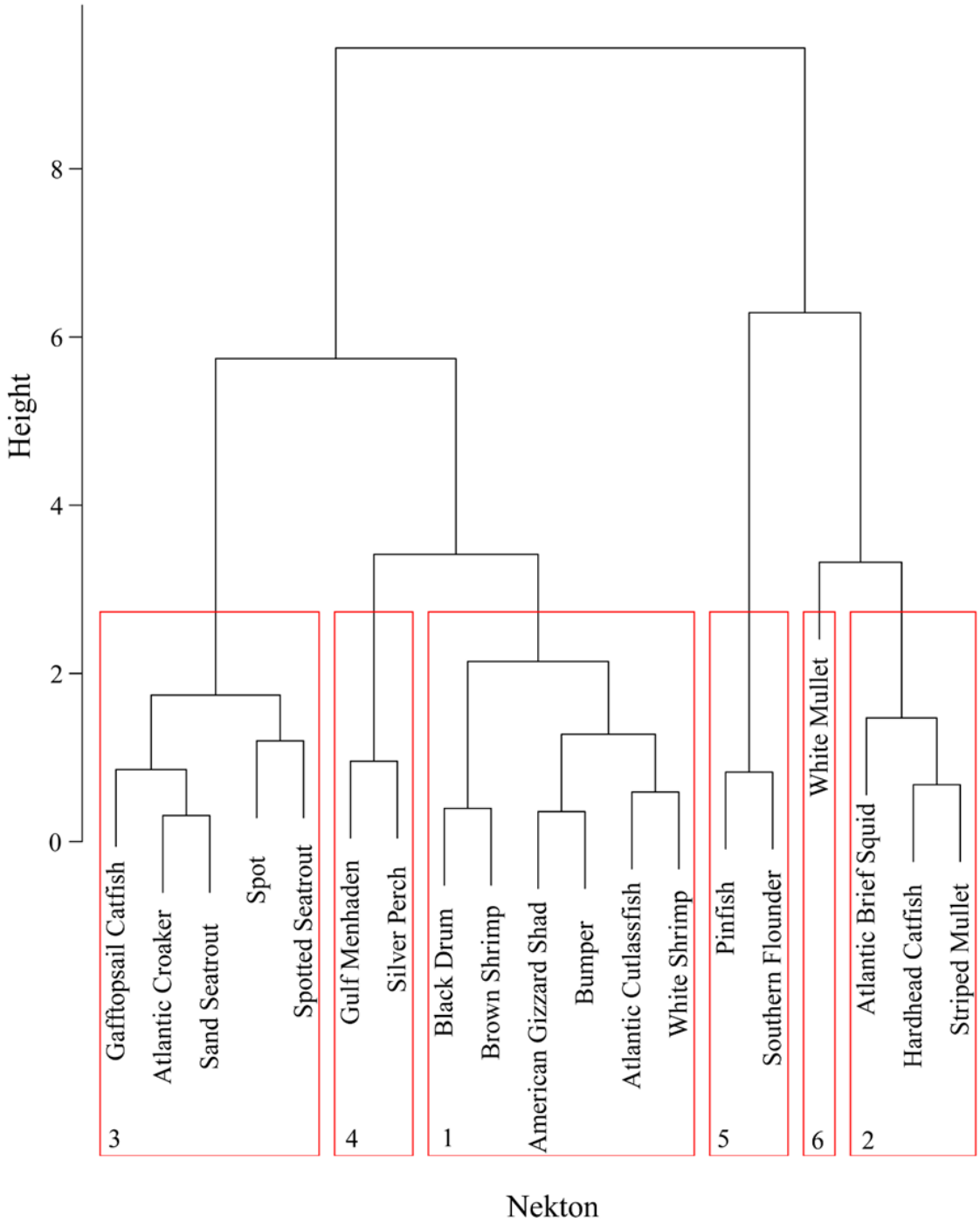

Figure 13:

Cluster dendrogram of Ward's hierarchical cluster analysis of 19 nekton species classified into six groups used for MixSIAR analysis based on similarity of average $\delta^{13}C$ and $\delta^{15}N$ tissue values. Numbers in bottom left of red boxes indicate group assignment.

Table 7:

Nekton trophic groupings based on $\delta^{13}C$ and $\delta^{15}N$ using Ward's hierarchical cluster analysis and their respective mean and standard deviations (SD) of $\delta^{13}C$ and $\delta^{15}N$.

Group	Common Name	Scientific Name	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	n
1	American Gizzard Shad[+]	*Dorosoma cepedianum*	-20.82	1.51	17.58	1.32	25
	Atlantic Cutlassfish	*Trichiurus lepturus*					
	Black Drum[*]	*Pogonias cromis*					
	Brown Shrimp[+]	*Farfantepenaeus aztecus*					
	Atlantic Bumper`	*Chloroscombrus chrysurus*					
	White Shrimp[+]	*Litopenaeus setiferus*					
2	Atlantic Brief Squid[+]	*Lolliguncula brevis*	-18.96	1.39	17.22	1.18	14
	Hardhead Catfish`	*Ariopsis felis*					
	Striped Mullet[+]	*Mugil cephalus*					
3	Atlantic Croaker`	*Micropogonias undulatus*	-23.02	2.19	18.19	1.84	118
	Gafftopsail Catfish`	*Bagre marinus*					
	Sand Seatrout`	*Cynoscion arenarius*					
	Spot`	*Leiostomus xanthurus*					
	Spotted Seatrout[+]	*Cynoscion nebulosus*					
4	Gulf Menhaden[+]	*Brevoortia patronus*	-21.18	1.21	15.71	2.08	6
	Silver Perch[+]	*Bairdiella chrysoura*					
5	Pinfish[+]	*Lagodon rhomboides*	-19.32	1.41	13.47	1.64	11
	Southern Flounder[+]	*Paralichthys lethostigma*					
6	White Mullet[+]	*Mugil curema*	-16.27	1.71	17.5	0.59	2

Data source: Barcenas (2013)[+], current study`, current study and Barcenas (2013) data combined[*]

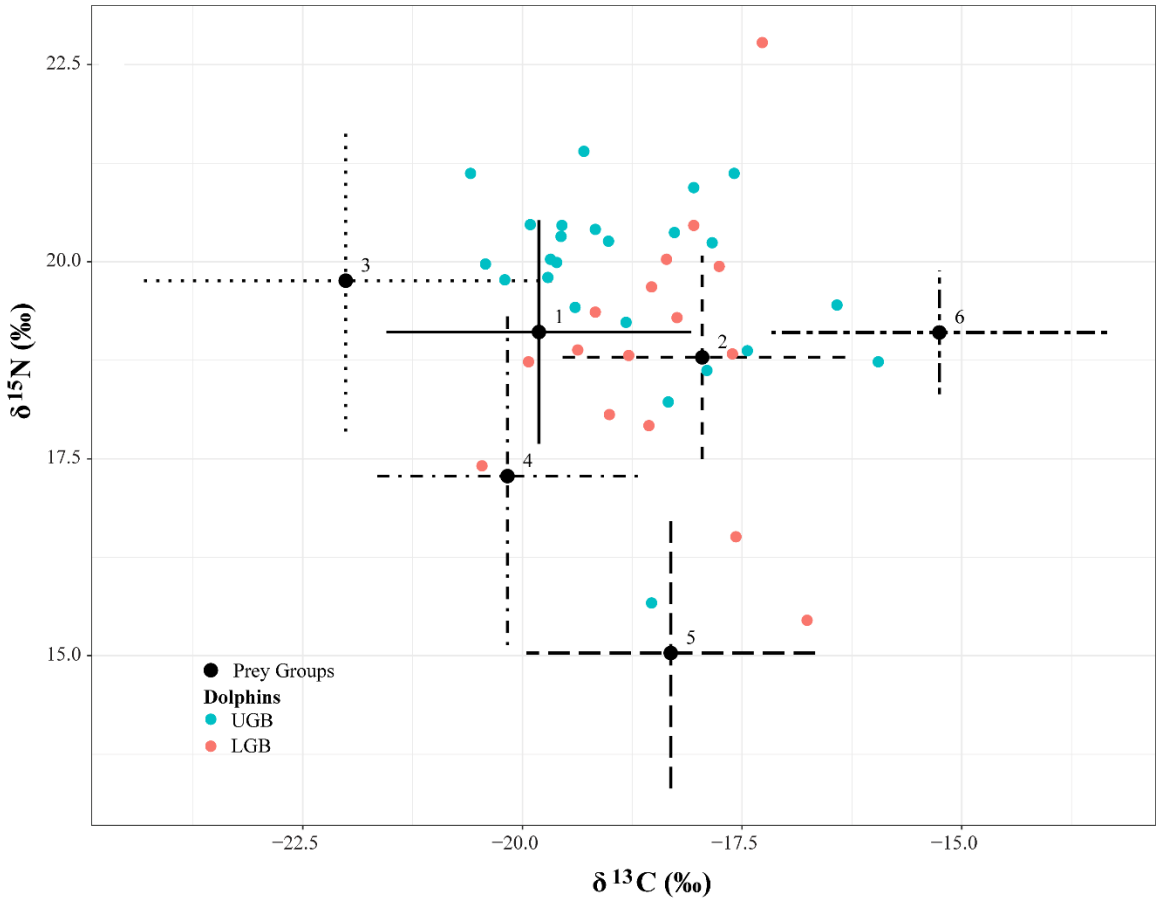

Figure 14:

Isospace plot of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of dolphins (colored dots) and average prey groups (black dots) by location (UGB: Upper Galveston Bay; LGB: Lower Galveston Bay). Error bars indicate combined prey and discrimination factor uncertainty ± one standard deviation: Group 1: American Gizzard Shad, Atlantic Cutlassfish, Black Drum, Brown Shrimp, Atlantic Bumper, White Shrimp; Group 2: Atlantic Brief Squid, Hardhead Catfish, and Striped Mullet; Group 3: Atlantic Croaker, Gafftopsail Catfish, Sand Seatrout, Spot, and Spotted Seatrout; Group 4: Gulf Menhaden and Silver Perch; Group 5: Pinfish and Southern Flounder; Group 6: White Mullet

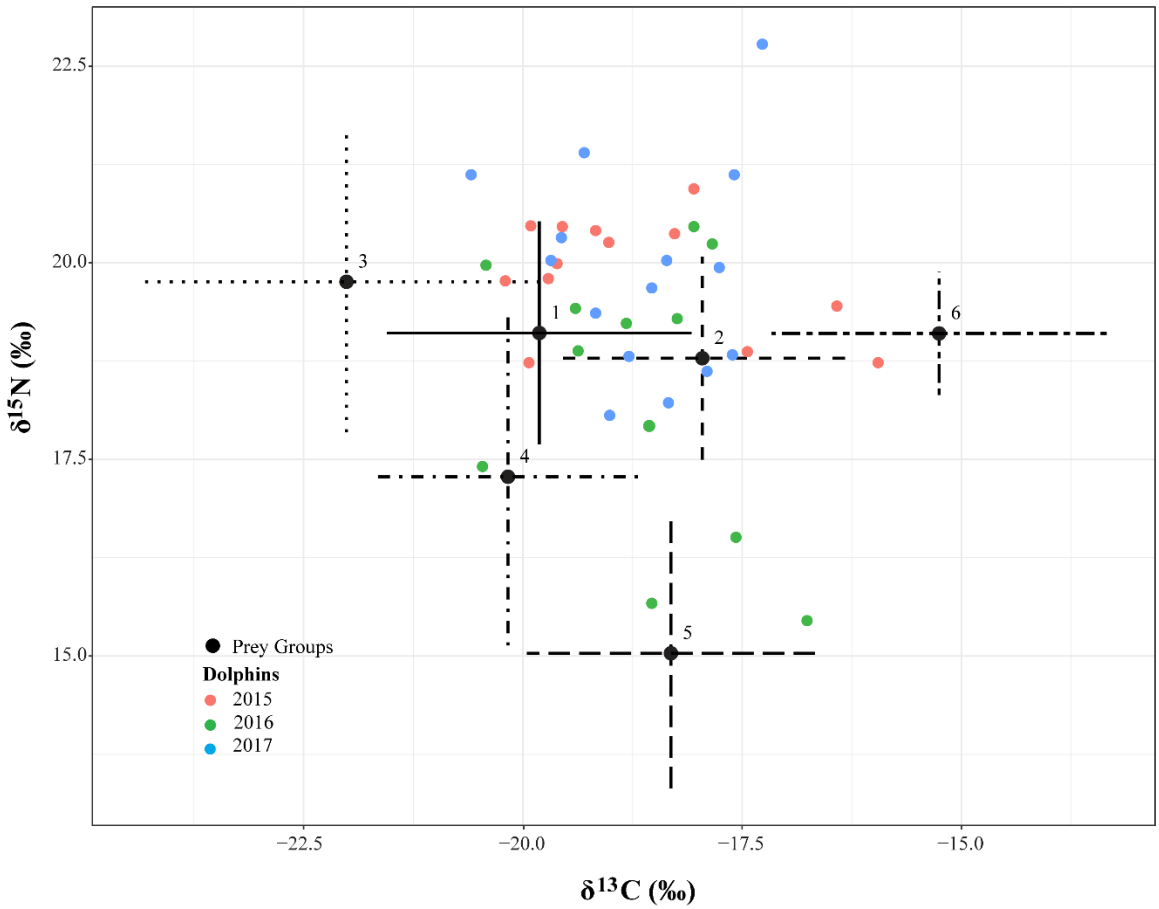

Figure 15:

Isospace plot of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of dolphins (colored dots) and average prey groups (black dots) by year. Error bars indicate combined prey and discrimination factor uncertainty ± one standard deviation: Group 1: American Gizzard Shad, Atlantic Cutlassfish, Black Drum, Brown Shrimp, Atlantic Bumper, White Shrimp; Group 2: Atlantic Brief Squid, Hardhead Catfish, and Striped Mullet; Group 3: Atlantic Croaker, Gafftopsail Catfish, Sand Seatrout, Spot, and Spotted Seatrout; Group 4: Gulf Menhaden and Silver Perch; Group 5: Pinfish and Southern Flounder; Group 6: White Mullet

Overall, group six which contained only one species, White Mullet, was estimated to contribute to the highest proportion of nekton prey consumed by dolphins (median: 25.3%) based on MixSIAR analyses (Figure 16). The second highest proportion consumed by dolphins overall was group two (Atlantic Brief Squid [*Lolliguncula brevis*], Hardhead Catfish, and Striped Mullet) at 21.0%. The third and fourth highest group consumed was group one (American Gizzard Shad [*Dorosoma cepedianum*], Atlantic Cutlassfish [*Trichiurus lepturus*], Black Drum [*Pogonias cromis*], Brown Shrimp, Atlantic Bumper [*Chloroscombrus chrysurus*], and White Shrimp) at 16.9% and group three (Atlantic Croaker, Gafftopsail Catfish [*Bagre marinus*], Sand Seatrout, Spot, and Spotted Seatrout) at 13.0%, respectively. Finally, the least consumed groups were four (Gulf Menhaden and Silver Perch [*Bairdiella chrysoura*]) and five (Pinfish and Southern Flounder) at 7.9% and 4.4%, respectively.

