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1 Acoustic backscatter at a Red Sea whale shark aggregation site

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Abstract

An aggregation of sexually immature whale sharks occurs at a coastal submerged reef near the Saudi Arabian Red Sea coast each spring. We tested the hypothesis that these megaplanktivores become attracted to a prey biomass peak coinciding with their aggregation. Acoustic backscatter of the water column at 120 kHz and 333 kHz – a proxy for potential prey biomass – was continuously measured spanning the period prior to, during, and subsequent to the seasonal whale shark aggregations. No peak in acoustic backscatter was observed at the time of the aggregation. However, we observed a decrease in acoustic backscatter in the last days of deployment, which coincided the trailing end of whale shark season. Organisms forming the main scattering layer performed inverse diel vertical migration, with backscatter peaking at mid-depths during the day and in the deeper half of the water column at night. Target strength analyses suggested the backscatter was likely composed of fish larvae. Subsurface foraging behavior of the whale sharks within this aggregation has not been described, yet this study does not support the hypothesis that seasonal peaks in local whale shark abundance correspond to similar peaks in prey availability.

Keywords

acoustic backscatter; diel vertical migration; Red Sea; target strength; whale shark aggregation

1. Introduction

The whale shark, *Rhincodon typus*, is an iconic marine megafauna, being the world's largest fish and a docile filter-feeder mainly targeting zooplankton and micronekton (Colman, 1997; Martin, 2007; Rowat and Brooks, 2012). The species is listed as endangered by the IUCN Red List due to exploitation by targeted fisheries and poaching driven by demand in Asian markets and from bycatch fisheries (Chen & Phipps 2002, Pierce & Norman 2016). Conservation efforts need to be based on knowledge such as the species' ecology and life history, but targeted research on the whale shark has historically been limited by diffuse distribution across a wide, pelagic range (Stevens 2007). Modern whale shark study is often confined to the dozen-or-so known coastal aggregation sites, areas these sharks gather in large numbers for a predictable period (Rowat & Brooks 2012). One explanation for these seasonal, localized aggregations is that the whale sharks seek out "foraging hotspots" (Kahl et al. 2010) where they encounter high densities of

their planktonic prey (de la Parra Venegas et al. 2011, Robinson et al. 2013). High productivity (e.g. upwelling), high prey abundance, and predictable food pulse (e.g. spawning) have been suggested as motivations for aggregations in Australia (Taylor 1996, Wilson et al. 2002, Meekan et al. 2009), Mexico (Hoffmayer et al. 2007, de la Parra Venegas et al. 2011), Belize (Heyman et al. 2001, Graham et al. 2006), New Zealand (Duffy 2002), and Qatar (Robinson et al. 2013).

A recently discovered aggregation site in Saudi Arabia (Berumen et al. 2014) has provided a new opportunity to study whale sharks for improving their conservation management (Cochran et al. 2016, Sun et al. 2016). From February to May, juvenile whale sharks aggregate at Shi'b Habil, a nearshore reef (20.11°N, 40.22°E) close to Al-Lith, Saudi Arabia (Berumen et al., 2014). Individuals spotted in Al-Lith actively feed at the surface and form the basis of ongoing research into the ecology of this aggregation (Cochran et al. 2016). Satellite tags revealed whale sharks tagged in Al-Lith spend a short time (16.7%) at the air-sea interface (Berumen et al. 2014), similar to observations in Mexico (31.1%) (Motta et al. 2010). Thus surface foraging does not encompass their entire food source and subsurface prey abundance is important. To test the hypothesis that Al-Lith is a foraging hotspot for whale sharks, like many other aggregation sites, this study investigated the subsurface potential prey abundance during whale shark aggregation season.

