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OBSERVATIONS OF A FEEDING AGGREGATION OF WHALE SHARKS, *RHINCODON TYPUS*, IN THE NORTH CENTRAL GULF OF MEXICO

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ABSTRACT On 26 June 2006 an aggregation of 16 whale sharks was observed for a period of 4 hr in the north central Gulf of Mexico (GOM). The sharks remained within an area about 1.0 km² in size and continuously ram filter fed at the surface. Visual analysis of a plankton sample collected from the study site revealed the presence of copious amounts of fish eggs in mid-embryonic development and a minor amount of other zooplankton. A second plankton sample (control) collected about 3.5 km from the study site in an area where no whale sharks were present contained few eggs, however other zooplankton were similar to the study site sample in species composition and abundance. Two egg morphs were identified, and samples of one of the morphs, which represented 98% of the eggs at the study site, were verified by genetic analysis as little tunny, *Euthynnus alletteratus*. The observed feeding behavior and the abundance of fish eggs at the study site indicated the whale sharks were feeding on recently spawned little tunny eggs. This represents the first confirmed observation of a feeding aggregation of whale sharks in the GOM.

RESUMEN El 26 de Junio del 2006 un agrupamiento de 16 tiburones ballena fue observado por un periodo de 4 horas en el centro norte del Golfo de Méjico (GOM). Los tiburones permanecieron dentro de un área alrededor de 1.0 km² y continuamente se desplazaron filtrando alimento en la superficie. Un análisis visual de una muestra de plankton colectada en el sitio de estudio revela la presencia de grandes cantidades de huevos de peces en un desarrollo intermedio del embrión y una pequeña cantidad de otro zooplancton. En un área donde no habían tiburones ballena, una segunda muestra de plancton (control) colectada (alrededor de 3.5 km. del sitio de estudio) presento pocos huevos de peces, sin embargo el otro zooplancton fue similar en composición de especies y abundancia con la muestra colectada en el sitio de estudio. Dos formas de huevos fueron identificadas, la forma que represento el 98% de los huevos en el sitio de estudio fue identificada mediante un análisis genético como bacoreta, *Euthynnus alletteratus*. El comportamiento de alimentación observado y la abundancia de huevos de peces en el área de estudio indicaron que los tiburones ballena se alimentaron de un desove de huevos reciente de bacoreta. Esto representa la primera observación confirmada de una agregación de tiburones ballena en el GOM.

INTRODUCTION

The whale shark, *Rhincodon typus* (Smith 1828), is the largest fish in the sea, is distributed circumglobally in tropical and subtropical marine waters except for the Mediterranean Sea (Compagno 2001), and is threatened (Stewart and Wilson 2005). Despite their large size and ubiquitous distribution, little is known about their biology and behavior. They are opportunistic filter feeders (Taylor et al. 1983, Colman 1997) that aggregate in areas of high localized productivity, e.g., mass spawning events identified near Ningaloo Reef, Australia (Colman 1997, Wilson et al. 2001), La Paz, Mexico (Clark and Nelson 1997), and Gladden Spit, Belize (Heyman et al. 2001).

Whale sharks were first reported in the Gulf of Mexico (GOM) by Gudger (1939), with subsequent sightings of solitary individuals off Texas (Baughman 1947, Gunter and Knapp 1951, Hoffman et al. 1981), Mississippi

(Springer 1957), and Florida (Clark and von Schmidt 1965). Also, the presence of whale shark aggregations in the region have been noted (Gudger, 1939, Hoffmayer et al. 2005, Burks et al. 2006), and these authors suggested that the aggregations occurred in response to feeding opportunities. We report on an opportunistic encounter with an aggregation of 16 whale sharks on 26 June 2006 in the GOM west of the Mississippi River Delta (Delta).