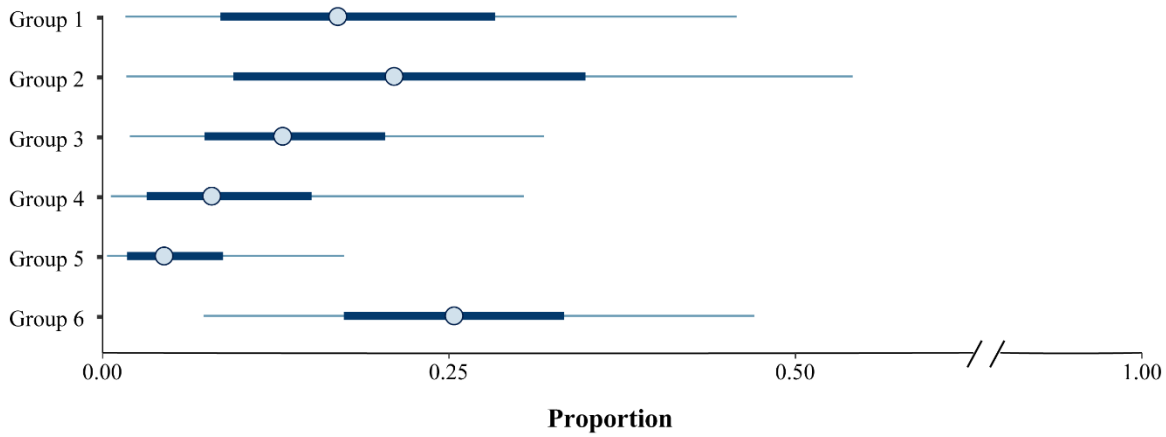

Figure 16:

Overall proportion estimates of prey consumed based on MixSIAR analysis (Group 1: American Gizzard Shad, Atlantic Cutlassfish, Black Drum, Brown Shrimp, Atlantic Bumper, and White Shrimp; Group 2: Atlantic Brief Squid, Hardhead Catfish, and Striped Mullet; Group 3: Atlantic Croaker, Gafftopsail Catfish, Sand Seatrout, Spot, and Spotted Seatrout; Group 4: Gulf Menhaden and Silver Perch; Group 5: Pinfish and Southern Flounder; Group 6: White Mullet) by dolphins overall: posterior medians (points), 50% credible intervals (thick bars), and 90% credible intervals (thin lines).

Group six was the highest proportion consumed by dolphins when analyzed by location (median: UGB: 26.3% and LGB: 25.4%) (Figure 17). The least and second least group consumed by dolphins in both UGB and LGB were groups four (Gulf Menhaden and Silver Perch, 4.1% and 7.9%, respectively) and five (Pinfish and Southern Flounder, 1.9% and 4.4%, respectively). However, the second, third, and fourth most consumed groups slightly differed by location. The second highest group consumed by dolphins in UGB was group one (American Gizzard Shad, Atlantic Cutlassfish, Black Drum, Brown Shrimp, Atlantic Croaker, and White Shrimp) at 20.3% while the second largest group consumed by dolphins in LGB was group two (Atlantic Brief Squid, Hardhead Catfish, and Striped Mullet) at 21.3%. The third most consumed group in UGB was group three (Atlantic Croaker, Gafftopsail Catfish, Sand Seatrout, Spot, and Spotted Seatrout) at 18.6%. The third most consumed group by dolphins in LGB was group one at 17.0%. The fourth most consumed group by UGB dolphins was two (16.0%) and for LGB dolphins group three (13.0%).

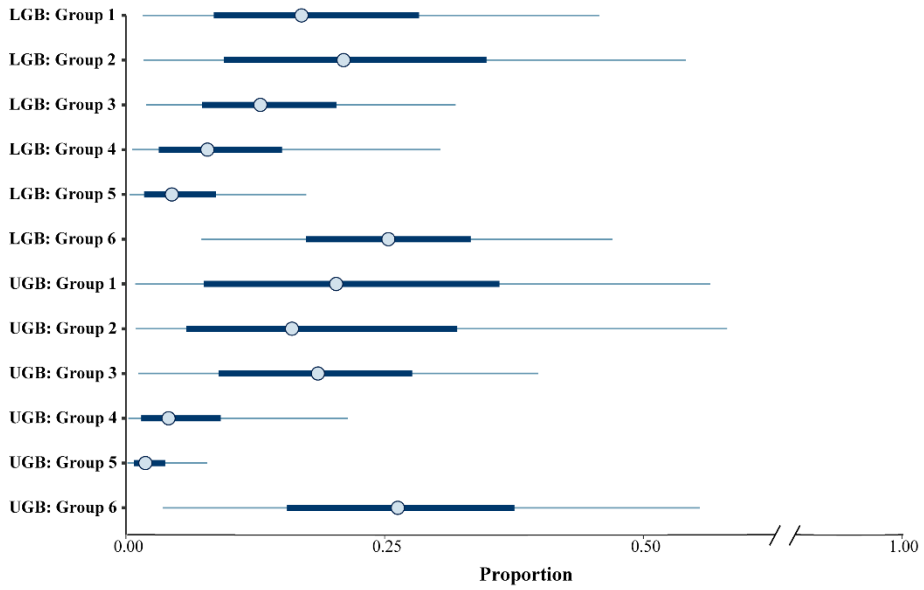

Figure 17:

Proportion of prey groups consumed by dolphins at each location (UGB: Upper Galveston Bay and LGB: Lower Galveston Bay) (Group 1: American Gizzard Shad, Atlantic Cutlassfish, Black Drum, Brown Shrimp, Atlantic Bumper, White Shrimp; Group 2: Atlantic Brief Squid, Hardhead Catfish, and Striped Mullet; Group 3: Atlantic Croaker, Gafftopsail Catfish, Sand Seatrout, Spot, and Spotted Seatrout; Group 4: Gulf Menhaden and Silver Perch; Group 5: Pinfish and Southern Flounder; Group 6: White Mullet) by dolphins: posterior medians (points), 50% credible intervals (thick bars), and 90% credible intervals (thin lines).

Proportion data analyzed by year resulted in the same order of groups for 2015 and 2017 (groups six, one, two, three, four, and five in descending order) (Figure 18). However, 2016 was slightly different in the proportion of groups. In 2015 and 2017 group six was the highest proportion of prey consumed (28.1% and 29.0%, respectively) while group two was the highest proportion consumed (21.3%) in 2016. The second highest prey group consumed in 2015 was the exact same for group one and two at 18.2%. The second highest group preyed on 2017 was one (19.0%) and was group six in 2016 (17.9%). The third highest group preyed on in 2017 was two (17.7%) and was group one in 2016 (15.5%).

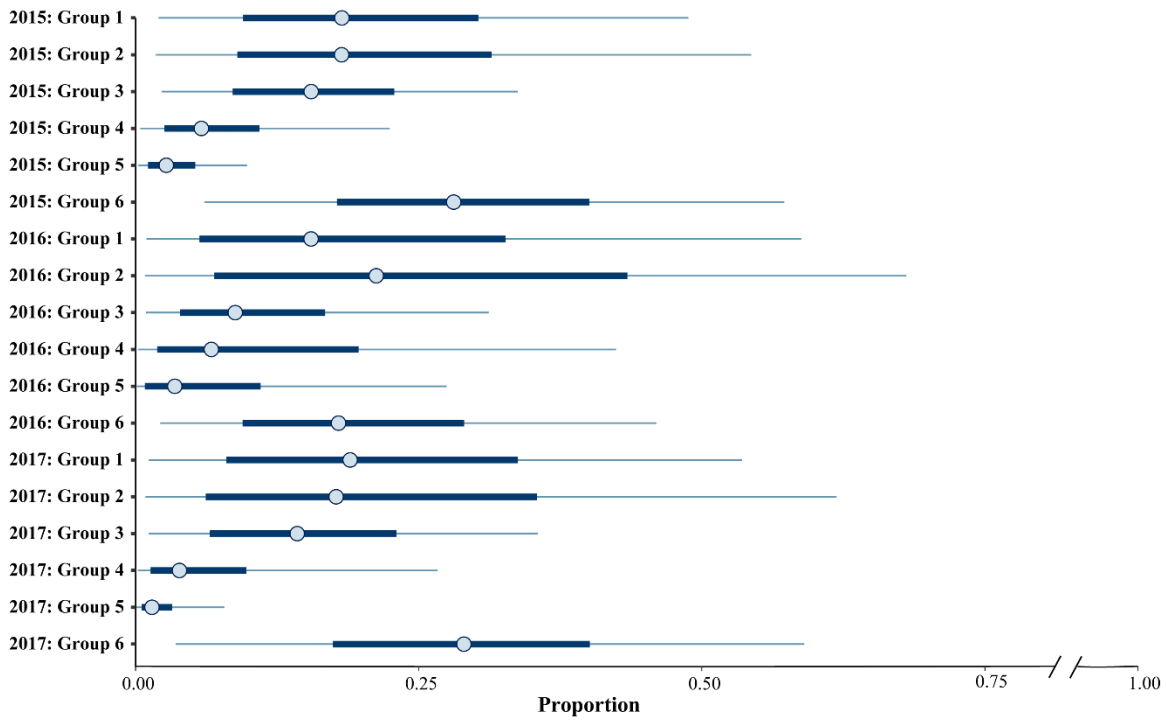

Figure 18:

Proportion of prey groups consumed by dolphins each year (Group 1: American Gizzard Shad, Atlantic Cutlassfish, Black Drum, Brown Shrimp, Atlantic Bumper, White Shrimp; Group 2: Atlantic Brief Squid, Hardhead Catfish, and Striped Mullet; Group 3: Atlantic Croaker, Gafftopsail Catfish, Sand Seatrout, Spot, and Spotted Seatrout; Group 4: Gulf Menhaden and Silver Perch; Group 5: Pinfish and Southern Flounder; Group 6: White Mullet) by dolphins: posterior medians (points), 50% credible intervals (thick bars), and 90% credible intervals (thin lines).

Discussion

The comparisons made by the different variables in GB of C and N are based on 40 dolphin biopsy samples collected over 2015-2017. The statistical tests conducted on the C and N values may indicate a stronger detectable difference if a larger sample size was obtainable (i.e., more statistical power).

It was expected that dolphins with lower $\delta^{13}C$ values were likely foraging in Trinity Bay or Upper Galveston Bay while dolphins with higher $\delta^{13}C$ values were likely

foraging in East Bay or Lower Galveston Bay based on findings from Barcenas (2013). Barcenas (2013) found that based on $\delta^{13}C$ analysis, few of the nekton species sampled assimilated one basal carbon source exclusively, instead a mixture of sources from each sub-bay were consumed. In the eastern part of the Galveston Bay system, the food web supporting most of the species was based on a mixture of phytoplankton and epiphytic algae and/or detritus while in the western part of the Galveston Bay system, epiphytic algae and/or detritus are very important (Barcenas 2013). No detectable differences were observed by year, location, habitat, or sex in dolphin $\delta^{13}C$ values during this study. This result implies that carbon sources in Galveston Bay are well mixed or dolphins that feed throughout the bay are not able to be distinguished.

It was expected that $\delta^{15}N$ values would be higher in UGB compared to LGB due to anthropogenic nitrogen loading from the Trinity and San Jacinto Rivers. The average $\delta^{15}N$ value for dolphins overall in Galveston Bay for this study was 19.43‰. This average is much higher than any other location that was found in the literature. Nitrogen values from dolphins near Spain were approximately 5-6‰ lower than dolphins from Galveston Bay (Giménez et al. 2017, Brotons et al. 2019). Nitrogen values in various locations in Florida were nearly 5-10‰ lower than the average of Galveston Bay (Barros et al. 2010, Browning et al. 2014b, Wilson et al. 2017). Sharks (fork length <150cm) collected around Galveston, Texas were analyzed for stable isotopes and the highest average $\delta^{15}N$ value was 16.43±0.09‰ for Blacktip sharks and the lowest average $\delta^{15}N$ value was 15.91±0.08‰ for Bonnethead (Plumlee & Wells 2016). Holt and Ingall (2000) found that the $\delta^{15}N$ levels from Spotted Seatrout collected in Galveston Bay were approximately 6‰ higher than the Upper Laguna Madre, suggesting the Spotted Seatrout in Galveston Bay feed at higher trophic levels, however, these results likely indicate the

contribution of anthropogenic nitrogen loading. Fry (1988) documented $\delta^{15}N$ values of 11.2-15.2‰ for piscivorous fish in George's Bank off of Massachusetts.

Fry (1988) documented a consistent fractionation rate of 3.6‰ increase in $\delta^{13}N$ for every increase in trophic level in a pelagic food web. However, Giménez et al. (2016) conducted a 350-day controlled experiment to determine fractionation factors and turnover rates in the skin of captive bottlenose dolphins and found 1.57± 0.52‰ (mean±sd) for $\delta^{15}N$ to be an appropriate estimate for free ranging dolphins.

Fine scale differences in diet between habitats were not detected during the current study using SIA. Although a significant difference in $\delta^{15}N$ or $\delta^{13}C$ was not detected between years using a Pairwise Wilcoxon Rank Sum Test, differences in proportions of prey consumed were observed using MixSIAR. In 2015 and 2017 group six represented the highest proportion of prey consumed while group two represented the highest proportion consumed in 2016. Most samples taken in 2015 were in UGB which may explain the differences between 2015 and 2016 or prey availability was different in 2016 than in 2015 and 2017. There was a significant difference of $\delta^{15}N$ (‰) between UGB and LGB (Wilcoxon Rank Sum Test, W=105.5, p-value $\leq$0.05) and this was reflected in the MixSIAR analysis with the second, third, and fourth most consumed groups being different by location.

Significant ($p < 0.01$) differences in isotopic signatures were not detected between male and female dolphins in Galveston Bay. Differences in prey composition between sexes have been observed in Florida (Barros & Odell 1990, Barros 1994). Barros and Odell (1990) documented female dolphins consumed mullet more frequently than males on the west coast of Florida. On the other side of Florida, Barros (1993) noted that male dolphins in the Indian River Lagoon preyed more frequently on Pinfish and Pigfish than females, and males from the Atlantic beachside consumed more squid than females.

During the current study there was a positive sampling bias toward male specimens due to our avoidance of mom and calf pairs. The target spot to biopsy on an adult dolphin is right where a calf would surface next to its mom. As a result, fewer females were sampled than males.

Isotopic signatures can vary spatially and temporally for any specific prey source. For this study, seasonality should not be a confounding factor when comparing dolphin tissue samples since most samples were collected between May-October over the study period. Over half of all dolphin samples were taken in August. Barcenas (2013) sampled Galveston Bay fishes from April-October in 2008 and in May 2009.

Freshwater pulses into Galveston Bay are important to changes of environmental variables such as salinity, nutrient and organic matter loading (Steichen 2018). However, one of the largest contributors to the impairment of river water quality in many states in the United States is the urban runoff and storm water with the highest eutrophic conditions observed in the Gulf of Mexico (45% of the estuaries surrounding the Gulf of Mexico) (Clement et al. 2001, Steichen 2018). Annual precipitation prior to sampling was 54.03 in, 44.93 in, 32.17 in for 2015, 2016, and 2017 respectively (TWDB 2020). Above average freshwater inflow occurred in 2015 and the relative abundance of fishes was higher compared to the drought year of 2011 (Steichen 2018). Atlantic Croaker and Gulf Menhaden were present in the highest relative abundance during times of lower salinity (Steichen 2018). When salinities increased throughout the bay, White Mullet and Spot abundance increased (Steichen 2018).

Differences in isotopic signatures can only be detected if individuals are selecting different prey or feeding in different locations. The complexity of foraging behaviors by fishes in an estuary makes it difficult to define trophic levels due to ontogenetic shifts and prey availability at a given location and time (Cowan Jr. et al. 2012). Inferences from

models such as MixSIAR are estimates of the assimilated diet, which can differ from the ingested diet (Phillips et al. 2014). For example, some dolphins from Mississippi and Florida eat catfish bodies leaving the heads behind and therefore may not be identifiable in stomach content analysis (Ronje et al. 2017). This study reflects assimilation generally for the warm months of the year.