Several oceanographic processes in the South Central Red Sea (SCRS) could create favorable conditions for zooplankton and micronekton aggregations to make Al-Lith a foraging hotspot. First, winter winds that converge near this latitude (19 °N) accumulate water, resulting in increased sea surface height (SSH) from December to February (Zhan, 2013; Zhan et al., 2014). Convergence zones and fronts can accumulate upward-swimming and positively buoyant organisms near the surface (Dandonneau et al., 2008) and provide a foraging hotspot for predators (Godø et al. 2012, Scales et al. 2014). At Ningaloo Reef in Western Australia, repeated whale shark sightings are made in frontal zones (Wilson et al., 2002). Second, winter mixing of Red Sea Deep Water (RSDW; Zhai and Bower, 2013) or water flowing into the Red Sea from the Indian Ocean (known as Gulf of Aden Intermediate Water (GAIW); Churchill et al., 2014; Sofianos and Johns, 2007; Yao et al., 2014) could bring nutrients into the upper water column and enhance biological productivity of the region. Additionally, anthropogenic input of nutrients and organic matter from a nearby local coastal aquaculture facility in Al-Lith (Hozumi et al. 2017) could increase

productivity. A prey biomass peak co-occurring with whale shark season would support the hypothesis that Al-Lith provides a high prey density area for effective foraging. Alternatively, the aquaculture wastewater might create an odor-plume, such as dimethyl sulfide, that may attract top marine predators (Dove, 2015; Hay, 2009; Martin, 2007; Savoca and Nevitt, 2014).

Acoustic backscatter was measured from mid-January to the end of April, covering most of whale shark aggregation season (late-February to mid-May). We assumed that the observed acoustic targets represent potential prey for whale sharks, and therefore are motivating factors for their aggregation. The objectives of this study were to 1) compare progression of acoustic backscatter abundance throughout the whale shark season, 2) describe the diel vertical variability of acoustic backscatter, 3) differentiate organisms responsible for the backscatter, 4) identify the taxonomic composition of zooplankton and their vertical distribution by net tows, and 5) investigate correlations between scatterers and environmental factors.

2. Materials and methods

2.1 Site selection

The annual whale shark aggregation near Al-Lith occurs mainly surrounding a reef known as Shi'b Habil (20.11°N, 40.22°E), located approximately 4 km offshore (Figure 1) (Berumen et al. 2014). The reef is 5 km long and 600 m wide with an eastward branching reef 1.8 km long on its northeast side. The inshore side of the reef is shallow and complex with steep coral structures dispersed on sandy bottom. Offshore of the reef the topography drops steeply from a plateau of a few meters depth to over 60 m depth. The echosounder was deployed at the northern end of this reef, where the highest whale shark detections occurred from a five-year acoustic tag effort (Cochran, unpublished data). The depth in the area around the echosounder deployment site is about 40 meters. The reef is ~4 km offshore from an aquaculture wastewater outfall. Detailed analysis of the aquaculture effluent before, during, and after the whale shark aggregation season would be necessary to assess any direct influence from the plume, and that aspect is not addressed in this paper.

2.2 Environmental data

An instrument package was used to profile physical and biological parameters at the northern end of Shi'b Habil every month during the echosounder deployment. The instrument package consisted of an Onset HOBO Water Level Data Logger for pressure (depth) and temperature, Seabird Microcat CTD 37SMP for temperature and conductivity (salinity), Sequoia Scientific Laser In-Situ Scattering and Transmissometry 100-X (LISST) for pressure (depth), particle size distribution, and particulate beam attenuation coefficient (c_p) (670 nm) with 5 cm path length, and WETlabs fluorometer/turbidity sensor (FLNTUB) for chlorophyll *a* fluorescence (chl_a) (ex 470/em 695 nm) and turbidity (700 nm). Measurements and sampling frequencies are summarized in Table 1. Data were merged according to their time stamps. The package descended one meter per 12 seconds to obtain one-meter resolution data. The chlorophyll and turbidity measurements were based on factory calibration and dark counts measured in the lab. To describe the stratification of the water column, mixed layer depths (MLD) were calculated with a density gradient criterion of 0.125 kg m^{-3} (Suga et al., 2004) and buoyancy frequency squared (N^2) was calculated:

$$N^2 = \frac{g}{\rho} \frac{\partial \rho}{\partial z}$$

where g is earth's gravitational acceleration, ρ is potential density, and z is depth. The environmental data was compared with the corresponding acoustic data.

2.3 Fixed moorings

To obtain a time series with higher temporal resolution than the monthly casts described above, two moorings collected environmental data ~3 km south of the echosounder mooring. They were fixed at ~10 m and ~6 m depth directly on the exposed and sheltered sides of Shi'b Habil, respectively (Figure 1). The moorings were equipped with CTDs for measuring temperature, conductivity, and pressure every 40 seconds and Fluorometer-Turbidity sensors (FLNTU), which measured chlorophyll *a* at excitation 470 nm/emission 695 nm and turbidity at 700 nm for particulate backscatter every hour for 20 seconds at 1Hz. Measurements were made continuously from January to May 2015. Data collection failed in 2014 due to battery failure and other instrument mishaps.