MATERIALS AND METHODS

An on-site investigation was conducted on 26 June 2006 to confirm recent reports by mariners of whale sharks aggregating in the north central GOM in the vicinity of the Delta. With a spotter aircraft searching a 129 km² area off the Delta, an aggregation of whale sharks was located ~70 km southwest of the mouth of the Mississippi River

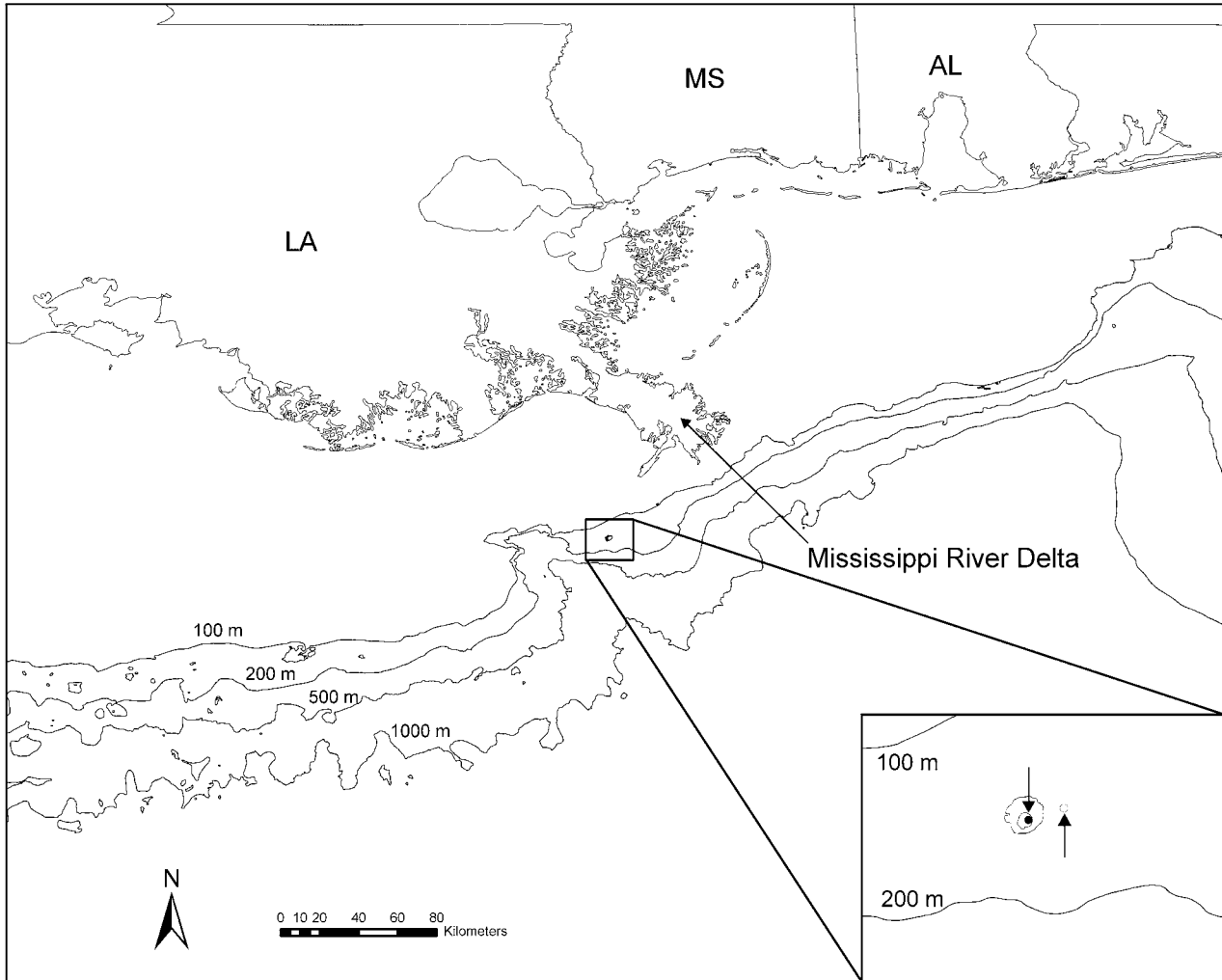


Figure 1. Map of the north central Gulf of Mexico showing the location of the study (closed circle) and control sites (open circle) (see inset). The study site was located in surface waters 78 meters (m) above the eastern edge of the crest of a topographic high, the base of which is located at 100 m water depth. Exact latitude and longitude of the study site can be obtained from the authors.

between 0800 to 1200 hr. The sharks remained in surface water within $\sim 1.0 \text{ km}^2$ area over the crest of a small-scale, shelf-edge topographic high (Rezak et al. 1983), henceforth referred to as the study site (Figure 1).