Six (Silver Perch, Black Drum, American Gizzard Shad, Atlantic Bumper, Atlantic Cutlassfish, Atlantic Brief Squid) of the 19 species chosen to be analyzed for potential dolphin prey were not in the top 30 abundant species captured by TPWD from 1992-2015, however, TPWD targeted juvenile and subadult nekton found close to the nearshore habitat. While dolphins may eat larger adult fish, the TPWD data represents abundant species present throughout the Galveston Bay ecosystem but may be missing species that do not use the nearshore habitat as juveniles or subadults and are not captured with the standard beach seine sampling method used for the long-term monitoring. Barros and Odell (1990) analyzed stomach contents from stranded dolphins near Galveston Bay and reported a mixed diet of fish, shrimp, and cephalopods with individual prey items often being small in size. They speculated that the diverse prey composition in the stomachs of these dolphins was due to foraging on discarded organisms from shrimp trawlers.

It is important to note that although White Mullet was the highest reported proportion consumed for dolphin diets, any prey having similar isotopic values (mean $\delta^{13}C$: -16.27±1.71 and mean $\delta^{15}N$: 17.5±0.59) that were not used in this study may be important dolphin prey. Although some groups minimally contribute to the overall proportion of dolphin diets, they are still important to include into the model to ensure all assumptions are met (dolphins bound by prey sources in isoplot). The model was run

several times with different prey sources in order to minimize variables but to also be inclusive of many prey isotopic spaces.

Fish and invertebrates are commonly used as bioindicators of overall health of an estuary (Bortone et al. 2005, Bortone 2005, Quigg & Steichen 2015, Steichen 2018, Steichen & Quigg 2018) and therefore dolphins which feed on these species can act as sentinels of estuarine health (Reif 2011). Data collected for this research can be used to estimate trophic positions and used in conjunction with mercury and organochlorine contaminant analysis (Monk et al. 2014) to examine trophic level biomagnification in the GB ecosystem (Barragán-Barrera et al. 2019). GDRP continues to monitor the population in GB. Long-term sighting data paired with SIA may contribute to determining if individuals are residents of the GB ecosystem or transients. Including the analysis of sulfur isotopes or fatty acids in future studies may facilitate population differentiation of the BSE dolphin stock and nearshore dolphin stocks (Barros et al. 2010, Browning et al. 2014b, Giménez et al. 2017).

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APPENDIX A:

UNIVERSITY OF HOUSTON-CLEAR LAKE INSTITUTIONAL ANIMAL CARE

AND USE PROTOCOL #F14.005

v.110323 **Protocol # F14.005**

Federal animal welfare regulations require that the Institutional Animal Care and Use Committee (IACUC) must review and approve all activities involving the use of vertebrate animals prior to initiation of such use. Once approved by the IACUC, any change(s) to the following protocol must be submitted in a written amendment for review and approval of the IACUC prior to implementation of the change(s).

1. Title of Project

"Ecology and Conservation of the Common Bottlenose Dolphin *(Tursiops truncatus)* in the Bay, Sound, Estuary and Near-shore Coastal Waters of Texas"

2. Principal Investigator:

Name:	George Guillen		
Title:	Associate Professor	Program:	Environmental Science
Phone:		Emergency Phone:	
Email:		Campus Mail:	EIH

Secondary Contact Person involved in the study:

Name:	Sherah Loe		
Email:		Emergency Phone:	

3. Project Type

☒ New Protocol ☐ Renewal ☐ Addendum Previous Protocol #			
If renewal:	☐ Annual Renewal ☐ Protocol Renewal **see Appendix E**		
If addendum:	☐ Personnel Change ☐ Minor Revision ☐ Major Revision **see Appendix E**		
Number of years project is expecting to continue:	☐ 1 year ☐ 2 years ☒ 3 years		
This protocol is for:	☐ Teaching ☒ Research ☐ Breeding		
If teaching:			
Course name and number:			
Frequency course is offered:			
If research:			

How will this project be funded:	Grant
If grant, this project is:	☒ Pending ☐ Funded – Federal ☐ Funded – Other
Grant title and/or contract number (if available):	
Has this project already received an independent scientific peer-review?	☒ Yes ☐ No
If yes, by whom?	Intrastate panel of dolphin experts

4. Location

Where will animals be housed?	☐ UHCL Animal Research Facility ☒ Field Study
☐ Other:*	
Where will animal use take place?	Galveston Bay
Will animals be kept for over 12 hours outside of housing area?	☐ Yes* ☒ No
If yes, give location and reason:	

*A standard operating procedure (SOP) to ensure proper welfare and housing of animals must be attached to this protocol in Appendix F. This does not apply to animals housed at other AAALAC accredited animal facilities (e.g., UH or NASA).

5. Lay Summary:

Describe the goals and intended benefits of the project in terms that can be understood by a non-scientist. Include the species and the number of animals to be used. This description should be no more than 250 words. Avoid the use of technical jargon and abbreviations.

The proposed research aims to establish a long-term monitoring program that will provide population distribution and abundance estimates, identify natural and human-generated risks and establish baseline health and life history parameters for Texas bay, sound, estuary and near-coastal bottlenose dolphins (*Tursiops truncatus*). The most recent abundance estimates for dolphins in this region (with the exception of the West Bay stock) come from aerial surveys performed in 1992. The numbers are outdated, and this method is now generally considered unreliable for bay, sound, and estuary dolphin stocks, leaving current estimates "Unknown". Without updated and ongoing data, it is impossible to meet the objectives of the Marine Mammal Protection Act with regards to maintaining an optimum sustainable population size or to take steps to replenish a stock should it fall below the optimum sustainable population. Data will be published and presented in a variety of scientific and popular venues to disseminate results and aid in management decisions. In the event of an environmental disturbance, such as an oil spill,

the underlying logistical structure and availability of baseline data will improve response efforts and allow us to characterize effects on Texas populations. Our methods include boat based field surveys, photographic identification, behavioral observation and remote biopsy darting (up to 100 dolphins/year subjected to biopsy sampling protocol). This research will not involve the capture or captive use of any species and will be conducted under a Federal permit for scientific research issued by the Marine Mammal Commission under the Marine Mammal Protection Act.

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6. Animal Use:
Provide the specifications for all of the animals requested for use in this protocol. List each strain separately.

Species (common name)	Breed/Strain	Vender/Source	Number Requested			
			Year 1	Year 2	Year 3	Total

☒ Not Applicable (this is a population field study of common bottlenose dolphins *Tursiops truncatus*)
If a population field study, check all vertebrate animals that are planned to be studied:
☐ Fish ☐ Amphibians ☐ Reptiles ☐ Birds ☒ Mammals / ☒ Cetaceans

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7. Personnel:
List all personnel having contact with animals, the species proposed and the years of experience the individual has with the species. List the specific roles the individual will have in the project and the date of last training received.

Name, Degree, Title	Species and Years of Experience	Specific Role in Project*	Training Date
George Guillen	2 yrs *Tursiops truncatus* – stranding networks	Principal Investigator	9/29/2014
Kristi Fazioli	25 yrs experience *Tursiops truncatus* and other cetacean species;	Management and supervision of protocol development, field surveys and data collection	9/29/2014 Also: in 2000 – Training for remote biopsy darting; will refresh training before commencing current project

Sherah Loe	1 year	Develop, lead and participate in field surveys and data collection	9/29/2014

* Examples include: supervision, care/handling, anesthesia, surgery, monitoring, post-procedural care, euthanasia in the stated species

☒ Student visitors will/may participate in this protocol and will be supervised by: Kristi Fazioli (students/visitors will participate in photographic identification and behavioral observation protocols only, and may observe, but not participate in remote biopsy darting)

8a. Literature Search

Using at least two different databases, perform literature searches to determine alternatives to procedures that may cause more than a momentary or slight pain or distress to the animals, and unnecessary duplication of research.

Search Database (e.g., Agricola)	**Date of Search** MM/DD/YYYY	**Years Covered** (e.g., 1980 – 2010)	**Keywords or Search Strategy Used in Search**
Google Scholar	09/08/2014	Any time	alternatives to remote biopsy darting *Tursiops truncatus*
Animal Welfare Information Center	09/08/2014	Any time	remote biopsy darting *Tursiops truncatus*

8b. Rationale and purpose of animal use

State the overall rationale, purpose, and significance of this project.

The proposed research intends to provide critically needed baseline data to characterize residency patterns, habitat use and population abundance trends for Texas dolphin populations by utilizing boat-based photo-identification surveys and tissue sampling through remote biopsy darting. Collection of baseline data is a crucial first step in assessing potential risks and evaluating the health of these strategic dolphin stocks. Delineation of biologically significant boundaries between dolphin communities and the dynamics of how they interact with each other is crucial to understanding the population as a whole. Tracking individuals using photo-identification surveys and analyzing genetic markers (Martien et al. , Sellas et al. 2005, Möller et al. 2007), stable isotope signatures (Barros et al. 2010, Wilson et al. 2013b, Browning et al. 2014b), and anthropogenic compounds (Hansen et al. 2004, Litz et al. 2007, Balmer et al. 2011) through remote biopsy samples will provide data for designating socially and biologically distinct population units, as opposed to current geographically defined management units.

Integrating data in this way from various lines of evidence will increase confidence in stock delineation over any single method (Mullin et al. 2007, Balmer et al. 2013, Vollmer & Rosel 2013). Managers can use this data for informed decisions regarding stock delineation and management of population units as functional elements of the ecosystem.

8c. Justification for animal use.
Explain why non-animal models such as isolated organ preparation, cell or tissue culture, or computer simulation cannot be used.

Direct study of the population is the only way to gather the data necessary for conservation management of the species.

8d. Justification for using this particular species.
Explain why the species and/or strain(s) requested is/are the most appropriate for this research. Statements that the planned species is traditionally used for the proposed research are not sufficient.

Large data gaps exist within Texas for managing this protected species.

8e. Alternatives to Potentially Distressful Procedures
Describe considerations of alternatives to procedures that may cause more than a momentary or slight pain or distress to the animals, and determination that alternatives were not available.
☐ Not Applicable (animals listed are only in USDA Category B or C)

The only alternative to collecting tissue samples for analysis of diet and contaminant loads in live, free-ranging cetaceans is through capture. This alternative is considerably more stressful and dangerous to the animal than the low-level startle response typically incurred during remote biopsy darting. While some studies utilize samples collected from stranded/dead animals, these samples have limitations due to tissue decay and lack of associated data from tracking of the individual's distribution through field survey and photo-identification.

8f. Assurance of Non-Duplication
☒ This experiment does not unnecessarily duplicate previous experiments.
Otherwise, provide justification of the necessity of experiments proposed.

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9. Justification of animal numbers.
Provide a detailed justification for the numbers of animals requested. Include number of animals per group and total number of animals. If power analysis was utilized, give

appropriate details. If the determination was based on prior experience, please cite reference. If a population study in the field give justification of sampling method.

For population field study, all dolphins sighted during boat surveys will be approached to within 6-20 m for photographic identification collection of behavioral and environmental data. This method is widely used in marine mammal population research and all personnel are trained to approach dolphins using protocol established to minimize disturbance. We will use well-established, standard boat-based photo-identification techniques, with protocols outlined in the Sarasota Dolphin Research Program manual for field research and laboratory activities (SDRP 2006), (Rosel et al. 2011) Photo-identification Workshop Report, and Urian et al. (2014) Recommendations for Photo-identification Methods.

For determination of biopsy sample sizes, we examined similar studies that analyzed bottlenose dolphin tissue for fine-scale population structure and contaminant loads utilizing samples from a variety of sources (remote biopsy, live capture and stranded animals). Sample sizes in these studies range from 18 – 300 depending on the extent of the area being studied and the type of analysis being performed (Litz et al. 2007, Kiszka et al. 2010a, Balmer et al. 2011, Kiszka et al. 2011, Kucklick et al. 2011, Wilson et al. 2013b). For example, Kucklick et al. (2011) examined persistent organic pollutants (POPs) at 14 Atlantic and U.S. GoM locations, with sample sizes ranging from 3 to 55 for each location; Balmer et al. (2011) examined 102 samples for POPs from two field sites along the Georgia coast; Litz analyzed 34 samples of individuals with photographic sighting histories within a Florida bay; and Kiszka et al. examined stable isotope signatures in two ecological niche focused studies with sample sizes of 91 and 105 (Kiszka et al. 2010a, Kiszka et al. 2011). Analysis of POPs for stock structure requires the use of only samples from males, resulting in additional effort since males and females cannot be distinguished in the field. Missed shots and non-viable or lost samples are included in our count and we are estimating an 80-90% success rate for our sampling attempts (based on Noren and Mocklin (2012). Our goal is to sample up to 100 dolphins per year by remote biopsy, based on both consideration of statistically viable sample sizes and logistical constraints of annual effort.

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10. Pain, Discomfort, and Distress.

a. USDA Pain/Distress Classification

Check the category that indicates the highest level of pain/distress the animals will experience during the course of these studies. (Refer to the Instructions, Section 10 for help.)

☐ Category B ☒ Category C ☐ Category D ☐ Category E

b. If Category E is selected, provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Samples are collected remotely from free-swimming dolphins. A small (10 mm wide x 25 mm deep) blubber and skin sample will be retrieved using a dart shot from a crossbow and animals will not be captured, restrained or handled in any way. Therefore, it is not possible to administer pain reliever or anesthetic at the biopsy location. Remote biopsy sampling of free-swimming dolphins has minimal risk of injury and typically elicits mild, short-term, startle responses (Weller et al. 1997, Krützen et al. 2002, Sellas et al. 2005, Gorgone et al. 2008, Kiszka et al. 2010b, Noren & Mocklin 2012, Tezanos-Pinto & Baker 2012).

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11. What will happen with animals at the end of their roles in the project?

a. Check all that apply and provide explanation if necessary.

☐ Placed for adoption
☐ Released into wild (field study)
☐ Euthanasia:

Rodents:

- ☐ CO_2- followed by secondary method (e.g., bilateral thoracotomy, cervical dislocation)
  State secondary method:
- ☐ Injectable agent *(Specify Agent, Route, Dose):*
- ☐ Inhalant agent *(Specify Agent, Dose):*
- ☐ Cervical Dislocation (rodents < 200 gm) w/ anesthesia- (S*pecify Agent, Route, Dose*):
- ☐ Decapitation/Guillotine w/ anesthesia- (S*pecify Agent, Route, Dose*):
- ☐ Exsanguination w/ anesthesia (S*pecify Agent, Route, Dose*):
- ☐ Anesthetic + Perfusion (S*pecify Agent, Route, Dose*):
  Type of perfusion:

Amphibians, Fish, Reptiles:

- ☐ CO_2
- ☐ Injectable agent *(Specify Agent, Route, Dose):*
- ☐ External or topical agent *(Specify Agent, Route, Dose):*
- ☐ Inhalant agent *(Specify Agent, Dose):*
- ☐ Decapitation and pithing
- ☐ Stunning and decapitation/pithing

☒ Other: Animals will not be captured (field study)

b. Explanation / Justification

Dolphins will not captured, restrained or handled in any way. Individuals sampled using remote biopsy darting will be identified using photographic identification techniques and healing of the biopsy wound will photographed and tracked as possible whenever that individual is sighted during monitoring surveys.

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12. Additional Forms Attached

Check all that apply and attach appropriate forms. If a form is not needed, delete the page from the protocol.