2.4 Echosounder deployment

High frequency echosounding is a non-intrusive sampling method that has the ability to observe zooplankton and micronekton (Axenrot et al., 2004). By deploying autonomous echosounder systems, such investigations can be carried out over extended periods of time. To obtain adequate temporal resolution for this study, we deployed bottom-mounted upward-looking echosounders to record patterns of zooplankton and micronekton abundance near the aggregation site prior to, during, and following the whale shark aggregation season. In this way we provide insight into the abundance and seasonality of acoustic backscatter, a proxy for prey populations, at this whale shark aggregation site.

A mooring with two SIMRAD EK 60 echosounders operating at 333 kHz and 120 kHz was deployed at the northern end of Shi'b Habil. The transceivers were mounted in a pressure-proof casing together with a computer; batteries were mounted in a separate container. The echosounders were operated on a pre-programmed duty cycle sampling for one hour every three hours to conserve battery power but still resolve the daily pattern of the vertical distribution. However, it appeared that the sampling did not strictly follow the pre-programmed duty cycle and sampled at random intervals; fortunately, the sampling still occurred with an appropriate resolution to achieve the objective. The mooring was deployed for deployment 1 (D1) on 15 January 2014, retrieved 10 March 2014 for battery charging, and re-deployed the next day (start of deployment 2 (D2)). Recording continued until the instrument package ran out of power on 30 April 2014, ending D2. Time was recorded in UTC (local time is UTC+3). Sampling rate was set to 1 ping s⁻¹, bin size 4.9 cm, and pulse length 0.256 ms. For deployment and retrieval purposes, the mooring was secured to an anchor and subsurface float. Mooring sites for D1 and D2 were located within 180 m of each other (Figure 1, 20.1337°N, 40.2089°E and 20.1326°N, 40.2102°E respectively), where D2 was (unintentionally) 100 m closer to the reef than D1. The instrument depths were at 44 and 38 m respectively. The echosounder was calibrated following SIMRAD protocol (Foote et al., 1987).

2.5 Data processing

The acoustic data was processed using the Sonar 5 Pro processing software (Balk and Lindem, 2002) and MATLAB (2012b). To compensate for periods without echoes as a consequence of the intermittent duty cycle, two weeks of data were averaged into bins of 30 seconds into one “daily” echogram. These averages were separated for the two deployments due to differences in deployment depths, giving

four averages for each deployment period. In the same way, a “daily” average echogram of each deployment was created to compare between D1 and D2.

Temporal variability of acoustic backscatter was analyzed. Data from the top 10 m were especially affected by wave-induced turbulence, therefore excluded from further analysis to minimize noise. The data from 10 m to bottom (the surface of the transducer) was in units of dB (log-scale) so it was back-transformed into linear values to calculate the mean and median for night and daytime, then log-transformed for comparison. Data collected an hour before sunrise to an hour before sunset was treated as daytime and the rest as nighttime. Since the data did not fit the assumptions of parametric tests, a Mann-Whitney U test was used to check whether there were significant differences between day and night acoustic backscatter and between deployments.

To obtain preliminary information on acoustic targets that formed a main, diel migrating scattering layer (SL), the target strength (TS) was measured when the layer resided near-bottom. The close proximity to the echosounder then enabled resolution of the acoustic data into individual targets. For this analysis we used Sonar5 Pro (Balk and Lindem, 2002) and non-averaged 120 kHz data. Isolated pings were captured using auto-mode with four to ten continuous pings and maximum one skipped ping. The compiled isolated pings (12718) were linear-transformed and calculated for mean, median, and standard deviation, and then log-transformed for comparison. The TS was analyzed separately for the two deployments and pings related to ascending and descending tracks were analyzed separately. A Mann-Whitney U test was used to test for differences in TS between the two deployments and between ascent and descent.