The total length (TL, m) of several sharks was estimated as they individually swam parallel with the 11 m vessel (Figure 2a). No gender data were recorded. Surface observations of the whale sharks' behavior were made visually from the vessel, and aspects of their behavior were documented with digital video and still photography. Surface water temperature ($^{\circ}\text{C}$), salinity (psu), and dissolved oxygen (mg/l) were recorded at the study site using a YSI meter (Model 85), and water depth (m) was recorded using a Furuno FE700 Echo Sounder. Surface plankton was collected at the study site using a 60 cm diameter plankton net (0.333 mm mesh) towed for 10 min (1040–1050 hr)

at a speed of 61.7 m/min. Following the same protocol, between 1325–1335 hr a second plankton sample (control) was collected 3.5 km east of the study site (Figure 1). Plankton samples were preserved in 95% ethanol and later examined in the laboratory. The volume of water filtered by the plankton net was calculated as $V = D \times A$, where V is the volume of the water filtered (m^3), D is the distance of the plankton tow (speed \times time, m), and A is the area of the plankton net mouth (m^2).

Plankton settled volume for the study and control sites were determined with a Motoda plankton splitter box by allowing a 1/16 split of the sample to settle into a 250 ml graduated cylinder for 24 hr. Egg counts were performed from a 1/256 split of the sample using an Olympus dissecting scope. The density of eggs for each tow was standardized to the number of eggs per m^3 of water filtered.



Figure 2. Images showing whale sharks, *Rhincodon typus*, feeding on 26 June 2006 in the north central Gulf of Mexico. A) Total lengths were estimated by aligning the 11 m vessel with the shark and estimating size. B) Whale shark swimming horizontally showing ram surface filter feeding. C) Close up of a whale shark mouth while surface ram filter feeding; upper jaw is above the water's surface while the lower jaw is submerged. D) An image of 2 whale sharks swimming adjacent and parallel in the foreground and background.

A subsample of the eggs was examined to determine size (diameter, mm) and developmental stage (Kendall et al. 1984) using an Olympus dissecting microscope equipped with a calibrated ocular micrometer. A gross microscopic survey of the zooplankton sample was performed to identify component species (Smith and Johnson 1996).

Eggs morphs (see below) were identified genetically via direct amplification and sequence analysis of the mitochondrial DNA 16S locus. Template DNA was isolated from individual eggs using GeneReleaser (BioVentures Inc.) following the manufacturers' protocol. An aliquot of this egg/GeneReleaser solution was used in a PCR reaction that amplified a portion of the mtDNA 16S rDNA using primers and conditions as described in Quattro et al. (2001). Purified amplification products were used as templates for ABI Big Dye Terminator cycle sequencing reactions. Fragments were analyzed on an Applied Biosystems 3730 automated DNA sequencer. Sequences were edited with SEQUENCHER (Gene Codes Corp.) and subjected to BLAST (Altschul et al. 1990) searches against the GenBank NR DNA database (Benson et al. 2005).

Finally, we observed schools of little tunny (*Euthynnus alletteratus*) and collected 2 specimens at the study site. Gonads were removed, preserved in 10% buffered formalin, and processed for histological examination, following standard histological procedures, to determine gonadal maturity. No fish were observed or caught at the control site.

RESULTS

The 16 whale sharks we observed were skimming the surface of the water as they swam with their lower jaw positioned slightly under the surface, an activity that was interspersed with periodic gulping and contraction of the buccal cavity which caused lateral displacement of the gill slits (Figures 2b, c). Additionally, "coughing" behavior was observed. Individual sharks swam continuously on a steady course at ~3.7 km/h for a few minutes and then changed course. Frequently, some of the sharks appeared to pair off and swim parallel and adjacent to each other (Figure 2d). The estimated lengths of the whale sharks ranged from 6.0 to 12.0 m TL, with most being >8.0 m TL.

Surface water quality conditions were typical of summer except depth was 78 m at the study site whereas it was 111.0 m at the control site. Each plankton net tow filtered ~174.4 m³ of surface water. Plankton settled volume was 5x higher in the study site sample (50 ml) than the control site sample (10 ml), with the primary difference being the high volume of eggs (40 ml) in the study site sample. The density of eggs was 106x higher in the study site sample (9,000 eggs m⁻³) than in the control sample (85 eggs m⁻³).