☐ Appendix A: Laboratory Research or Classroom
☐ Appendix B: Surgical Procedures
☒ Appendix C: Wild Animal and/or Field Research
☐ Appendix D: Safety
☐ Appendix E: Renewal / Addendum
☒ Appendix F: Additional Information / Standard Operating Procedures

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13. Check the following:

☒ I certify that the use of all animals involved in this project will be carried out within the provisions of the Animal Welfare Act, the Guide for Care and Use of Laboratory Animals, the PHS Policy on Humane Care and Use of Animals, the University of Houston Policy on Care and Use of Animals and related animal welfare rules and regulations as issued by state and/or federal agencies.

☒ I am aware that the Institutional Animal Care and Use Committee (IACUC) may make periodic inspections of all labs in which animals are used. I will permit unannounced inspections and observations of my animals and surgical techniques by a UH veterinarian or other representative of the IACUC.

☒ I am aware that the IACUC is empowered to stop any objectionable procedure or project. Where procedures have caused severe distress to an animal which cannot be alleviated, UH staff veterinarians are authorized to humanely euthanize that animal. I understand that every attempt will be made to contact me before any action is taken.

☒ I understand that I cannot start this project until I have received approval from the IACUC.

☒ I understand that I will make written notification to the IACUC of any proposed changes to the project. I understand that I will not be able to implement such changes until approval is received from the IACUC.

☒ I certify that the above statements are true and that I will make written notification to the IACUC of any changes in the proposed project prior to proceeding with any animal experiment.

George Guillen

_____

9/26/14

Signature of Principal Investigator or Instructor Date

☒ Submitted Electronically: Instead of signature, protocol is emailed from the PI's UHCL email address

Appendix C: Wild Animal and/or Field Research

1. How will the animals be captured?

N/A – No animals will be captured or restrained for this research

2. Will animals be maintained for any length of time, where they will be maintained (field and/or animal facility), and for how long?

N/A

3. Will animals be transported, and if so, how will stress be minimized?

N/A

4. How will the housing and nutritional needs of animals that are captured and detained for a research project be met?

N/A

5. What criteria will be used in determining whether the animals can be released after they have been captured (even if the animals are part of a capture and release project, and they will not be maintained for any length of time)?

N/A

6. How will pain and/or distress be monitored in these animals?

Individuals sampled using remote biopsy darting will be identified using photographic identification techniques and behavioral reactions will be recorded during and immediately following a biopsy shot. Healing of the biopsy wound will photographed and tracked as possible whenever that individual is sighted during future monitoring surveys.

Appendix F: Additional Information / Standard Operating Procedures
Use this page to add any additional information the committee may need for evaluation of this protocol. This can include standard operating procedures (SOP's) utilized.

Photo-Identification and behavioral observations – We will use well-established, standard boat-based photo-identification techniques, with protocols outlined in the Sarasota Dolphin Research Program manual for field research and laboratory activities (SDRP 2006), (Rosel et al. 2011) Photo-identification Workshop Report, and Urian et al. (2014) Recommendations for Photo-identification Methods. A variety of small outboard survey vessels will be used, with several vessels potentially surveying simultaneously in different regions due to the large overall study area. Vessels will cruise at standard slow planing speed (typically 18.5-32 km/hr) along a meandering route or line transect until dolphins are encountered. The vessel will typically approach the dolphins to within 6-20 m for photographs (dolphins may approach the boat more closely on their own). During an encounter, typically lasting 15-30 min (this will vary depending on group size and activity), the boat will slowly parallel the group, dolphin dorsal fins will be photographed with digital cameras with zoom-telephoto lenses, group number and composition will be estimated, activities, headings, and environmental conditions will be recorded, the location will be recorded by a GPS, and the occurrence of anthropogenic interactions will be recorded (Henningsen & Wursig 1992, Powell & Wells 2011). The vessel will then continue along the survey route until the next group of dolphins is encountered, and the process will be repeated.

In some cases, the photo-identification survey will be followed by sessions of focal-individual or -group follows involving behavioral sampling methods (Altmann 1974, Mann 1999). During focal follows, a selected individual or group of individuals will be observed from a distance sufficiently close to ensure reliable determination of behavioral state, but sufficiently far to minimize behavioral effects from the boat presence. Behavior will be systematically recorded over periods of 30 min to two hours, depending on the project.

In the laboratory, photo-analysis will include grading of photos for quality and distinctiveness of fins, matching of fins to previously identified dolphins and cataloging of individuals. Separate institutions will maintain their own catalogs, following standardized photo-analysis protocols, and will cross-match against other catalogs. Sighting and environmental data will be archived with the photographs using the FinBase Photo-Identification Database and Mapping Tool, allowing efficient submission to GoMDIS for collaboration throughout the Gulf Coast (Cush & Wells 2013).

Remote Biopsy Sampling – Biopsy darting is accepted as a safe technique for cetacean research, with minimal short-term behavioral reaction and minor injury to the animals

(Weller et al. 1997, Krützen et al. 2002, Noren & Mocklin 2012, Tezanos-Pinto & Baker 2012). Sampling will be conducted following standardized protocols developed by NOAA and the Chicago Zoological Society/SDRP (Krützen et al. 2002, Sellas et al. 2005, Wenzel et al. 2010). Biopsy samples will include skin and blubber from below the dorsal fin, collected using biopsy darts discharged from a crossbow (Barnett Panzer V or equivalent). Biopsy cutter heads measuring 10 mm wide x 25 mm deep are specially designed to penetrate only through the depth of the blubber, using a sterilized stainless-steel tip (developed by Dr. Finn Larson). Samples will be processed as needed for each particular project and in accordance with protocols established by NOAA and the National Institute of Standards and Technology (NIST). Typically, the sample will be divided into epidermis and blubber portions (using sterile forceps). Skin is further sectioned, and portions placed in labeled 20% DMSO/saturated NaCl vials for gender, genomic DNA and stable isotope analysis. The blubber for organic contaminant analysis is placed in a Teflon vial container with a cryogenic label, wrapped in foil and placed in a liquid nitrogen dry shipper while on the sampling vessel and then stored in the lab in a -80° C freezer prior to shipping (overnight in dry ice) or analysis.

During a biopsy survey, dolphin groups will be approached in a similar manner as during photo-identification and suitability for sample collection will be determined. Dolphin groups will be eliminated as sampling candidates if one or more neonates are in the group (identified by body length less than 50% of the mothers, presence of fetal folds, head-out surfacing, and surfacing in echelon position (Urian & Wells 1996), dolphins display evasive behavior (such as those defined by NOAA of "prolonged diving, underwater exhalation, underwater course changes, or rapid swimming at the surface"), or the group consists primarily of previously sampled dolphins. To limit disturbance of animals, no more than two biopsy samples will be collected per group of dolphins observed. Animals will be photographed during biopsy to ensure the integrity of photo-identification records for each animal and to avoid resampling. The sampler will shoot when the target animal is perpendicular to the dart impact at a typical distance of 5-10 m and not less than 4 m, aiming at the flank of the dolphin below the dorsal fin and above the lateral midline.

Personnel conducting biopsy surveys will be fully trained and accompanied by experienced personnel working under a National Marine Fisheries Service Scientific Research Permit and institutionally approved IACUC protocols prior to conducting any sample collection activity.

Anticipated Effects on Animals

Photo-identification and behavioral follows- Boat-based research surveys and follows are used successfully with cetaceans world-wide with little to no adverse effects. Short-term behavioral responses consistent with disturbance may include

avoidance of the research vessel and a change in group cohesiveness and activity (Nowacek et al. 2001, Christiansen et al. 2010).

Remote Biopsy Sampling- Remote biopsy sampling of free-swimming dolphins has minimal risk of injury and typically elicits mild, short-term, startle responses (Weller et al. 1997, Krützen et al. 2002, Sellas et al. 2005, Gorgone et al. 2008, Kiszka et al. 2010b, Noren & Mocklin 2012, Tezanos-Pinto & Baker 2012). Only one instance of dolphin mortality has been published due to biopsy sampling (Bearzi 2000), and was considered an anomalous event. Wounds are expected to heal quickly and without complications (Weller et al. 1997, Krützen et al. 2002).

Measures to Minimize Negative Effects

Photo-identification and behavioral follows- During boat-based surveys and follows, boat operators will consist only of personnel trained and experienced with maneuvering a vessel around dolphins in a manner that will reduce disturbance. To minimize harassment, dolphins will be approached based on NOAA's responsible viewing guidelines, and dolphins exhibiting behaviors consistent with annoyance or irritation (such as evasive behavior or continued chuffing and tail slapping) will be approached no more than three times before terminating the attempt. During photo-identification, sub-group survey efforts will not exceed 30 minutes. While conducting behavioral follows, the vessel will stay as far from the group as possible while still allowing observation and identification of the focal individual or group.

Remote Biopsy Sampling- Standard biopsy safety protocols will be strictly adhered to during any biopsy survey and only samplers who have demonstrated proficiency and completed training and apprenticeship under experienced samplers will perform sampling duties. Additional safety measures designed to

reduce injury include: 1) Only taking shots within a safe range of > 4 m, perpendicular to the target animal surfacing in a predictable manner; 2) Never attempting to sample a group with a neonate present and never targeting a dolphin calf that is known or estimated to be < 2 years of age (< 75% of the presumed mother's body length (Urian & Wells 1996) or target an adult in close association with a calf of any age; 3) All dart cutting heads will be sterilized before use and have a stopper to prevent deep penetration of the blubber layer; and 4) Never attempting to sample an individual that appears ill or emaciated.

To minimize the impact of multiple biopsy events, no individual dolphin will be sampled more than once in a year and no more than twice total. This will allow us to re-sample an individual if needed for comparison after an environmental disturbance. Photo-identification will be performed for each sampled animal and experienced crew members will make their best efforts to identify all targeted animals before making a biopsy attempt. No more than two dolphins per group will be sampled during any sampling event, with typically no more than three attempts made per group.

APPENDIX B:

NATIONAL MARINE FISHERIES PERMIT #18881

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

APR 2 2 2015

Timothy Tristan, D.V.M.
Texas Sealife Center
14225 S Padre Island Dr.
Corpus Christi, Texas 78418

Dear Dr. Tristan:

The National Marine Fisheries Service has issued Permit No. 18881 to the Texas Sealife Center, for research activities on marine mammals. This permit is effective upon your signature and valid through the expiration date indicated in Condition A.1.

Here's what you need to do to use your permit:

1. Read the permit, including attachments. If you have questions, call your permit analyst – Amy Hapeman or Howard Goldstein – at 301-427-8401 before signing the permit.
2. Sign and date the original signature page and the signature page marked "file copy."
3. Keep the original signature page with your permit. You need both as proof of your authorization to conduct the research activities.
4. Send the "file copy" signature page to our office as proof of your acceptance of the permit.

Please keep your email contact information current in our online database (https://apps.nmfs.noaa.gov/). You will receive automated email reminders of due dates for annual and final reports, and a notice prior to expiration of your permit.

Please return the signature page marked "file copy" to the Permits Division (F/PR1), 1315 East-West Highway, Silver Spring, MD 20910. You may also submit the "file copy" of the signature page by email or facsimile (FAX number: 301-713-0376) and confirm it by mail.

Sincerely,

Jolie Harrison
Chief, Permits and Conservation Division
Office of Protected Resources
(phone: 301-427-8401)

Enclosure

Printed on Recycled Paper

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

Permit No. 18881
Expiration Date: April 30, 2020
Reports Due: July 31st, annually

PERMIT TO TAKE PROTECTED SPECIES[1] FOR SCIENTIFIC PURPOSES

I. Authorization

This permit is issued to the Texas Sealife Center, 14225 S Padre Island Dr., Corpus Christi, TX 78418, (hereinafter "Permit Holder"), [Responsible Party: Dr. Timothy Tristan], pursuant to the provisions of the Marine Mammal Protection Act of 1972 as amended (MMPA; 16 U.S.C. 1361 *et seq.*) and the regulations governing the taking and importing of marine mammals (50 CFR Part 216).

II. Abstract

The objectives of the permitted activity, as described in the application, are to study bottlenose dolphins (*Tursiops truncatus*) in the bay, sound, estuary and near-shore coastal waters of Texas to: 1) develop and maintain standardized photo-identification catalogs; 2) characterize fine-scale population structure and dynamics; 3) estimate abundance for strategic stocks; 4) establish baseline patterns of distribution, habitat use, site-fidelity, diet, and contaminant loads; 5) analyze dolphin behavior in relation to anthropogenic activities; and 6) identify potential risks to the population.

III. Terms and Conditions

The activities authorized herein must occur by the means, in the areas, and for the purposes set forth in the permit application, and as limited by the Terms and Conditions specified in this permit, including attachments and appendices. Permit noncompliance constitutes a violation and is grounds for permit modification, suspension, or revocation, and for enforcement action.

A. Duration of Permit

1. Personnel listed in Condition C.1 of this permit (hereinafter "Researchers") may conduct activities authorized by this permit through April 30, 2020. This permit expires on the date indicated and is non-renewable. This permit may be extended by the Director, NMFS Office of Protected Resources, pursuant to applicable regulations and the requirements of the MMPA.

[1] "Protected species" include species listed as threatened or endangered under the ESA, and marine mammals.

2. Researchers must immediately stop permitted activities and the Permit Holder must contact the Chief, NMFS Permits and Conservation Division (hereinafter "Permits Division") for written permission to resume:

   a. If serious injury or mortality[2] of protected species occurs. See Condition E.2 for reporting requirements.

   b. If authorized take[3] is exceeded in any of the following ways:

      i. More animals are taken than allowed in Table 1 of Section B.1.

      ii. Animals are taken in a manner not authorized by this permit.

      iii. Protected species other than those authorized by this permit are taken.

      See Condition E.2 for reporting requirements.

3. The Permit Holder may continue to possess biological samples[4] acquired[5] under this permit after permit expiration without additional written authorization, provided the samples are maintained as specified in this permit.

B. Number and Kind(s) of Protected Species, Location(s) and Manner of Taking

1. The table in Appendix 1 outlines the number of protected species, by species and stock, authorized to be taken, and the locations, manner, and time period in which they may be taken.

2. Researchers working under this permit may collect visual images (e.g., photographs, video) in addition to the photo-identification or behavioral photo-documentation authorized in Appendix 1 as needed to document the permitted activities, provided the collection of such images does not result in takes.

3. The Permit Holder may use visual images and audio recordings collected under this permit, including those authorized in Table 1 of Appendix 1, in printed materials (including commercial or scientific publications) and presentations

[2] This permit does not allow for unintentional serious injury and mortality caused by the presence or actions of researchers. This includes, but is not limited to: deaths of dependent young by starvation following research-related death of a lactating female; deaths resulting from infections related to sampling procedures; and deaths or injuries sustained by animals during capture and handling, or while attempting to avoid researchers or escape capture. Note that a serious injury is defined by regulation as any injury that will likely result in mortality.

[3] By regulation, a take under the MMPA means to harass, hunt, capture, collect, or kill, or attempt to harass, hunt, capture, collect, or kill any marine mammal. This includes, without limitation, any of the following: the collection of dead animals, or parts thereof; the restraint or detention of a marine mammal, no matter how temporary; tagging a marine mammal; the negligent or intentional operation of an aircraft or vessel, or the doing of any other negligent or intentional act which results in disturbing or molesting a marine mammal; and feeding or attempting to feed a marine mammal in the wild.

[4] Biological samples include, but are not limited to: carcasses (whole or parts); and any tissues, fluids, or other specimens from live or dead protected species; except feces, urine, and spew collected from the water or ground.