2.6 Multinet tow and visual inspection

The echosounder data was supplemented with vertical net tows to potentially identify the biotic scatterers responsible for the acoustic signals recorded by the echosounders. Zooplankton was sampled from 10:00–11:30 UTC on April 14, 2014, using a multinet with 200- μ m mesh size at position D2. The multinet opened and closed to capture three depth ranges [bottom–25 m; 25–15 m; 15 m–surface] in one cast and this was done for three replicate series. The nine samples were preserved with 90% ethanol in 50 mL individual tubes with screw-on caps. In the laboratory, all samples were analyzed and enumerated according to genera, and to species when possible. Enumeration was performed using a dissecting

microscope, tally counters, and a glass counting chamber. The abundance data (Appendix A) was log-transformed then analyzed using Primer6 (Clarke and Gorley, 2006). ANOSIM was used to test for significant pairwise relations between the three depths, and PERMANOVA to test whether depth factor or replicate factor was significant in species abundance. We created multidimensional scaling (MDS) maps to illustrate vertical patterns in species abundance.

In an attempt to visually inspect the main SL, a diver dove to 25 m depth near position D2 during the daytime. This depth was chosen as the previously-analyzed data (from D1) showed that the main SL occupied mid-waters in daytime. This effort that was done in addition to the multinet tow and the non-invasive echosounder observations did not help to identify the acoustic targets.

3. Results

3.1 Environmental data

Mooring data with high temporal resolution showed that temperature steadily increased from late February to May (26.1 to 32.2 °C) (Figure 2a). Salinity decreased from late March ($S > 39$) until it stabilized in May ($S \leq 38.65$). The water column was well mixed from January to April until stratification became evident in May (Figure 2b). During the sampling period, the absolute values of chlorophyll were relatively low ($< 0.9 \mu\text{g l}^{-1}$). Values were highest prior to and subsequent to the whale shark aggregation season. In January and May, chlorophyll fluorescence was homogeneously distributed by depth, while from February to April, chlorophyll was higher near the bottom (Figure 2b). c_p , a proxy for suspended particles, was homogenous except just above the seafloor. This layer near the bottom with mass of high-suspended particles had markedly higher salinity than waters above. This is clearly seen in the vertical profiles, except March, as a peak in salinity and c_p or scattering (Figure 2b).

3.2 Acoustic data

The acoustic data was characterized by high backscatter in the deeper half of the water column at night while backscatter peaked in mid-depth during the day (Figure 3). The main SL ascended to mid-water around dawn with the lower edge of the ~16 m thick scattering layer staying approximately 8 to 15 m above the bottom during the daytime. Around dusk, this SL descended to near-bottom waters and occupied

the deeper half of the water column throughout the night. This inverse diel vertical migration (IDVM) of the prevailing acoustic targets was found throughout the registration period and at both frequencies, although backscatter was stronger at 120 kHz than at 333 kHz (Figure 4). Apart from the surface noise that occasionally penetrated deep (20 m depth), the acoustic backscatter was lower near the surface compared to the main SL.

The TS of individuals forming the main SL suggests they are small acoustic targets. The median TS of these individuals at 120 kHz measured during the first deployment (D1) was significantly lower during descent (−66.5 dB) than ascent (−64.8 dB) (Mann-Whitney U, $Z=21.9$, $P<0.0001$). Similarly, median TS was significantly lower during descent (−73.7 dB) than ascent (−72.3 dB) during the second deployment (D2) period (Mann-Whitney U, $Z=12.6$, $P<0.0001$). The mode TS were −64.1, −69.5, −72.4, and −73.9, for D1 ascent, D1 descent, D2 ascent, and D2 descent, respectively (Figure 5). The TS from D2 (−72.7 dB) were significantly lower than D1 (−65.9 dB) (Mann-Whitney U, $Z=82.4$, $P<0.0001$).

No increase in acoustic backscatter or fluctuations in total backscatter was observed during the registration period. The mean and median backscatter during the day was significantly higher than the nighttime (Mann-Whitney U, $Z=-2.74$ and -2.94 , $P<0.01$). However, acoustic backscatter decreased by 13 dB for the 120 kHz frequency in the last ten days of D2 (Figure 6). This drop was not observed in the 333 kHz data during the same registration period.

Another discrepancy between 120 and 333 kHz observations was the decrease in baseline from D1 to D2. The 120 kHz data showed a noticeable drop in its baseline from −60 to −65 dB (Figure 6) after the second deployment in a position 180 m away from the first (Mann-Whitney U, $Z=10.1$, $P<0.0001$). This did not occur at 333 kHz, where the baseline remained the same between D1 and D2 (Mann-Whitney U, $Z=0.55$, $P=0.58$).