Eggs from the study site were in mid-embryonic developmental stage (Kendall et al. 1984) and were represented by 2 egg morphs. Morph 1 represented 98% of the eggs collected and ranged 0.70 to 0.80 mm in diameter with a single oil globule which ranged 0.16 to 0.20 mm in diameter. Morph 2 ranged 0.56 to 0.63 mm in diameter with a single oil globule that ranged 0.18 to 0.20 mm in diameter. Eggs identified from the study site sample were identical in appearance and size but not density to eggs collected at the control site. Sequence analysis and subsequent DNA database searches revealed high homology between 16S rDNA sequences from egg morph 1 and egg morph 2 and sequences from little tunny (*Euthynnus alletteratus*) and crevalle jack (*Caranx hippos*), respectively. Homology in each case was very high: 561 of 561 bases compared were identical between our egg morph 1 sequences and little tunny (GenBank accession AB099716), while 563 of 565 bases compared were identical between our egg morph 2 sequences and a crevalle jack sequence (GenBank accession DQ532847) deposited in GenBank.

Histological assessments of gonadal tissue collected from little tunny (1 male, 1 female) at the study site showed them to be in spawning condition. Ovarian tissue contained post-ovulatory follicles (POF), indicative of recent spawning (<24 hr) (Brown-Peterson et al. 2001), and testes contained sperm ducts filled with sperm.

There was no obvious difference in the species composition of zooplankton between the study and control sites. Plankton samples collected from both sites revealed calanoid copepods, hyperiid amphipods, crab zoea, crab megalopae, and sergestid shrimp were the major constituents.

DISCUSSION

Whale shark aggregations have been reported in association with spawning of a variety of fishes (Gunn et al. 1992, Heyman et al. 2001), corals (Taylor 1994), crabs (Colman 1997), and copepods (Clark and Nelson 1997). The whale sharks observed in this study exhibited behaviors similar to those in Colman (1997) and Heyman et al. (2001) described as surface ram filter feeding, and by

Colman (1997) as 'coughing' to clear or flush gill rakers of accumulated food particles. These observations, when combined with the abundance of fish eggs at the study site and reported feeding of whale sharks on fish eggs by Heyman et al. (2001), indicate that whale sharks in our study area were feeding on the fish eggs. This represents the first confirmed observation of a feeding aggregation of whale sharks in the GOM.

Genetic analysis revealed that egg morph 1 (98% of eggs collected) was little tunny; a finding supported by gonad histology of little tunny caught at the study site. Eggs from little tunny and crevalle jack (2% of eggs collected) were in the mid-embryonic stage of development, indicating recent spawning had occurred at the study site which was located over the only significant shelf edge promontory in the area (Figure 1). Crevalle jack were not observed at the study site during the investigation but are common residents within the area during summer months (S. Schindler, pers. comm., Shore Thing Charters, Bay St. Louis, MS). The constant presence of feeding whale sharks, little tunny eggs, and little tunny in spawning condition over the topographic feature throughout our investigation strongly suggests this was the location of spawning activity which produced eggs collected at the study site.

Previous research has shown that whale sharks occur in areas of enhanced productivity (Iwasaki 1970, Arnborn and Papastavrou 1998, Duffy 2002) and may time their migrations to coincide with localized productivity events to increase feeding opportunities (Wilson et al. 2001). Interestingly, 2 other whale shark aggregations were reported in the vicinity of our study site about 2 weeks prior to (10 June, 15 sharks, D. Bouza, per. comm., Metairie, LA) and following (13 July, >50 sharks, M. Boatner, pers. comm., Tomball, TX) our investigation. The study site was in close proximity to the Mississippi River, which is the greatest source of nutrient input in the GOM (Lohrenz et al. 1990). The mixing of Mississippi River waters with oligotrophic northern GOM oceanic waters, combined with continental slope upwelling (Lohrenz et al. 1990), enhances primary productivity and creates a favorable environment for zooplankton (Grimes and Finucane 1991); a documented food of whale sharks (Clark and Nelson 1997, Colman 1997). The Mississippi River's highest discharge typically occurs during spring and summer (Dinnell and Wiseman 1986); a time that coincides with highest seasonal abundance of whale sharks in the GOM (Burks et al. 2006, Hoffmayer and Franks unpub. data). The north central GOM may provide the most consistent seasonal feeding location for whale sharks in the GOM and may represent a predictable area for their occurrence.

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