[5] Authorized methods of sample acquisition are specified in Appendix 1.

provided the images and recordings are accompanied by a statement indicating that the activity was conducted pursuant to a NMFS Permit. This statement must accompany the images and recordings in all subsequent uses or sales.

4. The Chief, Permits Division may grant written approval for personnel performing activities not essential to achieving the research objectives (e.g., a documentary film crew) to be present, provided:

   a. The Permit Holder submits a request to the Permits Division specifying the purpose and nature of the activity, location, approximate dates, and number and roles of individuals for which permission is sought.

   b. Non-essential personnel/activities will not influence the conduct of permitted activities or result in takes of protected species.

   c. Persons authorized to accompany the Researchers for the purpose of such non-essential activities will not be allowed to participate in the permitted activities.

   d. The Permit Holder and Researchers do not require compensation from the individuals in return for allowing them to accompany Researchers.

5. Researchers must comply with the following conditions related to the manner of taking:

   Counting and Reporting Takes

   a. Any "approach"[6] of a cetacean constitutes a take and must be counted and reported regardless of whether an animal reacts.

   b. During an approach, Researchers may attempt all procedures in a take table row once.

   c. For Level A procedures [biopsy sampling]:
   Each additional attempt to perform the suite of procedures during the same approach constitutes a new take and must be counted and reported against that row of takes.

   Attempts include:
   - misses,
   - successful hits, and
   - hits with no sample collected.

[6] An "approach" is defined as a continuous sequence of maneuvers involving a vessel, including drifting, directed toward a cetacean or group of cetaceans closer than 100 yards for baleen and sperm whales and 50 yards for all other cetaceans.

d. No individual animal may be taken more than 3 times in one day.

General

e. To minimize disturbance of the subject animals the Permit Holder must exercise caution when approaching animals and must retreat from animals if behaviors indicate the approach may be interfering with reproduction, feeding, or other vital functions.

f. Where females with calves are authorized to be taken, Researchers:

i. Must immediately terminate efforts if there is any evidence that the activity may be interfering with pair-bonding or other vital functions;

ii. Must not position the research vessel between the mother and calf;

iii. Must approach mothers and calves gradually to minimize or avoid any startle response; and

iv. Must not approach any mother or calf while the calf is actively nursing.

Biopsy Sampling

g. All biopsy tips must be disinfected between and prior to each use.

h. No calves of any age may be biopsy sampled.

i Before attempting to sample an individual, Researchers must take reasonable measures (e.g., compare photo-identifications) to avoid repeated sampling of any individual.

j. A biopsy attempt must be discontinued if an animal exhibits repetitive strong adverse reactions to the activity or the vessel.

k. In no instance will Researchers attempt to biopsy a cetacean anywhere forward of the pectoral fin.

6. The Permit Holder must comply with the following conditions and the regulations at 50 CFR 216.37, for biological samples acquired or possessed under authority of this permit.

a. The Permit Holder is ultimately responsible for compliance with this permit and applicable regulations related to the samples unless the samples

are permanently transferred according to NMFS regulations governing the taking and importing of marine mammals (50 CFR 216.37).

b. Samples must be maintained according to accepted curatorial standards and must be labeled with a unique identifier (e.g., alphanumeric code) that is connected to on-site records with information identifying the

   i. species and, where known, age and sex;

   ii. date of collection, acquisition, or import;

   iii. type of sample (e.g., blood, skin, bone);

   iv. origin (i.e., where collected or imported from); and

   v. legal authorization for original sample collection or import.

c. Biological samples belong to the Permit Holder and may be temporarily transferred to Authorized Recipients identified in Appendix 2 without additional written authorization, for analysis or curation related to the objectives of this permit. The Permit Holder remains responsible for the samples, including any reporting requirements.

d. The Permit Holder may request approval of additional Authorized Recipients for analysis and curation of samples related to the permit objectives by submitting a written request to the Permits Division specifying the

   i. name and affiliation of the recipient;

   ii. address of the recipient;

   iii. types of samples to be sent (species, tissue type); and

   iv. type of analysis or whether samples will be curated.

e. Sample recipients must have authorization pursuant to 50 CFR 216.37 prior to permanent transfer of samples and transfers for purposes not related to the objectives of this permit.

f. Samples cannot be bought or sold, including parts transferred pursuant to 50 CFR 216.37.

g. After meeting the permitted objectives, the Permit Holder may continue to possess and use samples acquired under this permit, without additional written authorization, provided the samples are maintained as specified in the permit and findings are discussed in the annual reports (See Condition E. 3).

C. Qualifications, Responsibilities, and Designation of Personnel

1. At the discretion of the Permit Holder, the following Researchers may participate in the conduct of the permitted activities in accordance with their qualifications and the limitations specified herein:

   a. Principal Investigator – Kristi Fazioli

   b. Co-Investigators – See Appendix 2 for list of names and corresponding activities.

   c. Research Assistants – personnel identified by the Permit Holder or Principal Investigator and qualified to act pursuant to Conditions C.2, C.3, and C.4 of this permit

2. Individuals conducting permitted activities must possess qualifications commensurate with their roles and responsibilities. The roles and responsibilities of personnel operating under this permit are as follows:

   a. The Permit Holder is ultimately responsible for activities of individuals operating under the authority of this permit. The Responsible Party is the person at the institution/facility who is responsible for the supervision of the Principal Investigator.

   b. The Principal Investigator (PI) is the individual primarily responsible for the taking, import, export and related activities conducted under the permit. The PI must be on site during activities conducted under this permit unless a Co-Investigator named in Condition C.1 is present to act in place of the PI.

   c. Co-Investigators (CIs) are individuals who are qualified to conduct activities authorized by the permit, for the objectives described in the application, without the on-site supervision of the PI. CIs assume the role and responsibility of the PI in the PI's absence.

   d. Research Assistants (RAs) are individuals who work under the direct and on-site supervision of the PI or a CI. RAs cannot conduct permitted activities in the absence of the PI or a CI.

3. Personnel involved in permitted activities must be reasonable in number and essential to conduct of the permitted activities. Essential personnel are limited to

   a. Individuals who perform a function directly supportive of and necessary to the permitted activity (including operation of vessels or aircraft essential to conduct of the activity);

b. Individuals included as backup for those personnel essential to the conduct of the permitted activity; and

c. Individuals included for training purposes.

4. Persons who require state or Federal licenses to conduct activities authorized under the permit (e.g., veterinarians, pilots) must be duly licensed when undertaking such activities.

5. Permitted activities may be conducted aboard vessels or aircraft, or in cooperation with individuals or organizations, engaged in commercial activities, provided the commercial activities are not conducted simultaneously with the permitted activities.

6. The Permit Holder cannot require or receive direct or indirect compensation from a person approved to act as PI, CI, or RA under this permit in return for requesting such approval from the Permits Division.

7. The Permit Holder or PI may add CIs by submitting a request to the Chief, Permits Division that includes a description of the individual's qualifications to conduct and oversee the activities authorized under this permit. If a CI will only be responsible for a subset of permitted activities, the request must also specify the activities for which they would provide oversight.

8. Where the Permit Holder is an institution/facility, the Responsible Party may request a change of PI by submitting a request to the Chief, Permits Division that includes a description of the individual's qualifications to conduct and oversee the activities authorized under this permit.

9. Submit requests to add CIs or change the PI by one of the following:
   - the online system at https://apps.nmfs.noaa.gov;
   - an email attachment to the permit analyst for this permit; or
   - a hard copy mailed or faxed to the Chief, Permits Division, Office of Protected Resources, NMFS, 1315 East-West Highway, Room 13705, Silver Spring, MD 20910; phone (301)427-8401; fax (301)713-0376.

D. Possession of Permit

1. This permit cannot be transferred or assigned to any other person.

2. The Permit Holder and persons operating under the authority of this permit must possess a copy of this permit when:

   a. engaged in a permitted activity;

b. a protected species is in transit incidental to a permitted activity; and

c. a protected species taken under the permit is in the possession of such persons.

3. A duplicate copy of this permit must accompany or be attached to the container, package, enclosure, or other means of containment in which a protected species or protected species part is placed for purposes of storage, transit, supervision or care.

E. Reports

1. The Permit Holder must submit annual, final, and incident reports containing the information and in the format specified by the Permits Division.

a. Reports must be submitted to the Permits Division by one of the following:

- the online system at https://apps.nmfs.noaa.gov;
- an email attachment to the permit analyst for this permit; or
- a hard copy mailed or faxed to the Chief, Permits Division.

b. You must contact your permit analyst for a reporting form if you do not submit reports through the online system.

2. Incident reports: must be submitted within two weeks of serious injury and mortality events or exceeding authorized takes, as specified in Condition A.2.

a. The incident report must include a complete description of the events and identification of steps that will be taken to reduce the potential for additional serious injury and research-related mortality or exceedence of authorized take.

b. In addition to the written report, the Permit Holder must contact the Permits Division by phone (301-427-8401) as soon as possible, but no later than within two business days of the incident.

c. The Permits Division may grant authorization to resume permitted activities based on review of the incident report and in consideration of the Terms and Conditions of this permit.

3. Annual reports describing activities conducted during the previous permit year (from May 1 to April 30 of the following year) must

a. be submitted by July 31st each year for which the permit is valid; and

b. include a tabular accounting of takes and a narrative description of activities and effects.

4. A final report summarizing activities over the life of the permit must be submitted by October 30, 2020, or, if the research concludes prior to permit expiration, within 180 days of completion of the research.

5. Research results must be published or otherwise made available to the scientific community in a reasonable period of time. Copies of technical reports, conference abstracts, papers, or publications resulting from permitted research must be submitted the Permits Division.

F. Notification and Coordination

1. The Permit Holder must provide written notification of planned field work to the applicable NMFS Region at least two weeks prior to initiation of each field trip/season. If there will be multiple field trips/seasons in a permit year, a single summary notification may be submitted per year.

a. Notification must include the

i. locations of the intended field study and/or survey routes,

ii. estimated dates of activities, and

iii. number and roles of participants (for example: PI, CI, veterinarian, boat driver, safety diver, animal restrainer, Research Assistant "in training").

b. Notification must be sent to the Southeast Assistant Regional Administrator for Protected Resources:

Southeast Region, NMFS, 263 13th Ave South, St. Petersburg, FL 33701; phone (727)824-5312; fax (727)824-5309

Email (*preferred*): nmfs.ser.research.notification@noaa.gov

2. To the maximum extent practical, the Permit Holder must coordinate permitted activities with activities of other Permit Holders conducting the same or similar activities on the same species, in the same locations, or at the same times of year to avoid unnecessary disturbance of animals. Contact the Regional Office listed in F.1.b for information about coordinating with other Permit Holders.

G. Observers and Inspections

1. NMFS may review activities conducted under this permit. At the request of NMFS, the Permit Holder must cooperate with any such review by:

a. Allowing an employee of NOAA or other person designated by the Director, NMFS Office of Protected Resources to observe permitted activities; and

b. Providing all documents or other information relating to the permitted activities.

H. Modification, Suspension, and Revocation

1. Permits are subject to suspension, revocation, modification, and denial in accordance with the provisions of subpart D [Permit Sanctions and Denials] of 15 CFR part 904.

2. The Director, NMFS Office of Protected Resources may modify, suspend, or revoke this permit in whole or in part

a. In order to make the permit consistent with a change made after the date of permit issuance with respect to applicable regulations prescribed under section 103 of the MMPA;

b. In a case in which a violation of the terms and conditions of the permit is found;

c. In response to a written request[7] from the Permit Holder;

d. If NMFS determines that the application or other information pertaining to the permitted activities (including, but not limited to, reports pursuant to Section E of this permit and information provided to NOAA personnel pursuant to Section G of this permit) includes false information; and

3. Issuance of this permit does not guarantee or imply that NMFS will issue or approve subsequent permits or amendments for the same or similar activities requested by the Permit Holder, including those of a continuing nature.

I. Penalties and Permit Sanctions

1. A person who violates a provision of this permit, the MMPA, or the regulations at 50 CFR 216 is subject to civil and criminal penalties, permit sanctions, and forfeiture as authorized under the MMPA, and 15 CFR part 904.

[7] The Permit Holder may request changes to the permit related to: the objectives or purposes of the permitted activities; the species or number of animals taken; and the location, time, or manner of taking or importing protected species. Such requests must be submitted in writing to the Permits Division in the format specified in the application instructions.

2. The NMFS Office of Protected Resources shall be the sole arbiter of whether a given activity is within the scope and bounds of the authorization granted in this permit.

   a. The Permit Holder must contact the Permits Division for verification before conducting the activity if they are unsure whether an activity is within the scope of the permit.

   b. Failure to verify, where the NMFS Office of Protected Resources subsequently determines that an activity was outside the scope of the permit, may be used as evidence of a violation of the permit, the MMPA, and applicable regulations in any enforcement actions.

J. Acceptance of Permit

1. In signing this permit, the Permit Holder

   a. Agrees to abide by all terms and conditions set forth in the permit, all restrictions and relevant regulations under 50 CFR Part 216 and all restrictions and requirements under the MMPA;

   b. Acknowledges that the authority to conduct certain activities specified in the permit is conditional and subject to authorization by the Office Director; and

   c. Acknowledges that this permit does not relieve the Permit Holder of the responsibility to obtain any other permits, or comply with any other Federal, State, local, or international laws or regulations.

Donna S. Wieting
Director, Office of Protected Resources
National Marine Fisheries Service

APR 2 1 2015
Date Issued

Tim Tristan, DVM
Texas Sealife Center
Responsible Party

Date Effective

FILE COPY

Appendix 1: Tables Specifying the Kinds of Protected Species, Locations, and Manner of Taking

Table 1: Authorized annual take of bottlenose dolphins during vessel surveys in the bay, sound, estuary and near-shore coastal waters of Texas. LOC = Letter of Confirmation.

LISTING UNIT/STOCK	LIFE STAGE	ANNUAL TAKES[8]	TAKES PER ANIMAL	TAKE ACTION	PROCEDURES	BEGIN DATE	DETAILS
Gulf of Mexico Western Coastal Stock	All	2,277	1	Harass	Count/survey; Observations, behavioral; Photo-id	5/1/2015	Includes focal follows; Some individuals may be subject to multiple takes annually
Gulf of Mexico Western Coastal Stock	Adult/ Juvenile	33	1	Harass/Sampling	Photo-id; Sample, skin and blubber biopsy	5/1/2015	Texas Coast from barrier islands to 20-m isobath
Gulf of Mexico Bay - Sound & Estuarine (BSE) Stocks	All	2,857	1	Harass	Count/survey; Observations, behavioral; Photo-id	5/1/2015	Includes focal follows; BSE stocks, including passes leading to the GoM. Some individuals may be taken multiple times annually
Gulf of Mexico Bay - Sound & Estuarine Stocks	All	774	6	Harass	Count/survey; Photo-id	5/1/2015	North Texas BSE waters - CMR surveys
Gulf of Mexico Bay - Sound & Estuarine Stocks	Adult/ Juvenile	65	1	Harass/Sampling	Photo-id; Sample, skin and blubber biopsy	5/1/2015	All Texas BSE waters, including passes leading to the Gulf of Mexico

[8] Takes = the **maximum** number of animals, not necessarily individuals, that may be targeted for research annually for the suite of procedures in each row of the table.

LISTING UNIT/STOCK	LIFE STAGE	ANNUAL TAKES[8]	TAKES PER ANIMAL	TAKE ACTION	PROCEDURES	BEGIN DATE	DETAILS
Gulf of Mexico Bay - Sound & Estuarine Stocks	All	2,120	1	Harass	Count/survey; Observations, behavioral; Photo-id	7/1/2019	Continuation of photo-id after LOC No. 18715 expires; Central and South Texas BSE waters, including passes leading to the GoM; Some individuals may be taken multiple times annually

Appendix 2: NMFS-Approved Personnel and Authorized Recipients for Permit No. 18881.