Likely fish schools (approximately −30 to −20 dB) were repeatedly observed, mostly below the main SL (Figure 7), causing apparent avoidance reactions among the organisms of the SL. There was no apparent pattern for their timing.

3.3 Zooplankton samples

Zooplankton data from multinet samples did not reveal clear vertical patterns. MDS plots did not form clusters (not shown) and ANOSIM and PERMANOVA results did not show differences between depth strata (ANOSIM Global $R=0.15$, $P=0.79$; PERMANOVA Pseudo- $F=1.1$, $P=0.40$). Looking separately at hard-shelled genera (most relevant acoustic targets) neither showed any difference between casts or depth cells. Copepods, pteropods, sergestid shrimps, euphausiids, chaetognaths, fish eggs, and fish larvae were present (Figure 8) with no distinct vertical distribution (Appendix A). To compare the vertical distribution of the acoustic backscatter with results from the net tow collected between 10:00–11:30 UTC April 14, 2014, we used echosounder data collected on April 14, 2014 11am UTC (not shown). This data showed the main SL was distributed from 10–35 m depths, which spans over all depth cells. The 15–25 m depth cell of the net tow best matches the main SL observed by the acoustic backscatter.

4. Discussion

We used echosounders to make continuous observations (mid-January to end of April) of acoustic backscatter, a proxy for prey biomass, over most of whale shark aggregation season (late-February to mid-May). Whale shark detections increased up to 16-fold at its peak in mid-April (Cochran, 2014), highlighting the relevance of the acoustic registration period. However, there was no apparent relation between acoustic backscatter and whale shark detections as the acoustic backscatter was consistent during D1 (mid-January to mid-March) and similarly, during D2 (mid-March to May), except for some trailing in backscatter at 120 kHz at the end of the registration period (Figure 6). Thus despite the oceanographic processes that could have contributed to a zooplankton and micronekton aggregation in Al-Lith (e.g. convergence zone, nutrient-rich water mass intrusion), we observed no increase in acoustic backscatter.

4.1 Backscatter below the neuston layer

Previous studies suggested whale sharks most likely forage at subsurface depth following prey distributions (Graham et al. 2006, Rowat & Gore 2007, Motta et al. 2010, Berumen et al. 2014). Tagged whale sharks in Al-Lith were subsurface (occupying depths of 2–50 m) ~56% of their time and dove regularly to 400 m when offshore (Berumen et al. 2014). They only spent ~16.7% of their time in 0–2 m depth (Berumen et al. 2014), where they were presumably feeding on the neuston layer. This is lower than

the 31.1% average spent at the surface (0–1 m depths) by tagged whale sharks off the Mexican Yucatan Peninsula, where the sharks dove to 2–6 m depths at nighttime most likely following DVM of local plankton (Motta et al. 2010). Off Ningaloo Reef, tagged whale sharks appeared to dive to forage on vertically migrating species seeking refuge at depths (>15 m) at night (Wilson et al., 2006). In Mozambique, tagged whale sharks spent nighttime in epipelagic depths and daytime in >200 m depths, as a response to the DVM of local prey (Brunnschweiler and Sims, 2011). These observations suggest the local prey's DVM influence the whale shark's vertical movement in the subsurface layers. Thus subsurface layers are important in their feeding ecology. But our results showed no peak in subsurface acoustic backscatter, a proxy for whale shark prey populations, during their aggregation season. This does not support our initial hypothesis that Al-Lith is a foraging hotspot due to high subsurface prey abundance.

The backscatter at 120 kHz became markedly reduced after recharge and redeployment for D2, which was unintentionally 100 m closer to the reef than D1. The TS of migrating individuals also decreased from D1 to D2 (Figure 5). Corresponding changes did not occur at 333 kHz. This suggests that a methodological problem between the deployments could have affected the measurements, although the two transducers were connected to the same batteries and computer and thus would have caused the same change in both frequencies, which was not our case. Alternatively, different responses at different frequencies could be explained biologically if more animals with swim bladder resonance (which at given sizes give strongly enhanced backscatter at given frequencies) at 120 kHz but not 333 kHz are present at the first deployment site than the second. This could be due to the different proximities of D1 and D2 from the reef and small scale patchiness that is pronounced at reef habitats (Pandolfi 2002). Regardless, this does not affect our conclusion that the temporal pattern of acoustic backscatter did not follow the temporal pattern of whale shark detections. The only possible relation was suggested by the decrease in backscatter at 120 kHz the last ten days of April (Figure 6), roughly coinciding with the trailing end of whale shark season. This decrease was only seen in the 120 kHz data and not in the 333 kHz data. If this were to be explained biologically, it would have related to a decrease in animals with specific resonance at 120 kHz but not at 333 kHz.