The following individuals are approved to act as Co-Investigators pursuant to the terms and conditions under Section C (Qualifications, Responsibilities, and Designation of Personnel) of this permit.

Name of Co-Investigator	Activities
Kristi Fazioli (PI)	All activities
Jason Allen	All activities
Aaron Barleycorn	All activities
Dara Orbach	All except biopsy sampling
Sarah Piwetz	All except biopsy sampling
Bernd Wursig	All except biopsy sampling
Andreas Fahlman	All except biopsy sampling
George Guillen	All except biopsy sampling
Christopher Marshall	All except biopsy sampling
Danielle Kleinhenz	All except biopsy sampling
Sherah Loe	All except biopsy sampling
William McGlaun	All except biopsy sampling
Linda Price May	All except biopsy sampling

Biological samples authorized for collection or acquisition in Table 1 of Appendix 1 may be transferred to the following Authorized Recipients for the specified disposition, consistent with Condition B.6 of the permit:

Sample Type	Disposition	Authorized Recipient
Skin and blubber	Analysis and curation	NMFS Southeast Fisheries Science Center, Marine Mammal Molecular Genetics Lab, Lafayette, LA
Blubber	Analysis	National Institute of Standards and Technology Laboratory (NIST), Charleston, SC
Skin	Analysis	University of Georgia, Center for Applied Isotope Studies, Athens, GA
Skin and blubber	Curation	University of Houston, Clear Lake
Skin and blubber	Analysis and curation	Chris Marshall, Texas A&M University, Galveston, Department of Marine Biology

APPENDIX C:

UNIVERSITY OF HOUSTON-CLEAR LAKE INSTITUTIONAL ANIMAL CARE AND USE PROTOCOL #11.001.R1

v.110323 Protocol # _11.001. R1

University of Houston-Clear Lake
Institutional Animal Care and Use Protocol

Federal animal welfare regulations require that the Institutional Animal Care and Use Committee (IACUC) must review and approve all activities involving the use of vertebrate animals prior to initiation of such use. Once approved by the IACUC, any change(s) to the following protocol must be submitted in a written amendment for review and approval of the IACUC prior to implementation of the change(s).

1. Title of Project
Long term changes in freshwater, estuarine and marine fish populations in Texas due to biological competition and alteration of habitat and water quality.

2. Principal Investigator:

Name:	George Guillen		
Title:	Associate Professor	Program:	EIH
Phone:	[redacted]	Emergency Phone:	[redacted]
Email:	[redacted]	Campus Mail:	[redacted]

Secondary Contact Person involved in the study:

Name:	Jenny Oakley		
Email:	[redacted]	Emergency Phone:	[redacted]

3. Project Type

☐ New Protocol	☒ Renewal	☐ Addendum	Previous Protocol # 11.001
If renewal:	☒ Annual Renewal	☐ Protocol Renewal	**see Appendix E**
If addendum:	☒ Personnel Change	☒ Minor Revision ☐ Major Revision	**see Appendix E**
Number of years project is expecting to continue:	☐ 1 year	☒ 2 years	☐ 3 years
This protocol is for:	☐ Teaching	☒ Research	☐ Breeding
If teaching:			
Course name and number:			
Frequency course is offered:			
If research:			
How will this project be funded:	NOAA, HGAC, Harris County Flood Control District, TPWD		
If grant, this project is:	☐ Pending	☒ Funded – Federal	☒ Funded – Other
Grant title and/or contract number (if available):			
Has this project already received an independent scientific peer-review?	☒ Yes	☐ No	
If yes, by whom?	NOAA		

4. Location

Where will animals be housed?	☐ UHCL Animal Research Facility	☒ Field Study	
☐ Other:*			
Where will animal use take place?	Texas (Freshwater and Marine Ecosystems).		
Will animals be kept for over 12 hours outside of housing area?	☐ Yes*	☒ No	
If yes, give location and reason:			

*A standard operating procedure (SOP) to ensure proper welfare and housing of animals must be attached to this protocol in Appendix F. This does not apply to animals housed at other AAALAC accredited animal facilities (e.g., UH or NASA).

Page **1** of **11**

5. Lay Summary:
Describe the goals and intended benefits of the project in terms that can be understood by a non-scientist. Include the species and the number of animals to be used. This description should be no more than 250 words. Avoid the use of technical jargon and abbreviations.

This ongoing research project will investigate the relative role that habitat, invasive species and water quality plays in the structuring fish populations that inhabit freshwater, estuarine and marine ecosystems in, south central and southeast Texas, and the upper coast of the Gulf of Mexico. Fish communities, water quality and habitat will be sampled in streams, reservoirs, marshes, bayous, open bays and the near-shore Gulf of Mexico to determine the relationships between fish diversity, population parameters, and the abundance of alien species, habitat and water quality. This research project will incorporate the collection of fish in the field for taxonomic identification and population assessment. Sampling methods will include the collection of fish using standardized seines nets, plankton nets, traps, gillnets, and electrofishing gear (Murphy and Willis 1996. Fisheries Techniques). It is difficult to accurately estimate the total number of fish that will be collected. In fact that is one of our primary study objectives. However, based on historical data we expect to collect 15 to 65 species (average 21) per site. In general, we expect that on average fewer than 1000 fish per species will be collected at each site during the study period each year. For approximately 50% of the species sampled, we expect to collect less than 30 specimens per site. Depending on ultimate funding and the resulting number of sites monitored, the maximum total number of fish that could be collected annually from 38 sites would be between 650,000 – 2,470,000 fish. Due to the high fecundity of fish and small sample size relative to the waterbodies studied (Southeast Texas estuaries and nearshore Gulf of Mexico); these samples would however represent a minute fraction of the total population of any given species (Martinez-Andrade et al. 2005; Donaldson et al. 1996). Invertebrate species will also be collected. Approximately 95% of all individuals of all fish collected for the study will be euthanized. These fish will be sacrificed with MS222 immediately after collection. The remaining fish (5%) will be released back into the water body unharmed. It is necessary to retain these specimens for identification due to inherent difficulty in identifying this taxonomically complex group and their small size, and need to critically examine physical features necessary for correct species identification. A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA. A few live specimens of stingrays may be transported for use by other researchers under their approved animal care protocol.

State or federally listed protected species will not be retained. The principal investigator has all required government collecting permits. The long range objective of my study is to develop a predictive habitat/fish model useful for management of fisheries resources and water quality including development of biocriteria (e.g. state and EPA biological surface water regulatory criteria).

6. Animal Use:
Provide the specifications for all of the animals requested for use in this protocol. List each strain separately.

Species (common name)	Breed/Strain	Vender/Source	Number Requested			
			Year 1	Year 2	Year 3	Total

☒ Not Applicable (this is a population field study)
If a population field study, check all vertebrate animals that are planned to be studied:
☒ Fish ☐ Amphibians ☐ Reptiles ☐ Birds ☐ Mammals / ☐ Cetaceans

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7. Personnel:
List all personnel having contact with animals, the species proposed and the years of experience the individual has with the species. List the specific roles the individual will have in the project and the date of last training received.

Name, Degree, Title	Species and Years of Experience	Specific Role in Project*	TRAINING DATE
George Guillen, Assoc. Professor Biology, Ph.D.	Fish, 30	Principal Investigator	5/4/2011
Greg Knothe, B.S.Grad student	Fish, 4	Field Monitoring	5/6/2011
Jenny Wrast, M.S.,Senior Research Assoc.	Fish, 10	Field Coordinator	5/6/2011
Colby Lawrence, B.S. Res. Assoc.	Fish 10	Field Monitoring	5/6/2011
Emma Clarkson, B.S. Grad Student	Fish 4	Field Monitoring	5/6/2011
Abby Marlow, B.S. Grad Student	Fish 4	Field Monitoring	5/6/2011
Alex Miller, B.S. Grad Student	Fish 4	Field Monitoring	5/6/2011
Khem Paudel, B.S.Grad Student	Fish 1	Field Monitoring	5/6/2011

Misty Shepard, B.S. Research Assoc.	Fish 1	Field Monitoring	5/6/2011
Kristen Vale, B.S. Research Assoc.	Fish 2	Field Monitoring	NA
Sybil Glenos	Fish 2	Field Monitoring	NA
Laila Melendez	Fish 2	Field Monitoring	NA
Up to 4 new students - future	Fish 1-2	Field Monitoring	NA

* Examples include: supervision, care/handling, anesthesia, surgery, monitoring, post-procedural care, euthanasia in the stated species

☒ Student visitors will/may participate in this protocol and will be supervised by: George Guillen

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8a. Literature Search

Using at least two different databases, perform literature searches to determine alternatives to procedures that may cause more than a momentary or slight pain or distress to the animals, and unnecessary duplication of research.

Search Database (e.g., Agricola)	**Date of Search** MM/DD/YYYY	**Years Covered** (e.g., 1980 – 2010)	**Keywords or Search Strategy Used in Search**
* UHCL One Search	3-29-12	pre-2012 47,785 references	Alternative approaches to site specific fish population sampling
* *Google Scholar	3-29-12	pre-2012 60,400 references	Alternative approaches to site specific fish population sampling

* No articles found providing alternative methods.

** No articles found providing alternative methods.

8b. Rationale and purpose of animal use

State the overall rationale, purpose, and significance of this project.

Native fish communities are at risk due to changing climate, habitat degradation and ongoing water quality impairment and invasive species. Recent studies have shown that alteration of native fish communities in Texas may be occurring due to an interaction of various factors including long term changes in habitat quality reduced freshwater inflow, global warming and introductions of invasive species. Management agencies are considering closure of certain areas to fishing to reduce damage to habitat from fishing and trawling. In order to assess the extent of this degradation and responses to ongoing management actions it is necessary to conduct analyses of time series data from before and after management actions or alterations in the environment. Fortunately, data sets exist that can be used to compare current population data with previous data collected during the 1970-1990's. My research will focus on the quantification of habitat and water quality and their affect on marine, estuarine and freshwater fishes. Fish communities, water quality and habitat will be sampled in streams, reservoirs, marshes, bayous, open bays and the near-shore Gulf of Mexico to determine the relationship of diversity, population parameters, and these variables. In addition, changes in these environmental variables and fishing pressure will be used to develop predictive models of fish abundance over a wide salinity regime.

The collection and identification of fish is a necessary component of my research study. One of the primary objectives is to estimate the density and abundance of individual species populations and overall community structure. This data will be combined with historical data collected various researchers and agencies in the 1980's and 1990's have to evaluate temporal and spatial trends in diversity and community composition. This study will facilitate the investigation of the relative role of habitat and water quality in structuring of native fish populations that inhabit freshwater, estuarine and marine ecosystems across a salinity gradient.

All of these past studies, including those conducted by state and federal agencies, have used standardized gear and effort. Therefore we will use the same gear to facilitate comparison of our data with historical data. Some of our sampling will occur in boats or in remote isolated waterbodies accessible only by foot. We plan to monitor up to 38 sites in the Galveston and East Mataagorda Bay watershed and nearshore Gulf of Mexico over a three year period. Most of these sites will be monitored 1-2 times a year, while a few (10) may be monitored quarterly. Depending on gear used and time of year, a variety of species may be collected. It is difficult to accurately estimate the total number of fish that will be collected. In fact that is one of our primary study objectives.

However, based on historical data we expect to collect 15 to 65 species (average 21) per site. In general, we expect that on average fewer than 1000 fish per species will be collected at each site during the study period each year. For approximately 50% of the species sampled, we expect to collect less than 30 specimens per site. Depending on ultimate funding and the resulting number of sites monitored, the maximum total number of fish that could be collected annually from 38 sites would be between 650,000 – 2,470,000 fish. Due to the high fecundity of fish and small sample size relative to the waterbodies studied (Southeast Texas estuaries and nearshore Gulf of Mexico); these samples would however represent a minute fraction of the total population of any given species (Martinez-Andrade et al. 2005; Donaldson et al. 1996). Invertebrate species will also be collected. Approximately 95% of all individuals of all fish collected for the study will be euthanized. These fish will be sacrificed with MS222 immediately after collection. The remaining fish (5%) will be released back into the water body unharmed. Based on the primary sampling gear that we will be using (trawls, seines, plankton nets, and back-pack electroshockers), the majority of species that we will be collecting will be small (< 6 inches) or juvenile and larval fish. State or federally listed protected species will not be retained. The principal investigator has all required government collecting permits.

Due to extremely low water clarity, and the presence of density gradients and bottom obstructions, it is impossible to use visual or acoustic census techniques that would reduce the need to retain specimens. In most cases, due to the small size and/or taxonomic diversity of fish there is a need to utilize taxonomic keys that emphasize meristic (e.g. fin ray counts, morphological measurements, etc.) internal and external characters, to identify fish reliably. Due to the high diversity of fishes collected, their small size, and need to obtain in some cases hard structure samples (scales and otoliths) for aging, it is nearly impossible to analyze and release all fish back into the field. We must bring them back for identification and/or processing. In addition, large amounts of debris including submerged grass, mud and invertebrates (e.g., shrimp, crabs, etc.) are occasionally collected as well, increasing processing time. Finally, due to the patchy nature of fish distribution due to schooling and migration, we occasionally can be overwhelmed with a large catch, which of course reduces processing time. However, we plan to release whenever possible, larger more easily identified specimens. In all other cases, fish will be collected, euthanized, and subsequently preserved for later identification and/or removal of otoliths.

Population and community statistics that would be generated include density, catch per unit effort, absolute numbers, community diversity, size and age composition (commercially and recreationally important species), and by calculation growth and mortality of the population. Therefore primary variables like density must be estimated using catch statistics and/or mark recapture methods. Other variables are derived from measurements of annuli (annual growth rings) on scales, spines, otoliths (ear bones), or length and weight. In addition, we plan to extract tissue from a subsample of recently

euthanized dead fish. This tissue will be analyzed for one or all of the following condition biomarkers including length-weight condition factors, RNA/DNA ratios (larvae), liver condition/weight indices, hematocrit/leucocrit levels and level of parasitism.

A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA Training on the proper care of animal specimens and safety will be provided to all participants using this protocol.

8c. Justification for animal use.
Explain why non-animal models such as isolated organ preparation, cell or tissue culture, or computer simulation cannot be used.

Since populations and communities of fish vary geographically based on known patterns of biogeography and land use it is necessary to evaluate the response of local populations to adjust for this source of variation when evaluating waterbodies. The central question of this study is how fish communities have varied both spatially and temporally within Texas coastal systems, including freshwater tributaries. Fish population analysis requires the evaluation of fish populations and vital statistics. This requires the collection of fish density data, growth information estimated from bony structures and length data, and spawning as estimated from gonad developmental stage, and overall condition (weight and length data). There are no other animal models in ecology that can substitute for this group, since we are trying to evaluate local endemic population trends.

8d. Justification for using this particular species.
Explain why the species and/or strain(s) requested is/are the most appropriate for this research. Statements that the planned species is traditionally used for the proposed research are not sufficient.

The purpose of the study is to evaluate local populations and community structure and their response to changing climate, habitat change and freshwater flow regime. Sponsoring agencies need site specific data.

8e. Alternatives to Potentially Distressful Procedures
Describe considerations of alternatives to procedures that may cause more than a momentary or slight pain or distress to the animals, and determination that alternatives were not available.
☒ Not Applicable (animals listed are only in USDA Category B or C)

8f. Assurance of Non-Duplication
☒ This experiment does not unnecessarily duplicate previous experiments.
Otherwise, provide justification of the necessity of experiments proposed.