4.2 Inverse diel vertical migration by main scattering layer

The acoustic data revealed the IDVM of the main SL (Figure 3) through the use of non-intrusive sampling with bottom mounted upward-facing echosounders (Kaartvedt et al. 2009). IDVM is hypothesized to represent predator avoidance from animals performing normal DVM (Ohman et al., 1983). The net sampling and diving did not give clues to the taxonomic composition of the layer, suggesting that small organisms (not seen by divers) may be avoiding the net. The TS was reasonably strong, suggesting organisms with a swim bladder. Fish larvae are likely candidates, and such visual predators may ascend to forage in mid-waters at day and descend to apparent deeper and safer waters at night when they are not foraging. For comparison, the results on TS during D1 would correspond to 1 cm length perch larvae (*Perca fluviatilis* L.) with developing swim bladders which ranges from -70 to -63 dB (Frouzova and Kubecka, 2004), and higher than 3–4 cm length krill (*Euphausia superba*) which has -81 to -74 dB at 120 kHz (Figure 5) (Foote et al., 1990).

Whale sharks could have dynamic DVM in order to follow local prey DVM (Graham et al. 2006, Martin 2007, Rowat & Gore 2007). IDVM by basking sharks, *Cetorhinus maximus*, (Sims et al., 2005) and porbeagle sharks, *Lamna nasus* (Pade et al. 2009) have been observed where prey populations perform IDVM. These sharks demonstrated plasticity and switched to normal DVM when foraging in deeper stratified open water where the local SL performed normal DVM. In Al-Lith, two tagged whale sharks surfaced around dusk and dawn, and otherwise occupied the deeper depths (Sun et al. 2016). This short-term observation (22 hr) suggests whale sharks perform DVM that surfaces during the so-called antipredation window at dusk and dawn (Clark & Levy 1988). The antipredation window is a result of the trade-off between successful foraging and predator avoidance at intermediate light levels (Clark & Levy 1988, Scheuerell & Schindler 2003). Since whale sharks are unlikely avoiding predators, it is probable they follow prey populations that surface during dusk and dawn. Identifying a scattering layer or species that migrates in the antipredation window during whale shark aggregation season will be an interesting future research question. Multinet sampling with the aid of fluid imaging technology (e.g. FlowCam) or use of video plankton recorders to characterize the species composition at higher depth resolution for a daily cycle (i.e. sampling at midnight, sunrise, midday, sunset) may reveal species surfacing during the antipredation window and or identify any spawning or biological phenomenon that may attract whale sharks. Coupling

this with echosounder monitoring and a thermistor chain will characterize the vertical distribution of acoustic backscatter and temperature at higher temporal resolutions to complement the casts.

Additionally, investigating the frequency of subsurface feeding will help us understand the role of DVM on whale shark's diet. Previous studies have successfully monitored subsurface pelagic feeding for grey seals (*Halichoerus grypus*) through temperature loggers in the stomach (Austin et al. 2006), and examining stomach content for broadnose sevengill shark (*Notorynchus cepedianus*) using non-lethal stomach flushing methods (Barnett et al. 2010). But these methods require capturing and or anaesthetizing individuals, which will be difficult to implement on whale sharks. Use of tags measuring bulk electrical impedance inside the stomach successfully measured feeding and digestion events of captive tiger sharks (*Galeocerdo cuvier*) and sandbar sharks (*Carcharhinus plumbeus*) (Meyer & Holland 2012). Deploying these tags in parallel with tags that have been used previously to record depth (Brunnschweiler & Sims 2011, Sun et al. 2016), may be more applicable to resolve when and at what depths whale sharks feed at this aggregation.