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9. Justification of animal numbers.
Provide a detailed justification for the numbers of animals requested. Include number of animals per group and total number of animals. If power analysis was utilized, give appropriate details. If the determination was based on prior experience, please cite reference. If a population study in the field give justification of sampling method.

This is a population and community study. The research question requires we collect these organisms and identify them in the laboratory due to difficulty taxonomy. Based on past studies

we expect that on the average fewer than 250 fish per species will be collected at each site during the study period each year. For greater than 70% of the species sampled, less than 30 specimens will be collected. Approximately 95% of all individuals of each species collected will be euthanized for the study. These fish will be sacrificed with MS222 immediately after collection. The remaining fish (5%) will be released back into the water body unharmed. Based on past years a maximum number of 17,000 fish per /year may be collected study-wide. This number represents a minute percentage of the total number of fish present in regional waterbodies based on extrapolation of density of fish per stream distance. The methods used include seining and electrofishing which are identified in the TCEQ guidance manual as the standard protocol required for studies that will be used for regulatory purposes. Also, this method allows comparison with past data.

Animals sacrificed in the field will be used to estimate various population parameters including density, mortality, growth, and age. Density will be obtained from counting the number of organisms collected by unit area or volume. Mortality, growth and age data will be obtained from a variety of methods including, reductions in catch per unit effort (CPUE) over various age groups, length or weight frequency analysis (e.g., statistical mixture analysis), analysis of otolith (ear bones) and scale growth rings (ear bones), and examination of RNA/DNA ratios. These statistical methods are outlined in various fisheries population analysis texts including Pauly (1984), Hilborn and Walters (1991), and Quinn (1998). Due to the nature of ecological research it is difficult to project absolute numbers of fish that will be collected. In fact that is one of our primary objectives of our study. However, based on historical data, we expect to collect 15 to 65 species (average 21) per site. In general, we expect that on average fewer than 1000 fish per species will be collected at each site during the study period each year. For approximately 50% of the species sampled, we expect to collect less than 30 specimens. A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA

Depending on future funding and the resulting number of sites monitored, the maximum total number of fish that could be collected annually from 38 sites would be between 650,000 – 2,470,000 fish. During the first year less than 1000 fish were collected. Due to the high fecundity of fish and small sample size relative to the waterbodies studied (Southeast Texas estuaries and nearshore Gulf of Mexico); these samples would however represent a minute fraction of the total population of any given species (Martinez-Andrade et al. 2005; Donaldson et al. 1996). . State or federally listed protected species will not be retained. The principal investigator has all required government collecting permits.

Martinez-Andrade, F., P. Campbell and B. Fuls. 2005. Trends in relative abundance and size of selected finfishes and shellfishes along the Texas coast: November 1975-December 2003. TPWD Management Data Series 232. Austin, Texas

Donaldson, D., N. Sanders Jr., R. Minkler and P. Thompson. 1996. SEAMAP Environmental and Biological Atlas of the Gulf of Mexico, 1993. Gulf States Maine Fisheries Commission. Ocaan Springs, MS.

10. Pain, Discomfort, and Distress.

a. USDA Pain/Distress Classification

Check the category that indicates the highest level of pain/distress the animals will experience during the course of these studies. (Refer to the Instructions, Section 10 for help.)

☐ Category B ☒ Category C ☐ Category D ☐ Category E

b. If Category E is selected, provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

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11. What will happen with animals at the end of their roles in the project.

a. Check all that apply and provide explanation if necessary.

☐ Placed for adoption
☐ Released into wild (field study)
☒ Euthanasia:

Rodents:

- ☐ CO_2- followed by secondary method (e.g. bilateral thoracotomy, cervical dislocation)
  State secondary method:
- ☐ Injectable agent *(Specify Agent, Route, Dose):*
- ☐ Inhalant agent *(Specify Agent, Dose):*
- ☐ Cervical Dislocation (rodents < 200 gm) w/ anesthesia- (*Specify Agent, Route, Dose*):
- ☐ Decapitation/Guillotine w/ anesthesia- (*Specify Agent, Route, Dose*):
- ☐ Exsanguination w/ anesthesia (*Specify Agent, Route, Dose*):
- ☐ Anesthetic + Perfusion (*Specify Agent, Route, Dose*):
  Type of perfusion:

Amphibians, Fish, Reptiles:

- ☐ CO_2
- ☐ Injectable agent *(Specify Agent, Route, Dose):*
- ☐ External or topical agent *(Specify Agent, Route, Dose):*
- ☒ Inhalant agent *(Specify Agent, Dose):* MS-222, 1,000 mg/L
- ☐ Decapitation and pithing
- ☒ Stunning and decapitation/pithing

☐ Other:

b. Explanation / Justification

MS-222 will be used in a dip bath to euthanize smaller fish retained for species identification. Larger fish that are not identified in the field and are retained for i.d. in the lab will be euthanized by a sharp blow to the head. Death is confirmed by cessation of operculum movement.

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12. Additional Forms Attached

Check all that apply and attach appropriate forms. If a form is not needed, delete the page from the protocol.

☐ Appendix A: Laboratory Research or Classroom
☐ Appendix B: Surgical Procedures
☒ Appendix C: Wild Animal and/or Field Research
☐ Appendix D: Safety
☒ Appendix E: Renewal / Addendum
☐ Appendix F: Additional Information / Standard Operating Procedures

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13. Check the following:

☒ I certify that the use of all animals involved in this project will be carried out within the provisions of the Animal Welfare Act, the Guide for Care and Use of Laboratory Animals, the PHS Policy on Humane Care and Use of Animals, the University of Houston Policy on Care and Use of Animals and related animal welfare rules and regulations as issued by state and/or federal agencies.

☒ I am aware that the Institutional Animal Care and Use Committee (IACUC) may make periodic inspections of all labs in which animals are used. I will permit unannounced inspections and observations of my animals and surgical techniques by a UH veterinarian or other representative of the IACUC.

☒ I am aware that the IACUC is empowered to stop any objectionable procedure or project. Where procedures have caused severe distress to an animal which cannot be alleviated, UH staff veterinarians are authorized to humanely euthanize that animal. I understand that every attempt will be made to contact me before any action is taken.

☒ I understand that I cannot start this project until I have received approval from the IACUC.

☒ I understand that I will make written notification to the IACUC of any proposed changes to the project. I understand that I will not be able to implement such changes until approval is received from the IACUC.

☒ I certify that the above statements are true and that I will make written notification to the IACUC of any changes in the proposed project prior to proceeding with any animal experiment.

George Guillen 3/28/12

Signature of Principal Investigator or Instructor Date

☒ Submitted Electronically: Instead of signature, protocol is emailed from the PI's UHCL email address

Appendix C: Wild Animal and/or Field Research
Delete page if not needed.

1. How will the animals be captured?

Seine, trawls, gillnets, long-line, and electrofishing. Seine distances will be less than 30 ft per haul to reduce stress and mortatlity. Trawls will be towed for less than 10 minutes to reduce mortality and stress. Electroshocking will be done using a Smith Root electroshocker designed for collection of fisheries data. Total electroshocking effort duration will be less than 90 seconds of effort. All collections methods will follow protocols outlined in:

Gillnets and long-lines will not be deployed longer than 2 hrs to reduce mortality. Larger fish will be released within 5 minutes. A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems.

Nickum, J.G., H. L. Bart, Jr, P. R. Bowser, I. E. Greer, C. Hubbs, J. A. Jenkins, J., R. MacMillan, J. W. Rachlin, J. D. Rose, P. W. Sorensen, and J. R. Tomasso. 2004. Guidelines for the Use of Fishes in Research. Published by: American Fisheries Society, American Institute of Fishery Research Biologists, American Society of Ichthyologists and Herpetologists.. American Fisheries Society. Bethesda, M.D.

2. Will animals be maintained for any length of time, where they will be maintained (field and/or animal facility), and for how long?

Most will be euthanized within 2-5 minutes. Those retained up to 10 minutes will be kept in a large bucket with aeration prior to identification, length and weight measurement and release. Larger fish will be released within 5-10 minutes to reduce stress.

3. Will animals be transported, and if so, how will stress be minimized?

No

4. How will the housing and nutritional needs of animals that are captured and detained for a research project be met?

Not applicable. All organisms will be euthanized unless resleased immediately

5. What criteria will be used in determining whether the animals can be released after they have been captured (even if the animals are part of a capture and release project, and they will not be maintained for any length of time)?

Normal gill operculum movement and response to tactile stimulus

6. How will pain and/or distress be monitored in these animals?

Primarily through observation of operculum respiration rate.

Appendix E: Renewal / Addendum
Delete page if not needed.

1. Please check all that apply:

☒ Renewal	☒ Addendum
If renewal:	☐ Annual Renewal ☐ Protocol Renewal (3 yr) ☐ No Changes in Protocol
If addendum:	☐ Personnel Change ☐ Minor Revision ☐ Major Revision

2. Current Status
☒ Active ☐ Temporarily Inactive ☐ Never Started
If the study is temporarily inactive, or never started please explain why:

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3. Personnel Change
Please list personnel added or removed from the protocol. Additionally, the personnel list along with experience, role and training should be updated on the original protocol to reflect current project personnel.

Add	Remove
Kristen Vale	Kevin Young
Sybil Glenos	Amanda Moss
Laila Melendez	Rosaleen March
	Jonathan Barton

4. Methodological Changes
Has there been, or do you anticipate in the next 12 months, any change in your protocol?
☒ Yes ☒ No
This includes change of species, change in numbers of animals used, change of techniques, change of anesthesia or analgesia, change in drugs or test chemicals being used, changes in euthanasia methods, etc. **Briefly** summarize changes below. In addition, edit the original protocol submission and indicate any changes by underlining new text.

A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine and marine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA.

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5. Problems
Did any of the animals used have an unanticipated adverse reaction(s)?
☐ Yes ☒ No
If yes, please describe:

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6. Progress
Describe the progress and/or any significant findings relative to this project. Projects which have not realized any progress should provide an explanation. Please include citations of abstracts, publications, etc. (full text is not needed).
Project activities were completed through 2011. We are in process of completing interim report report. Students will be using part of this data for M.S. thesis projects. Project will continue 2 more years at least.

APPENDIX D: ENVIRONMENTAL PARAMETERS BIOPSY SURVEYS

Year	Parameter	n	Min.	Max.	Mean	Med.	Std. Dev.
	Air Temp. (°C)	18	19.30	32.20	26.20	26.75	3.68
	Water Temp. (°C)	44	24.23	32.05	28.73	29.52	2.04
	Salinity (psu)	44	13.59	18.71	16.43	16.84	1.54
2015	*pH*	2	7.72	7.86	7.79	7.79	0.10
	DO (%)	28	65.80	280.80	118.47	110.65	42.73
	DO (mg/L)	28	4.63	18.15	8.35	7.99	2.76
	Secchi	20	0.22	0.52	0.38	0.39	0.08
	Air Temp. (°C)	39	8.60	34.40	28.09	29.40	5.50
	Water Temp. (°C)	93	10.40	33.14	28.19	30.20	6.13
	Salinity (psu)	39	1.03	30.75	12.01	10.52	8.05
2016	*pH*	35	7.71	8.56	8.18	8.17	0.23
	DO (%)	36	55.00	133.60	100.29	104.10	19.56
	DO (mg/L)	36	3.76	12.09	7.67	7.60	2.13
	Secchi	34	0.10	0.58	0.33	0.35	0.12
	Air Temp. (°C)	11	28.60	33.20	30.75	31.10	1.53
	Water Temp. (°C)	25	2.00	32.63	30.17	31.12	5.89
	Salinity (psu)	25	0.00	32.86	19.42	16.42	7.93
2017	*pH*	12	7.59	8.22	8.03	8.12	0.21
	DO (%)	12	56.10	131.10	95.89	97.35	20.94
	DO (mg/L)	12	3.82	8.66	6.35	6.12	1.37
	Secchi	8	0.31	0.47	0.38	0.38	0.05

APPENDIX E: FISH LOCATION, WEIGHT, LENGTH, AND ISOTOPIC VALUES

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Elops saurus	Ladyfish	8/24/2015	2551	UGB	29.70762	-95.02324	260.0	277.0	-22.72	19.20
Ariopsis felis	Hardhead Catfish	8/8/2016	1390	LGB	29.34684	-94.76831	252.5	248.0	-20.90	17.88
Ariopsis felis	Hardhead Catfish	8/8/2016	1390	LGB	29.34684	-94.76831	273.2	243.0	-20.59	17.61
Ariopsis felis	Hardhead Catfish	8/8/2016	1390	LGB	29.34684	-94.76831	127.7	202.0	-21.22	18.03
Ariopsis felis	Hardhead Catfish	8/9/2016	2555	UGB	29.54232	-94.97413	107.5	186.0	-27.80	16.18
Ariopsis felis	Hardhead Catfish	8/9/2016	2555	UGB	29.54232	-94.97413	57.0	151.0	-25.08	17.89
Bagre marinus	Gafftopsail Catfish	8/24/2015	2551	UGB	29.70762	-95.02324	740.0	330.0	-23.04	18.73
Bagre marinus	Gafftopsail Catfish	8/24/2015	1394	UGB	29.74709	-94.72837	-	415.0	-21.54	20.88
Bagre marinus	Gafftopsail Catfish	8/8/2016	1391	LGB	29.52712	-94.80524	16.3	103.0	-22.80	17.46
Bagre marinus	Gafftopsail Catfish	8/8/2016	1391	LGB	29.52712	-94.80524	11.3	89.0	-22.38	20.67
Bagre marinus	Gafftopsail Catfish	8/8/2016	1391	LGB	29.52712	-94.80524	11.2	88.0	-21.05	20.33
Bagre marinus	Gafftopsail Catfish	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	19.1	104.0	-21.95	18.47
Bagre marinus	Gafftopsail Catfish	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	14.9	95.0	-21.86	17.86
Bagre marinus	Gafftopsail Catfish	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	13.9	94.0	-26.62	17.78

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Bagre marinus	Gafftopsail Catfish	8/9/2016	1393	UGB	29.66258	-94.86980	12.3	92.0	-18.94	16.66
Bagre marinus	Gafftopsail Catfish	8/9/2016	1393	UGB	29.66258	-94.86980	11.3	89.0	-20.05	16.68
Bagre marinus	Gafftopsail Catfish	8/9/2016	1393	UGB	29.66258	-94.86980	8.0	80.0	-21.77	16.52
Cynoscion arenarius	Sand Seatrout	6/23/2015	2552	LGB	29.48447	-94.82158	12.8	94.0	-23.24	19.39
Cynoscion arenarius	Sand Seatrout	6/23/2015	2552	LGB	29.48447	-94.82158	20.0	94.0	-18.88	15.20
Cynoscion arenarius	Sand Seatrout	6/23/2015	2552	LGB	29.48447	-94.82158	30.0	105.0	-25.44	17.37
Cynoscion arenarius	Sand Seatrout	6/23/2015	1391	LGB	29.52712	-94.80524	40.0	116.0*	-24.22	18.75
Cynoscion arenarius	Sand Seatrout	6/23/2015	1391	LGB	29.52712	-94.80524	30.0	137.0*	-24.11	19.01
Cynoscion arenarius	Sand Seatrout	6/23/2015	1391	LGB	29.52712	-94.80524	50.0	152.0*	-24.37	18.75
Cynoscion arenarius	Sand Seatrout	6/24/2015	2555	UGB	29.54232	-94.97413	30.0	117.0	-27.34	17.56
Cynoscion arenarius	Sand Seatrout	6/24/2015	2555	UGB	29.54232	-94.97413	40.0	124.0	-23.86	19.66
Cynoscion arenarius	Sand Seatrout	6/24/2015	2555	UGB	29.54232	-94.97413	40.0	132.0	-24.05	19.17
Cynoscion arenarius	Sand Seatrout	6/24/2015	1392	UGB	29.58397	-94.98043	150.0	202.0	-23.84	19.61
Cynoscion arenarius	Sand Seatrout	6/24/2015	1392	UGB	29.58397	-94.98043	90.0	184.0	-26.00	17.30