4.3 Anthropogenic water intrusion

Monthly profiles of temperature, salinity, and chlorophyll suggest that the water column was physically well-mixed throughout our deployments. The integrated water column of chlorophyll fluorescence did not peak during the observation period. We observed no GAIW or RSDW temperature and salinity signatures during the deployment. Thus neither of these natural water masses, which might motivate whale shark aggregation through stimulation of phytoplankton biomass and growth, was observed. Instead, the plume water from the large coastal aquaculture facility, discharged from an outfall approximately 4.7 km away, was detected as the halocline associated with high concentrations of suspended particles near the bottom. This anthropogenic effluent has significantly different physico-chemical properties from the coastal water (Hozumi et al. 2017); concentrations such as dimethyl sulfide, that may attract top marine predators (Hay 2009, Dove 2015), should be measured to test whether the plume could alter whale shark distribution.

5. Conclusions

In conclusion, the autonomous acoustic echosounders monitored the temporal and vertical variability of biomass at a whale shark aggregation site in Al-Lith before, during, and subsequent to the whale shark season. No clear relation with whale shark abundance was evident, suggesting subsurface prey abundance did not increase during the aggregation season. Further work to resolve the times and depths of foraging by whale sharks in Al-Lith, to identify species performing DVM patterns that match whale shark foraging pattern (i.e. antipredation window), and to quantify dimethyl sulfide concentration in the aquaculture plume is recommended. The prevailing acoustic targets performed IDVM throughout the registration period and were most likely composed of larval fish, large zooplankton, or hard-shelled organisms. We reject the hypothesis that subsurface acoustic backscatter and whale shark detection peaks synchronously at this site, but foraging is still believed to be an important part of their motivation behind the seasonal aggregation. Foraging may be restricted to the surface at Shib Habil, and subsurface feeding may occur elsewhere. Future work should identify the surface plankton being targeted by actively feeding whale sharks. The responsible driver for the Al Lith whale shark aggregation remains unclear.

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Figure Captions

Fig. 1 A map of the Red Sea highlighting wind and oceanographic features relevant to Al-Lith, Saudi Arabia (the site of a seasonal aggregation of whale sharks), is shown. The anticyclonic eddy may upwell Red Sea Deep Water, particles (e.g., prey) may accumulate at the winter winds convergence zone, and Gulf of Aden Intrusion Water can supply nutrient-rich water along this coast. The locations of Shi'b Habil, a submerged reef in the Al-Lith area, the echosounder mooring sites, and the fixed mooring locations are illustrated in the inset

Fig.2 Environmental data recorded during echosounder deployments over the four monthly surveys associated with a whale shark aggregation. The (a) temperature and salinity time-series from fixed moorings show the seasonal variability at higher temporal resolution and b) the profiles of temperature, salinity, and optical measurements show homogenous water column until the stratification in May and optical measurements reflect this homogeneity except near the bottom where higher suspended particles are measured

Fig.3 Snapshots of the inverse diel vertical migration by a main scattering layer are presented. Echograms of ascent, vertical distribution during daytime, descent at dusk, and vertical distribution at night are shown in high resolution from the 120 kHz frequency data from D1. The time on the horizontal axis is in UTC and local time is UTC+3

Fig.4 Average daily echograms of the deployments, for 120 (a, c) and 333 (b, d) kHz echosounder data are shown. Strength of acoustic backscatter (dB) is shown as color-scheme. Data are recorded in UTC time (local time is UTC+3) and binned every 30 seconds. The two deployments have different depths, 44m and 38m for D1 (a, b) and D2 (c, d), respectively

Fig.5 A histogram of target strengths of individuals ascending and descending into the main scattering layer near dawn and sunset is represented for a) D1 and b) D2 for the 120 kHz dataset. The target strengths for a) 1 cm perch larvae (−70 to −63 dB; Frouzova and Kubecka, 2004) and for b) 3–4 cm krill (−81 to −74 dB; Foote, Everson, Watkins, and Bone, 1990) are shaded for comparison

Fig.6 A time-series of the mean and median of acoustic backscatter of 120 kHz (top) and 333 kHz (bottom) frequency are shown. Day and nights were 03:00–14:59 and 15:00–02:59 UTC, respectively. The acoustic backscatter between 10 m to bottom was included in the analysis. The two deployments are graphed continuously with a vertical line separating the two

Fig.7 Echograms of the 120 kHz data on January 17, 2014 that was one of the few observations made of the unidentified fauna not part of the main SL. It usually appeared below the main SL with approximately −30 to −20 dB acoustic backscatter (right). The main SL is seen avoiding this unknown target (left)

Fig.8 Abundance in counts of genera and species identified in multinet samples taken near Shi'b Habil in the Saudi Arabian Red Sea. Copepod *sp.* (e.g., Calanoid and Cyclopoid) were most abundant in number. Others include fish larvae, polychaete, crab larvae, ostracoda, etc.