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Cynoscion arenarius	Sand Seatrout	6/24/2015	1392	UGB	29.58397	-94.98043	100.0	189.0	-23.88	19.30
Cynoscion arenarius	Sand Seatrout	8/24/2015	1393	UGB	29.66258	-94.86980	138.0	36.7	-22.54	18.97
Cynoscion arenarius	Sand Seatrout	8/24/2015	1393	UGB	29.66258	-94.86980	143.0	41.5	-18.82	17.56
Cynoscion arenarius	Sand Seatrout	8/24/2015	1393	UGB	29.66258	-94.86980	185.0	100.5	-21.00	18.83
Cynoscion arenarius	Sand Seatrout	8/8/2016	Back Bay	LGB	29.33837	-94.88008	71.2	170.0	-18.07	14.43
Cynoscion arenarius	Sand Seatrout	8/8/2016	Back Bay	LGB	29.33837	-94.88008	53.3	153.0	-19.33	16.08
Cynoscion arenarius	Sand Seatrout	8/8/2016	Back Bay	LGB	29.33837	-94.88008	26.9	116.0	-18.96	14.77
Cynoscion arenarius	Sand Seatrout	8/8/2016	2553	LGB	29.47280	-94.73910	51.0	137.0	-24.85	18.54
Cynoscion arenarius	Sand Seatrout	8/8/2016	2553	LGB	29.47280	-94.73910	46.4	140.0	-25.15	18.32
Cynoscion arenarius	Sand Seatrout	8/8/2016	2553	LGB	29.47280	-94.73910	19.4	101.0	-22.95	18.38
Cynoscion arenarius	Sand Seatrout	8/8/2016	1391	LGB	29.52712	-94.80524	20.1	106.0	-23.69	21.91
Cynoscion arenarius	Sand Seatrout	8/8/2016	1391	LGB	29.52712	-94.80524	12.0	85.0	-25.95	18.58
Cynoscion arenarius	Sand Seatrout	8/8/2016	1391	LGB	29.52712	-94.80524	15.5	94.0	-25.23	22.88
Cynoscion arenarius	Sand Seatrout	8/9/2016	2555	UGB	29.54232	-94.97413	82.7	172.0	-21.02	16.85

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Cynoscion arenarius	Sand Seatrout	8/9/2016	2555	UGB	29.54232	-94.97413	133.2	193.0	-25.13	18.00
Cynoscion arenarius	Sand Seatrout	8/9/2016	2555	UGB	29.54232	-94.97413	66.1	156.0	-23.80	15.93
Cynoscion arenarius	Sand Seatrout	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	73.1	160.0	-26.46	17.82
Cynoscion arenarius	Sand Seatrout	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	81.7	166.0	-25.53	17.46
Cynoscion arenarius	Sand Seatrout	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	65.5	153.0	-24.90	17.72
Cynoscion arenarius	Sand Seatrout	8/9/2016	1393	UGB	29.66258	-94.86980	24.6	115.0	-24.02	17.19
Cynoscion arenarius	Sand Seatrout	8/9/2016	1393	UGB	29.66258	-94.86980	18.2	107.0	-23.80	16.99
Cynoscion arenarius	Sand Seatrout	8/9/2016	1393	UGB	29.66258	-94.86980	19.9	110.0	-23.95	17.08
Cynoscion arenarius	Sand Seatrout	8/9/2016	2551	UGB	29.70762	-95.02324	16.3	102.0	-21.02	16.70
Cynoscion arenarius	Sand Seatrout	8/9/2016	2551	UGB	29.70762	-95.02324	6.7	75.0	-20.13	15.86
Cynoscion arenarius	Sand Seatrout	8/9/2016	2551	UGB	29.70762	-95.02324	4.5	64.0	-22.07	16.04
Leiostomus xanthurus	Spot	6/3/2015	2554	LGB	29.30845	-94.94170	90.0	139.0	-20.53	18.54
Leiostomus xanthurus	Spot	6/3/2015	2554	LGB	29.30845	-94.94170	120.0	164.0	-21.29	15.80
Leiostomus xanthurus	Spot	6/22/2015	1390	LGB	29.34684	-94.76831	14.0	88.0	-21.88	19.35

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Leiostomus xanthurus	Spot	6/22/2015	1390	LGB	29.34684	-94.76831	16.8	88.0	-20.69	18.68
Leiostomus xanthurus	Spot	6/22/2015	1390	LGB	29.34684	-94.76831	14.5	90.0	-19.43	17.54
Leiostomus xanthurus	Spot	6/23/2015	2552	LGB	29.48447	-94.82158	6.6	68.0	-22.98	19.56
Leiostomus xanthurus	Spot	6/24/2015	2555	UGB	29.54232	-94.97413	80.0	140.0	-24.02	18.10
Leiostomus xanthurus	Spot	6/24/2015	2555	UGB	29.54232	-94.97413	50.0	125.0	-22.13	19.28
Leiostomus xanthurus	Spot	6/24/2015	2555	UGB	29.54232	-94.97413	90.0	135.0	-21.91	18.97
Leiostomus xanthurus	Spot	6/24/2015	1392	UGB	29.58397	-94.98043	90.0	149.0	-23.27	19.21
Leiostomus xanthurus	Spot	8/24/2015	2551	UGB	29.70762	-95.02324	19.2	95.0	-22.43	18.97
Leiostomus xanthurus	Spot	8/24/2015	2551	UGB	29.70762	-95.02324	17.4	90.0	-21.48	21.76
Leiostomus xanthurus	Spot	8/24/2015	2551	UGB	29.70762	-95.02324	23.4	101.0	-23.42	19.80
Leiostomus xanthurus	Spot	8/8/2016	Back Bay	LGB	29.33837	-94.88008	29.0	98.0	-20.95	20.16
Leiostomus xanthurus	Spot	8/8/2016	Back Bay	LGB	29.33837	-94.88008	17.5	88.0	-18.89	17.84
Leiostomus xanthurus	Spot	8/8/2016	Back Bay	LGB	29.33837	-94.88008	18.1	91.0	-20.56	20.09
Leiostomus xanthurus	Spot	8/8/2016	2553	LGB	29.47280	-94.73910	26.0	95.0	-25.78	22.20

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Leiostomus xanthurus	Spot	8/8/2016	2553	LGB	29.47280	-94.73910	26.0	94.0	-22.20	19.50
Leiostomus xanthurus	Spot	8/8/2016	2553	LGB	29.47280	-94.73910	21.6	91.0	-22.75	17.53
Leiostomus xanthurus	Spot	8/9/2016	2551	UGB	29.70762	-95.02324	85.5	155.0	-18.23	17.49
Leiostomus xanthurus	Spot	8/9/2016	2551	UGB	29.70762	-95.02324	19.0	96.0	-20.67	20.36
Leiostomus xanthurus	Spot	8/9/2016	2551	UGB	29.70762	-95.02324	26.1	101.0	-18.56	17.03
Micropogonias undulatus	Atlantic Croaker	6/22/2015	1390	LGB	29.34684	-94.76831	15.8	98.0	-26.79	19.62
Micropogonias undulatus	Atlantic Croaker	6/22/2015	1390	LGB	29.34684	-94.76831	15.9	96.0	-20.29	19.25
Micropogonias undulatus	Atlantic Croaker	6/22/2015	1390	LGB	29.34684	-94.76831	15.2	95.0	-23.49	19.15
Micropogonias undulatus	Atlantic Croaker	6/23/2015	2552	LGB	29.48447	-94.82158	7.9	78.0	-21.80	19.42
Micropogonias undulatus	Atlantic Croaker	6/23/2015	2552	LGB	29.48447	-94.82158	6.3	72.0	-25.68	17.57
Micropogonias undulatus	Atlantic Croaker	6/23/2015	2552	LGB	29.48447	-94.82158	20.0	88.0	-26.19	17.53
Micropogonias undulatus	Atlantic Croaker	6/23/2015	1391	LGB	29.52712	-94.80524	60.0	164.0*	-20.03	18.09
Micropogonias undulatus	Atlantic Croaker	6/23/2015	1391	LGB	29.52712	-94.80524	60.0	158.0*	-24.61	17.82
Micropogonias undulatus	Atlantic Croaker	6/23/2015	1391	LGB	29.52712	-94.80524	17.7	102.0	-23.06	18.68

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Micropogonias undulatus	Atlantic Croaker	6/24/2015	2555	UGB	29.54232	-94.97413	40.0	120.0	-23.89	17.74
Micropogonias undulatus	Atlantic Croaker	6/24/2015	2555	UGB	29.54232	-94.97413	50.0	135.0	-23.64	18.13
Micropogonias undulatus	Atlantic Croaker	6/24/2015	2555	UGB	29.54232	-94.97413	40.0	113.0	-23.94	17.17
Micropogonias undulatus	Atlantic Croaker	6/24/2015	1392	UGB	29.58397	-94.98043	80.0	158.0	-21.83	18.87
Micropogonias undulatus	Atlantic Croaker	6/24/2015	1392	UGB	29.58397	-94.98043	90.0	160.0	-23.99	20.30
Micropogonias undulatus	Atlantic Croaker	6/24/2015	1392	UGB	29.58397	-94.98043	60.0	142.0	-24.11	20.60
Micropogonias undulatus	Atlantic Croaker	8/24/2015	1393	UGB	29.66258	-94.86980	6.5	71.0	-22.46	9.94
Micropogonias undulatus	Atlantic Croaker	8/24/2015	1393	UGB	29.66258	-94.86980	5.4	68.0	-22.54	15.70
Micropogonias undulatus	Atlantic Croaker	8/24/2015	1393	UGB	29.66258	-94.86980	4.9	67.0	-28.39	18.34
Micropogonias undulatus	Atlantic Croaker	8/24/2015	2551	UGB	29.70762	-95.02324	23.9	110.0	-23.10	19.29
Micropogonias undulatus	Atlantic Croaker	8/24/2015	2551	UGB	29.70762	-95.02324	17.9	99.0	-23.41	19.75
Micropogonias undulatus	Atlantic Croaker	8/24/2015	2551	UGB	29.70762	-95.02324	46.6	137.0	-22.40	18.54
Micropogonias undulatus	Atlantic Croaker	8/8/2016	Back Bay	LGB	29.33837	-94.88008	50.5	140.0	-22.60	17.60
Micropogonias undulatus	Atlantic Croaker	8/8/2016	Back Bay	LGB	29.33837	-94.88008	53.0	138.0	-20.80	14.79

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Micropogonias undulatus	Atlantic Croaker	8/8/2016	Back Bay	LGB	29.33837	-94.88008	11.4	84.0	-23.27	17.57
Micropogonias undulatus	Atlantic Croaker	8/8/2016	1390	LGB	29.34684	-94.76831	23.6	105.0	-21.33	18.42
Micropogonias undulatus	Atlantic Croaker	8/8/2016	1390	LGB	29.34684	-94.76831	16.5	99.0	-20.35	17.69
Micropogonias undulatus	Atlantic Croaker	8/8/2016	1390	LGB	29.34684	-94.76831	8.3	78.0	-21.32	17.23
Micropogonias undulatus	Atlantic Croaker	8/8/2016	2553	LGB	29.47280	-94.73910	7.2	71.0	-27.90	17.71
Micropogonias undulatus	Atlantic Croaker	8/8/2016	2553	LGB	29.47280	-94.73910	8.3	74.0	-26.63	18.88
Micropogonias undulatus	Atlantic Croaker	8/8/2016	2553	LGB	29.47280	-94.73910	6.7	72.0	-25.81	19.02
Micropogonias undulatus	Atlantic Croaker	8/8/2016	1391	LGB	29.52712	-94.80524	44.4	132.0	-25.21	17.82
Micropogonias undulatus	Atlantic Croaker	8/8/2016	1391	LGB	29.52712	-94.80524	51.7	138.0	-24.75	21.72
Micropogonias undulatus	Atlantic Croaker	8/8/2016	1391	LGB	29.52712	-94.80524	8.8	76.0	-24.66	22.03
Micropogonias undulatus	Atlantic Croaker	8/9/2016	2555	UGB	29.54232	-94.97413	44.0	124.0	-24.30	16.41
Micropogonias undulatus	Atlantic Croaker	8/9/2016	2555	UGB	29.54232	-94.97413	54.8	136.0	-24.34	16.91
Micropogonias undulatus	Atlantic Croaker	8/9/2016	2555	UGB	29.54232	-94.97413	45.9	125.0	-22.97	16.24
Micropogonias undulatus	Atlantic Croaker	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	55.6	142.0	-24.23	19.78

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Micropogonias undulatus	Atlantic Croaker	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	7.2	72.0	-22.73	18.78
Micropogonias undulatus	Atlantic Croaker	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	6.5	67.0	-23.41	11.33
Micropogonias undulatus	Atlantic Croaker	8/9/2016	1393	UGB	29.66258	-94.86980	43.6	135.0	-23.71	17.30
Micropogonias undulatus	Atlantic Croaker	8/9/2016	1393	UGB	29.66258	-94.86980	39.7	130.0	-19.15	15.53
Micropogonias undulatus	Atlantic Croaker	8/9/2016	1393	UGB	29.66258	-94.86980	7.1	74.0	-22.84	17.83
Micropogonias undulatus	Atlantic Croaker	8/9/2016	2551	UGB	29.70762	-95.02324	48.2	145.0	-23.57	17.44
Micropogonias undulatus	Atlantic Croaker	8/9/2016	2551	UGB	29.70762	-95.02324	11.5	83.0	-18.36	15.92
Micropogonias undulatus	Atlantic Croaker	8/9/2016	2551	UGB	29.70762	-95.02324	8.0	75.0	-19.34	16.98
Pogonias cromis	Black Drum	8/24/2015	2551	UGB	29.70762	-95.02324	1750.0	423.0	-22.94	18.05
Trichiurus lepturus	Atlantic Cutlassfish	8/8/2016	1391	LGB	29.52712	-94.80524	12.8	200.0	-20.38	17.17
Trichiurus lepturus	Atlantic Cutlassfish	8/8/2016	1391	LGB	29.52712	-94.80524	5.2	139.0	-22.84	18.68
Trichiurus lepturus	Atlantic Cutlassfish	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	8.5	181.0	-26.37	17.69
Trichiurus lepturus	Atlantic Cutlassfish	8/9/2016	Sylvan Bay	UGB	29.63340	-94.98370	11.4	124.0	-23.24	16.48
Chloroscombrus chrysurus	Atlantic Bumper	8/8/2016	1390	LGB	29.34684	-94.76831	48.2	139.0	-21.25	17.81

*Total Length (mm)

Scientific name	Common name	Date	Site	Location	Latitude	Longitude	Weight (g)	Standard Length (mm)	$\delta^{13}C$	$\delta^{15}N$
Chloroscombrus chrysurus	Atlantic Bumper	8/8/2016	1390	LGB	29.34684	-94.76831	6.2	75.0	-20.54	18.36
Chloroscombrus chrysurus	Atlantic Bumper	8/8/2016	1390	LGB	29.34684	-94.76831	5.8	68.0	-20.59	17.86