Table 1 Measured variables and sampling frequencies of each instrument used for monthly hand casts at position D1

Monthly profile measurements		
Instrument	Variables measured	Sampling Frequency
Onset HOBO Water Level Data Logger	absolute pressure (kPa); temperature (°C)	1 Hz
Seabird Microcat CTD	temperature (°C); conductivity (s/m)	1/6 Hz
Sequoia LISST	particle size distribution; particulate beam attenuation coefficient (m^{-1}); temperature (°C)	1 Hz
WETlabs FLNTUB	chlorophyll <i>a</i> fluorescence ($\mu\text{g/l}$); turbidity (NTU)	1 Hz

Appendix A: zooplankton identification from multinet vertical net sampling

Zooplankton enumeration using microscopy revealed no vertical structure between the three depth cells sampled. Abundance (counts) of each identified species/genera/family is listed below. The replicate number of the cast is listed in the first column and the depth range sampled in the second column. Copepods were the most abundant in number.

Sample ID	Depth range (m)	Calanoid	Cyclopoid	Cyclopoid eggs	Oithana (Cyclopoid)	Cyclopoid (veins)	Ocartia (calanoid)	Mollusca	Appendicularia (Chordata)	Flatworm	Terapod	Chaetognata	Ghost shrimp	Crab larvae	Cirripod	Crustacean larvae	Jelly
C1D1	0-15	193	181	7	25	84	43	31	260	36	17	24	2	1	10	68	232
C2D1	0-15	487	117	3	26	210	43	26	605	21	27	28	8	8	7	170	4
C3D1	0-15	1648	286	3	40	503	82	73	1566	42	48	207	15	8	15	478	849
C1D2	15-25	681	1313	126	27	126	60	13	207	128	58	80	8	40	16	94	92
C2D2	15-25	293	98	2	15	18	61	13	209	18	12	15	3	2	1	27	67
C3D2	15-25	627	99	4	31	66	90	53	1029	25	44	84	14	12	15	159	312
C1D3	25-40	370	521	131	19	91	28	16	47	36	16	14	6	27	15	60	25
C2D3	25-41	1027	568	77	166	209	66	48	456	43	33	94	7	28	65	138	159
C3D3	25-42	373	122	7	26	48	61	17	345	25	11	39	3	14	36	62	51

Sample ID	Depth range (m)	Protozoan radiolaria	Single egg	Echinoderm	Brachiopoda	Polychaete	Ostracoda	Fish larvae	Centropages (calanoid)	Chiridius (calanoid)	Microcalanus (calanoid)	Harpacticoid (H)	Harpacticoid (D)	Izopod	Megalopa (larvae)	Paracalanus	Onychocorycaeus
C1D1	0-15	30	45	1	0	3	1	1	17	30	5	2	0	0	0	0	0
C2D1	0-15	76	69	4	6	11	10	4	18	28	2	1	2	1	0	0	8
C3D1	0-15	140	252	6	32	47	6	9	28	118	1	4	0	4	0	0	0
C1D2	15-25	72	8	2	4	5	2	5	8	22	1	0	0	0	0	0	0
C2D2	15-25	10	8	0	0	0	19	4	3	7	0	2	0	0	0	0	0
C3D2	15-25	98	48	4	0	16	44	13	5	22	4	0	0	0	0	0	0
C1D3	25-40	7	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C2D3	25-41	171	9	0	0	3	93	6	3	96	19	2	0	1	1	0	0
C3D3	25-42	18	12	0	0	1	34	2	2	46	12	1	0	0	0	1	0

b)

January

April

February

May

March

120 kHz Deployment 1

333 kHz Deployment 1

120 kHz Deployment 2

333 kHz Deployment 2

a) 120 kHz Acoustic backscatter from Jan 15- Apr 30, 2014

b) 333 kHz Acoustic backscatter from Jan 15- Apr 30, 2014